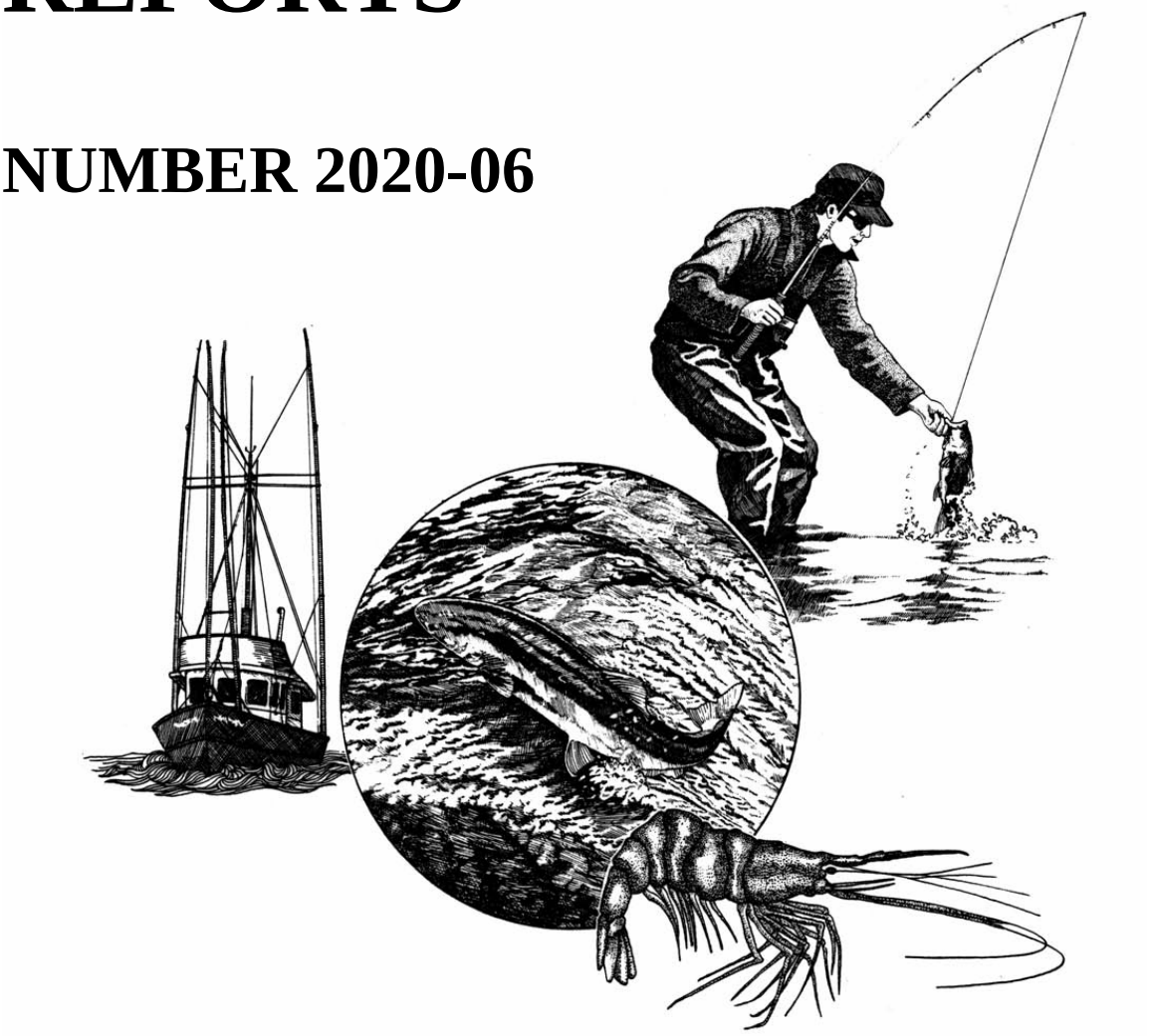
INFORMATION REPORTS

NUMBER 2020-06



FISH DIVISION

Oregon Department of Fish and Wildlife

An evaluation of “early” and “late” run alleles in Rogue River Chinook salmon (*Oncorhynchus tshawytscha*)

An evaluation of “early” and “late” run alleles in Rogue River Chinook salmon (*Oncorhynchus tshawytscha*)

Prepared by

Kathleen G. O’Malley, Oregon State University
Dan Van Dyke, Oregon Department of Fish and Wildlife
Peter A. Samarin, Oregon Department of Fish and Wildlife
Sandra Bohn, Oregon State University
Shaun Clements, Oregon Department of Fish and Wildlife

Oregon Department of Fish and Wildlife
4034 Fairview Industrial Drive SE
Salem, OR 97302

CONTENTS

BACKGROUND 1
METHODS 2
RESULTS 5
CONCLUSIONS 13
ACKNOWLEDGMENTS 15
REFERENCES 15
SUPPLEMENTARY INFORMATION 16

BACKGROUND

The Rogue River in Oregon supports one of the largest remaining coastal runs of wild spring Chinook salmon in the Pacific Northwest and California, as well as a robust run of fall Chinook salmon. In the Rogue basin, the Oregon Department of Fish and Wildlife (ODFW) manages spring and fall Chinook salmon as distinct species management units[1] (SMUs). Spring Chinook salmon are currently defined as those adult Chinook salmon that enter freshwater during the period February through mid-July. The management goals and strategies for naturally produced spring Chinook salmon (NPCHS) in the Rogue SMU are documented in the Rogue Spring Chinook Salmon Conservation Plan (hereafter referred to as "Plan"), which was adopted by the ODFW Fish and Wildlife Commission on September 7, 2007 and updated in 2019. The desired biological status for NPCHS was defined during a stakeholder process associated with the development of the Plan and includes targets for abundance, migration time, age structure, spawner distribution and composition, and persistence.

To achieve desired status, the Plan outlines several management strategies and actions to address primary factors that limit the population of NPCHS and thereby achieve desired levels of various measurable criteria (abundance, spawner distribution, spawner composition, persistence). A key objective for the Plan is to restore and maintain, at sustainable levels of abundance, the historical life history characteristics of NPCHS. This was an important preference coming from the advisory committee (public stakeholders and agency representatives) that aided ODFW with Plan development. The advisory committee was particularly concerned about the early run NPCHS because this component of the run was disproportionately impacted by the construction and operation of William Jess Dam and Lost Creek Reservoir, and experienced higher harvest rates than other run components. Historically, early run NPCHS migrated the farthest upstream into areas that were blocked by construction of the dam. In addition, the increased water temperatures associated with reservoir heating in winter appear to have accelerated embryo development in the spawning reaches below the dam. This disproportionately impacts early run NPCHS because they spawn earlier than other parts of the run and are therefore at risk of emerging from their redds during periods when river conditions are poor for fry survival. Additionally, flow augmentation from the reservoir in summer, intended to improve pre-spawn survival of early run NPCHS, has allowed fish expressing the late run life history phenotype to migrate further upriver, resulting in increased overlap in spawning distribution between spring and fall run fish. Given this, it is important for ODFW to understand the current nature of genetic interactions between spring and fall Chinook salmon to better inform management decisions.

There have been significant advances over the last five years in the field of genetics and our understanding of the genetic basis of run timing in anadromous salmonids. In the Rogue River, two genetic markers appear to be highly diagnostic for run timing (Thompson *et al.* 2019). Spring Chinook salmon have two copies (homozygous

[1] A Species Management Unit is a collection of populations from a common geographic region that share similar genetic and ecological characteristics.

spring) of the "early" run allele while fall Chinook salmon have two copies (homozygous fall) of the "late" run allele. Chinook salmon that have one copy of the "early" run allele and one copy of the "late" run allele (heterozygous) tend to have intermediate run timing (Thompson *et al.* 2019). These diagnostic run-timing markers provide a useful tool to evaluate the current status and effectiveness of management actions with respect to many of the metrics described in the Plan. Additionally, understanding the genetic status of spawning Chinook salmon is likely to provide clarity to previous research, like Thompson *et al.* (2019), and the validity of assumptions regarding temporal and spatial spawning behavior of spring and fall Chinook salmon in the upper Rogue River.

The analysis in Thompson *et al.* (2019) noted spatial and temporal overlap in homozygous spring, heterozygous, and homozygous fall fish in the primary spawning reaches below William Jess Dam based on samples collected by ODFW in 2014. However, there are some important caveats to the inference that can be drawn from these data because of the sample collection design. Specifically, tissue samples in 2014 were collected during a period (Sept 22-Oct 29) that did not include carcasses from the first three weeks of spawning, when early returning NPCHS typically predominate. Additionally, in 2014 surveyors were intentionally selecting fish based on appearance of the carcass (versus simply sampling every 4th fish encountered, as in 2016-18). Last, the small sample size in 2014 likely does not adequately capture diversity present within time periods or sites, and thus cannot be considered representative of the run (see Supplemental Information for Summary of 2014 sampling).

To address these limitations of previous work and provide a baseline assessment of the spatial and temporal distribution of Rogue River Chinook salmon based on the run-timing genetic markers, ODFW implemented a project beginning in 2016 to collect and analyze samples from across the entire spawning period in survey reaches below Cole Rivers Hatchery. Samples were also collected from a subset of the 2018 Cole Rivers Hatchery spring Chinook broodstock to determine the genetic composition of the fish based on run-timing markers.

Objectives

1. Evaluate the spatial and temporal patterns of "early" and "late" run alleles in Chinook salmon spawning below William Jess Dam in the Rogue River watershed.

2. Determine the number of homozygous spring, heterozygous, and homozygous fall Chinook salmon in a subsample of the 2018 Cole Rivers Hatchery broodstock.

