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EVALUATING SPRING CHINOOK SALMON RELEASES ABOVE FALL CREEK DAM USING GENETIC PARENTAGE ANALYSIS

Prepared for:

U. S. ARMY CORPS OF ENGINEERS
PORTLAND DISTRICT – WILLAMETTE VALLEY PROJECT
333 SW First Ave.
Portland, Oregon 97204

Prepared by:

Kathleen G. O'Malley, Cristín K. Fitzpatrick, Kevin C. Olsen

State Fisheries Genomics Lab,
Coastal Oregon Marine Experiment Station
Department of Fisheries, Wildlife, and Conservation Sciences,
Hatfield Marine Science Center,
Oregon State University
2030 SE Marine Science Drive
Newport, Oregon 97365

Fall Creek Genetic Parentage Analysis

SUMMARY

Background

Upper Willamette River spring Chinook salmon (*Oncorhynchus tshawytscha*) are listed as threatened under the U.S. Endangered Species Act. Fall Creek, a tributary of the Middle Fork Willamette River, provides critical spawning habitat for threatened spring Chinook salmon. However, recovery of spring Chinook salmon is limited by Fall Creek Dam, which blocks access to historical spawning habitat for this species. Beginning in 1998, primarily hatchery-origin (HOR; adipose fin removed) spring Chinook salmon were released above Fall Creek Dam with the aim of re-establishing natural production on historical spawning and rearing grounds. Since June 2009, only unmarked, natural-origin (NOR; adipose fin intact) spring Chinook salmon have been reintroduced above the dam.

Population productivity above Fall Creek Dam

Previous research has used genetic parentage analysis to evaluate the contribution of spring Chinook salmon released above Fall Creek Dam to subsequent adult salmon recruitment to the river (O'Malley et al. 2018). Here, we extend the genetic parentage analysis by assigning the 2016 – 2020 adult salmon returns to salmon reintroduced above Fall Creek Dam in 2011 – 2017. We found that the assignment rates of returning adult Chinook salmon to salmon previously reintroduced above Fall Creek Dam increased from 92% (391/423) in 2016 to 99% (803/809) in 2020. The inferred age structure based on the genetic parentage analysis results indicates that most salmon return to Fall Creek at age-3 and age-4 with few age-5 salmon.

Above Fall Creek Dam in 2011 – 2015, about 13% of Chinook salmon, on average, produced at least one adult offspring each year that was detected through genetic parentage analysis. The number of adult offspring produced by an individual salmon ranged from a low of 0 – 15 in 2014 to a high of 0 – 54 in 2012. Accordingly, the mean TLF was lowest in 2014 and highest in 2012. We found that length was a significant predictor of TLF, in that TLF increased with increasing length of fish. Total cohort replacement rate (CRR_{total}) was less than one in most years from 2011 – 2015, indicating that the reintroduced population above Fall Creek Dam is not replacing itself. However, in 2012, CRR_{total} was 1.49 indicating that replacement had been met. The minimum CRR_{total} (0.21) was observed in 2014.

Variation in the effective number of breeders (N_b) above Fall Creek Dam from 2011 – 2015 tracked with the number of candidate parents that produced one or more adult offspring ($n_{success}$). However, these two values did not correspond to the number of candidate parents (n_{cand}) released above Fall Creek Dam. For instance, as n_{cand} increased, $n_{success}$ and N_b decreased, except in 2015. This is reflected in N_b: N_{cand} ratio which was lowest in 2014 (0.07) when the second largest number of salmon (n = 453) were reintroduced above Fall Creek Dam. Overall, N_b ranged from 25.1 in 2015 to 50.0 in 2012.

Interestingly, population productivity of Chinook salmon above Fall Creek Dam from 2011 – 2015 resembled productivity in two of the other three Chinook salmon reintroduction programs in the UWR basin. For example, productivity of salmon reintroduced above dams in the North and South Santiam Rivers was also highest in 2012 and lowest in 2014 while productivity of Chinook salmon reintroduced above Cougar Dam on the South Fork McKenzie did not follow this trend. Furthermore, preliminary TLF estimates for the 2016 cohort in Fall Creek and the North and South Santiam Rivers are quite high. These findings suggest that basin-wide and/or ocean conditions may have been primary factor(s) influencing productivity in three of the four river systems from 2011 – 2016.

Release day above Fall Creek Dam

While the day of first release for salmon reintroduced above Fall Creek Dam shifted from May 5 in 2011 to April 13 in 2015, the day of first release for salmon reintroduced above the dam from 2016 – 2020 did not follow the same trend. Instead, we found that Chinook salmon release day was similar in 2016, 2018, and 2020, while all other cross-year comparisons to 2011 – 2020 were significantly different.

