STATE OF WASHINGTON February 2020

# Population genetic analysis of Chehalis River watershed coho salmon (*Oncorhynchus kisutch*)

by Todd R. Seamons, Curt Holt, Lea Ronne, Amy Edwards, and Mike Scharpf

*Washington Department of*
*FISH AND WILDLIFE*
*Fish Program*

FPT 20-02

# Population genetic analysis of Chehalis River watershed coho salmon (*Oncorhynchus kisutch*)

Todd R. Seamons[1], Curt Holt[2], Lea Ronne[2], Amy Edwards[2], Mike Scharpf[2]

29 July 2019

Author information

[1]Washington Department of Fish and Wildlife, 600 Capitol Way N., Olympia, WA 98501

[2]Washington Department of Fish and Wildlife Region 6, 48 Devonshire Road, Montesano, WA 98563

## Acknowledgements

We greatly appreciate the efforts of Garrett Moulton, Megan Tuttle, Patrick Landback and the rest of WDFW District 17 spawning ground survey crew members who collected the samples. Thanks also to the hatchery staff at the Humptulips, Wishkah, Lake Aberdeen, Bingham Creek, and Skookumchuck hatcheries for their cooperation and assistance in genetic sampling. Thanks to Todd Kassler in the Washington Department of Fish and Wildlife Molecular Genetics Laboratory for managing contracts. And thank you to Alicia Terepocki for doing a fantastic job producing the genetic data.

This work was funded by the Washington State Legislature in Second Engrossed House Bill 1115 (Chapter 3, Laws of 2015) designated for study, analysis, and implementation of flood control projects in the Chehalis River Basin. Projects under this funding were selected to fill key information gaps identified in the Aquatic Species Enhancement Plan Data Gaps Report published in August 2014. Project funding was administered by the Washington State Recreation Conservation Office.

Cover photos courtesy of Keith Brady.

# Contents

# List of Tables

# List of Figures

## Executive summary

Understanding the population structure of wild salmon and steelhead (*Oncorhynchus* spp.) in the Chehalis Basin 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 coho salmon (*O. kisutch*) in the Chehalis River basin. Specifically, our objectives were to determine the genetic relationship of Chehalis Basin coho with available reference baseline coho collections in Washington State, to examine the spatial and temporal (among cohort) genetic structure of coho among the Chehalis River sub-basins, and to evaluate genetic relationships of coho with early and late return timing.

Samples were collected in 2017 and 2018 from throughout the Chehalis Basin, including fish from all major spawning tributaries and from the mainstem Chehalis River upstream of the proposed dam site at river mile 108.2. These samples represented fish that spawned mainly in 2014 and 2015. Genetic diversity was generally high, though late coho hatchery programs had lower estimated effective population sizes ($N_e$) and weak indication of Hardy-Weinberg and linkage disequilibrium typical of smaller (i.e., fewer broodstock) hatchery programs observed in other salmonid species.

Genetic data revealed that Chehalis Basin coho salmon are very different from Puget Sound coho salmon (the only available reference collections). This difference allowed us to detect two Puget Sound coho, one each in the Skookumchuck and Newaukum rivers. These fish likely represented unintentional releases of Puget Sound coho, which are temporarily reared at the Skookumchuck Hatchery.

Within the Chehalis Basin, population structure was complex with Chehalis coho displaying genetic structure related to cohort (brood year), spawning location, and run timing. Hatchery practices likely greatly influenced observed structure, especially for populations where hatchery broodstock were collected and direct plants of hatchery produced fish occurred. Most populations had differences among cohorts, i.e., 2017 collections were different from 2018 collections. This suggests that few jacks return to the Chehalis, that jacks have poor reproductive success, or both, and in the case of populations with hatchery programs, it suggests that those hatchery programs spawn few jacks as broodstock. Indeed, the jack fraction of hatchery broodstock for all programs was, on average, less than the target 10% for recent years (including 2014 and 2015, which produced the adults sampled in 2017 and 2018), but it was not zero for programs with available data. Spatially, most spawning tributaries were different from other spawning tributaries, including the upper Chehalis coho, which were collected from upstream of the proposed dam site. Other researchers have also noted that “upper Chehalis coho” were different from other Chehalis coho, though the collection location of their samples was either unknown or from tributaries downstream of the proposed dam site. Exceptions to the general pattern of spatial structure were mainly the late coho in rivers with late coho hatchery programs, i.e., the Humptulips, Satsop, and Skookumchuck rivers. In those locations, the late coho were genetically similar, likely a legacy of long-term releases and propagation of Satsop late coho at the Humptulips and Skookumchuck hatcheries. These locations, along with the Wishkah River, were also the only locations

where early and late timed coho were consistently genetically different, again, likely a legacy of hatchery practices. In the Wishkah River, the source broodstock of the early hatchery program is unknown, but could include fish from Puget Sound or the north Washington coast. Results hint of an out of Chehalis basin influence, especially for the 2018 cohort, but our ability to provide a more precise test was limited by a lack of a comprehensive reference baseline.

This study represents a comprehensive survey and genetic analysis of Chehalis Basin coho salmon populations, but some improvements could be made in the future. Sample sizes could be increased, particularly from smaller spawning tributaries. Given that among cohort differences were observed, sampling the third cohort for all locations could be required, especially if the future use of genetic stock identification was needed for within the Chehalis Basin. Improvements could be made in the spatial coverage of collections as well, particularly from tributaries of South Grays Harbor and mid-Chehalis tributaries. The Washington Department of Fish and Wildlife Molecular Genetics Laboratory is in the process of developing a statewide coho reference baseline. Once this reference baseline is expanded beyond Puget Sound and the Chehalis Basin, particularly to other systems near the Chehalis Basin (e.g., Quinault River to the north and Willapa River to the south), reanalysis of Chehalis Basin coho with the reference baseline would improve our understanding of how coho are related on a broader spatial scale and in particular how Chehalis Basin coho are related to other coho in Washington State. In addition, future analyses could incorporate power analysis to determine the ability to use genetic stock identification to assign coho of unknown origin to their source population among populations within the Chehalis Basin, but also among major systems or on a regional scale.

# Introduction

Understanding the population structure of 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 (*O. mykiss*) in the Chehalis Basin 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 Basin 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 coho (*O. kisutch*) in the Chehalis River basin. Previous genetic analyses in which Chehalis River coho salmon tissue collections were included were conducted using markers with limited power (allozymes or microsatellites), did not include all known or suspected spawning populations in the Chehalis Basin, and did not include temporally replicate collections to evaluate temporal stability (Beacham et al. 2001; Beacham et al. 2019; Beacham et al. 2017; Beacham et al. 2011; Teel et al. 2003; Van Doornik et al. 2007; Weitkamp et al. 1995). Here we examine genetic population structure of coho salmon 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, with most sampled in two separate years, and with collections from putative early (normal) and late spawning populations.

## Objectives

1. Evaluate the genetic relationship of Chehalis Basin coho salmon to other coho salmon populations in Washington State from where data are available.
2. Evaluate the genetic population structure of coho salmon within the Chehalis River basin with regard to spawning location (sub-basin).
3. Evaluate the genetic population structure of early and late runs of coho within the Chehalis River basin.

# Methods

## Study site

The Chehalis River basin 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). Coho salmon spawn throughout the watershed in small and medium sized rivers and streams (< 55 m channel width). Over the past decade, spawner abundance of coho throughout the Chehalis River basin has averaged 52,400 (19,300 – 88,000), which is above the escapement goal of 28,506 spawners (M. Scharpf, WDFW, unpublished data).

The Chehalis Basin has a long history of hatchery coho production that is ongoing. Over two million hatchery produced coho smolts are released each year (Table 1) comprising roughly 40% of all coho that outmigrated from the Chehalis Basin to the Pacific Ocean in 2015 and 2016 (brood years 2014 and 2015; Zimmerman 2015; Zimmerman 2016). Broodstock are collected and spawned in five different sub-basins and separate hatchery programs for early spawning and late spawning coho exist in three of those locations. Hatchery produced coho are released throughout the Chehalis River basin from hatchery locations where broodstock are collected and in many other locations where broodstock are not collected, including net pen releases in the lower mainstem and Grays Harbor and remote site incubators in small tributaries (Table 1). All WDFW Chehalis Basin coho hatchery programs are officially considered "integrated" (WDFW Future Brood Documents), meaning both hatchery- and natural-origin fish are intended for use as broodstock. Thus, for the purposes of analysis we assumed that hatchery- and natural-origin fish belonged to the same population, and hatchery- and natural-origin fish collected in the same place and time were considered part of the same collection for collection level analyses.

## Chehalis coho salmon tissue collections

Fin or opercle tissue was collected from live or dead adult or live juvenile coho throughout the Chehalis River watershed in 2017 and 2018 (Table 2). Based on previously published and unpublished coho salmon 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, collection efforts were focused on known spawning tributaries of the Chehalis River and the larger Chehalis Basin (i.e., including the Humptulips River) and not on the mainstem Chehalis River downstream of Pe Ell, Washington. Those tributaries were the Humptulips River, Hoquiam River, Wishkah River, Wynoochee River, Satsop River, Cloquallum Creek, Black River, Skookumchuck River, Newaukum River, South Fork Chehalis River (SF Chehalis), and the upper Chehalis River (Figure 1). Additional samples from small tributaries to the mid-Chehalis were also collected. Sample sizes from each of these locations were small, so they were all grouped together under the name Mid-Chehalis Tributaries (not shown on Figure 1 map). Though collected during the same efforts, Cloquallum Creek and Mid-Chehalis Tributary collections were genotyped under a different project, but were made available for this analysis.

In each tributary location, adult and occasionally jack salmon were captured in a weir trap (at hatchery locations), angled live, or found as spawned out carcasses and tissue sampled. In four locations, Black River, Newaukum River, SF Chehalis, and the Mid-Chehalis Tributaries, efforts of finding and sampling adult coho salmon carcasses failed to produce an adequate sample size for genetic analysis, so the collections were augmented by capturing juvenile coho via electrofishing. When electrofishing was used, sampling efforts were spread out in space as much as possible 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. Assignments of samples to either the early (normal) or late populations were made in the field based on the date with carcasses found before the last week of November called “early” and those found after called “late”. Early and late coho exist throughout the Chehalis River basin, so juveniles could be offspring of either group. Thus, juveniles were not assigned to early or late populations, but were considered unknown and likely mixed.

From each sampled fish, biological data including origin (hatchery or wild), sex (if possible), and fork length were obtained. Origin was determined by the presence or absence of the adipose fin (present in wild fish, absent from hatchery-produced fish) or coded wire tag (absent from wild fish and present in some hatchery-produced fish). Scales were taken for confirming origin and estimating age, and a small section of caudal fin was excised and immediately placed in 100% ethanol or on 3 mm Whatman chromatography paper. Juvenile and live adult fish were released back into the location from where they were captured. Fin clips in ethanol and on blotter paper were accessioned to the WDFW Molecular Genetics Laboratory archive and stored at room temperature.

## Non-Chehalis collections

Available non-Chehalis reference baseline coho data were limited to Puget Sound rivers and streams (Table 3). Collections were available from throughout Puget Sound, from South Puget Sound to north and included one collection from Hood Canal.

## Genetics laboratory processing

Chehalis coho samples were genotyped at the current WDFW statewide coho panel of 257 SNPs (Table 4) using a cost effective method based on custom amplicon sequencing called Genotyping in Thousands (GTseq; Campbell et al. 2015). This panel was ascertained using Lower Columbia River coho and the GTseq panel was developed by geneticists from the Columbia River Inter-Tribal Fish Commission (Campbell et al. 2017).

## Genotyping

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. Then, 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.

## Evaluation of loci/diversity metrics

To evaluate genetic qualities of loci, we quantified several genetic parameters in the Chehalis Basin coho salmon 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). A measure of the fractional reduction in heterozygosity due to inbreeding in individuals within a subpopulation and an additional indicator of systematic issues, $F_{IS,}$ 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. An additional indicator of deviations from HWE expectations are $F_{IS}$ values significantly greater or less than zero. 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 using GDA (Lewis and Zaykin 2001). Effective population size ($N_e$) was calculated using the linkage disequilibrium methods employed in the software NEESTIMATOR (LDNE; 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. $N_e$ estimated using LDNE is an estimate of the size of an ideal population with the same amount of genetic drift as the sampled population (variance $N_e$).

## Population genetic analysis

Population structure of coho salmon has been shown to be hierarchical, that is, genetic structure of salmonid populations exists at several hierarchical levels typically defined geographically (e.g., Beacham et al. 2011). Thus, our approach to evaluating and interpreting the population structure of Chehalis Basin coho populations was hierarchical. First, Chehalis Basin coho collections were compared to Puget Sound reference baseline collections. Second, population structure (among years and between early and late runs) within Chehalis Basin coho sub-basins (putative populations) was evaluated. Third, population structure among Chehalis Basin sub-basins was evaluated.

Population genetic analyses of Chehalis Basin coho 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 Basin coho 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 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, which is a commonly used metric that estimates subpopulation differentiation. These estimates were calculated and statistical significance was estimated by permutation tests using the *popStructTest* command of package STRATAG in R with 10,000 permutations (Archer et al. 2017; R Development Core Team 2017). As another measure of population structure, we calculated a pairwise 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. Cavalli-Sforza chord genetic distances assume that divergence is entirely due to genetic drift, i.e., no mutation, which is plausible. No available genetic distance model captures all reasonable assumptions of our data and the biology of coho salmon. 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 salmon and steelhead 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. Chehalis Basin Chinook salmon (*O. tshawytscha*) and steelhead both showed some evidence of isolation by distance (Brown et al. 2017; Seamons et al. 2017). In order to test the hypothesis of isolation by distance among coho 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. Because the Mid-Chehalis samples were taken from coho found in many different tributaries, Mid-Chehalis collections were left out of isolation by distance analyses. Since collections represented major tributaries of the Chehalis watershed, 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 there was evidence of genetic differences among cohorts (i.e., 2017 collections were different than 2018 collection in many locations; see Results), analyses were carried out separately by cohort. In addition, in a review of Grays Harbor coho hatchery programs, the Hatchery Scientific Review Group (HSRG) stated that Humptulips and Skookumchuck late coho were introduced from the Satsop River (HSRG 2004). Thus, analyses were conducted both with and without the Humptulips, Satsop, and Skookumchuck late coho collections.