METHODS

Sampling

ODFW collected tissue samples from every 4th Chinook salmon carcass found in 2016 (N = 445), 2017 (N = 485), and 2018 (N = 485). Carcass samples were collected from 9 survey reaches along a 31.5 mile stretch of the Rogue River between the old Gold Ray Dam site and Cole Rivers Hatchery. Previous tagging studies have shown that this area is the only habitat currently used by NPCHS for spawning in the mainstem Rogue River (Table 1, Figure 1). Sampling occurred between Sept 10 and Nov 9, which represented the entire known spawning season. Tissue samples collected from carcasses were folded in paper and stored dry in coin envelopes. Carcasses are assumed to represent the composition of Chinook salmon spawning in the reach they were collected based on previous marking experiments in which there was minimal movement out of a reach under normal flow conditions. Only three carcasses were adipose fin clipped: sample 451 and 452 collected on Oct 19 and sample 475 collected on Oct 29 in 2018. Three envelopes from 2016 (one each from BB-RE, SC-TAK, and DB-CC) and one envelope from 2018 (from SC-TAK) did not contain fin clips. Therefore, the total number of fin clips collected in each year was as follows: 2016 (N = 442), 2017 (N = 485), and 2018 (N = 484). Samples were also collected from 1,575 of the 4,302 Chinook salmon used as broodstock in 2018 at the Cole Rivers Hatchery (Table 3). Broodstock tissue samples were batch sampled and stored in 95% ethanol.

Table 1. Ten sites that mark the beginning and end of the nine survey reaches sampled during the upper Rogue River Chinook salmon spawning ground surveys. Abbreviations are used to indicate the sites in Figure 1.

Abbreviation	Description	River Mile
CRH	Cole Rivers Hatchery	157
BB	Big Butte Creek	155.5
RE	Rogue Elk Park	152
TC	Trail Creek	148.6
SC	Shady Cove	146.2
TAK	Takelma Park	142.2
DB	Dodge Bridge	138.6
CC	Constance Creek	134.7
TV	TouVelle Park	131.5
GR	Gold Ray Dam site	125.5

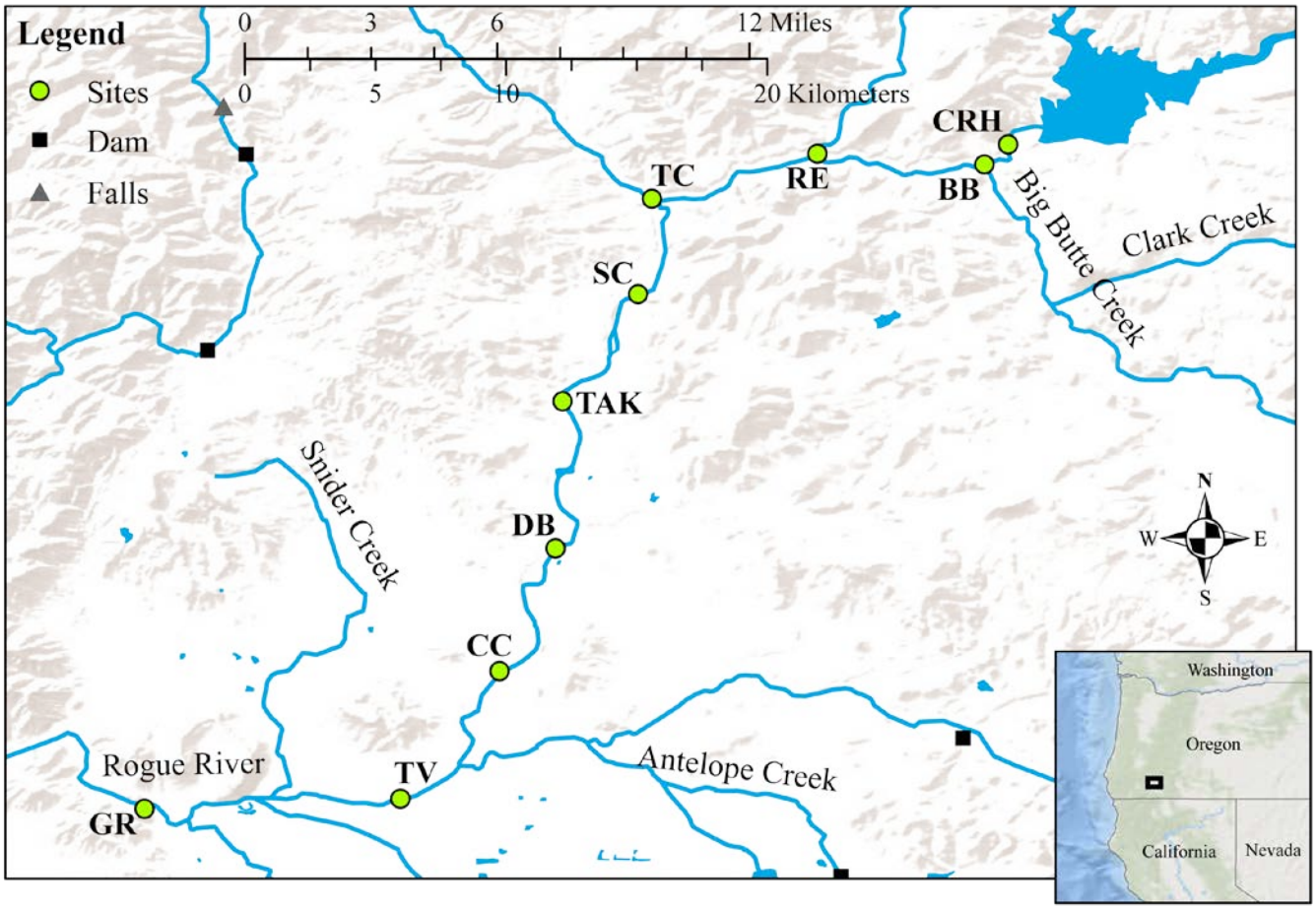


Figure 1. Map of the nine survey reaches sampled during the Rogue River Chinook salmon spawning ground surveys (P. Samarin, pers. comm.). Site names indicating the beginning and end of each reach are listed in Table 1.

DNA extraction and genotyping

DNA was extracted from the samples using the method of Ivanova *et al.* (2006). Using the GT-seq method (Campbell *et al.* 2015), all samples were genotyped at 298 single nucleotide polymorphisms (SNPs) (Hess *et al.* 2015), a sex marker (Hess *et al.* 2015), and two SNPs (positions 640165 and 670329) located ~30 kb apart and just upstream of the *Greb1L* gene (Thompson *et al.* 2019). These two SNPs are hereafter referred to as *Greb1L* SNP1 and SNP2, respectively.

Twelve pairs of loci align within 10,000 bp of each other. To avoid possible linkage disequilibrium, we removed the locus from each pair with a lower number of effective alleles calculated in GENALEX (Peakall and Smouse 2012). In addition, we removed 28 loci that had more than 20% missing data and 15 loci that were monomorphic in the carcass and broodstock samples. The final marker set included 243 SNPs previously developed by Hess *et al.* (2015), the sex marker (Hess *et al.* 2015) and the two *Greb1L* SNPs (Thompson *et al.* 2019).

Carcass samples that were not successfully genotyped using the GT-seq method due to tissue sample degradation were genotyped at *Greb1L* SNP1 using the qPCR protocol described in Thompson *et al.* (2019). *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.). Each qPCR plate contained known spring, fall, and heterozygous samples (provided by T. Thompson) and negative controls to check for contamination. All known samples had the expected genotype and no negative controls showed amplification.

Statistical analyses

Conformance to Hardy–Weinberg proportions (HWP) was examined using GENEPOP version 3.3 (Raymond and Rousset 1995). Linkage equilibrium was examined in GENEPOP and PLINK version 1.9 (Chang *et al.* 2015). We corrected for multiple tests using false discovery rate (FDR) following Benjamini and Hochberg (1995). Expected and observed heterozygosities and fixation indices were calculated using GENETIX version 4.02 (Belkhir 2000). Fixation indices were tested for significance using 5,000 bootstrap permutations.

Exact tests for differences in genic and genotypic frequencies among samples were performed using GENEPOP. Tests were conducted with specified Markov chain parameters of 5,000 dememorization steps followed by 500 batches of 2,000 iterations per batch. Estimates of F_{ST} were calculated using GENETIX and the data permuted 5,000 times to allow hypothesis testing. We corrected for multiple testing using FDR following Benjamini and Hochberg (1995).

Carcass samples were checked for the presence of genetically distinct groups using the clustering method of STRUCTURE (Pritchard *et al.* 2000; Hubisz *et al.* 2009). Samples were analyzed with and without *Greb1L* SNP1 (Thompson *et al.* 2019). Structure runs had a burn-in of 50,000 iterations followed by 50,000 data collection iterations and assumed admixture and correlated allele frequencies. The number of clusters (k) was allowed to range from 1 to 5, and 20 replicates were completed for each k.

RESULTS

Genotyping

As a result of tissue sample degradation, many of the carcass samples failed to genotype using the GT-seq method (Table 2). Overall genotyping success was higher for *Greb1L* SNP1 because samples were re-genotyped using the qPCR approach outlined in Thompson *et al.* (2019). One marker, *Ots_111312-435*, significantly deviated from HWP in all carcass samples after FDR correction. Both *Greb1L* SNP1 and SNP2 significantly deviated from HWP after FDR correction in 2018, but not in 2016 or 2017. Genotyping success rate was much higher for the 2018 Cole Rivers Hatchery broodstock collection (Table 3). Thirty-five loci deviated from HWP in the broodstock samples after FDR correction. The 2018 carcass samples had the lowest observed heterozygosity of

the carcass samples (Table 4). The 2018 Cole Rivers Hatchery broodstock had similar genetic diversity to the 2018 carcass samples.

Table 2. Carcass samples genotyped from each survey reach by year. The number of samples genotyped is reported for the 243 SNPs and *Greb1L* SNP1 and SNP2. SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.).