From 2011 – 2020, the median release day of males and females was significantly different in four of the years. For instance, in 2013 and 2019, release day for males was significantly later than female release day, whereas in 2016 and 2018, male release day was significantly earlier than female release day.

Comparing across years, we found that early released Chinook salmon in 2011 were significantly different from early released salmon in five years (2012, 2015, 2016, 2017, 2020) based on variation at *Ots515NWFSC.* In addition, early released salmon in 2017 were significantly different from early released salmon in most years based on variation at *OmyFbxw11*. The

magnitude of differentiation at these two run timing markers (i.e., *Ots515NWFSC* and *OmyFbxw11*) was substantially greater than that of neutral microsatellite markers, suggesting these differences may reflect adaptive genetic changes over the time span evaluated.

INTRODUCTION

Upper Willamette River (UWR) spring Chinook salmon (*Oncorhynchus tshawytscha*) are currently listed as threatened under the U.S. Endangered Species Act (NMFS 2008). To facilitate access to historical habitats and increase the abundance and distribution of naturally produced fish, spring Chinook salmon are released above U.S. Army Corps of Engineers (USACE) dams throughout the UWR basin. Fall Creek, a tributary of the Middle Fork Willamette River, provides critical spawning and rearing habitat for threatened spring Chinook salmon. Beginning in 1998, primarily hatchery-origin (HOR; adipose fin removed) adult spring Chinook salmon were released above Fall Creek Dam with the aim of re-establishing natural production on historical spawning grounds. Since 2009, only unmarked, natural-origin (NOR; adipose fin intact) adult spring Chinook salmon have been reintroduced above the dam. Beginning in 2011, tissue samples for genetic parentage analysis have been collected from adult spring Chinook salmon prior to release above Fall Creek Dam.

The first evaluation of the Fall Creek reintroduction program focused on adult spring Chinook salmon returning in 2011 – 2015. O'Malley and Bohn (2018) determined that 10% (46/453) of the salmon returning in 2014 and 87% (224/257) of the salmon returning in 2015 were offspring of salmon previously reintroduced above Fall Creek Dam. Note, these assignments are likely underestimates because potential parents that returned in 2009 and 2010 were not sampled. Based on age-3 and age-4 offspring only, the preliminary fitness estimates for salmon reintroduced above the dam in 2011 averaged 0.77 (± 3.45 SD; range = 0 – 40) offspring. In this report, we extend the evaluation of the Fall Creek reintroduction program by genotyping adult spring Chinook salmon that returned to the dam in 2016 – 2020 and evaluating five complete cohorts reintroduced above the dam in 2011 – 2015.

Interestingly, there was a marked shift in the return timing of spring Chinook salmon to Fall Creek Dam between 2011 and 2015. For instance, in 2011, only 18% (66/364) of the total spring Chinook salmon run were released above Fall Creek Dam before June 1, whereas in 2015, 71% (183/257) of the total run were released before June 1 (Table A1). In 2016 – 2020, return timing was variable with 19% to 51% of the total run released above the dam before June 1 (Table A1). On average, 40% of the adult run was released above Fall Creek Dam before June 1 during this five-year period.

Given that previous studies have documented an association between circadian clock genes and adult return timing in salmonid fishes (O'Malley *et al.* 2007, O'Malley *et al.* 2010, O'Malley *et al.* 2013, O'Malley *et al.* 2014), O'Malley and Bohn (2018) tested for differences at these genes between the first third and the last third of the Chinook salmon run released above Fall Creek Dam in 2011 – 2015. Within three of the five years (2011, 2014, and 2015), there was evidence for potentially adaptive genetic differentiation. By comparing the first third of the run across the five years (2011 – 2015), O'Malley and Bohn (2018) also found evidence for potentially adaptive genetic differentiation. For instance, the first third of the spring Chinook salmon run in 2011 was significantly differentiated from the first third of the run in all other years (2012, 2013, 2014, and 2015) based on variation at the circadian clock genes. In this report, we extend the evaluation of circadian clock gene variation in Chinook salmon returning to Fall Creek Dam in 2016 – 2020 and test for potentially adaptive genetic differences within and across years.

This work will address the information needs of the following Reasonable and Prudent Alternatives (RPAs) described in the Willamette Project Biological Opinion (NMFS 2008):

- RPA 4.1 (Adult Chinook Salmon Outplanting)
- RPA 4.3 (Willamette Fish Operations Plan)
- RPA 4.7 (Adult Fish Release Sites Above Dams)
- RPA 4.8.1 (Fall Creek Drawdown)
- RPA 4.11 (Assess Downstream Juvenile Fish Passage through Dams)
- RPA 6.2.3 (Continue Adult Chinook Outplanting Program)
- RPA 9.3 (Fish Passage RM&E)
- RPA 9.5.1 (Spring Chinook)

OBJECTIVES

1. Determine the number and proportion of the unmarked adult spring Chinook salmon sampled at Fall Creek Dam in 2016 – 2020 that can be assigned as offspring of salmon released above the dam in 2011 – 2017.