# Results

## Tissue collections

A total of 1,006 samples from marked and unmarked coho salmon in Chehalis Basin tributaries were collected and processed as part of this project (Table 2). Genotyping success was fairly high (87.2%) resulting in 877 genotyped samples available for analysis. Collections with lower genotyping success were those mishandled in the field. Genotypes from an additional 123 Chehalis Basin coho salmon, obtained through a separate project, were also available (Table 2), increasing our spatial and temporal coverage of coho populations. Most tissues were taken from adults during their spawning season and most sampled adults were unmarked. With the exception of a single fish collected in the Newaukum River, hatchery-produced fish were sampled only where hatchery broodstock are collected, i.e., Humptulips, Wishkah, Satsop, and Skookumchuck rivers (hatchery broodstock are also collected in the Wynoochee River, but are not released there). Of collections taken in those locations, the Wishkah collections were notable in that the 2017 and 2018 early collections consisted almost entirely of hatchery produced fish (only one unmarked adult was taken for the 2018 early collection). In contrast, the Wishkah 2018 late collection consisted entirely of unmarked, naturally produced fish. Collections from four locations were augmented with juvenile samples taken by electrofishing (Table 2). Many of the collections with small sample sizes were pooled prior to analysis resulting in 34 different collections for analysis.

## Evaluation of loci/within-collection diversity

Samples were genotyped at 257 SNP loci. Of those, five loci were removed from further analysis because they were invariant, or nearly invariant (i.e., only one instance in one individual of a second allele), in all Washington coho genotyped to-date (Chehalis and Puget Sound). Another 21 loci were removed from analysis due to poor amplification of samples from all Chehalis River collections. Two loci, Oki_RAD37493-51 and Oki_RAD59556-32, showed statistically significant linkage disequilibrium in nearly every collection, suggesting that these loci are physically linked in Chehalis coho salmon. Both loci were left in the analysis. No other systematic scoring issues were identified. Analysis was conducted with the remaining 231 SNP loci.

Within collection genetic diversity was variable and reasonably high. The number of fixed alleles per collection varied from 1 to 21, but was correlated with collection sample size ($R^2$ = 0.45; Table 5). Within-collection average observed and expected heterozygosity varied from 0.342 to 0.382, with an overall mean of 0.363. Collections from locations where hatchery broodstock were collected, in particular collections from late fish, had higher counts of loci out of HWE and pairs of loci in LD before correcting for multiple tests (Table 5; HWE averages: hatchery late – 5.08%, hatchery early – 3.97%, all others – 3.49%; LD averages: hatchery late – 4.79%, hatchery early – 4.47%, all others – 3.72%), which is common in salmonid hatchery collections (WDFW, unpublished). Using ANOVA, we tested for differences among hatchery late collections, hatchery early collections, and all other collections combined; no statistically significant difference was found ($P$ = 0.18) and differences largely disappeared after correction for multiple tests (Table 5).

Estimates of $N_e$, an important indicator of the genetic health of a population ranged from -3,214.1 to 1,653.8 among collections within the Chehalis Basin (Table 5). Negative estimates of $N_e$ can occur when little to no linkage disequilibrium exists (i.e., no genetic drift) and are interpreted as infinitely large. Non-infinite estimates of effective population size ranged from 27.5 to 1,653.8. The 95% confidence intervals (95%CI) of most estimates also had infinite upper bounds. Average $N_e$ estimates of coho collections from tributaries where early hatchery broodstock is collected and from tributaries where no hatchery broodstock is collected were negative (i.e., infinite), while the average $N_e$ estimate for late coho collections where broodstock are collected was just 286.1. Indeed, five of seven late hatchery coho collections had $N_e$ estimates that were non-negative and whose 95%CI did not include infinity. The average $N_e$ of these five collections was 166.0 and the largest 95%CI upper bound was 447.9. Sample sizes for some collections were small enough to impact the accuracy and precision of the $N_e$ estimate. There was some support for pooling some collections within tributaries based on population structure results (see below). In particular, Hoquiam and Cloquallum collections had low and non-significant $F_{ST}$s among collections within the tributaries (Table 6), no within-tributary structure was revealed with individual based analyses (not shown), and there was high bootstrap support for the nodes separating these collections from all others (see below). Thus, for these two tributaries, collections were pooled and $N_e$ was re-estimated using the larger datasets. The updated estimate for Hoquiam River coho was 145.2 (104.8 – 232.9) and for Cloquallum Creek coho was 837.0 (249.0 – Inf).

## Genetic population structure

*Statewide* – PCA analysis with Chehalis and baseline coho revealed that Chehalis Basin coho clustered together separate from Puget Sound coho (Figure 2). This strong separation was also supported by individual analysis using STRUCTURE (Figure 3), by the large pairwise $F_{ST}$ estimate between Puget Sound

and Chehalis Basin coho collections (average Puget Sound-Chehalis pairwise $F_{ST}$ = 0.059 compared to average within Chehalis Basin pairwise $F_{ST}$ = 0.019), and by 100% bootstrap support for the node separating Puget Sound coho from Chehalis Basin coho in a dendrogram constructed using Cavalli-Sforza genetic chord distances (Figure 4).

PCA analysis also revealed two Chehalis coho samples (one adult sampled in the Skookumchuck River and one adult sampled in the Newaukum River) as Puget Sound origin fish. Rather than being Puget Sound fish that strayed into the Chehalis Basin, these fish were likely unintentionally released Puget Sound coho that were reared for a short time at the Skookumchuck Hatchery, which happens every year. Because the mechanism by which they ended up in the Chehalis Basin was unknown, both fish were left in the dataset.

*Chehalis Basin* – Chehalis Basin coho salmon had complex genetic structure, within which hatcheries figured strongly. The first two axes of PCA analysis showed three clusters, two of which had some overlap (Figure 5). Though there was some overlap, Axis 1 separated late coho from rivers where late coho hatchery broodstock are collected and where hatchery late coho are released (i.e., Humptulips/Satsop/Skookumchuck; hereafter "late hatchery coho") from all other Chehalis coho collections, early or late run. Axis 2 fairly cleanly separated Wishkah 2018 early run hatchery coho from all other Chehalis coho collections, including from the Wishkah 2017 early run hatchery coho. This same structure plus additional genetic structure was evident in clustering analysis using the algorithms of STRUCTURE (Figure 6). Nine clusters (K = 9) was the most strongly supported cluster number using the methods of Evanno et al. (2005). At K = 9, the most obvious clusters were the 2017 cohort of late hatchery coho, the 2018 cohort of late hatchery coho, the Wishkah 2017 early hatchery coho, and the Wishkah 2018 early hatchery coho. The remaining clusters, which were less clear, were Hoquiam River coho (early and late), upper Chehalis coho, Newaukum/South Fork/Mid-Chehalis tributary coho (all cohorts, and early and late), Satsop early coho (all three cohorts), an undefined ninth cluster.

These general clusters were also supported using genetic distances visualized in a neighbor-joining dendrogram (Figure 7). Temporal and spatial stability was evident in most locations where there is no hatchery broodstock collection including the Black River, Newaukum River, South Fork Chehalis River, and upper Chehalis River. Cloquallum Creek 2018 early and late coho clustered together, as did Hoquiam River 2018 early and late coho, both with strong bootstrap support. All Satsop River early collections clustered together with good bootstrap support as did all of the late hatchery coho, clustered together on one branch with each cohort (2010/2017 and 2018) on separate branches. Interestingly, the 2017 cohort of Wishkah early coho clustered with the 2017 cohort of Humptulips early coho (with strong bootstrap support), as did the 2018 cohorts of Wishkah and Humptulips early coho (with poor bootstrap support).

Isolation by distance among Chehalis River basin coho populations was evident (Figure 8). The positive relationship of genetic and geographic distance was stronger in 2017 ($P$ = 0.028, $R^2$ = 0.088) than in 2018 ($P$ = 0.103, $R^2$ = 0.02) and removing collections from sub-basins where late hatchery broodstock are collected improved the relationship in 2017 ($P$ = 0.008, $R^2$ = 0.25) but not in 2018 ($P$ = 0.07, $R^2$ = 0.03).

*Early and late run structure* – Early and late run coho were genetically different only in tributaries where late run coho hatchery broodstock are collected, i.e., the Humptulips, Satsop, and Skookumchuck rivers, and in the Wishkah River. In these locations, late coho clustered separately from early coho from the same year in the same location (Figure 6), nodes on the dendrogram separating early coho from the late

coho in the same year and location had strong bootstrap support (Figure 7), and pairwise $F_{ST}$s for early and late coho from the same year and location were relatively large and statistically significant (Table 6). Of the remaining locations, in those where early and late collections for the same cohort were taken (Hoquiam, Wynoochee, Cloquallum, Newaukum, and upper Chehalis), no genetic difference of early coho from late coho was detected. Support for this conclusion was indicated by no support for differences between early and late coho in clustering analysis (i.e., only one cluster detected and complete overlap of early and late coho in PCA analysis, per location-cohort; data not shown) and small, statistically non-significant pairwise $F_{ST}$s per location-cohort (Table 6). In the upper Chehalis River, though the pairwise $F_{ST}$ was small, it was statistically significant. In the South Fork Chehalis River, juvenile fish comprised the majority of the 2017 collection and thus the run timing of their parents was unknown. No structure was revealed with clustering analysis (not shown), but the degree to which the juvenile collection captured early and late run timing parents was unknown. Poor sample sizes of early and late coho in the Mid-Chehalis tributaries precluded comparison.

## Discussion

Using SNP genotypes, we 1) determined the genetic relationship of Chehalis Basin coho salmon to the available reference baseline collections (Puget Sound); 2) determined the genetic population structure of coho salmon among the sub-basins of the Chehalis watershed; and 3) determined the genetic relationship of early (normal) and late run coho salmon within the Chehalis Basin.

*Statewide* – Similar to what has been found before using different collections and markers, Chehalis Basin coho were genetically distinct from Puget Sound coho. For example, analysis using allozymes or microsatellites found the few Chehalis Basin coho populations analyzed had a relatively high genetic distance from Puget Sound coho and were clustered on distinct branches of the dendrogram (Weitkamp et al. 1995). More recent analysis using microsatellites and MHC loci found the same pattern (Beacham et al. 2001; Beacham et al. 2011; Van Doornik et al. 2002). Although their values of $F_{ST}$ estimates are not directly comparable to our results because different markers were used, Beacham et al. (2011) found, using microsatellites, the same higher pairwise Puget Sound – Chehalis average $F_{ST}$s than within-Chehalis pairwise $F_{ST}$s that we found using SNP markers. These previous analyses were limited in their coverage of populations within the Chehalis Basin. They appear to have been mainly from hatchery populations (for example, listed alternately as Simpson Hatchery or Bingham), or certainly from the sub-basins that produce most of the hatchery fish (Humptulips and Satsop), and no distinction was made between early or late runs, so direct comparison is difficult, but the pattern was consistent.

This clear distinction between Puget Sound and Chehalis Basin coho allowed us to detect at least two Puget Sound ancestry adults among the sampled Chehalis coho. These two fish were found one in the Skookumchuck River and one in the Newaukum River, which both receive hatchery fish from the Skookumchuck Hatchery. Puget Sound origin fish (mainly from the Wallace Hatchery, Snohomish Basin) destined for Squaxin Tribe net pens in south Puget Sound are reared for some time at the Skookumchuck Hatchery. Though we cannot be certain, it seems more likely that these fish represent some unknown number of Puget Sound fish unintentionally released in the Skookumchuck or Newaukum rivers rather than fish that failed to find their way home to Puget Sound.

*Chehalis Basin* – Within the Chehalis Basin, coho were structured by cohorts (brood years), by space among spawning tributaries, and by run timing. Differences among cohorts can arise when there is low

to no variability in age at maturity, i.e., no interbreeding among age classes. Coho in Washington tend to have mainly only two ages at maturity: total age 2, which are likely exclusively males (jacks), and total age 3, which are comprised of males and females (Weitkamp et al. 1995). If jacks are rare or reproduce at a very low rate, cohorts can become genetically distinct. Van Doornik et al. (2002) found that the effective contribution of jacks in two Puget Sound populations was ~35%, but speculated that given their results and frequency dependent selection (Gross 1985) that the contributions from jacks could be highly variable among years. Differences among cohorts in Chehalis Basin coho, and elsewhere have been previously observed (Van Doornik et al. 2002). In our study, differences among cohorts were most pronounced among populations with hatchery programs, but weren't consistent. Distinct cohorts were seen in the Humptulips and Wishkah early coho and in the late hatchery coho (Humptulips, Satsop, and Skookumchuck), but all three cohorts of Satsop early coho were more similar to one another than to other collections. The most pronounced differences among cohorts were seen in the Wishkah early coho and the late hatchery coho. The strength of all of these cohort differences suggests that the use of jacks in hatchery broodstock is very low. It was a common practice to exclude jacks from hatchery spawning in the past, which likely exacerbated any natural cohort distinction (e.g., Smith et al. 2015). Jack salmon also more easily slip through bars of weirs making them difficult to capture for use in spawning. With WDFW hatchery reform activities, a 10% jack use target was established for Chehalis coho hatchery programs. Available data show that the use of jacks in the Humptulips, Satsop, and Skookumchuck early and late programs is variable and below the 10% target on average, but the patterns of jack use as broodstock do not exactly fit the genetic data. For example, there is no clear difference in the percent of spawners that were jacks between Satsop early and Satsop late programs, but Satsop early cohorts are not distinct from one another while Satsop late cohorts are distinct from one another. Collections from the putative third cohort were only available for Satsop early and late coho (2010 collections). The 2010 Satsop late coho collection clustered with the 2017 late coho collection. Whether or not this is a general pattern in Chehalis Basin coho is unknown.

Genetic structure of Chehalis Basin coho among spawning tributaries was also present. Spatial structure arises because of the homing behaviors of salmonids. This allows evolutionary processes that can create differences among populations, such as selection or genetic drift, to occur. Chehalis Chinook salmon, chum salmon (*O. keta*), and steelhead showed variable levels of spatial genetic structure. Chehalis Basin Chinook salmon and chum salmon both lacked a clear signal of distinct spawning populations among spawning tributaries, but did show isolation by distance (Brown et al. 2017; Small et al. 2019). Chehalis Basin steelhead showed isolation by distance and clear genetic differences among tributaries originating in the three different mountain ranges drained by the Chehalis River: the Willapa Hills, Cascade Range, and Olympic Range (Seamons et al. 2017). Chehalis Basin coho showed structure among spawning tributaries, but the structure was inconsistent with known basin features and showed strong effects of hatchery practices. Coho from spawning locations without ongoing hatchery broodstock collection or hatchery fish releases, i.e., coho from the Hoquiam River, Cloquallum Creek, Black River, South Fork Chehalis River, and the upper Chehalis River, were different from each other and coho from all other locations to some degree. Satsop early coho were different from coho from other locations, but Satsop late coho were not. The Newaukum River, which receives hatchery produced coho from the Skookumchuck Hatchery, but does not yet have hatchery broodstock collection, had a mixed signal. Newaukum 2018 late coho clustered with Newaukum 2017 unknown run timing coho, but the Newaukum 2018 early coho clustered near the Satsop early coho and Skookumchuck 2017 early coho. The Wynoochee River, from which broodstock are collected, but which does not receive direct plants of

hatchery fish, also failed to show consistent structure with the 2017 early fish clustering with Hoquiam (early and late) and Wishkah 2018 late coho and the 2017 late fish clustering with the Black River collections. The Humptulips and Wishkah rivers, in which broodstock are collected and which receive direct plants of hatchery fish, clustered together or near one another depending on whether or not Puget Sound collections were included in the analysis. These patterns of spatial structure strongly suggest an impact of hatchery practices.