(a) 2016

Survey Reach	Collected	243 SNPs	*Greb1L* SNP1	*Greb1L* SNP2
CRH-BB	29	12	26	12
BB	31	6	30	6
BB-RE	67	22	61	22
RE-TC	46	15	39	14
TC-SC	51	13	49	13
SC-TAK	92	29	84	29
TAK-DB	69	17	62	17
DB-CC	21	5	19	5
CC-TV	28	8	24	8
TV-GR	8	1	7	1
Total	442	128	401	127

(b) 2017

Survey Reach	Collected	243 SNPs	*Greb1L* SNP1	*Greb1L* SNP2
CRH-BB	34	17	33	16
BB	53	15	51	15
BB-RE	82	24	76	24
RE-TC	62	8	56	8
TC-SC	70	16	67	16
SC-TAK	95	34	92	34
TAK-DB	55	20	53	19
DB-CC	16	5	16	5
CC-TV	13	2	12	2
TV-GR	5	4	5	4
Total	485	145	461	143

(c) 2018

Survey Reach	Collected	243 SNPs	*Greb1L* SNP1	*Greb1L* SNP2
CRH-BB	67	32	63	31
BB	60	23	59	22
BB-RE	59	19	54	19
RE-TC	66	14	61	14
TC-SC	75	21	71	20
SC-TAK	99	29	91	29
TAK-DB	47	9	44	8
DB-CC	5	1	5	1
CC-TV	4		4	
TV-GR	2		2	
Total	484	148	454	144

(d) Total

Survey Reach	Collected	243 SNPs	*Greb1L* SNP1	*Greb1L* SNP2
CRH-BB	130	61	122	59
BB	143	42	140	43
BB-RE	209	65	191	65
RE-TC	174	35	156	36
TC-SC	196	50	187	49
SC-TAK	286	91	267	92
TAK-DB	171	46	159	44
DB-CC	42	11	40	11
CC-TV	45	10	40	10
TV-GR	15	5	14	5
Total	1411	421	1316	414

Table 3. Cole Rivers Hatchery broodstock samples genotyped for each collection date. For each collection date, we report the origin (hatchery-origin, HOR; natural-origin, NOR; or both, Mix), number of samples we attempted to genotype, the number of samples successfully genotyped, the number of duplicate samples identified, and the final number of samples genotyped for the 243 SNPs and *Greb1L* SNP1 and SNP2. SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.).

							Greb1L	
Jar #	Date	Origin	Attempted	Genotyped	Duplicate	243 SNPs	SNP1	SNP2
1	5/15	NOR	1	1	0	1	1	1
2	5/15	HOR	57	56	0	56	53	56
3	5/16	Mix	93	88	1	87	86	87
4	5/23	HOR	139	123	0	123	123	123
5	5/23	NOR	9	8	0	8	8	8
6	5/30	Mix	148	147	1	146	144	146
7	6/6	Mix	148	140	0	140	140	140
8	6/13	Mix	148	142	4	138	133	137
9	6/20	Mix	148	137	3	134	130	134
10	6/27	Mix	44	38	1	37	35	37
11	7/3	Mix	102	101	7	94	92	94
12	7/11	Mix	137	131	7	124	119	121
13	7/18	Mix	24	24	3	21	21	21
14	7/25	Mix	39	38	0	38	35	38
15	8/1	Mix	48	46	6	40	38	38
16	8/15	Mix	111	110	4	106	95	101
17	8/18	Mix	112	111	5	106	100	105
18	8/22	Mix	31	26	0	26	23	26
19	8/29	Mix	31	28	0	28	24	27
20	9/5	Mix	5	4	0	4	4	4
Total			1575	1499	42	1457	1404	1444

Table 4. Measures of genetic diversity for each year of carcass samples and the 2018 Cole Rivers Hatchery broodstock samples. Sample sizes (n), average number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F) are listed for 243 SNPs and *Greb1L* SNP1 which is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.). Fixation indices that significantly deviated from 0 are indicated in **bold-faced** type.

Year	Sample type	n	N_A	H_O	H_E	F
2016	Carcass	128	1.980	0.2954	0.3001	0.0196
2017	Carcass	145	1.963	0.2964	0.3009	**0.0185**
2018	Carcass	148	1.963	0.2898	0.2981	**0.0316**
2018	Hatchery broodstock	1457	1.975	0.2887	0.2988	**0.0343**

Population structure

None of the survey reaches were significantly different from each other based on the exact tests for genic or genotypic differentiation ($p > 0.9$). Similarly, pairwise F_{ST} estimates were not significant after correcting for multiple comparisons (Table S1). We did not see a pattern of isolation by distance within the extent of the sampling area, and the program STRUCTURE found no evidence of population structure. Removing *Ots_111312-435* and/or *Greb1L* SNP1 from the dataset did not affect the STRUCTURE results. There was also no evidence of population structure within the 2018 Cole Rivers Hatchery broodstock samples or between the Cole Rivers Hatchery broodstock and the carcass samples (2016-2018).

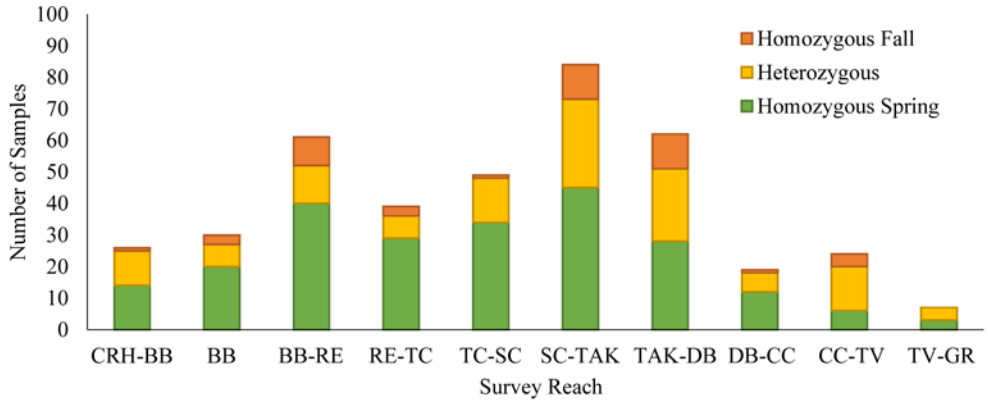
Temporal and spatial distribution of Greb1L genotypes among carcass samples (2016-2018)

Across all three sample years, homozygous spring fish were frequently found in upstream survey reaches from Cole Rivers Hatchery to Takelma Park (SNP1: Figure 2; SNP2: Figure S1). Homozygous spring and heterozygous fish were primarily (85.5% of total) sampled earlier in the season (i.e. weeks 37 to 41; ~Sept 10 – Oct 8) in these upstream survey reaches. Most homozygous fall fish (96.6% of total) were sampled in week 40 (~Oct 1) or later (SNP1: Figure 3; SNP2: Figure S2). When examining the distribution of *Greb1L* genotypes across time within each survey reach, we found that the frequency of homozygous fall fish increased later in the sampling period (i.e. week 40 or later) across all three sample years (SNP1: Figure 4; SNP2: Figure S3).

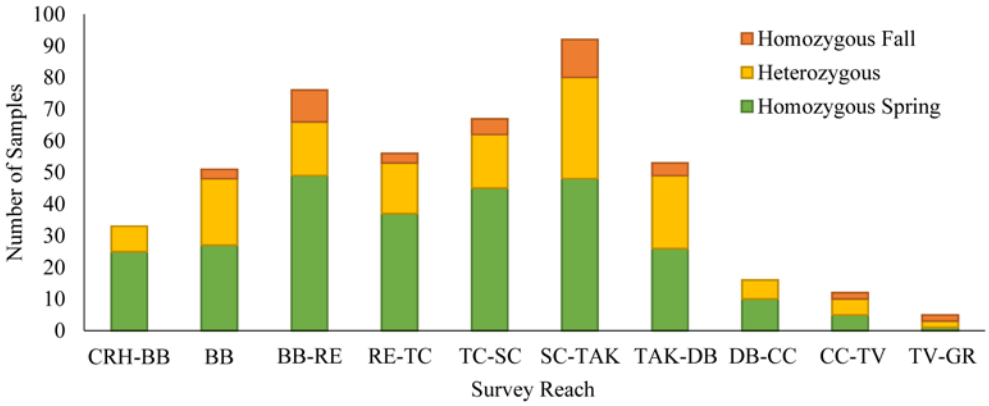
Temporal distribution of Greb1L genotypes in the Cole Rivers Hatchery broodstock (2018)

Most of the 2018 Cole Rivers Hatchery broodstock samples that were genotyped were homozygous for the spring *Greb1L* allele (SNP1: 88.2%; SNP2: 83.9%). However, some fish were heterozygous (SNP1: 11.5%; SNP2: 15.4%) and a small percentage of the broodstock were homozygous fall (SNP1: 0.3%; SNP2: 0.7%). The homozygous fall fish were collected after Aug 15 (Figure 5).

(a) 2016



(b) 2017



(c) 2018

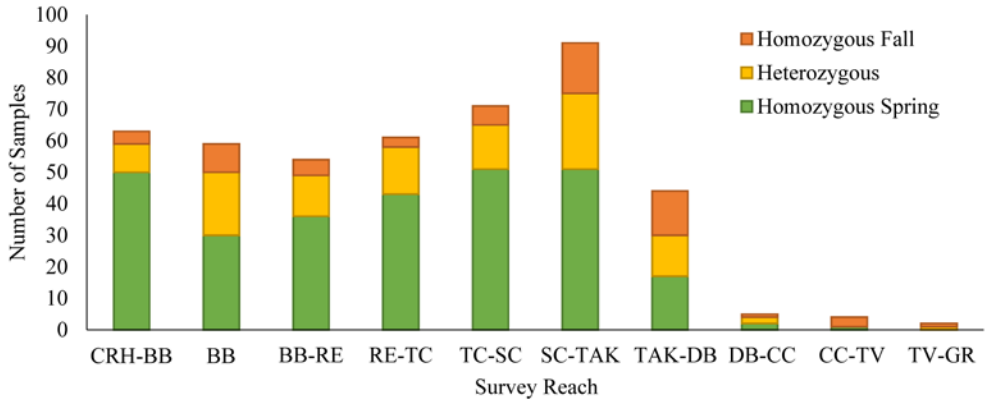


Figure 2. Distribution of *Greb1L* SNP1 genotypes across survey reaches. *Greb1L* SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.). Survey reach names are included in Table 1.

