**Methods**

*Sampling*

Fin clips or operculum punches were taken from natural origin Chinook salmon sampled in the lower Rogue by seining, creel surveys and volunteer anglers from April 5th to September 30th, 2020. Sample locations ranged from the Bay upstream to Quosatana Creek (figure 1).

Map

Description automatically generated

Figure 1: Sample locations throughout the lower Rogue River.

Volunteer anglers were asked to take tissue samples from any naturally produced Chinook salmon. Volunteer anglers were issued sampling kits containing written instructions, individually labeled vials containing ethanol, and a paper hole punch for extracting caudal fin tissue.

Additionally, the kits contained the following instructions:

* Minimize handling (keep in net on side of boat in/very close to the water surface)
* Work with a partner (one person holds fish, other holds net and collects sample)
* Use paper hole punch to extract fin tissue sample from caudal fin (Imagine the tail fin is a piece of paper)
* Gently release fish back into river
* Very carefully remove tissue sample from the hole punch’s “chip guard” and place into sample vial. Be sure that sample is completely immersed in the liquid and close the cap snugly.
* Place vial into resealable bag. With a sharpie, record date, angler (collector), and location on bag’s exterior.
* Rinse the hole punch in clean water
* Store sample in safe place away from heat for the duration of the fishing trip
* Drop samples off with Gold Beach ODFW staff directly or at one of the five drop boxes\* and then notify ODFW \*Drop boxes were located at Rogue Outdoor Store, ODFW Gold Beach office, Mill Site, Lobster Creek Campground, and Quosatana Creek Campgrounds.

The Drop boxes were setup prior to March 1. ODFW staff collected samples from the boxes daily, or, at a minimum, weekly during the study period. The returned sample vials were organized chronologically and stored at the Gold Beach ODFW office for the duration of the project. All samples were stored at room temperature away from sunlight. The ethanol in each vial was replaced one week after samples were collected. At that time, each vial was labeled externally with a sample-ID number. Additionally, a small piece of Rite-in-the-Rain paper with the sample-ID number recorded with pencil was placed inside the vial with the tissue sample. Data records for each sample were entered in to an Excel spreadsheet and included sample-ID number, collection date, collection area, collector, and collection method. All volunteer angler-caught individuals were sampled from April 5th to June 20th.

Creel survey samples were taken from the fish cleaning station at the Port of Gold Beach and all samples were reported to have been captured in the bay from July 8th to July 19th. Seined individuals were sampled by ODFW staff at Huntley Park from July 21st to September 30th.

*Genotyping*

Tissue samples were transferred to the State Fisheries Genomics Lab in Newport where DNA was extracted from the fin tissue samples using the method of Ivanova et al. (2006). Using the Genotyping-in-Thousands by sequencing method (GT-seq; Campbell et al. 2015), all samples were genotyped at 353 single nucleotide polymorphisms (SNPs) (Hess *et al.* 2016), including a sex marker and two SNPs (Ots37124\_12277401 and Ots37124\_12310649) that are ~33 kb apart and located in the intergenic region between GREB1L and ROCK1 . These latter two SNPs are hereafter referred to as Greb1L SNP1 and SNP2, respectively. Greb1L SNP1 is reportedly more diagnostic of adult migration phenotype than Greb1L SNP2 in Rogue River and Klamath River populations of Chinook salmon (T. Thompson, pers. comm.).

The genotyping protocol followed Campbell *et al*. (2015), except the second polymerase chain reaction (PCR) used Ultra II Q5 master mix (New England Biolabs) to add i5 and i7 adapters. Amplicons were sequenced on an Illumina NextSeq 2000 at University of Oregon. We also genotyped negative controls and replicates. We used genotyping scripts previously developed by Campbell *et al.* (2015) which are available at https://github.com/GTseq/GTseq-Pipeline/. Genotype quality control was assessed using fastqc, replicate samples and negative controls.

Briefly, we filtered genotypes on the basis of missingness, sample duplication and the individual fuzziness index (IFI), which estimates the amount of cross-contamination in a given. We also removed sites with poorly calibrated allele correction values or more than three clusters of allele ratios suggestive of a paralogous sequence variant. We took an iterative approach to missingness and IFI filtering and recalculated missingness for all individuals and genotypes between each step (O'Leary *et al.* 2018). We began filtering by removing negative controls and replicate individuals (retained replicate with highest number of on-target reads). Then we removed individuals with more than 30% missing data, then SNPs with greater than 50% data, and individuals with IFI greater than 10 (i.e. greater than 10% putative background reads). In our second round of filtering, we removed individuals with more than 10% missing data, then removed SNPs with greater than 20% missing data, and individuals with IFI greater than 2.5. We then examined any SNP with greater than 10% missing data, and skewed or high variance in allele ratios among uncalled and heterozygous samples by plotting corrected read counts of alternative alleles. SNPs with a strong bias towards one allele among heterozygotes, more than three clusters of allele ratios, or indistinct clusters of allele ratios were removed from the dataset. After genotype quality filtering was complete, we removed monomorphic loci. Of the 353 SNPs genotyped, we focused on only GREB1L SNP1 and SNP2 for this report.