*Early versus Late* – Genetic differences among collections of fish with different run timing but that spawn in the same place may arise if there is reproductive isolation between the two groups (isolation by time, analogous to isolation by distance; Hendry and Day 2005). Strong selection may decrease the temporal overlap of fish spawning in the same location or run-timing could be correlated with small spatial scales such that temporal and spatial overlap are reduced resulting in reproductive isolation. In the Chehalis Basin, early and late run coho are recognized to exist throughout the basin. A passing survey of available redd count data from throughout the Chehalis spawning tributaries suggests that there could be a combination of isolation by time and space within sub-basins depending on the sub-basin, but more research is needed to better understand the data and whether or not the patterns seen in the data have meaning. The criterion for designating early or late in the field consists primarily of whether or not the fish spawned before or after the third or fourth week of November (~Thanksgiving holiday). Field designations match genetic patterns only in rivers where late hatchery coho broodstock are collected, i.e., the Humptulips, Satsop, and Skookumchuck rivers. In other locations, early and late coho do not appear to be genetically isolated such that only one genetic stock exists so a comparison with field designations is not applicable.

Humptulips, Satsop, and Skookumchuck late run coho were thought to be derived from Satsop River late run coho and genetic analysis agree; late coho collections from those locations are more similar to each other (by cohort) than they are to early coho in the same rivers or to late coho anywhere else in the basin. Elsewhere in the basin the early and late coho from the same location are not genetically different, so it is interesting that in the Humptulips, Satsop, and Skookumchuck rivers the differences between early and late coho are maintained. Hatchery spawning protocols could have narrowed the return and spawn timing of late coho producing reproductive isolation: late coho hatchery programs have stricter criteria that ends early coho spawning on roughly the same date as field designation but have a 28 day window between spawning of early and late coho from the same location (data queried from WDFW Hatcheries Headquarters Database: Adults.accdb on 29 April 2019). However, new redds are seen throughout this 28-day window even in the Humptulips, Satsop, and Skookumchuck rivers (C. Holt, WDFW, unpublished data), so it seems that in order to maintain the genetic differences in these rivers there must be spatial or other non-temporal isolating mechanisms. Late coho hatchery programs were small, i.e., spawned relatively few fish, typically less than half the number of spawners of early coho hatchery programs, and most broodstock were hatchery origin in all years for which data were available (average proportion of natural origin broodstock < 0.35; WDFW HEAT unit, personal communication). However, currently, the proportion of hatchery fish on the spawning grounds is fairly low, so overall the hatchery influence should be low (high proportionate natural influence [PNI], a metric that includes the proportion of hatchery fish spawning in the wild and the proportion of natural origin fish included as broodstock). Humptulips, Satsop, and Skookumchuck late coho consistently had smaller $N_e$ estimates than those of early coho from the same rivers and smaller than those of early or late coho from almost all other sampled sub-basins, and there was some weaker evidence that the late

hatchery coho collections had more deviations from HWE and a higher incidence of LD than did other populations. This was likely related to the relatively low number of broodstock used and the smaller $N_e$. This suggests that there has consistently been an increased probability for divergence due to genetic drift.

Wishkah River early coho collections were genetically different from the Wishkah River late collection. The Wishkah Hatchery early coho program (a.k.a., Mayr Brothers COOP) is a long running hatchery program whose original broodstock source is unknown, but could include fish from many different rivers within Puget Sound or from rivers on the north coast of Washington (HSRG 2004). The Wishkah 2018 early coho collection was quite different from all other Chehalis Basin coho collections, including the Wishkah 2017 early coho collection and Wishkah 2018 late coho collection. The Wishkah 2017 early collection was also different from all other coho collections, but not as much as the 2018 early collection. One possible cause for this difference could be that the current Wishkah early coho retained the genetic signature of their source population, especially the 2018 cohort. We did not have enough spatial coverage in our reference baseline to adequately test all possible source populations, but we did have Puget Sound collections. When compared to the Puget Sound collections, there was some indication that the Wishkah 2018 early cohort may have some Puget Sound influence; when Puget Sound collections were included, the Wishkah 2018 early collection, among all Chehalis coho collections, clustered closest to Puget Sound collections hinting at some influence of Puget Sound or other coho populations in the Wishkah 2018 early coho cohort. The 2017 and 2018 Wishkah early coho collections are also notable for consisting almost completely of hatchery-produced fish. To make sampling easier, early timed fish were sampled at the hatchery, justified by an *a priori* assumption that since the hatchery program was classified as integrated, hatchery-produced fish should be genetically indistinguishable from naturally spawning fish. If the Wishkah hatchery program was integrated as intended (i.e., unmarked fish are used as broodstock), then hatchery- and naturally produced fish should not be genetically different from one another. Unfortunately, we did not have the unmarked samples necessary to test this assumption. However, the 2018 early collection was quite different from the 2018 late collection, suggesting that, similar to the late programs in the Humptulips, Satsop, and Skookumchuck rivers and unlike in other spawning tributaries, spatial or other non-temporal isolating mechanisms must be occurring in the Wishkah River.

*Summary and future considerations* – This study represents a comprehensive survey and genetic analysis of Chehalis Basin coho salmon populations, but some improvements could be made in the future. Sample size of collections from many locations and for run timing were fairly small, often small enough that samples had to be combined for analysis (e.g., Mid-Chehalis tributaries and Newaukum River) or that juveniles with unknown run timing had to be sampled instead of adults (e.g., Black River and South Fork Chehalis). In addition, given that cohort differences existed in Chehalis Basin coho among the two sampled cohorts, a complete genetic survey needs to include collections from the third possible cohort and better cohort coverage for some of the populations surveyed for this study. Improvements could also be made in spatial coverage of spawning populations, in particular, tributaries of the south Grays Harbor (e.g., Johns River) and mid-Chehalis mainstem, which were not sampled. Given that we found some spatial genetic structure, we may be missing distinct components of the overall Chehalis Basin coho metapopulation. WDFW Molecular Genetics Laboratory is in the process of developing a WA statewide coho reference baseline. Once this reference baseline is expanded beyond Puget Sound and the Chehalis Basin, particularly to other systems near the Chehalis Basin (e.g., Quinault River to the

north and Willapa River to the south), reanalysis of the Chehalis Basin coho with the reference baseline would improve our understanding of how coho are related on a broader spatial scale and in particular how Chehalis Basin coho are related to other coho in Washington State. In addition, future analyses could incorporate power analysis to determine the ability to use genetic stock identification to assign coho of unknown origin to their source population among populations within the Chehalis Basin, but also among major systems or on a regional scale.

# References

Archer, F. I., P. E. Adams, and B. B. Schneiders. 2017. stratag: An r package for manipulating, summarizing and analysing population genetic data. Mol Ecol Resour 17(1):5-11.

Beacham, T. D., and coauthors. 2001. Evaluation and Application of Microsatellite and Major Histocompatibility Complex Variation for Stock Identification of Coho Salmon in British Columbia. Transactions of the American Fisheries Society 130(6):1116-1149.

Beacham, T. D., and coauthors. 2019. Comparison of coded-wire tagging with parentage-based tagging and genetic stock identification in a large-scale coho salmon fisheries application in British Columbia, Canada. Evolutionary Applications 12:230-254.

Beacham, T. D., and coauthors. 2017. Population and individual identification of Coho Salmon in British Columbia through parentage-based tagging and genetic stock identification: an alternative to coded-wire tags. Canadian Journal of Fisheries and Aquatic Sciences 74:1391-1410.

Beacham, T. D., M. Wetklo, L. Deng, and C. MacConnachie. 2011. Coho Salmon Population Structure in North America Determined from Microsatellites. Transactions of the American Fisheries Society 140(2):253-270.

Brown, S. K., T. R. Seamons, C. Holt, S. Ashcraft, and M. Zimmerman. 2017. Population genetic analysis of Chehalis River basin Chinook salmon (*Oncorhynchus tshawytscha*). Washington Department of Fish and Wildlife, FPT 17-13, Olympia, Washington.

Campbell, N. R., S. A. Harmon, and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15(4):855-867.

Campbell, N. R., C. Kamphaus, K. Murdoch, and S. R. Narum. 2017. Patterns of genomic variation in Coho salmon following reintroduction to the interior Columbia River. Ecology and Evolution 7(23):10350-10360.

Do, C., and coauthors. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size ($N_e$) from genetic data. Molecular Ecology Resources 14(1):209-214.

Earl, D., and B. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611 - 2620.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package). Department of Genetics, University of Washington, Seattle, WA.

Garza, J. C., and coauthors. 2014. Population Structure of Steelhead in Coastal California. Transactions of the American Fisheries Society 143(1):134-152.

Gross, M. R. 1985. Disruptive selection for alternative life histories in salmon. Nature 313:47-48.

Heath, D. D., S. Pollard, and C. Herbinger. 2001. Genetic structure and relationships among steelhead trout (*Oncorhynchus mykiss*) populations in British Columbia. Heredity 86:618-627.

Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: isolation-by-time and adaptation-by-time. Molecular Ecology 14(4):901-916.

HSRG. 2004. Hatchery Scientific Review Group Puget Sound and Coastal Washington Hatchery Reform Recommendations Grays Harbor.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801-1806.

Lewis, P. O., and D. Zaykin. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Pages Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html *in*.

Pritchard, J., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945 - 959.

R Development Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49(6):1280-1283.

Rosenberg, N. A. 2004. distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4(1):137-138.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8(1):103-106.

Seamons, T. R., C. Holt, S. Ashcraft, and M. Zimmerman. 2017. Population genetic analysis of Chehalis River watershed winter steelhead (*Oncorhynchus mykiss*). Washington Department of Fish and Wildlife, Olympia, Washington.

Small, M. P., and coauthors. 2019. Chum salmon population structure in the Chehalis Basin. Washington Dept. of Fish & Wildlife.

Smith, C. T., and coauthors. 2015. Eliminating variation in age at spawning leads to genetic divergence within a single salmon population. Journal of Fish and Wildlife Management 6(1):n/a-n/a.

Teel, D. J., D. M. Van Doornik, and D. Kuligowski. 2003. Genetic analysis of juvenile coho salmon (Oncorhynchus kisutch) off Oregon and Washington reveals few Columbia River wild fish. Fishery Bulletin 101:640-652.

Van Doornik, D. M., M. J. Ford, and D. J. Teel. 2002. Patterns of temporal genetic variation in coho salmon: estimates of the effective proportion of 2-year-olds in natural and hatchery populations. Transactions of the American Fisheries Society 131:1007-1019.

Van Doornik, D. M., D. J. Teel, D. R. Kuligowski, C. A. Morgan, and E. Casillas. 2007. Genetic analyses provide insight into the early ocean stock distribution and survival of juvenile coho salmon off the coasts of Washington and Oregon. North American Journal of Fisheries Management 27:220-237.

Verhoeven, K. J. F., K. L. Simonsen, and L. M. McIntyre. 2005. Implementing false discovery rate control: increasing your power. Oikos 108(3):643-647.

Wang, J. 2013. An improvement on the maximum likelihood reconstruction of pedigrees from marker data. Heredity 111:165-174.

Wang, J., and A. W. Santure. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 181(4):1579-1594.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6):1358-1370.

Weitkamp, L. A., and coauthors. 1995. Status review of coho salmon from Washington, Oregon, and California. U.S. Dept. Commer., NMFS-NWFSC-24.

Zimmerman, M. 2015. 2016 Wild coho forecasts for Puget Sound, Washington Coast, and Lower Columbia. Washington Dept. of Fish and Wildlife.

Zimmerman, M. 2016. 2017 Wild coho forecasts for Puget Sound, Washington Coast, and Lower Columbia. Washington Dept. of Fish and Wildlife.

Table 1. Details of coho salmon hatchery activity in the Chehalis River basin. Data come from the Future Brood Documents for 2014 and 2015 (https://wdfw.wa.gov/fishing/management/hatcheries/future-brood).

| Facility | Operator[a] | Program spawn timing[b] | Broodstock source | Release river[c] | Avg PNI[d] | Release n[e] |
|---|---|---|---|---|---|---|
| Humptulips Hatchery | WDFW | early | Humptulips early | Humptulips | 0.47 | 400,000 |
| Humptulips Hatchery | WDFW | late | Humptulips late | Humptulips | 0.33 | 100,000 |
| Bingham Creek Hatchery | WDFW | early | Satsop early | Satsop | 0.47 | 150,000 |
| Satsop Springs Hatchery | WDFW | early | Satsop early | Satsop | NA | 450,000 |
| Friends Landing net pens | COOP | early | Satsop early | Lower Chehalis | NA | 25,000 |
| Westport net pens | COOP | early | Satsop early | Lower Chehalis | NA | 100,000 |
| Bingham Creek Hatchery | WDFW | late | Satsop late | Satsop | 0.80 | 150,000 |
| Skookumchuck Hatchery | WDFW | early | Skookumchuck early | Skookumchuck | 0.54 | 50,000 |
| Carlisle Lake | COOP | early | Skookumchuck early | SF Newaukum | 0.78 | 50,000 |
| Byrd Creek Project | COOP | early | Skookumchuck early | NF Newaukum | NA | 25,000 |
| Skookumchuck Hatchery | WDFW | late | Skookumchuck late | Skookumchuck | 0.49 | 50,000 |
| Carlisle Lake | COOP | late | Skookumchuck late | SF Newaukum | 0.87 | 50,000 |
| Eight Creek Pond | WDFW | late | Skookumchuck late | Upper Chehalis | NA | 100,000 |
| Mayr Brothers Rearing Pond | COOP | early | Wishkah early | Wishkah | 0.03 | 300,000 |
| Buzzard Creek | COOP | early | Wishkah early | Lower Chehalis | NA | 25,000 |
| Grays Harbor Gillnet | COOP | early | Wishkah early | Lower Chehalis | NA | 190,000 |
| Lake Aberdeen Hatchery | WDFW | early | Wynoochee early | Van Winkle Creek | 1.00 | 30,000 |
| | | | | | sum | 2,245,000 |