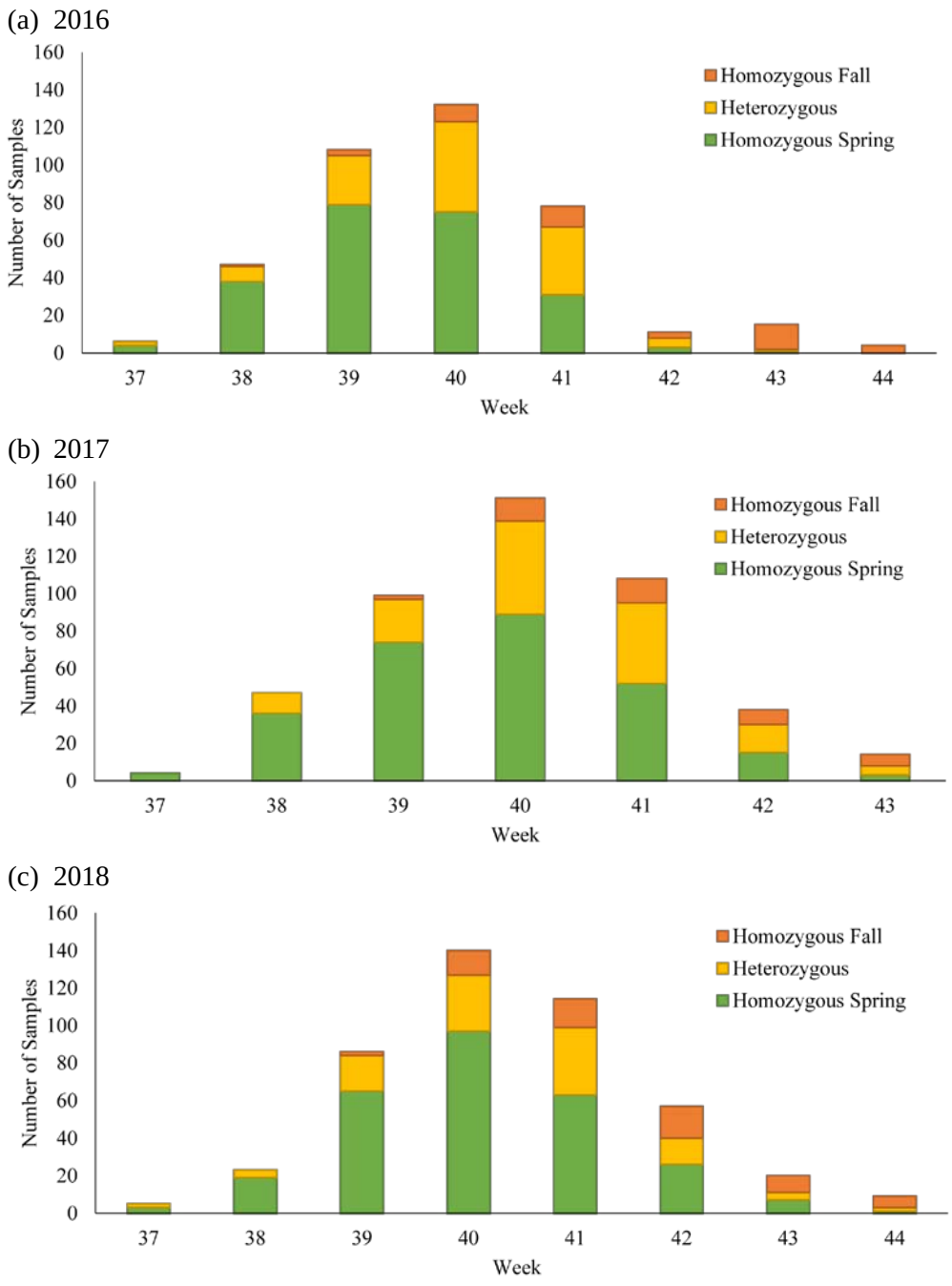


Figure 3. Distribution of *Greb1L* SNP1 genotypes by Julian week when carcass samples were collected, ranging from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4). SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.).

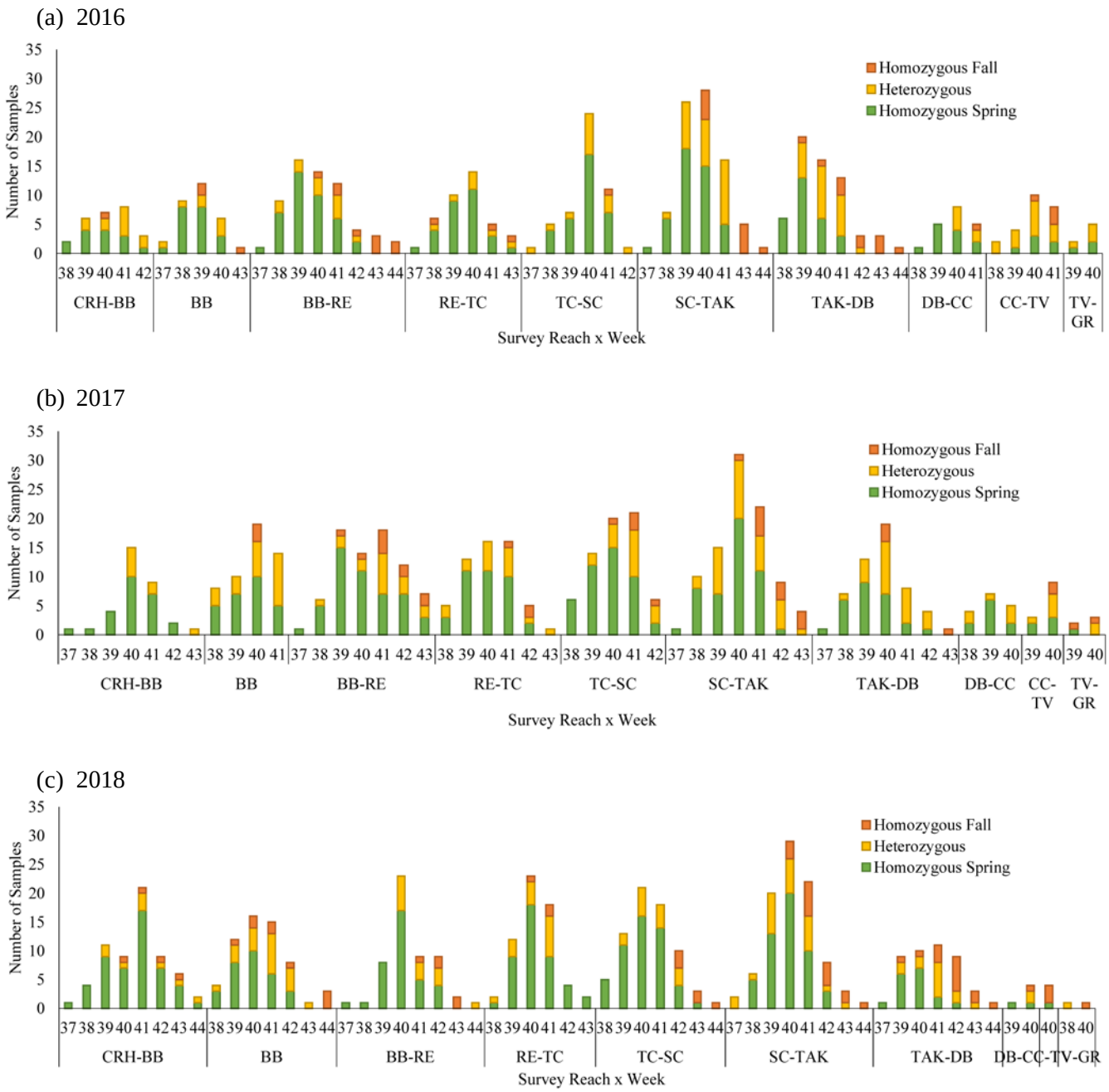
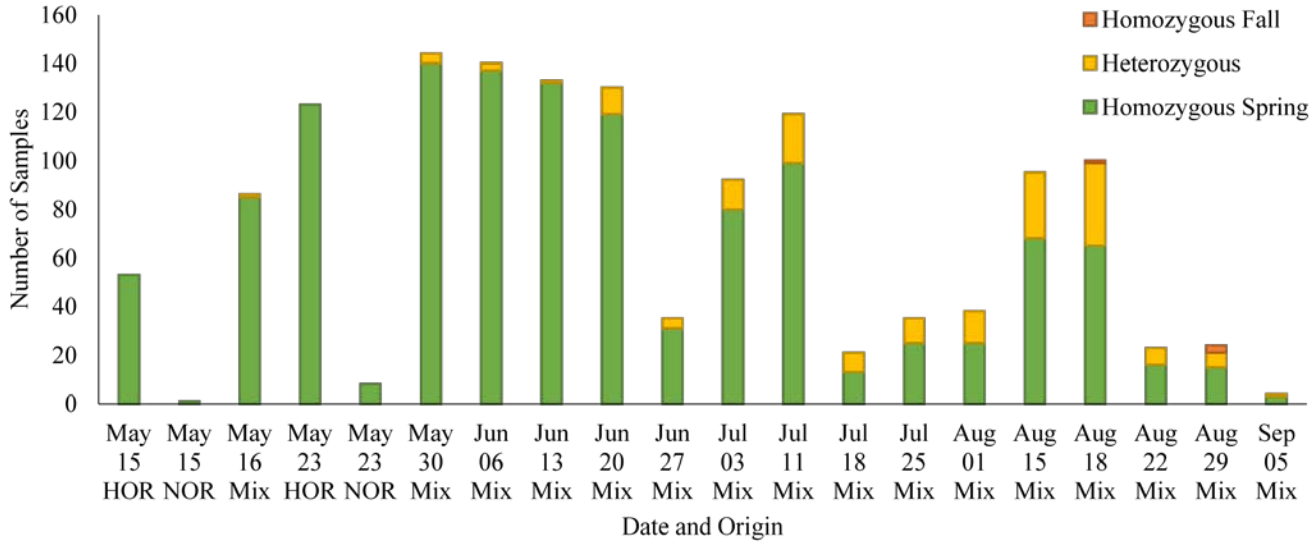


Figure 4. Distribution of *Greb1L* SNP1 genotypes across survey reaches and time within each year (2016, 2017 and 2018). The Julian week when carcass samples were collected is on the x-axis and ranges from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4), grouped by survey reach. Survey reach names are included in Table 1.