2. Estimate and report the annual abundance and age structure of adult Chinook salmon that returned to Fall Creek Dam in 2016 – 2020 and can be confidently assigned to parents through genetic parentage analysis.

3. Estimate the total lifetime fitness (TLF) of spring Chinook salmon released above Fall Creek Dam in 2011 – 2015.

4. Evaluate potential predictors of TLF for adult Chinook salmon released above Fall Creek Dam in 2011 – 2015.

5. Estimate cohort replacement rate (CRR), or "the number of future spawners produced by a spawner" for spring Chinook salmon released above Fall Creek Dam in 2011 – 2015.

6. Estimate the effective number of breeders (N_b) for the adult salmon population released above Fall Creek Dam in 2011 – 2015.

7. Test for allele frequency differences at the four circadian clock genes and 11 microsatellite markers between early (release day before July 1) and late (release day August 1 and after) spring Chinook salmon in years with adequate sample sizes.

8. Test for allele frequency differences at the four circadian clock genes and 11 microsatellite markers between male and female spring Chinook salmon within each year (2016 – 2020).

9. Test for inter-annual allele frequency differences at the four circadian clock genes and 11 microsatellite markers in spring Chinook salmon released before June 1 (2016 – 2020).

METHODS

Study System

Fall Creek Dam is a 62.5 meter-high rockfill embankment dam located in Fall Creek, Oregon (43.94663N – 122.75787W; Figure 1). Fall Creek Dam was established in 1965 and is operated and maintained by the Portland District of the U.S. Army Corps of Engineers (USACE). Since June 2009, primarily NOR adult spring Chinook salmon have been transported above the dam

and released at “Site C”. Site C is approximately 2.5 river km upstream of Fall Creek Reservoir at full pool elevation. To facilitate juvenile Chinook salmon passage and survival through the dam, the USACE lowers the reservoir elevation to above the regulating outlet entrance. In 2015, during the reservoir drawdown, the USACE constructed new intakes to supply water to an existing fish ladder. In addition, a newly constructed Adult Fish Collection Facility became fully operational in 2018.