**[a]- COOPs are public/private cooperatives operated by WA State citizens with WDFW help and oversight.**

**[b]- Unlike calls in the field, spawn timing in the hatcheries is strictly controlled with an average of 28 days between the last day of early spawning and the first day of late spawning**

**[c]- More detailed release sites are available, but they are within the listed sub-basins.**

**[d]- PNI stands for Proportionate Natural Influence, which is a function of the proportion of hatchery fish on the spawning grounds and the proportion of hatchery broodstock that are of natural origin. PNI ranges from 0 to 1, with a zero meaning all hatchery fish and a one meaning all wild fish. These data were obtained from WDFW HEAT Unit, Personal Communication.**

**[e]- Release n are planned release numbers for each program and do not precisely reflect actual release numbers. These numbers were obtained from the Future Brood Documents for 2014 and 2015, the spawn years that produced the adult coho returning in 2017 and 2018. (https://wdfw.wa.gov/fishing/management/hatcheries/future-brood)**

Table 2. List of Chehalis Basin coho salmon tissue collections used in genetic analysis.

| Chehalis sub-basin | WDFW MGL codes | Life stage | Spawn year | Spawn timing[a] | N processed | N genotyped | Genotyping success (%) |
|---|---|---|---|---|---|---|---|
| Humptulips | 17KM, 17OG | Adult | 2017 | early | 17 | 14 | 82.4 |
| Humptulips | 18EM, 18EV | Adult | 2018 | early | 32 | 26 | 81.3 |
| Humptulips | 17KU | Adult | 2017 | late | 46 | 42 | 91.3 |
| Humptulips | 18EM, 18EV | Adult | 2018 | late | 46 | 42 | 91.3 |
| Hoquiam | 18PN | Adult | 2018 | early | 12 | 12 | 100.0 |
| Hoquiam | 18PN | Adult | 2018 | late | 16 | 16 | 100.0 |
| Wishkah | 17KZ | Adult | 2017 | early | 46 | 46 | 100.0 |
| Wishkah | 18EY, 18MY | Adult | 2018 | early | 46 | 44 | 95.7 |
| Wishkah | 18MY | Adult | 2018 | late | 17 | 17 | 100.0 |
| Wynoochee | 17KJ | Adult | 2017 | early | 16 | 14 | 87.5 |
| Wynoochee | 17KJ | Adult | 2017 | late | 25 | 24 | 96.0 |
| Satsop | 17KI, 17KW | Adult | 2017 | early | 46 | 43 | 93.5 |
| Satsop | 18ER | Adult | 2018 | Early | 46 | 39 | 84.8 |
| Satsop | 17KV, 17KW | Adult | 2017 | Late | 46 | 37 | 80.4 |
| Satsop | 18ER, 18EW | Adult | 2018 | Late | 46 | 38 | 82.6 |
| Black | 18ET | Adult | 2018 | Early | 32 | 7 | 21.9 |
| Black | 18ET | Adult | 2018 | Late | 6 | 2 | 33.3 |
| Black | 18PQ | Juvenile | 2017 | Unknown | 40 | 40 | 100.0 |
| Skookumchuck | 17KN, 17KY | Adult | 2017 | early | 47 | 44 | 93.6 |
| Skookumchuck | 17KT, 17KY | Adult | 2017 | late | 47 | 44 | 93.6 |
| Skookumchuck | 18EN, 18EX | Adult | 2018 | late | 48 | 40 | 82.8 |
| Newaukum | 17KO | Adult | 2017 | early | 1 | 1 | 100.0 |
| Newaukum | 18EO | Adult | 2018 | early | 15 | 12 | 80.0 |
| Newaukum | 17KS | Adult | 2017 | late | 10 | 10 | 100.0 |
| Newaukum | 18EO | Adult | 2018 | late | 24 | 24 | 100.0 |
| Newaukum | 18EH | Juvenile | 2017 | Unknown | 25 | 23 | 92.0 |
| SF Chehalis | 17KP | Adult | 2017 | early | 1 | 1 | 100.0 |
| SF Chehalis | 18EU | Adult | 2018 | early | 3 | 3 | 100.0 |

| Chehalis sub-basin | WDFW MGL codes | Life stage | Spawn year | Spawn timing[a] | N processed | N genotyped | Genotyping success (%) |
|---|---|---|---|---|---|---|---|
| SF Chehalis | 17KP | Adult | 2017 | late | 1 | 1 | 100.0 |
| SF Chehalis | 18EP, 18EU | Adult | 2018 | late | 58 | 48 | 82.8 |
| SF Chehalis | 18EI | Juvenile | 2017 | Unknown | 23 | 23 | 100.0 |
| Upper Chehalis | 18EQ | Adult | 2018 | early | 26 | 21 | 80.8 |
| Upper Chehalis | 17KQ | Adult | 2017 | late | 48 | 34 | 70.8 |
| Upper Chehalis | 18EQ | Adult | 2018 | late | 48 | 45 | 93.8 |
| Additional Chehalis Basin coho salmon collections available for use in this project | | | | | | | |
| Satsop | 10LC | Adult | 2010 | early | NA | 31 | 96.9 |
| Satsop | 10LF | Adult | 2010 | late | NA | 21 | 65.6 |
| Cloquallum | 17KH | Adult | 2017 | early | NA | 1 | 50.0 |
| Cloquallum | 18PO | Adult | 2018 | early | NA | 10 | 90.9 |
| Cloquallum | 17KH | Adult | 2017 | late | NA | 5 | 83.3 |
| Cloquallum | 18EU, 18PO | Adult | 2018 | late | NA | 9 | 81.8 |
| Mid-Chehalis tributaries | 18EU | Adult | 2018 | early | NA | 3 | 75.0 |
| Mid-Chehalis tributaries | 18PP | Juvenile | 2017 | Unknown | NA | 11 | 100.0 |
| Mid-Chehalis tributaries | 17OF | Adult | 2017 | late | NA | 2 | 50.0 |
| Mid-Chehalis tributaries | 18EU | Adult | 2018 | late | NA | 30 | 68.2 |

**[a]- Spawn timing is a designation made in the field based on a rough cutoff date around the fourth week in November. All juvenile samples were designated unknown spawn timing.**

Table 3. Puget Sound baseline reference coho salmon collections available for analysis.

| Population | River Basin | location | WDFW code | Sampling year | N |
|---|---|---|---|---|---|
| Nooksack River - Skookum Creek Hatchery | Nooksack | North Puget Sound | 03JZ | 2003 | 19 |
| Goodell Creek | Skagit | North Puget Sound | 03MG | 2003 | 28 |
| Skagit River | Skagit | North Puget Sound | 17PT | 2017 | 140 |
| Stillaguamish River | Stillaguamish | North Puget Sound | 17LB | 2017 | 57 |
| Sunset Falls - Skykomish River | Snohomish | North Puget Sound | 17HM | 2017 | 98 |
| Skykomish, Snoqualmie rivers | Snohomish | North Puget Sound | 17HM, 17LA | 2017 | 118 |
| Johns Creek | Johns Creek | South Puget Sound | 03KD | 2003 | 26 |
| John Creek | Hamma Hamma | Hood Canal | 03JR | 2003 | 23 |

Table 4. List of loci from the coho GTseq SNP panel (Campbell et al. 2017) used in analysis. Twenty-six additional loci were dropped from analysis because they were invariant in Chehalis and Puget Sound coho or because of low genotyping success.

| Locus name | Locus name | Locus name |
|---|---|---|
| Oki_100771-83 | Oki_gshpx-152 | Oki_RAD65388-37 |
| Oki_100974-293 | Oki_hsc713-56 | Oki_RAD65610-58 |
| Oki_101419-103 | Oki_hsc71p-313 | Oki_RAD65902-30 |
| Oki_101770-525 | Oki_hsf1b-85 | Oki_RAD66265-54 |
| Oki_102195-92 | Oki_itpa-85 | Oki_RAD66663-68 |
| Oki_102267-166 | Oki_nips-159 | Oki_RAD66994-58 |
| Oki_102457-67 | Oki_pigh-33 | Oki_RAD67081-48 |
| Oki_102801-511 | Oki_pop5-265 | Oki_RAD67114-64 |
| Oki_103271-161 | Oki_RAD100310-36 | Oki_RAD67674-60 |
| Oki_103577-70 | Oki_RAD100331-48 | Oki_RAD68190-55 |
| Oki_103713-182 | Oki_RAD100388-66 | Oki_RAD69161-64 |
| Oki_104515-99 | Oki_RAD100479-50 | Oki_RAD69355-42 |
| Oki_104519-45 | Oki_RAD100507-58 | Oki_RAD70262-64 |
| Oki_105105-245 | Oki_RAD101032-66 | Oki_RAD70338-46 |
| Oki_105115-49 | Oki_RAD101136-60 | Oki_RAD70600-60 |
| Oki_105132-169 | Oki_RAD101478-57 | Oki_RAD70820-47 |
| Oki_105407-161 | Oki_RAD101607-49 | Oki_RAD70963-47 |
| Oki_106172-60 | Oki_RAD104335-44 | Oki_RAD71346-63 |
| Oki_106313-353 | Oki_RAD104946-41 | Oki_RAD71442-69 |
| Oki_106419-292 | Oki_RAD106191-62 | Oki_RAD71948-56 |
| Oki_106479-278 | Oki_RAD106666-44 | Oki_RAD72095-45 |
| Oki_106747-503 | Oki_RAD109528-59 | Oki_RAD72101-67 |
| Oki_107607-213 | Oki_RAD111744-32 | Oki_RAD72759-48 |
| Oki_107974-46 | Oki_RAD115799-69 | Oki_RAD72979-40 |
| Oki_109525-359 | Oki_RAD16167-62 | Oki_RAD73094-68 |
| Oki_109651-152 | Oki_RAD17541-50 | Oki_RAD73130-59 |
| Oki_109894-418 | Oki_RAD23788-32 | Oki_RAD73234-42 |
| Oki_110078-191 | Oki_RAD25212-35 | Oki_RAD75909-38 |
| Oki_110381-77 | Oki_RAD27801-45 | Oki_RAD75911-69 |
| Oki_111312-141 | Oki_RAD29028-42 | Oki_RAD76218-42 |
| Oki_111681-407 | Oki_RAD29136-50 | Oki_RAD77207-61 |
| Oki_113457-324 | Oki_RAD34432-38 | Oki_RAD77210-64 |
| Oki_113979-170 | Oki_RAD345-59 | Oki_RAD77803-60 |
| Oki_114250-187 | Oki_RAD35219-62 | Oki_RAD77883-62 |
| Oki_114448-101 | Oki_RAD35990-63 | Oki_RAD78112-64 |
| Oki_114587-309 | Oki_RAD36669-48 | Oki_RAD78543-33 |
| Oki_115987-366 | Oki_RAD37278-54 | Oki_RAD79761-66 |
| Oki_116362-411 | **Oki_RAD37493-51**[a] | Oki_RAD80460-54 |
| Oki_117043-374 | Oki_RAD37537-45 | Oki_RAD80645-70 |

| Locus name | Locus name | Locus name |
|---|---|---|
| Oki_117144-64 | Oki_RAD40179-68 | Oki_RAD80982-68 |
| Oki_117742-259 | Oki_RAD41030-31 | Oki_RAD81387-37 |
| Oki_117815-369 | Oki_RAD41603-39 | Oki_RAD82856-48 |
| Oki_118152-314 | Oki_RAD42204-39 | Oki_RAD83766-63 |
| Oki_118654-330 | Oki_RAD44268-51 | Oki_RAD83875-36 |
| Oki_120024-226 | Oki_RAD44444-52 | Oki_RAD84577-58 |
| Oki_121006-412 | Oki_RAD45691-45 | Oki_RAD85448-48 |
| Oki_122138-111 | Oki_RAD45878-53 | Oki_RAD85949-47 |
| Oki_123044-68 | Oki_RAD46160-48 | Oki_RAD86627-60 |
| Oki_123921-90 | Oki_RAD46744-47 | Oki_RAD87141-55 |
| Oki_124162-62 | Oki_RAD46974-68 | Oki_RAD87446-62 |
| Oki_125998-340 | Oki_RAD49111-64 | Oki_RAD87621-67 |
| Oki_126160-142 | Oki_RAD49348-51 | Oki_RAD87777-48 |
| Oki_126619-265 | Oki_RAD51428-47 | Oki_RAD88551-51 |
| Oki_127645-235 | Oki_RAD51585-47 | Oki_RAD89259-51 |
| Oki_128302-547 | Oki_RAD52040-63 | Oki_RAD89374-40 |
| Oki_128693-70 | Oki_RAD52785-52 | Oki_RAD91362-68 |
| Oki_128757-232 | Oki_RAD53121-66 | Oki_RAD91430-44 |
| Oki_128851-185 | Oki_RAD53655-42 | Oki_RAD91470-66 |
| Oki_129870-552 | Oki_RAD53703-50 | Oki_RAD91478-52 |
| Oki_130113-304 | Oki_RAD53750-45 | Oki_RAD91907-38 |
| Oki_130295-48 | Oki_RAD54417-49 | Oki_RAD92875-31 |
| Oki_130524-184 | Oki_RAD54918-40 | Oki_RAD93028-59 |
| Oki_131147-353 | Oki_RAD55090-49 | Oki_RAD94215-66 |
| Oki_131460-243 | Oki_RAD55690-46 | Oki_RAD94241-30 |
| Oki_131906-261 | Oki_RAD56094-43 | Oki_RAD96072-42 |
| Oki_94903-192 | Oki_RAD57307-33 | Oki_RAD96498-69 |
| Oki_95318-100 | Oki_RAD57956-47 | Oki_RAD97325-35 |
| Oki_96158-278 | Oki_RAD58310-55 | Oki_RAD97993-40 |
| Oki_96376-63 | Oki_RAD59054-54 | Oki_RAD98280-45 |
| Oki_97660-149 | **Oki_RAD59556-32**[a] | Oki_RAD99931-47 |
| Oki_afp4-10 | Oki_RAD59920-68 | Oki_sast-230 |
| Oki_arp-105 | Oki_RAD59945-45 | Oki_SECC22-67 |
| Oki_aspAT-273 | Oki_RAD60246-68 | Oki_srp09-107 |
| Oki_bcAKal-274 | Oki_RAD61746-62 | Oki_sys1-141 |
| Oki_ca050-17 | Oki_RAD61821-61 | Oki_taf12-40 |
| Oki_gdh-189 | Oki_RAD64084-65 | Oki_thyK-100 |
| Oki_gh-183 | Oki_RAD64627-67 | Oki_txnip-35 |