(a) SNP1



(b) SNP2

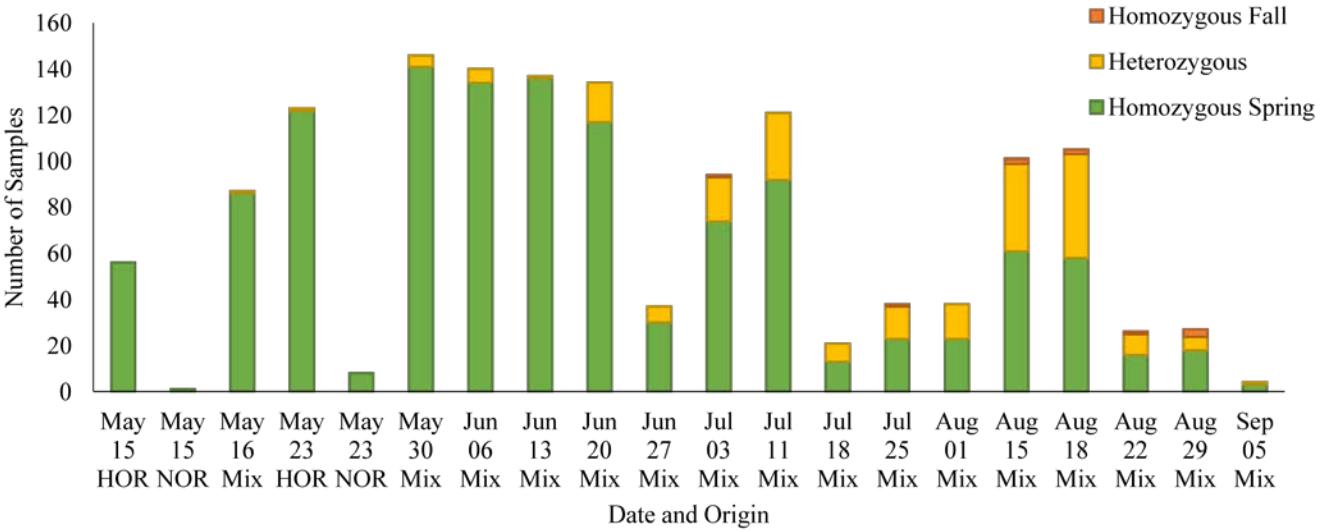


Figure 5. Distribution of *Greb1L* genotypes in the 2018 Cole Rivers Hatchery broodstock by date of collection. Samples are listed as hatchery-origin (HOR) or natural-origin (NOR) if known, and mixed (Mix) if unknown.

Extent of reproductive isolation of homozygous spring Chinook salmon

In most reaches per weeks (98 of 132), homozygous spring Chinook salmon constituted ≥50% of the spawners, though in any given reach per week these fish typically represented a small (<4%) proportion of the total population of homozygous spring Chinook salmon spawning in the basin that year.

Across all three years, we consistently saw that homozygous spring Chinook salmon constituted >70% of the Chinook spawners in the majority (~65%) of reaches per weeks. The fall Chinook salmon escapement for 2016-2018 was 27,278, 90,674, and 39,497 respectively. The spring Chinook salmon escapement for 2016-2018 was 9,573, 10,240, and 10,353 respectively.

CONCLUSIONS

These results provide the first comprehensive, multi-year analysis of the spatial and temporal distribution of *Greb1L* alleles across the entire spawning period for Chinook salmon in the upper Rogue River (River Mile 125.5-157). ODFW sampled carcasses from every fourth fish in each year (2016-2018). In total, tissue samples were collected from 1,411 fish spanning the 9 survey reaches with sampling beginning on Sept 10 each year and concluding on Nov 4.

Across all three sample years (2016-2018), homozygous spring fish were frequently found in the upstream survey reaches from Cole Rivers Hatchery to Takelma Park. Within each year and survey reach, homozygous spring fish were more frequently found earlier in the season while homozygous fall fish were more frequently found later in the season. The data suggest there is significant temporal and spatial separation among spring and fall Chinook salmon spawning, and, to a lesser extent, among spring Chinook salmon and heterozygotes in the upper Rogue River. In most instances (~65%), spring fish constitute >70% of all spawners collected within a reach per week across all three years.

Our results differ from those of Thompson *et al.* (2019), which suggested greater spatial and temporal overlap in homozygous spring, heterozygous, and homozygous fall fish. However, because of the small sample size (N=86) in 2014 and the numerous differences in sampling protocol between the 2014 and 2016-18 studies, significant caution should be used when making comparisons between the two studies (see summary in the Supplement and Figure S4). Sampling methods used in our study provide a clearer, more comprehensive picture of the genetic composition of spawning Chinook salmon in the upper Rogue River than has previously been available.

The majority of the 2018 Cole Rivers Hatchery broodstock samples were homozygous spring fish (SNP1: 88.2%) while only a small fraction were homozygous fall fish (SNP1: 0.3%). The frequency of heterozygous fish increased later in the collection period and homozygous fall fish were only collected after Aug 15. The hatchery

program mitigates for production of spring Chinook salmon that was lost when William Jess Dam was constructed, blocking access to the upper mainstem, the South Fork and the Middle Fork Rogue River. Similar to genetic results in 2004, ODFW believes that the results of this study show that the hatchery program continues to adequately reflect the pre-dam life history of Rogue spring Chinook salmon. The broodstock program is managed to maintain the pre-dam life history and fisheries managers will use this research to ensure that fall Chinook salmon are not used as broodstock.

Implications for monitoring

ODFW fish management staff estimate the abundance of NPCHS by counting the carcasses of Sept spawners. The estimates are derived from weekly surveys that encompass the entirety of known Rogue spring Chinook spawning habitat from river mile 125.5 to river mile 157 and the lower mile of Big Butte Creek (see Table 1, Figure 1). This count includes carcasses collected through Oct 9 to account for post-spawn longevity. Based on this three-year study, the majority (63.5%) of fish included in the annual estimate of abundance were homozygous spring fish and 29.4% were heterozygous fish. While some homozygous fall Chinook salmon are included in the count (7.1%), this is likely offset by the fact that some homozygous spring Chinook salmon are not collected by Oct 9. Regardless, these data could be used to adjust future abundance estimates. Additionally, these data establish a baseline against which periodic assessments could be conducted to monitor the trend in spatial and temporal overlap between homozygous spring, heterozygous, and homozygous fall fish.

Implications for management

The data suggest that homozygous spring Chinook salmon persist in the Rogue River in relatively large numbers and are generally spatially and temporally isolated during spawning from heterozygotes and homozygous fall Chinook salmon. Implementation of the Plan, because of its emphasis on restoring early run NPCHS, is expected to further protect and enhance homozygous spring Chinook salmon. Key management actions currently implemented include: Lost Creek Reservoir flow management with spring Chinook salmon as the highest priority; fishery management that protects early run wild spring Chinook salmon from direct harvest as the population builds; and added fishing opportunity to target early run fall Chinook salmon in the upper Rogue. The first year of post-plan implementation was 2008. The first returns from the collective stewardship of the Plan began in approximately 2012. ODFW will continue to monitor and report on Plan progress, incorporating periodic genetic monitoring, such as this research, to assess trends in spatial and temporal separation among the run timing genotypes.

Acknowledgments

We thank the Oregon State University Center for Genome Research and Biocomputing for sequencing our samples. This report benefitted from review by Steve Mazur and Chris Lorion of ODFW. This project was funded by a U.S. Army Corps of Engineers grant awarded to K. O'Malley and by the State Fisheries Genomics Lab.

References

Belkhir K (2000) GENETIX, logiciel sous Windows pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpelier.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)* 57: 289-300.

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. *Molecular Ecology Resources* 15: 855-867.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* 4:s13742–015–0047–8, https://doi.org/10.1186/s13742-015-0047-8.

Hess JE, Campbell NR, Matala AP, Hasselman DJ, Narum SR. (2015) GENETIC ASSESSMENT OF COLUMBIA RIVER STOCKS, 4/1/2014 -3/31/2015 Annual Report, 2008-907-00, https://www.critfc.org/wp-content/uploads/2016/04/16-03.pdf.

Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proceedings of the Royal Society B-Biological Sciences* 283: 20153064.

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9: 1322–1332.

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

Peakall R, Smouse, PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Prince DJ, O'Rourke SM, Thompson TQ, *et al.* (2017) The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Science Advances* 3: e1603198.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

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

Thompson TQ, Bellinger MR, O'Rourke SM, Prince DJ, Stevenson AE, Rodrigues AT, Sloat MR, Speller CF, Yang DY, Butler VL, Banks MA, Miller MR (2019) Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. *Proceedings of the National Academy of Sciences of the United States of America* 116: 177-186.

Supplementary Information

Summary of 2014 Sampling

In Sept 2014, ODFW received a request from UC Davis researchers (Dr. Miller *et al*.) to collect tissue samples from Chinook salmon in the Rogue River. The goal of sampling was to collect tissue samples from fish with a known run time phenotype (early and late) to test the diagnostic power of two genetic markers (snp640165 and snp670329). In support of this request, ODFW staff (Dr. Marc Johnson) developed a sampling protocol (below) that was intended to achieve this goal.

Proposed sampling protocol:

(presumed) Spring Chinook salmon:

- N = 10-36 samples from unmarked (presumed wild) fish that volitionally entered Cole Rivers Hatchery during the spring and summer of 2014. Sample collection dates to be Sept 10, 17, 24 of 2014
- N = 10-36 samples from fresh, unmarked (presumed wild) salmon carcasses encountered in upper reaches of the Rogue River on Sept 8, 15, 22, 29 of 2014

(presumed) Fall Chinook salmon:

- N = 24-36 from spawned-out fish in late Oct/Nov of 2014 from the Grants Pass area—in all likelihood, these will be fall Chinook salmon
- As available, a number samples from angler-caught fall Chinook salmon, collected at Huntley Park (lower Rogue River) in mid- to late-Sept

In season modifications to sampling protocol:

Sampling was carried out by ODFW staff from the Central Point office under direction of Pete Samarin. The following modifications were made to the proposed sampling:

1) Collecting samples from fresh carcasses proved challenging, as spring Chinook salmon carcasses deteriorate and are scavenged rapidly after death from spawning. As a result, samplers intentionally targeted fresh carcasses rather than using a random sampling design.