**Results**

*Sample Collection*

Volunteer anglers sampled 67 naturally produced Chinook salmon throughout the lower Rogue River, 12 individuals sampled in Rogue Bay were collected through creel surveys and 132 individuals were sampled at Huntley Park through seining (table 1). Compared to the previous year’s report, no high water events precluded angler sampling effort, and volunteer angler provided samples were collected consistently from April 5th to June 20th (figure 2).

|  | **Sampling Method** | | |
| --- | --- | --- | --- |
|  | **Angler**, N = 67 | **Creel Survey**, N = 12 | **Seine**, N = 132 |
| **Location** |  |  |  |
| Huntley Park | 0 (0%) | 0 (0%) | 132 (100%) |
| Lobster Creek | 25 (37%) | 0 (0%) | 0 (0%) |
| Old Mill Site | 16 (24%) | 0 (0%) | 0 (0%) |
| Elephant Rock | 4 (6.0%) | 0 (0%) | 0 (0%) |
| Quosatana Creek | 22 (33%) | 0 (0%) | 0 (0%) |
| Rogue Bay | 0 (0%) | 12 (100%) | 0 (0%) |
| **Age** |  |  |  |
| Adult | 67 (100%) | 7 (58%) | 132 (100%) |
| Jack | 0 (0%) | 5 (42%) | 0 (0%) |

Table 1: Sample sizes, locations and age among the three sampling methods



Figure 2: Discharge (cfs) and river temperature (°C) at USGS Agness gauge (number 14372300), and number of spring Chinook salmon sampled by anglers per week in 2019 (previous information report) and 2020

*Genotyping*

Two of the 211 samples were excluded during filtering. Of the remaining 209 samples, four samples had discordant genotypes across the two GREB1L SNPs we focus on in this report. For instance, an individual was genotyped as heterozygous at GREB1L SNP1 and homozygous late at the SNP2. Of the remaining 205 samples, 49 samples were genotyped homozygous early, 35 samples were genotyped heterozygous, and 121 samples were genotyped homozygous late (figure 3).



Figure 3. Number of Greb1L SNP1 (Ots37124\_12277401) and SNP2 (Ots37124\_12310649) genotypes of Chinook salmon caught in the lower Rogue River in 2020 by Julian week from April 5th to September 30th.

Greb1L SNP1 is reportedly more diagnostic of adult migration phenotype than Greb1L SNP2 in Rogue River and Klamath River Chinook salmon populations (T. Thompson, pers. comm.). Focusing only on SNP1, 50 individuals were homozygous early, 37 were heterozygous, and 122 were homozygous late (Figure 4). The first heterozygous sample was collected on May 5th (week 18). The first homozygous late sample was collected on June 9th (week 23). Samples first shifted from majority homozygous early to majority heterozygous in the week from June 4th to June 10th (week 23) and from majority heterozygous to majority homozygous late in the week from August 6th to August 12th (week 32). July 19th (week 28) was latest that a homozygous early individual was observed and September 4th (week 35) was the latest that an individual bearing an early-migration allele at SNP1 (heterozygote) was observed. 50% of all early-migration alleles in the dataset are observed before julian day 142 (May 21st). 80% of all early-migration alleles in the dataset are observed before julian day 169 (June 17th).



Figure 4. Greb1L SNP1 (Ots37124\_12277401) genotypes of Chinook salmon caught in the lower Rogue River in 2020 by Julian week from April 5th to September 30th.

Campbell NR, Harmon SA, Narum SR (2015) Genotyping-in-thousands by sequencing (gt-seq): A cost effective snp genotyping method based on custom amplicon sequencing. *Mol Ecol Resour* **15**, 855-867.

Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proc Biol Sci* **283**.

Ivanova NV, Dewaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* **6**, 998-1002.

O'Leary SJ, Puritz JB, Willis SC, Hollenbeck CM, Portnoy DS (2018) These aren't the loci you'e looking for: Principles of effective snp filtering for molecular ecologists. *Mol Ecol*.