**[a]-These two loci were in statistical linkage disequilibrium in nearly all collections suggesting they are physically linked.**

*Table 5. Genetic metrics and statistics for Chehalis Basin coho salmon collections*

| Population or stock[a] | Spawn year | Spawn timing | n | Fixed loci (%) | Mean $N_A$ | He | Ho | $F_{IS}$ | HWE p<0.05 | HWE p<0.05 | LD p<0.05 | LD p<0.05 | LDNe[b] (95%CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Humptulips | 2017 | Early | 14 | 8 (3.5) | 1.94 | 0.375 | 0.357 | 0.047 | 0.94 | 0.00 | 3.11 | 0.000 | 1653.8 (165.2 - Inf) |
| Humptulips | 2017 | Late | 42 | 5 (2.2) | 1.95 | 0.374 | 0.363 | 0.033 | 7.14 | 0.00 | 4.87 | 0.004 | 1289.6 (435.4 - Inf) |
| Humptulips | 2018 | Early | 26 | 1 (0.4) | 1.97 | 0.376 | 0.359 | 0.046 | 7.69 | 0.00 | 3.75 | 0.004 | -324.8 (-1784.2 - Inf) |
| Humptulips | 2018 | Late | 40 | 6 (2.6) | 1.95 | 0.358 | 0.346 | 0.034 | 5.45 | 0.45 | 5.37 | 0.008 | 122.4 (101.1 - 153.9) |
| Hoquiam | 2018 | Early | 12 | 13 (5.7) | 1.92 | 0.372 | 0.374 | -0.008 | 2.39 | 0.00 | 2.86 | 0.000 | 62.9 (42.8 - 114.0) |
| Hoquiam | 2018 | Late | 16 | 12 (5.2) | 1.92 | 0.375 | 0.351 | 0.050 | 2.86 | 0.00 | 3.17 | 0.000 | -100.1 (-197.0 - Inf) |
| Wishkah | 2017 | Early | 46 | 6 (2.6) | 1.95 | 0.361 | 0.355 | 0.014 | 5.41 | 0.00 | 4.94 | 0.004 | 166.9 (135.3 - 215.7) |
| Wishkah | 2018 | Early | 44 | 3 (1.3) | 1.96 | 0.369 | 0.370 | -0.003 | 1.81 | 0.00 | 5.07 | 0.020 | 158.2 (128.2 - 204.7) |
| Wishkah | 2018 | Late | 17 | 7 (3.0) | 1.94 | 0.373 | 0.363 | 0.019 | 2.75 | 0.00 | 4.65 | 0.000 | 27.5 (23.9 - 32.1) |
| Wynoochee | 2017 | Early | 14 | 9 (3.9) | 1.93 | 0.377 | 0.382 | -0.019 | 4.29 | 0.00 | 3.12 | 0.004 | 208.6 (94.2 - Inf) |
| Wynoochee | 2017 | Late | 24 | 7 (3.0) | 1.94 | 0.375 | 0.369 | 0.021 | 2.74 | 0.00 | 4.20 | 0.004 | 431.6 (192.6 - Inf) |
| Satsop | 2010 | Early | 31 | 7 (3.0) | 1.94 | 0.362 | 0.356 | 0.010 | 1.83 | 0.00 | 4.42 | 0.006 | -1910.8 (609.6 - Inf) |
| Satsop | 2010 | Late | 21 | 9 (3.9) | 1.93 | 0.350 | 0.370 | -0.054 | 0.48 | 0.00 | 3.40 | 0.000 | -116.5 (-218.5 - Inf) |
| Satsop | 2017 | Early | 43 | 6 (2.6) | 1.95 | 0.360 | 0.356 | 0.006 | 3.65 | 0.91 | 4.38 | 0.004 | -1159.5 (1760.9 - Inf) |
| Satsop | 2017 | Late | 37 | 2 (0.9) | 1.96 | 0.369 | 0.354 | 0.034 | 5.78 | 0.00 | 5.34 | 0.004 | 173.6 (133.1 - 246.0) |
| Satsop | 2018 | Early | 39 | 7 | 1.94 | 0.361 | 0.352 | 0.023 | 3.20 | 1.37 | 4.18 | 0.004 | 1072.2 |

| Population or stock[a] | Spawn year | Spawn timing | n | Fixed loci (%) | Mean $N_A$ | He | Ho | $F_{IS}$ | HWE p<0.05 | HWE p<0.05 | LD p<0.05 | LD p<0.05 | LDNe[b] (95%CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | (3.0) | | | | | | | | | (394.6 - Inf) |
| Satsop | 2018 | Late | 38 | 3 | 1.96 | 0.366 | 0.369 | -0.015 | 5.36 | 0.00 | 4.55 | 0.004 | 169.8 |
| | | | | (1.3) | | | | | | | | | (130.8 - 238.5) |
| Cloquallum | 2018 | Early | 11 | 8 | 1.94 | 0.366 | 0.365 | 0.000 | 1.95 | 0.00 | 1.74 | 0.000 | NA |
| | | | | (3.5) | | | | | | | | | |
| Cloquallum | 2018 | Late | 14 | 11 | 1.92 | 0.375 | 0.364 | 0.025 | 0.95 | 0.00 | 3.18 | 0.000 | 704.1 |
| | | | | (4.8) | | | | | | | | | (142.8 - Inf) |
| Mid Chehalis tributaries | 2018 | Mixed | 14 | 10 | 1.93 | 0.365 | 0.364 | -0.007 | 2.42 | 0.00 | 3.30 | 0.000 | 34.6 |
| | | | | (4.3) | | | | | | | | | (27.8 - 45.0) |
| Mid Chehalis tributaries | 2018 | Late | 32 | 4 | 1.95 | 0.369 | 0.347 | 0.053 | 4.55 | 0.00 | 4.15 | 0.004 | -280.1 |
| | | | | (1.7) | | | | | | | | | (-682.3 - Inf) |
| Black | 2017 | Unknown | 40 | 6 | 1.95 | 0.369 | 0.351 | 0.045 | 6.79 | 0.00 | 5.72 | 0.012 | 365 |
| | | | | (2.6) | | | | | | | | | (219.0 - 1026.9) |
| Black | 2018 | Mixed | 9 | 21 | 1.87 | 0.377 | 0.349 | 0.062 | 1.06 | 0.00 | 1.60 | 0.000 | NA |
| | | | | (9.1) | | | | | | | | | |
| Skookumchuck | 2017 | Early | 44 | 7 | 1.94 | 0.361 | 0.354 | 0.018 | 7.27 | 0.45 | 5.92 | 0.004 | 84.5 |
| | | | | (3.0) | | | | | | | | | (73.3 - 99.0) |
| Skookumchuck | 2017 | Late | 43 | 8 | 1.94 | 0.353 | 0.342 | 0.031 | 5.96 | 0.00 | 5.41 | 0.004 | 101.9 |
| | | | | (3.5) | | | | | | | | | (87.4 - 121.5) |
| Skookumchuck | 2018 | Late | 39 | 3 | 1.96 | 0.367 | 0.349 | 0.048 | 5.41 | 0.00 | 4.58 | 0.004 | 262.2 |
| | | | | (1.3) | | | | | | | | | (183.6 - 447.9) |
| Newaukum | 2017 | Mixed | 34 | 5 | 1.95 | 0.374 | 0.361 | 0.029 | 4.07 | 0.45 | 4.39 | 0.004 | 901.4 |
| | | | | (2.2) | | | | | | | | | (333.5 - Inf) |
| Newaukum | 2018 | Early | 12 | 11 | 1.92 | 0.374 | 0.356 | 0.046 | 2.91 | 0.00 | 2.53 | 0.000 | -451.7 |
| | | | | (4.8) | | | | | | | | | (207.7 - Inf) |
| Newaukum | 2018 | Late | 24 | 4 | 1.95 | 0.372 | 0.355 | 0.038 | 5.45 | 0.00 | 3.92 | 0.004 | -180.1 |
| | | | | (1.7) | | | | | | | | | (-379.8 - Inf) |
| SF Chehalis | 2017 | Mixed | 25 | 8 | 1.94 | 0.364 | 0.353 | 0.027 | 4.57 | 0.00 | 4.14 | 0.004 | -3214.1 |
| | | | | (3.5) | | | | | | | | | (391.1 - Inf) |
| SF Chehalis | 2018 | Mixed | 49 | 3 | 1.96 | 0.366 | 0.354 | 0.031 | 4.55 | 0.00 | 5.25 | 0.012 | 398.2 |
| | | | | (1.3) | | | | | | | | | (261.1 - 810.1) |
| Upper Chehalis | 2017 | Late | 34 | 7 | 1.94 | 0.363 | 0.348 | 0.035 | 5.16 | 0.00 | 4.09 | 0.008 | -153.3 |

| Population or stock[a] | Spawn year | Spawn timing | n | Fixed loci (%) | Mean $N_A$ | He | Ho | $F_{IS}$ | HWE p<0.05 | HWE p<0.05 | LD p<0.05 | LD p<0.05 | LDNe[b] (95%CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | (3.0) | | | | | | | | | (-229.1 - Inf) |
| Upper Chehalis | 2018 | Early | 21 | 9 | 1.93 | 0.366 | 0.358 | 0.022 | 3.69 | 0.46 | 4.18 | 0.004 | 155.0 |
| | | | | (3.9) | | | | | | | | | (97.9 - 352.6) |
| Upper Chehalis | 2018 | Late | 45 | 5 | 1.95 | 0.367 | 0.356 | 0.025 | 3.15 | 0.00 | 4.40 | 0.008 | -243.5 |
| | | | | (2.2) | | | | | | | | | (-371.0 - Inf) |

**[a]- Some collections listed in Table 2 were combined for collection level genetic analysis**

**[b]- Negative $N_e$ estimates are produced when little to no linkage disequilibrium exists (i.e., there is no genetic drift) and can be interpreted as infinite**

*Table 6. Estimated pair-wise* $F_{ST}$ *values for Chehalis Basin coho salmon below diagonal. Bold type indicates statistical non-significance after correction for multiple tests. Above diagonal are corrected* P *values (α = 0.05) via permutation testing. An asterisk indicates a corrected* P *values less than the table-wide* P *value. NS = Not Significant. Collection names include the location, an abbreviated year (2017 or 2018), and an indicator of run timing (E = early, L = late, Unk = unknown, Mixed = mixed early and late).*

| | Humptulips_17_E | Humptulips_17_L | Humptulips_18_E | Humptulips_18_L | Hoquiam_18_E | Hoquiam_18_L | Wishkah_17_E | Wishkah_18_E | Wishkah_18_L |
|---|---|---|---|---|---|---|---|---|---|
| Humptulips_17_E | - | * | * | * | * | * | * | * | * |
| Humptulips_17_L | 0.019 | - | * | * | * | * | * | * | * |
| Humptulips_18_E | 0.015 | 0.005 | - | * | * | * | * | * | * |
| Humptulips_18_L | 0.034 | 0.027 | 0.015 | - | * | * | * | * | * |
| Hoquiam_18_E | 0.031 | 0.025 | 0.024 | 0.045 | - | NS | * | * | * |
| Hoquiam_18_L | 0.013 | 0.009 | 0.009 | 0.037 | **0.007** | - | * | * | NS |
| Wishkah_17_E | 0.017 | 0.028 | 0.021 | 0.042 | 0.044 | 0.026 | - | * | * |
| Wishkah_18_E | 0.031 | 0.028 | 0.022 | 0.048 | 0.038 | 0.027 | 0.037 | - | * |
| Wishkah_18_L | 0.026 | 0.017 | 0.013 | 0.033 | 0.019 | **0.007** | 0.032 | 0.030 | - |
| Wynoochee_17_E | 0.013 | 0.009 | 0.009 | 0.026 | 0.022 | **0.006** | 0.020 | 0.025 | 0.010 |
| Wynoochee_17_L | 0.018 | 0.012 | 0.007 | 0.026 | 0.029 | 0.013 | 0.026 | 0.028 | 0.009 |
| Satsop_10_E | 0.021 | 0.020 | 0.018 | 0.035 | 0.034 | 0.021 | 0.021 | 0.030 | 0.018 |
| Satsop_10_L | 0.035 | 0.017 | 0.022 | 0.038 | 0.053 | 0.032 | 0.041 | 0.042 | 0.028 |
| Satsop_17_E | 0.020 | 0.016 | 0.013 | 0.032 | 0.035 | 0.017 | 0.016 | 0.032 | 0.019 |
| Satsop_17_L | 0.017 | 0.005 | 0.012 | 0.025 | 0.034 | 0.016 | 0.030 | 0.029 | 0.019 |
| Satsop_18_E | 0.017 | 0.017 | 0.013 | 0.033 | 0.028 | 0.016 | 0.021 | 0.030 | 0.021 |
| Satsop_18_L | 0.021 | 0.012 | 0.010 | 0.017 | 0.028 | 0.017 | 0.030 | 0.027 | 0.018 |
| Cloquallum_18_E | 0.024 | 0.018 | 0.011 | 0.032 | 0.031 | 0.018 | 0.026 | 0.037 | 0.021 |
| Cloquallum_18_L | 0.017 | 0.008 | 0.007 | 0.036 | 0.024 | 0.009 | 0.025 | 0.024 | 0.012 |
| Mid-Chehalis_17_Unk | 0.028 | 0.026 | 0.027 | 0.041 | 0.045 | 0.024 | 0.036 | 0.037 | 0.023 |
| Mid-Chehalis_18_L | 0.021 | 0.017 | 0.010 | 0.033 | 0.026 | 0.017 | 0.029 | 0.028 | 0.014 |
| Black_18_Mixed | 0.011 | 0.010 | **0.005** | 0.031 | 0.028 | **0.005** | 0.021 | 0.022 | **0.011** |
| Black_17_Unk | 0.014 | 0.014 | 0.007 | 0.030 | 0.035 | 0.014 | 0.021 | 0.026 | 0.020 |
| Skookumchuck_17_E | 0.018 | 0.016 | 0.011 | 0.031 | 0.032 | 0.015 | 0.019 | 0.028 | 0.018 |
| Skookumchuck_17_L | 0.031 | 0.014 | 0.022 | 0.039 | 0.049 | 0.035 | 0.043 | 0.043 | 0.032 |
| Skookumchuck_18_L | 0.022 | 0.014 | 0.007 | 0.020 | 0.031 | 0.016 | 0.033 | 0.027 | 0.016 |
| Newaukum_17_Mixed | 0.019 | 0.013 | 0.009 | 0.027 | 0.030 | 0.016 | 0.027 | 0.026 | 0.015 |
| Newaukum_18_E | 0.008 | 0.008 | **0.005** | 0.024 | 0.016 | **0.005** | 0.017 | 0.029 | 0.014 |
| Newaukum_18_L | 0.017 | 0.016 | 0.010 | 0.026 | 0.019 | 0.012 | 0.032 | 0.029 | 0.020 |
| SF_Chehalis_17_Unk | 0.017 | 0.021 | 0.015 | 0.031 | 0.034 | 0.017 | 0.033 | 0.033 | 0.019 |
| SF_Chehalis_18_L | 0.015 | 0.018 | 0.011 | 0.032 | 0.032 | 0.015 | 0.025 | 0.030 | 0.017 |
| Upper_Chehalis_17_L | 0.030 | 0.029 | 0.025 | 0.045 | 0.048 | 0.025 | 0.039 | 0.046 | 0.036 |
| Upper_Chehalis_18_E | 0.022 | 0.022 | 0.014 | 0.035 | 0.036 | 0.019 | 0.028 | 0.037 | 0.020 |
| Upper_Chehalis_18_L | 0.018 | 0.020 | 0.015 | 0.032 | 0.030 | 0.019 | 0.026 | 0.035 | 0.020 |