2) The samples of late run fish were intended to be obtained from the Grants Pass area of the Rogue in Oct and Nov. However, during the month of Sept it became apparent to ODFW supervisors that the survey crew would not have time to survey the Grants Pass area for the fall Chinook salmon samples. Instead, effort was redirected to collect fall Chinook salmon samples from carcasses found higher in the system during Oct. Carcasses sampled as presumed fall Chinook salmon were identified by physical characteristics such as freshly deceased and very little skin rot, indicative of fall Chinook salmon that experience less temporal exposure to fresh water (than spring Chinook salmon).

As a result of this combination of factors, sampling of carcasses occurred from the week of Sept 22 through the week of Oct 29. Samples were collected across a 30 mile area below Lost Creek Dam (see Table S3 in Thompson *et al.* 2019 PNAS 116: 177-186).

Note: Because of the way these samples were collected, and the lack of sample collection during the early part of the run, any inference from the data beyond marker validation is limited to qualitative descriptions during the period of data collection. These data should not be used to make comparisons between years.

Table S1. Weir and Cockerham (1984) F_{ST} was calculated in GENETIX from 243 SNPs and *Greb1L* SNP1, the more diagnostic SNP in Rogue River Chinook salmon (T. Thompson, pers. comm.). Sample sizes (n) are reported for each survey reach within each year (2016, 2017, and 2018). Survey reaches with n < 5 were grouped with neighboring survey locations. No comparisons were significant after FDR correction.

(a) 2016

		BB	BB-RE	RE-TC	TC-SC	SC-TAK	TAK-DB	DB-CC	CC-GR
CRH-BB	n = 12	0.0000	0.0008	0.0007	0.0038	0.0000	0.0003	0.0000	0.0041
BB	n = 6		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
BB-RE	n = 22			0.0062	0.0001	0.0000	0.0015	0.0000	0.0000
RE-TC	n = 15				0.0040	0.0013	0.0005	0.0000	0.0062
TC-SC	n = 13					0.0000	0.0009	0.0000	0.0000
SC-TAK	n = 29						0.0000	0.0000	0.0000
TAK-DB	n = 17							0.0000	0.0000
DB-CC	n = 5								0.0000
CC-GR	n = 9								

(b) 2017

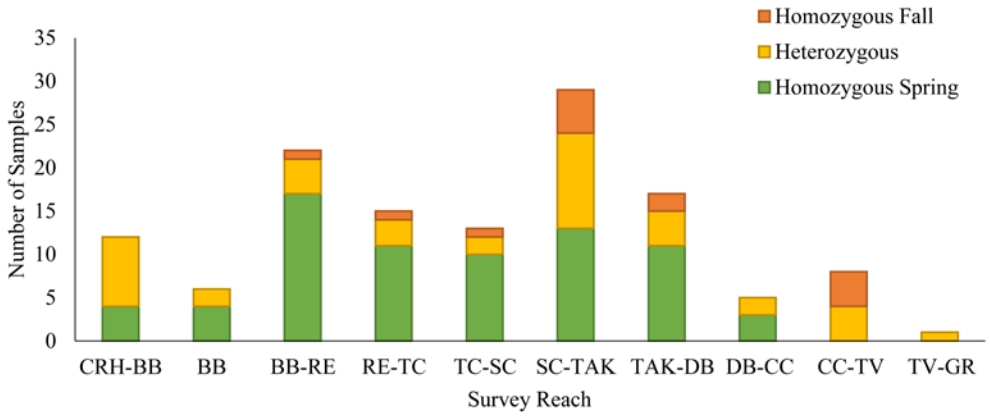
		BB	BB-RE	RE-TC	TC-SC	SC-TAK	TAK-DB	DB-CC	CC-GR
CRH-BB	n = 17	0.0068	0.0049	0.0000	0.0017	0.0023	0.0047	0.0095	0.0026
BB	n = 15		0.0039	0.0061	0.0028	0.0037	0.0038	0.0013	0.0014
BB-RE	n = 24			0.0000	0.0043	0.0000	0.0000	0.0105	0.0045
RE-TC	n = 8				0.0000	0.0000	0.0000	0.0212	0.0053
TC-SC	n = 16					0.0013	0.0015	0.0000	0.0000
SC-TAK	n = 34						0.0000	0.0097	0.0033
TAK-DB	n = 20							0.0066	0.0000
DB-CC	n = 5								0.0127
CC-GR	n = 6								

(c) 2018

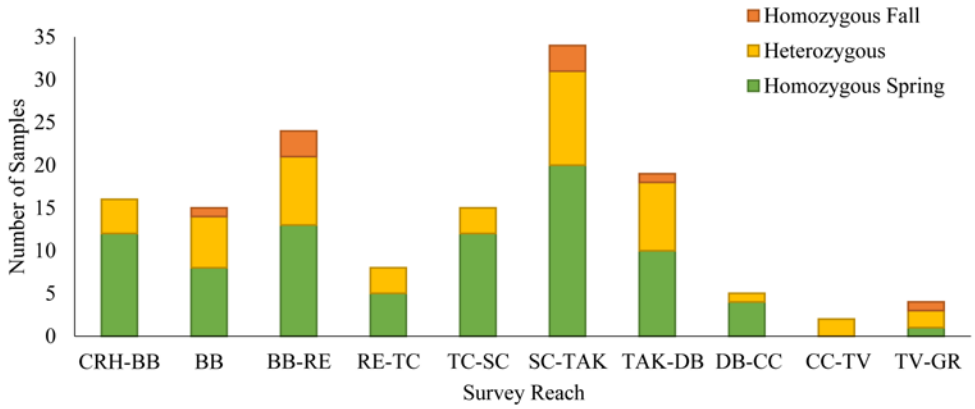
		BB	BB-RE	RE-TC	TC-SC	SC-TAK	TAK-CC
CRH-BB	n = 32	0.0010	0.0011	0.0040	0.0000	0.0021	0.0041
BB	n = 23		0.0001	0.0047	0.0009	0.0000	0.0000
BB-RE	n = 19			0.0023	0.0035	0.0000	0.0000
RE-TC	n = 14				0.0001	0.0007	0.0014
TC-SC	n = 21					0.0000	0.0010
SC-TAK	n = 29						0.0000
TAK-CC	n = 10						

Figure S1. Distribution of *Greb1L* SNP2 genotypes across survey reaches. Location names are included in Table 1.

(a) 2016



(b) 2017



(c) 2018

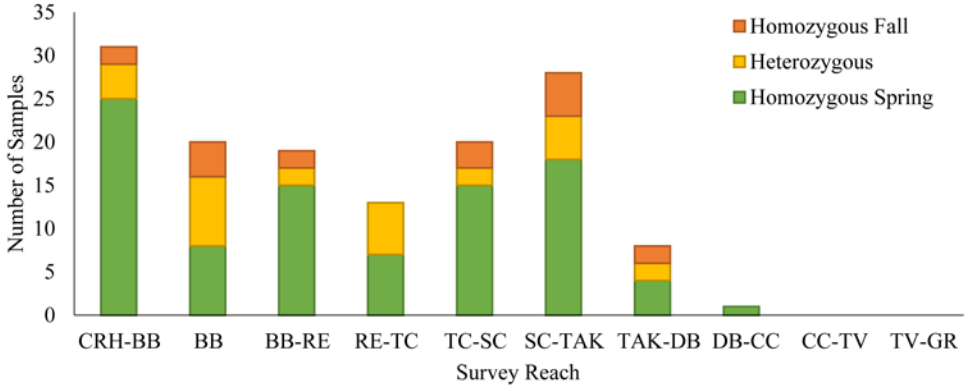
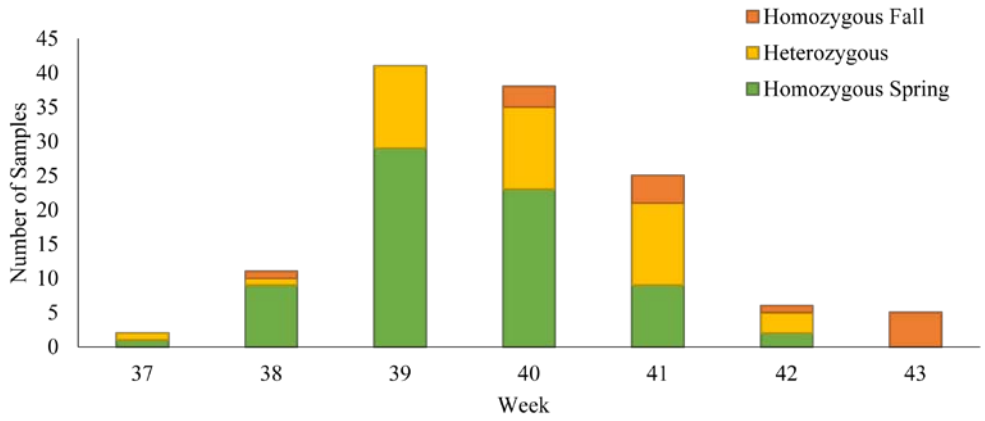
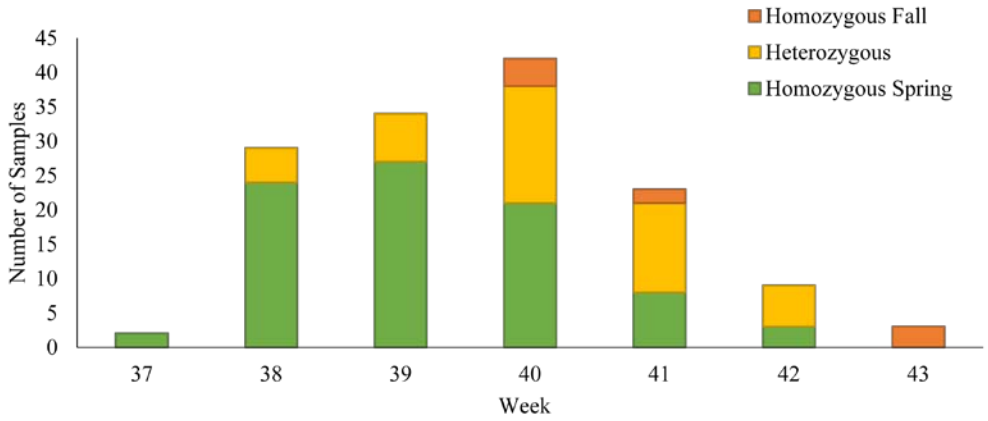


Figure S2. Distribution of *Greb1L* SNP2 genotypes by Julian week when carcass samples were collected, ranging from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4).