*Table 6. cont'd*

| | Wynoochee_17_E | Wynoochee_17_L | Satsop_10_E | Satsop_10_L | Satsop_17_E | Satsop_17_L | Satsop_18_E | Satsop_18_L |
|---|---|---|---|---|---|---|---|---|
| Humptulips_17_E | * | * | * | * | * | * | * | * |
| Humptulips_17_L | * | * | * | * | * | * | * | * |
| Humptulips_18_E | * | * | * | * | * | * | * | * |
| Humptulips_18_L | * | * | * | * | * | * | * | * |
| Hoquiam_18_E | * | * | * | * | * | * | * | * |
| Hoquiam_18_L | NS | * | * | * | * | * | * | * |
| Wishkah_17_E | * | * | * | * | * | * | * | * |
| Wishkah_18_E | * | * | * | * | * | * | * | * |
| Wishkah_18_L | * | * | * | * | * | * | * | * |
| Wynoochee_17_E | - | NS | * | * | * | * | * | * |
| Wynoochee_17_L | **0.004** | - | * | * | * | * | * | * |
| Satsop_10_E | 0.007 | 0.014 | - | * | * | * | * | * |
| Satsop_10_L | 0.024 | 0.028 | 0.029 | - | * | * | * | * |
| Satsop_17_E | 0.009 | 0.015 | 0.004 | 0.031 | - | * | * | * |
| Satsop_17_L | 0.010 | 0.009 | 0.018 | 0.010 | 0.016 | - | * | * |
| Satsop_18_E | 0.010 | 0.013 | 0.007 | 0.029 | 0.003 | 0.014 | - | * |
| Satsop_18_L | 0.011 | 0.012 | 0.016 | 0.020 | 0.015 | 0.007 | 0.014 | - |
| Cloquallum_18_E | 0.021 | 0.016 | 0.016 | 0.034 | 0.013 | 0.021 | 0.014 | 0.026 |
| Cloquallum_18_L | 0.012 | **0.005** | 0.013 | 0.022 | 0.013 | 0.011 | 0.011 | 0.017 |
| Mid-Chehalis_17_Unk | 0.026 | 0.020 | 0.022 | 0.035 | 0.024 | 0.020 | 0.019 | 0.025 |
| Mid-Chehalis_18_L | 0.012 | 0.008 | 0.014 | 0.032 | 0.014 | 0.017 | 0.014 | 0.014 |
| Black_18_Mixed | 0.007 | **0.000** | 0.011 | 0.021 | 0.012 | 0.008 | 0.013 | 0.013 |
| Black_17_Unk | 0.009 | 0.007 | 0.012 | 0.022 | 0.010 | 0.012 | 0.013 | 0.017 |
| Skookumchuck_17_E | 0.008 | 0.009 | 0.010 | 0.025 | 0.005 | 0.017 | 0.010 | 0.014 |
| Skookumchuck_17_L | 0.029 | 0.022 | 0.031 | 0.030 | 0.027 | 0.012 | 0.023 | 0.019 |
| Skookumchuck_18_L | 0.012 | 0.010 | 0.016 | 0.027 | 0.015 | 0.009 | 0.015 | 0.003 |
| Newaukum_17_Mixed | 0.010 | 0.007 | 0.016 | 0.019 | 0.014 | 0.011 | 0.014 | 0.012 |
| Newaukum_18_E | **0.000** | **0.005** | **0.005** | 0.025 | **0.002** | 0.009 | **0.003** | 0.010 |
| Newaukum_18_L | 0.008 | 0.007 | 0.018 | 0.026 | 0.017 | 0.011 | 0.013 | 0.010 |
| SF_Chehalis_17_Unk | 0.013 | 0.009 | 0.018 | 0.037 | 0.018 | 0.016 | 0.014 | 0.017 |
| SF_Chehalis_18_L | 0.015 | 0.005 | 0.017 | 0.027 | 0.016 | 0.015 | 0.014 | 0.016 |
| Upper_Chehalis_17_L | 0.023 | 0.021 | 0.027 | 0.047 | 0.027 | 0.029 | 0.027 | 0.031 |
| Upper_Chehalis_18_E | 0.017 | 0.011 | 0.011 | 0.038 | 0.006 | 0.020 | 0.008 | 0.018 |
| Upper_Chehalis_18_L | 0.011 | 0.011 | 0.014 | 0.034 | 0.012 | 0.021 | 0.009 | 0.016 |

*Table 6. cont'd.*

| | Cloquallum_18_E | Cloquallum_18_L | Mid-Chehalis_17_Unk | Mid-Chehalis_18_L | Black_18_Mixed | Black_17_Unk | Skookumchuck_17_E | Skookumchuck_17_L | Skookumchuck_18_L |
|---|---|---|---|---|---|---|---|---|---|
| Humptulips_17_E | * | * | * | * | * | * | * | * | * |
| Humptulips_17_L | * | * | * | * | * | * | * | * | * |
| Humptulips_18_E | * | * | * | * | NS | * | * | * | * |
| Humptulips_18_L | * | * | * | * | * | * | * | * | * |
| Hoquiam_18_E | * | * | * | * | * | * | * | * | * |
| Hoquiam_18_L | * | * | * | * | NS | * | * | * | * |
| Wishkah_17_E | * | * | * | * | * | * | * | * | * |
| Wishkah_18_E | * | * | * | * | * | * | * | * | * |
| Wishkah_18_L | * | * | * | * | NS | * | * | * | * |
| Wynoochee_17_E | * | * | * | * | * | * | * | * | * |
| Wynoochee_17_L | * | NS | * | * | NS | * | * | * | * |
| Satsop_10_E | * | * | * | * | * | * | * | * | * |
| Satsop_10_L | * | * | * | * | * | * | * | * | * |
| Satsop_17_E | * | * | * | * | * | * | * | * | * |
| Satsop_17_L | * | * | * | * | * | * | * | * | * |
| Satsop_18_E | * | * | * | * | * | * | * | * | * |
| Satsop_18_L | * | * | * | * | * | * | * | * | * |
| Cloquallum_18_E | - | NS | * | * | * | * | * | * | * |
| Cloquallum_18_L | **0.006** | - | * | NS | NS | NS | * | * | * |
| Mid-Chehalis_17_Unk | 0.039 | 0.023 | - | * | * | * | * | * | * |
| Mid-Chehalis_18_L | 0.014 | **0.002** | 0.021 | - | NS | * | * | * | * |
| Black_18_Mixed | 0.016 | **-0.002** | 0.011 | **0.002** | - | NS | * | * | * |
| Black_17_Unk | 0.011 | **0.003** | 0.017 | 0.008 | **0.003** | - | * | * | * |
| Skookumchuck_17_E | 0.015 | 0.010 | 0.020 | 0.009 | 0.010 | 0.007 | - | * | * |
| Skookumchuck_17_L | 0.041 | 0.026 | 0.032 | 0.029 | 0.026 | 0.027 | 0.029 | - | * |
| Skookumchuck_18_L | 0.019 | 0.012 | 0.022 | 0.008 | 0.005 | 0.010 | 0.013 | 0.021 | - |
| Newaukum_17_Mixed | 0.018 | **0.004** | 0.020 | 0.006 | **0.002** | 0.007 | 0.013 | 0.023 | 0.009 |
| Newaukum_18_E | **0.008** | **-0.002** | 0.011 | **-0.002** | **0.003** | **0.003** | **0.004** | 0.016 | 0.008 |
| Newaukum_18_L | 0.018 | 0.011 | 0.023 | 0.007 | 0.011 | 0.009 | 0.014 | 0.030 | 0.008 |
| SF_Chehalis_17_Unk | 0.022 | 0.012 | 0.024 | 0.009 | 0.005 | 0.014 | 0.013 | 0.029 | 0.012 |
| SF_Chehalis_18_L | 0.020 | 0.009 | 0.014 | 0.005 | **0.000** | 0.009 | 0.009 | 0.028 | 0.010 |
| Upper_Chehalis_17_L | 0.033 | 0.024 | 0.036 | 0.021 | 0.018 | 0.021 | 0.023 | 0.043 | 0.026 |
| Upper_Chehalis_18_E | 0.016 | 0.013 | 0.028 | 0.013 | **0.002** | 0.014 | 0.011 | 0.030 | 0.015 |
| Upper_Chehalis_18_L | 0.020 | 0.011 | 0.021 | 0.013 | 0.012 | 0.015 | 0.010 | 0.029 | 0.016 |

*Table 6. cont'd.*

| | Newaukum_17_Mixed | Newaukum_18_E | Newaukum_18_L | SF_Chehalis_17_Unk | SF_Chehalis_18_L | Upper_Chehalis_17_L | Upper_Chehalis_18_E | Upper_Chehalis_18_L |
|---|---|---|---|---|---|---|---|---|
| Humptulips_17_E | * | * | * | * | * | * | * | * |
| Humptulips_17_L | * | * | * | * | * | * | * | * |
| Humptulips_18_E | * | NS | * | * | * | * | * | * |
| Humptulips_18_L | * | * | * | * | * | * | * | * |
| Hoquiam_18_E | * | * | * | * | * | * | * | * |
| Hoquiam_18_L | * | NS | * | * | * | * | * | * |
| Wishkah_17_E | * | * | * | * | * | * | * | * |
| Wishkah_18_E | * | * | * | * | * | * | * | * |
| Wishkah_18_L | * | * | * | * | * | * | * | * |
| Wynoochee_17_E | * | NS | * | * | * | * | * | * |
| Wynoochee_17_L | * | NS | * | * | * | * | * | * |
| Satsop_10_E | * | NS | * | * | * | * | * | * |
| Satsop_10_L | * | * | * | * | * | * | * | * |
| Satsop_17_E | * | NS | * | * | * | * | * | * |
| Satsop_17_L | * | * | * | * | * | * | * | * |
| Satsop_18_E | * | NS | * | * | * | * | * | * |
| Satsop_18_L | * | * | * | * | * | * | * | * |
| Cloquallum_18_E | * | NS | * | * | * | * | * | * |
| Cloquallum_18_L | NS | NS | * | * | * | * | * | * |
| Mid-Chehalis_17_Unk | * | * | * | * | * | * | * | * |
| Mid-Chehalis_18_L | * | NS | * | * | * | * | * | * |
| Black_18_Mixed | NS | NS | * | * | NS | * | NS | * |
| Black_17_Unk | * | NS | * | * | * | * | * | * |
| Skookumchuck_17_E | * | NS | * | * | * | * | * | * |
| Skookumchuck_17_L | * | * | * | * | * | * | * | * |
| Skookumchuck_18_L | * | * | * | * | * | * | * | * |
| Newaukum_17_Mixed | - | NS | * | * | * | * | * | * |
| Newaukum_18_E | **0.003** | - | NS | * | NS | * | * | * |
| Newaukum_18_L | 0.005 | **0.002** | - | * | * | * | * | * |
| SF_Chehalis_17_Unk | 0.010 | 0.007 | 0.012 | - | NS | * | * | * |
| SF_Chehalis_18_L | 0.007 | **0.005** | 0.008 | **0.003** | - | * | * | * |
| Upper_Chehalis_17_L | 0.020 | 0.025 | 0.014 | 0.024 | 0.021 | - | * | * |
| Upper_Chehalis_18_E | 0.015 | 0.008 | 0.015 | 0.013 | 0.015 | 0.023 | - | * |
| Upper_Chehalis_18_L | 0.014 | 0.009 | 0.011 | 0.009 | 0.013 | 0.016 | 0.005 | - |