(a) 2016



(b) 2017



(c) 2018

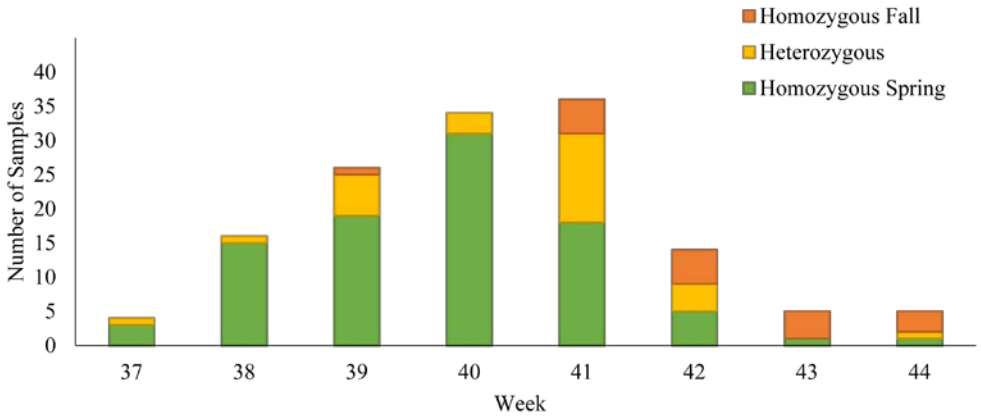
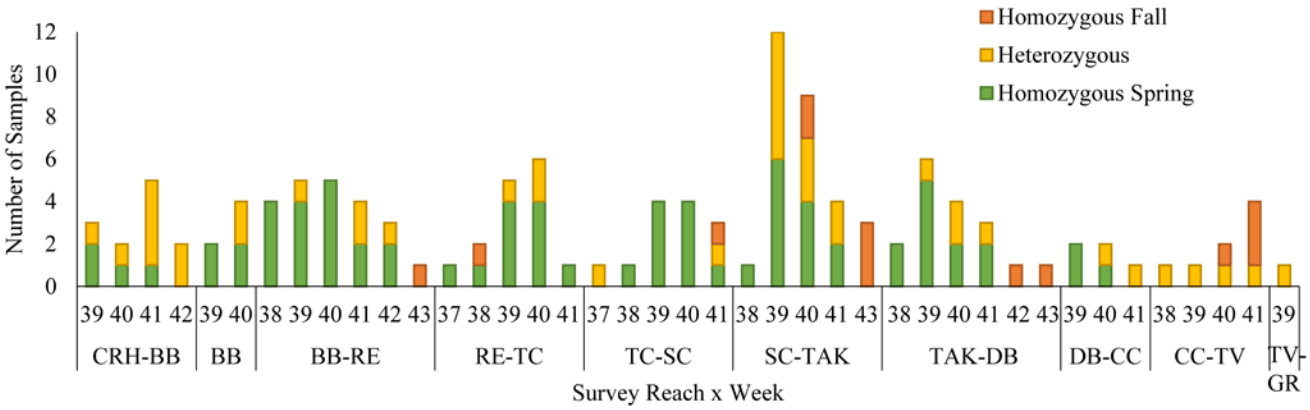
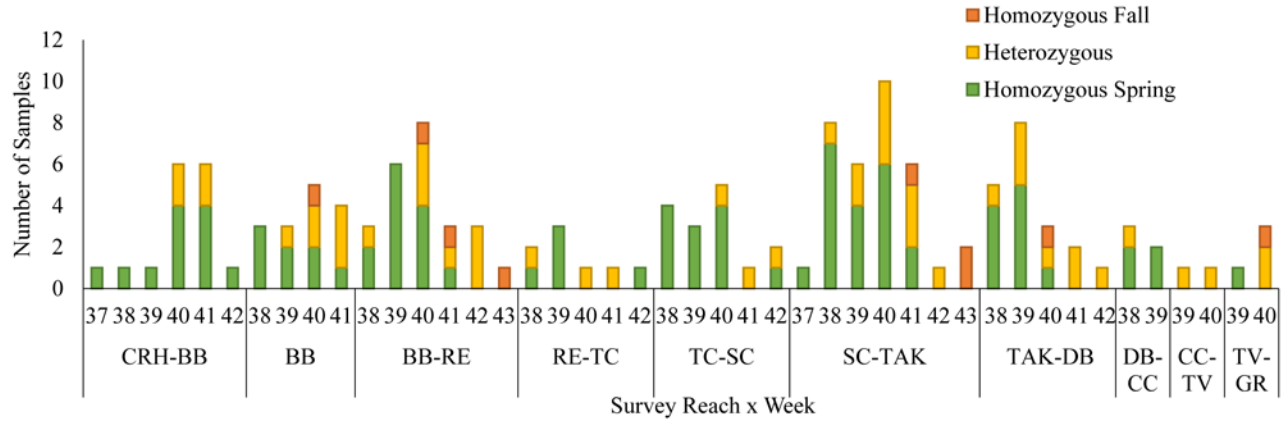


Figure S3. Distribution of *Greb1L* SNP2 genotypes across survey reaches and times. The Julian week when carcass samples were collected is on the x-axis and ranges from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4), grouped by survey reach. Survey reach names are included in Table 1.

(a) 2016



(b) 2017



(c) 2018

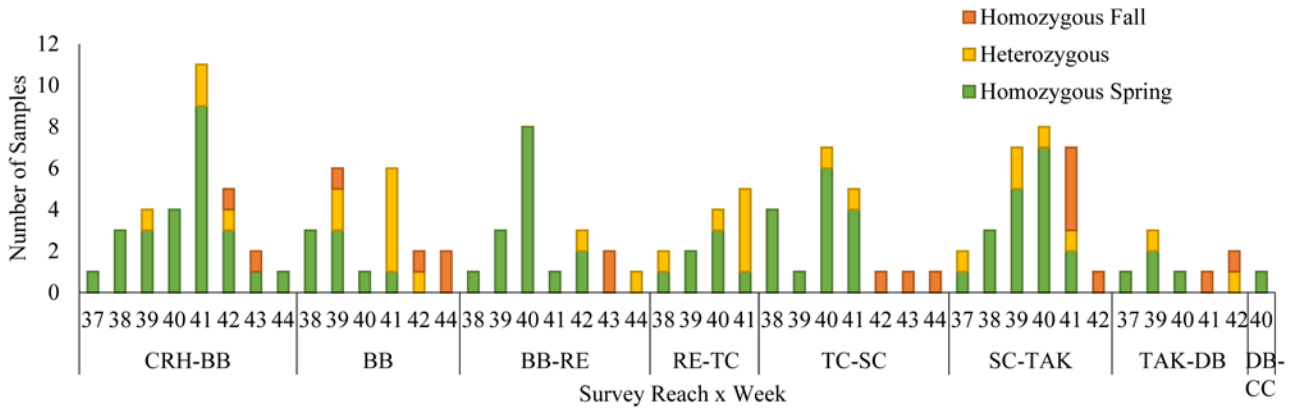
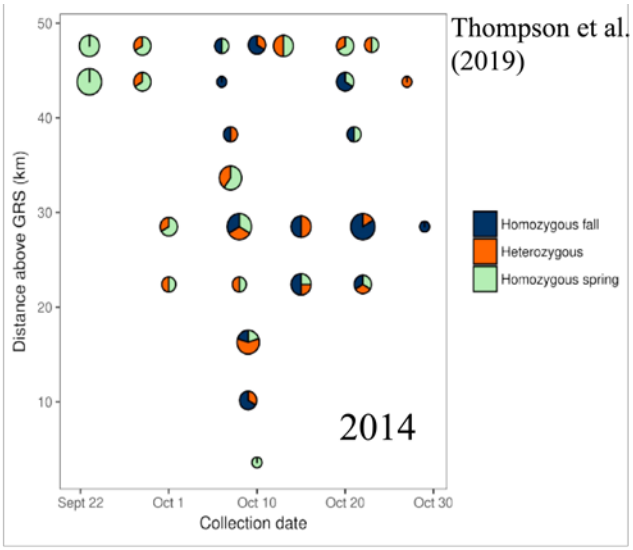
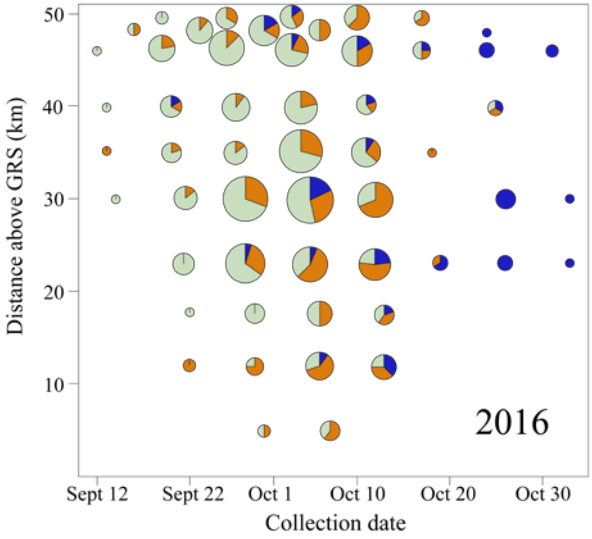


Figure S4. *Greb1L* SNP1 genotype frequencies across space and time in the upper Rogue River, modeled after Thompson *et al.* (2019) Figure S2. Pie charts represent genotype frequencies for each survey reach and date of collection. Pie chart size is proportional to sample size. Distance above the old Gold Ray Fish Counting Station (GRS) is measured from the center of each survey reach. (a) Thompson *et al.* (2019) 2014 results (range: 1-6 carcasses) (b) 2016 results (range: 1-28 carcasses) (c) 2017 results (range: 1-31 carcasses) (d) 2018 results (range: 1-29 carcasses).

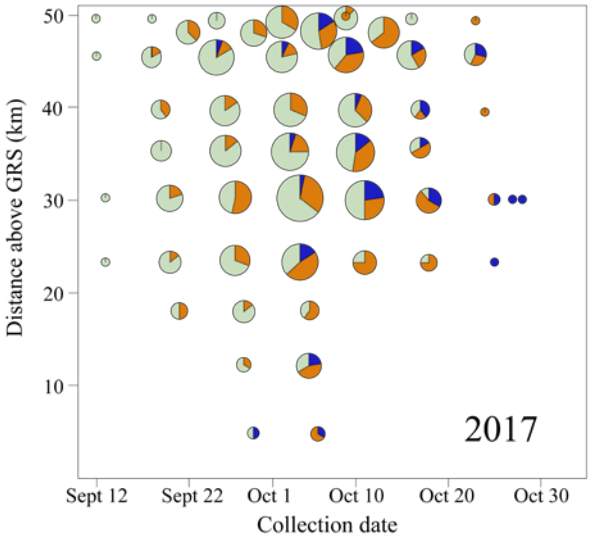
(a) 2014



(b) 2016



(c) 2017



(d) 2018

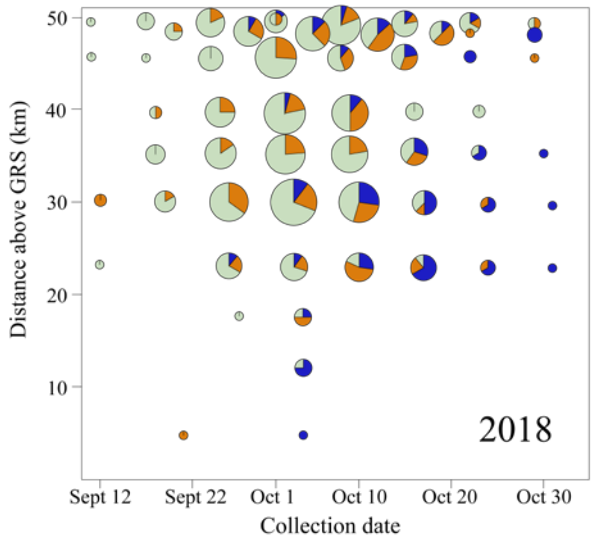


Figure S5. Distribution of *Greb1L* SNP1 genotypes across survey reaches with all years combined (2016 - 2018). *Greb1L* SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.). Survey reach names are included in Table 1.

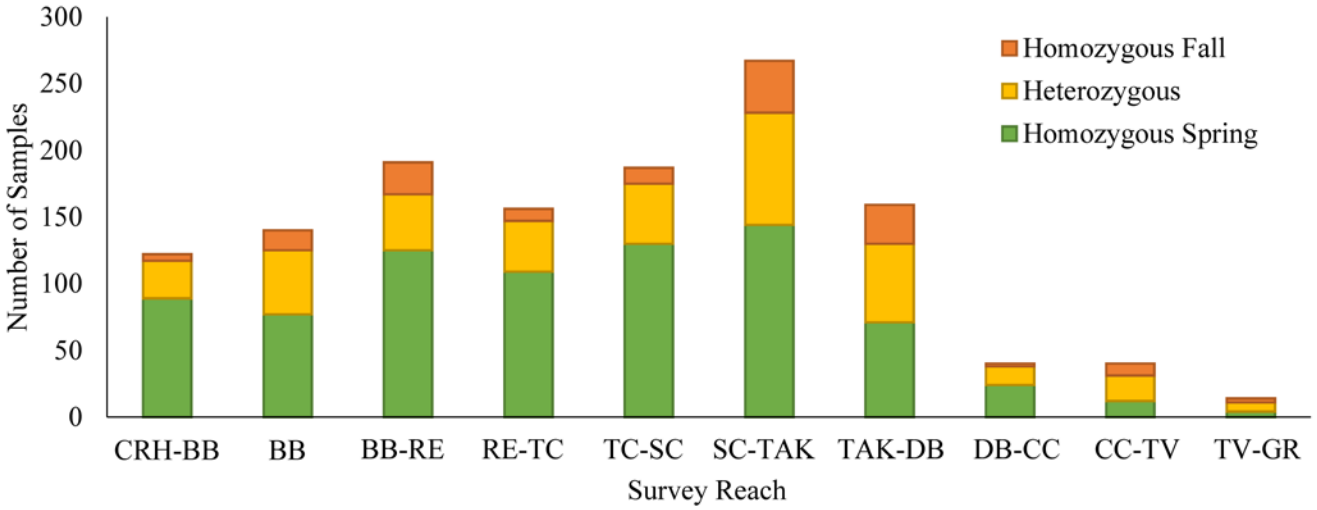


Figure S6. Distribution of *Greb1L* SNP1 genotypes across time with all years combined (2016 - 2018). *Greb1L* SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.). The Julian week when carcass samples were collected is on the x-axis and ranges from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4).

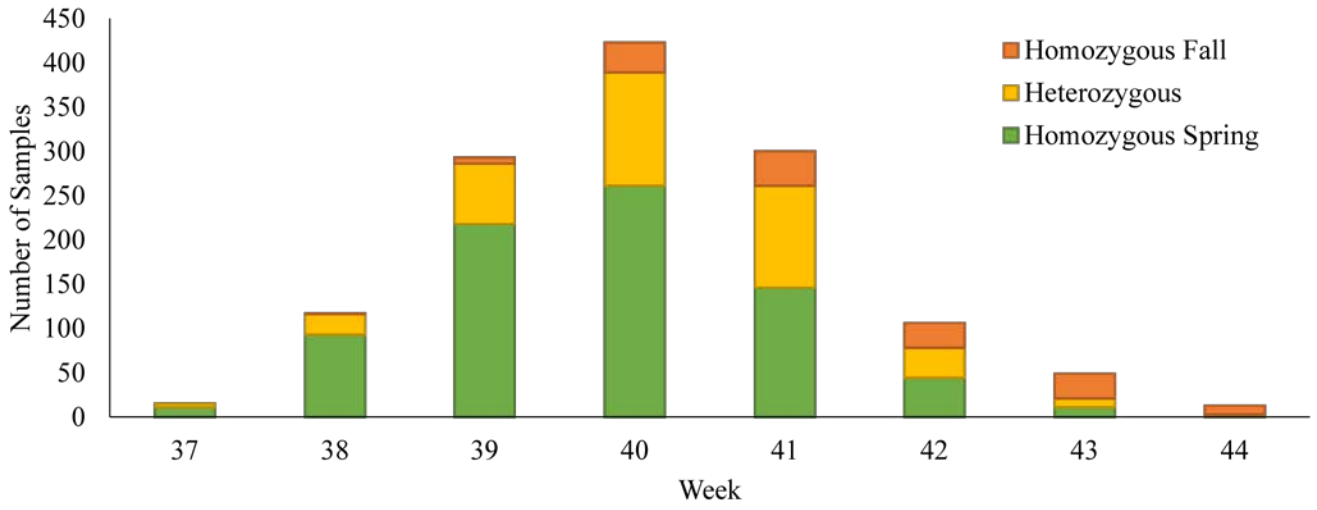
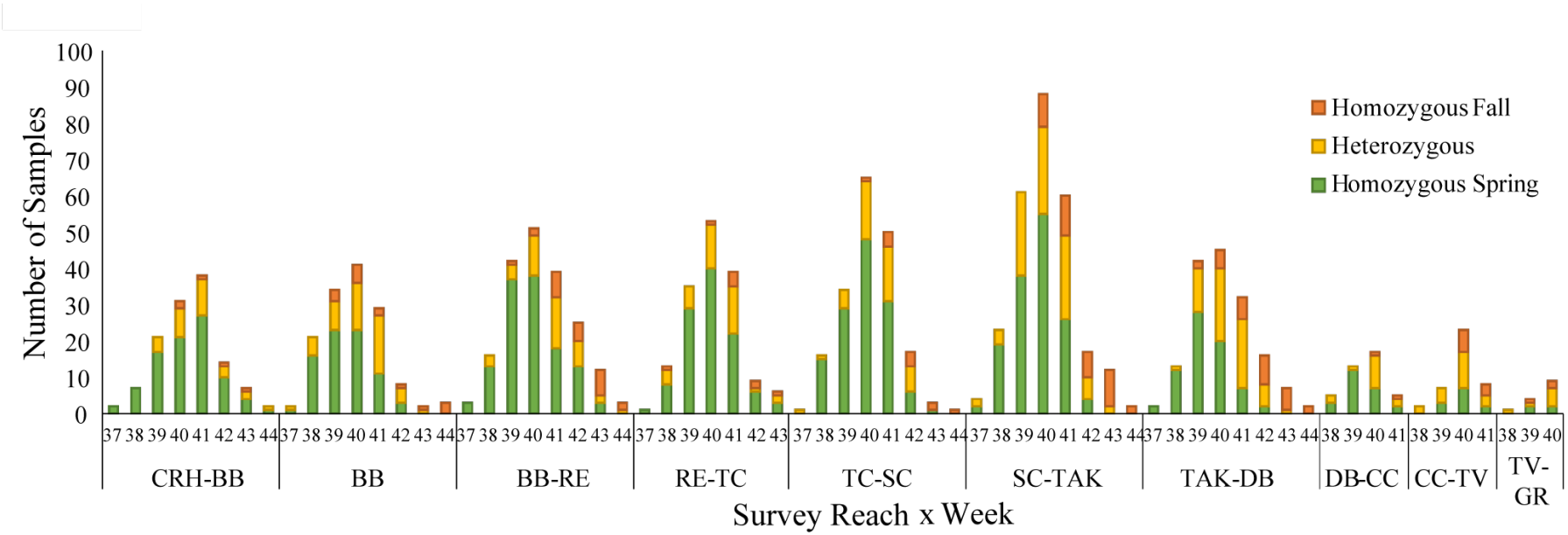


Figure S7. Distribution of *Greb1L* SNP1 genotypes across survey reaches and time with all years combined (2016 - 2018). *Greb1L* SNP1 is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (T. Thompson, pers. comm.). The Julian week when carcass samples were collected is on the x-axis and ranges from 37 (Sept 10 – 16) to 44 (Oct 28 – Nov 4), grouped by survey reach. Survey reach names are included in Table 1.



4034 Fairview Industrial Drive SE
Salem, OR 97302