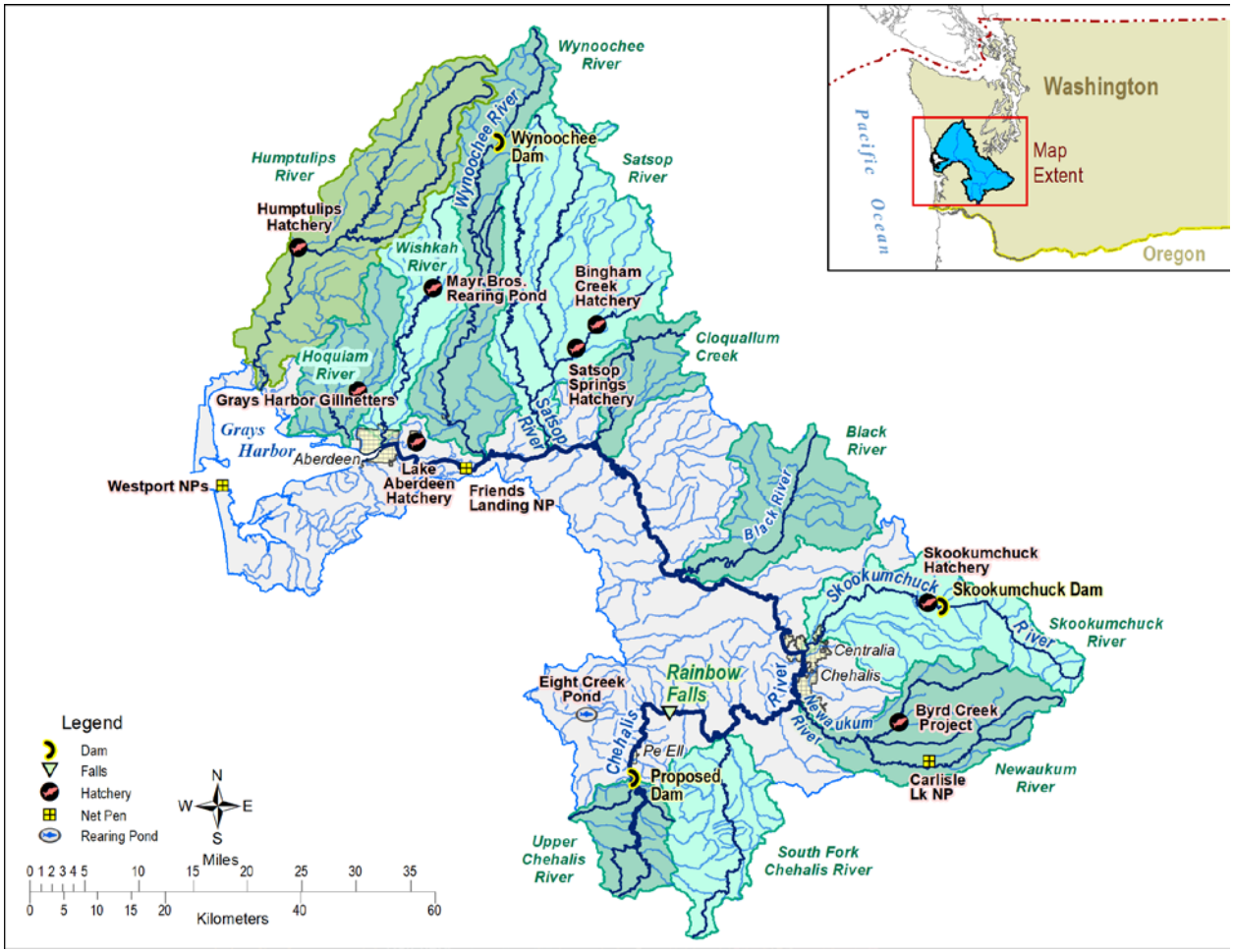


*Figure 1. Map showing the Chehalis River basin highlighting sampled coho salmon spawning tributaries, hatchery facilities where coho salmon are propagated, sites from which hatchery produced coho salmon are released, and existing and proposed dam sites. The Mid-Chehalis tributary collections consist of samples taken from coho carcasses found in many of the un-highlighted tributaries (colored gray). These were left un-highlighted intentionally to make visual interpretation easier. Release sites operated by cooperative agreements (COOPs) often change annually. Those shown here were those found in the WDFW Future Brood Documents for 2014 and 2015, which are the cohorts that produced the fish returning in 2017 and 2018, the main spawn years analyzed in this report.*

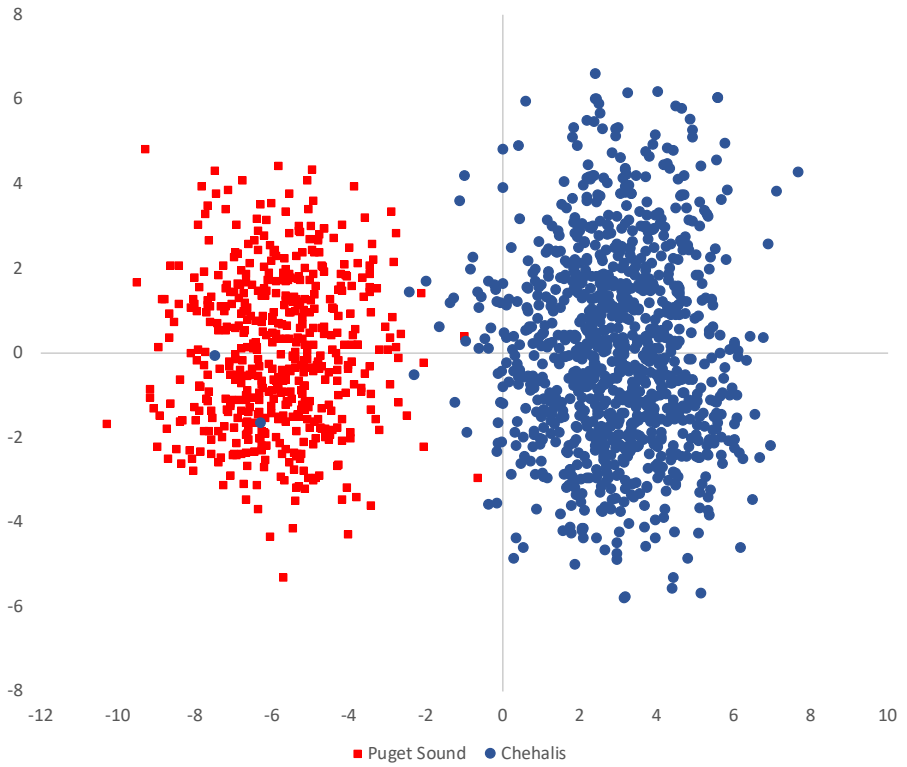


*Figure 2. PCA analysis of Chehalis and Puget Sound coho salmon. Axis 1 separates Puget Sound coho (red) from Chehalis coho (blue). Two adult fish sampled in the Chehalis (one each from the Skookumchuck and Newaukum rivers) can be seen clustering with the Puget Sound coho salmon. Puget Sound origin coho destined for Squaxin Tribe netpen releases in South Puget Sound are reared for a short time at the Skookumchuck Hatchery. These two fish likely represent some unknown number of these Puget Sound fish unintentionally released in the Chehalis Basin.*

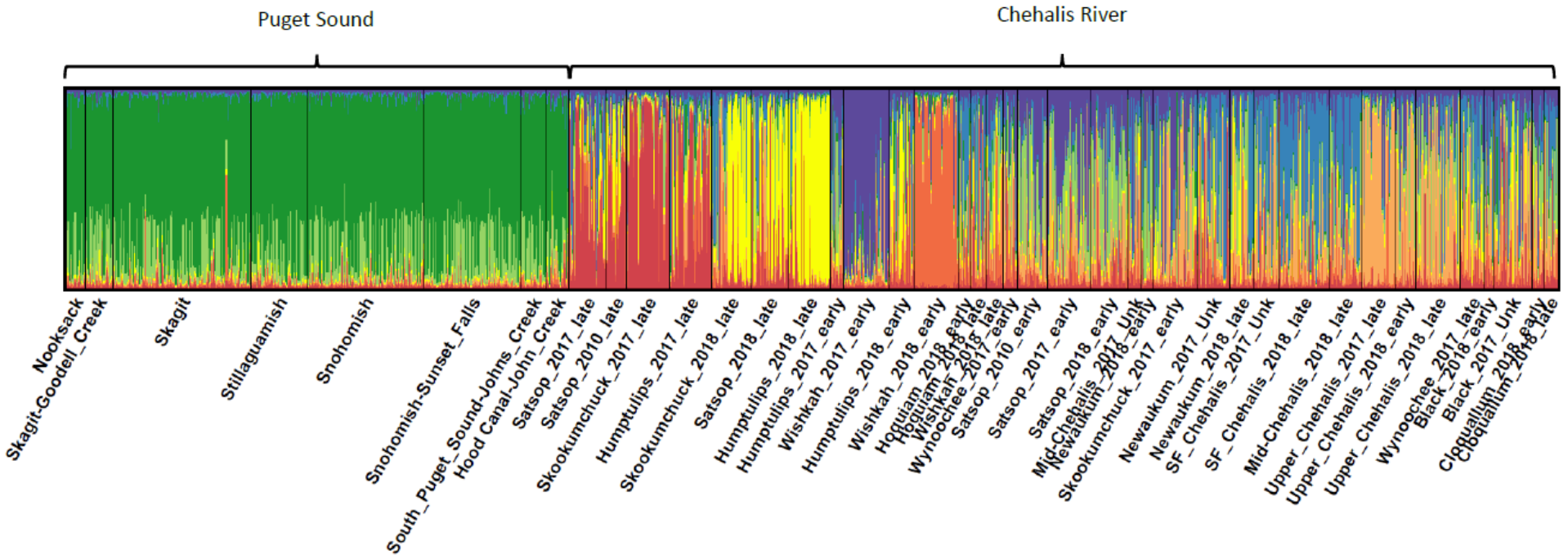


*Figure 3. Plots of the results of STRUCTURE analysis of coho salmon collections from Puget Sound and the Chehalis Basin, which represent the current extent of WDFW's genotyped coho salmon reference baseline colletions. The inferred number of clusters plotted above is K = 8, which was supported by the ΔK method of Evanno et al. (2005). K = 2 was very strongly supported, but simply separated the Puget Sound collections from those from the Chehalis Basin. With K = 8, no internal structure among Puget Sound collections was shown. A separate analysis of Puget Sound coho collections (Brown et al., WDFW unpublished) showed very weak structure among the Puget Sound populations analyzed. Among Chehalis coho collections, the most obvious identified clusters are the 2017 late coho from rivers where late broodstock are collected (dark red), the 2018 late coho from rivers where late broodstock are collected (yellow), the 2017 Wishkah early collection (purple), the 2018 Wishkah early collection (dark orange), the Hoquiam, South Fork Chehalis and Mid-Chehalis 2018 late collections (blue), and the Upper Chehalis collections (light orange). All three Satsop early collections showed weak clustering (light green). All remaining collections were not inferred to belong to strongly or weakly differentiated clusters at K = 8 and instead showed mixed membership of the other inferred clusters.*

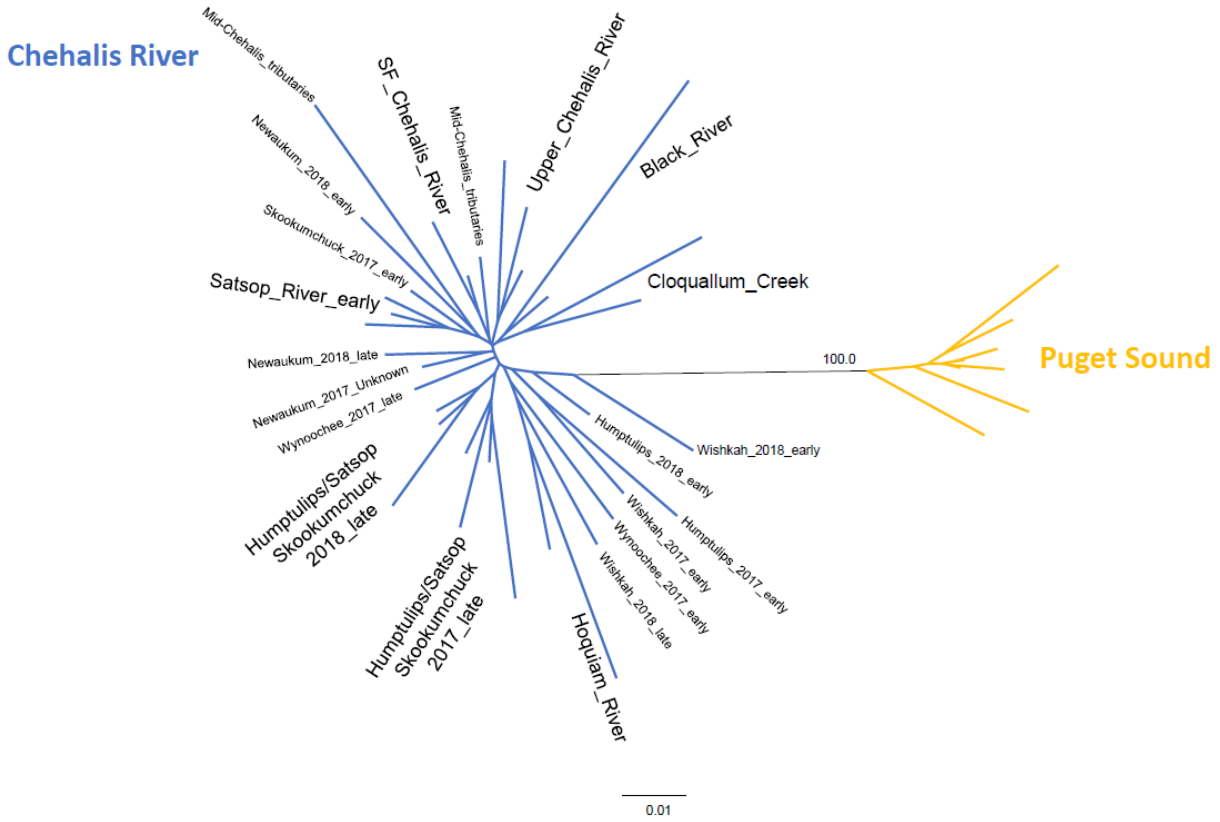


*Figure 4. Unrooted neighbor-joining dendrogram constructed with Cavalli-Sforza genetic distance values of coho salmon collections taken in Puget Sound and the Chahalis River basin. The bootstrap value (% of 10,000 boostraps) is shown only for the node separating Puget Sound coho from Chehalis coho, which are strongly differentiated and well supported by the data. In order to simplify this figure, names of many of the specific collections have either been removed (Puget Sound) or combined (Chehalis) where all members of a branch are similar in some way.*

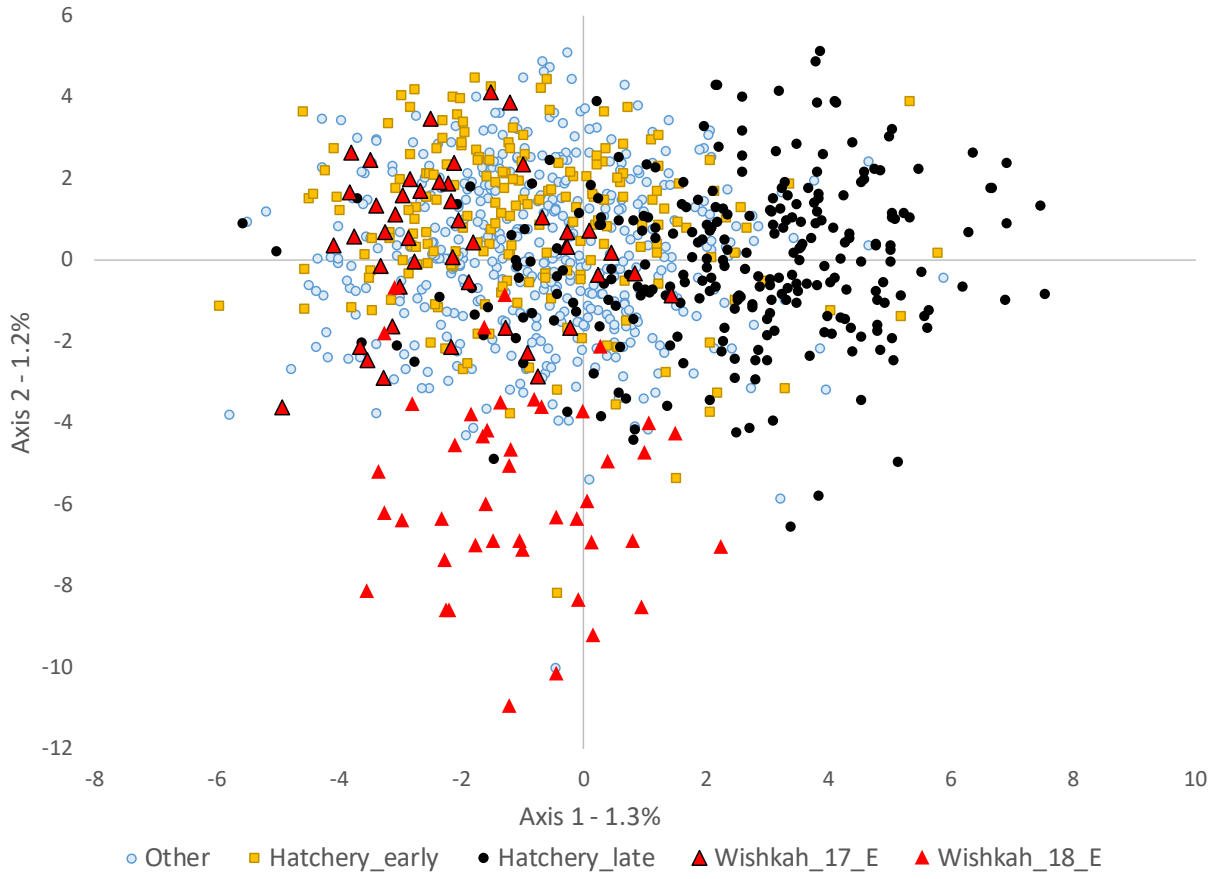


*Figure 5. Results of PCA analysis of Chehalis Basin coho salmon samples. While overlap was evident among individuals, three clusters were evident. Axis 1 separated late coho from rivers where late hatchery broodstock are collected ("Hatchery_late", i.e., Humptulips, Satsop, and Skookumchuck; in black circles) from all other samples; and Axis 2 separated the Wishkah 2018 early coho collection ("Wishkah_18_E", red triangles) from all other samples. The Wishkah 2017 early coho samples ("Wishkah_17_E", red triangles with black margins), the coho from rivers where early hatchery broodstock are collected (except the Wishkah; "Hatchery_early", i.e., Humptulips, Satsop, and Skookumchuck; yellow squares), and all other coho samples from all other locations, early and late run ("Other", light blue circles) completely overlap.*

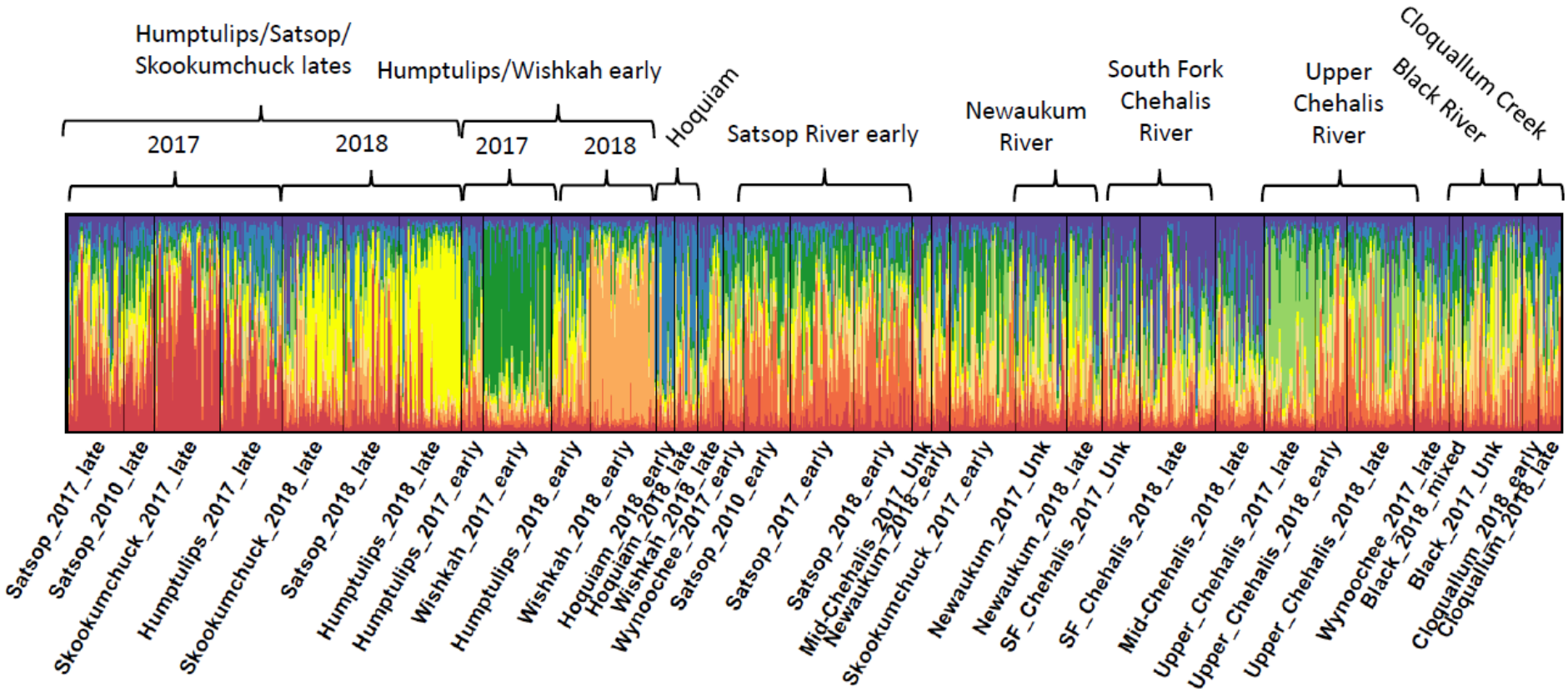


*Figure 6. Results of clustering analysis of Chehalis Basin coho salmon using STRUCTURE (Pritchard et al. 2000). The inferred number of clusters plotted above is K = 9, which was supported by the ΔK method of Evanno et al. (2005). K = 2 and K = 4 were also weakly supported (not shown). At K = 2, the clusters consisted of the Humptulips/Satsop/Skookumchuck late collections and the rest of the Chehalis Basin collections. At K = 4, the four clusters consisted of the Humptulips/Satsop/Skookumchuck late collections, the Wishkah 2017/Satsop/Skookumchuck early collections, the Wishkah 2018 early collection and the rest of the Chehalis Basin collections. At K = 9 (above), the Humptulips/Satsop/Skookumchuck late collections split into two cohort clusters, 2017 (red) and 2018 (yellow), the Wishkah 2018 early remained its own cluster (light orange), and remaining two clusters at K = 4 separated into the Wishkah 2017 early collection (green), the Hoquiam collections (blue), Satsop early collections (dark orange) the South Fork Chehalis and Mid-Chehalis 2018 late collections (purple), and the Upper Chehalis collections, particularly the 2017 late collection (light green). All remaining collections were not inferred to belong to strongly or weakly differentiated clusters at K = 9 and instead showed mixed membership of the other inferred clusters.*

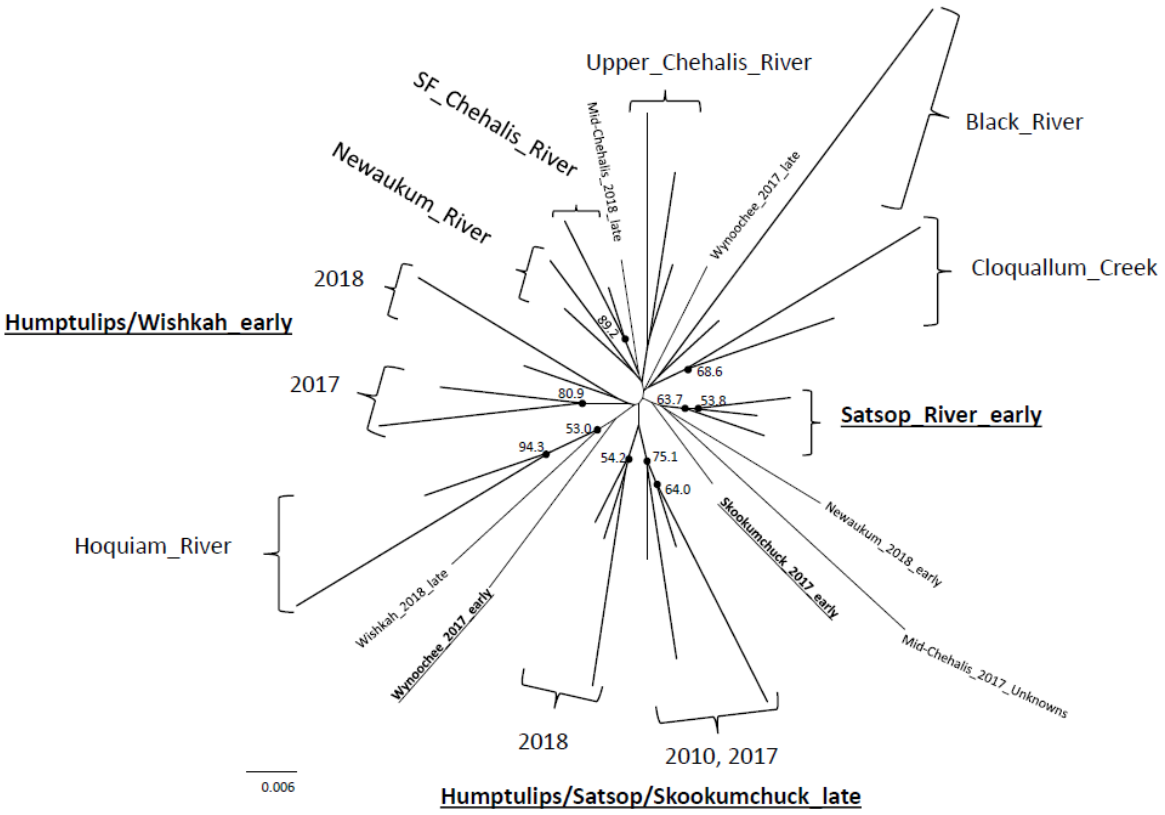


*Figure 7. Unrooted neighbor-joining dendrogram contructed from Cavalli-Sforza genetic distances calculated using PHYLIP (Felsenstein 1993). Nodes with greater than 50% (of 10,000 bootstraps) are noted and labeled with the percent bootstrap support. Collections from sub-basins where hatchery broodstock are collected are in bold and underlined. Most collections from sub-basins with replicate collections (temporal replicates and/or early vs. late) cluster with the other collections from the same sub-basin. Exceptions occur mainly where hatchery broodstock are collected, in particular collections from sub-basins that have late coho programs cluster together, and cluster at the next less-inclusive level by spawn year. There is high bootstrap support for the node separating Hoquiam and South Fork Chehalis collections each from all other collections. There is also strong bootstrap support for the node separating the 2017 collections of Wishkah and Humptulips early coho from all other collections. Nodes separating Cloquallum coho and Satsop early coho each from all other collections are also well supported.*

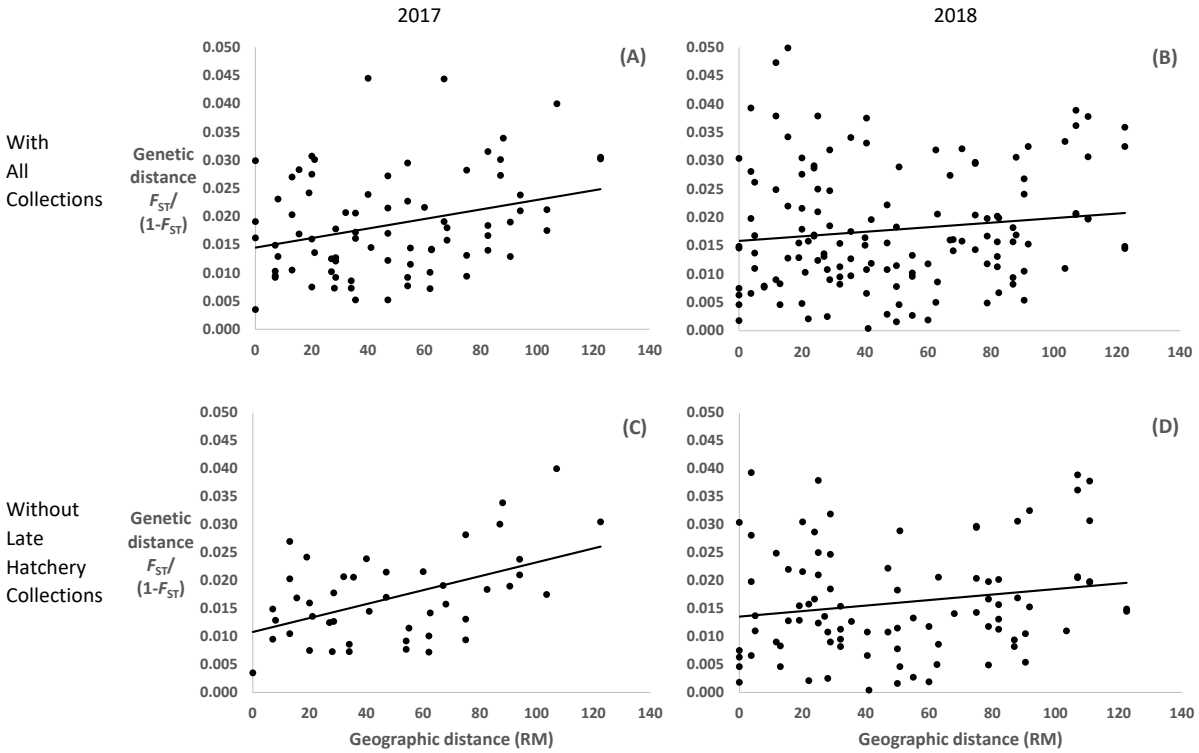


*Figure 8. Regression (Mantel's test) of pairwise genetic and geographic distances among 2017 (A and C) and 2018 (B and D) collections of Chehalis Basin coho salmon with (A and B) and without (C and D) late coho collections from sub-basins where late coho hatchery broodstock are collected (Humptulips, Satsop, and Skookumchuck rivers). Mid-Chehalis collections were not included. The positive relationship of genetic and geographic distance observed in 2017 was statistically significant (*P *= 0.028,* $R^2$ *= 0.09) and improved when late hatchery coho collections were removed (*P *= 0.008,* $R^2$ *= 0.24). The positive relationship of genetic and geographic distance observed in 2018 was marginally non-significant with (*P *= 0.103,* $R^2$ *= 0.02) or without (*P *= 0.07,* $R^2$ *= 0.03) late hatchery coho collections.*