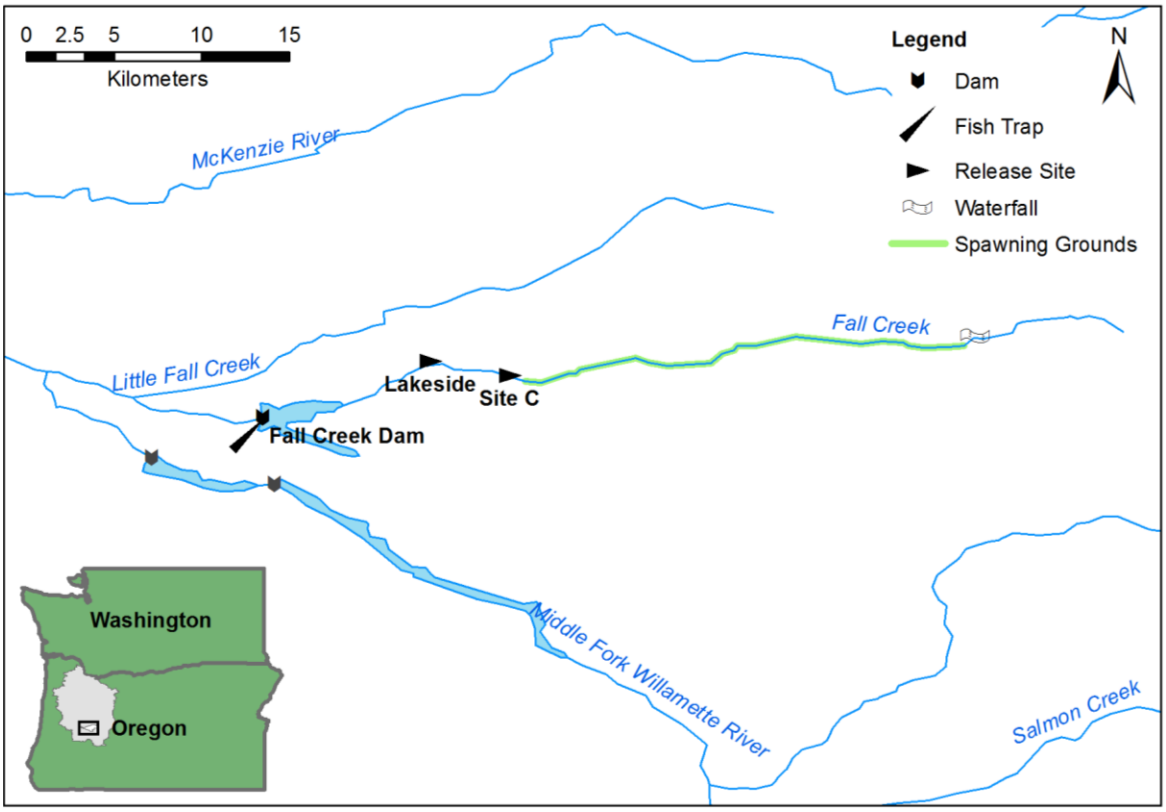


Figure 1. A map of Fall Creek and its surrounding area. The inset shows its position in the state of Oregon and within the Willamette River Basin, indicated in light gray. The Lakeside release site was only used for a small portion of returning adults in 2011. All other adults were released at Site C. The spring Chinook salmon spawning grounds are highlighted in light green.

Sample collection

In 2011 – 2020, tissue samples (i.e., fin clip) were collected from adult spring Chinook salmon prior to release above Fall Creek Dam by the USACE (Figure 1). In addition, tissue samples were collected from carcasses encountered on the spawning grounds above the dam in 2018 and 2020. All tissue samples were preserved in 95% ethanol. Sampling date, phenotypic sex, and fork length were recorded.

Microsatellite marker and circadian clock gene genotyping

Consistent with the previously published report (O'Malley and Bohn 2018), whole genomic DNA was isolated from the tissue samples using the protocol of Ivanova *et al.* (2006). Each sample was then genotyped at 11 microsatellite markers: *Ogo2*, *Ogo4*, *Ots201*, *Ots211*, *Ots212*, *Ots215*, *OtsG249*, *OtsG311*, *OtsG409*, *OtsG474*, and *Ssa408* (Olsen *et al.* 1998, Cairney *et al.* 2000, Naish and Park 2002, Williamson *et al.* 2002, Greig *et al.* 2003), four circadian clock genes: *Ots515NWFSC*, *Omy1009UW*, *OmyFbxw11*, and *OtsClock1b* (Naish and Park 2002; Spies *et al.* 2005; O'Malley *et al.* 2007), and the sex-linked marker, *Oty3* (Brunelli *et al.* 2008). Loci were amplified using polymerase chain reaction (PCR), PCR products were visualized on an ABI 3730XL DNA analyzer, and allele sizes were scored using GENEMAPPER software (Version 5.0, Applied Biosystems, Inc., Foster City, CA).

In total, 1880 tissue samples were collected from salmon prior to release above the dam in 2016 – 2020 (Table 1). Of these, we genotyped 1868, as well as tissue samples collected from carcasses above the dam in 2018 (n = 8) and 2020 (n = 60) (Table 1). We also genotyped juvenile Chinook salmon sampled during their outmigration in 2016 (n = 88).

Individuals genotyped at less than eight microsatellite markers were excluded from the parentage analysis (Table 1). Multilocus genotypes were then compared among individuals within each release year using GenAlEx v.6.503 (Peakall and Smouse 2012) to identify salmon that could have been sampled more than once. Duplicates could be the result of sampling error (e.g., fish sampled twice on the same day), or they could be indicative of "fallbacks" (i.e., fish that spill over the dam following reintroduction). "Fallbacks" may be re-trapped and re-released above the dam, and therefore re-sampled later. Genotypes identical at all markers were assumed to represent a single individual and redundant genotypes were removed from the dataset. Additionally, genotypes that matched at all, but one marker were also consolidated, as the probability of observing a near-identical multilocus genotype at 14 of the markers used in this study was extremely low in each year. If individuals failed to genotype at the sex-linked marker *Oty3*, phenotypic information was used to infer sex. If no phenotypic sex information was available, the individual was excluded from the parentage analysis. Markers were checked for

departures from Hardy-Weinberg proportions (HWP) and linkage equilibrium in Genepop Version v4.7.5 (Raymond and Rousset 1995).

Table 1. Summary of genotype quality filtering for Chinook salmon sampled in Fall Creek. Individuals were removed from analyses if they were genotyped at < 8 markers (missingness filtered), or if they represented a duplicate multilocus genotype (duplicate filtered). Most salmon were sampled at the dam prior to release while carcasses were also sampled in 2018 and 2020. Samples previously genotyped by O'Malley and Bohn (2018) are indicated (*).

Year	Tissues Genotyped	Missingness Filtered	Successful Genotypes	Duplicate Filtered	Final Males	Final Females	Total
2011	366*	2	364	0	208	156	364
2012	329*	3	326	2	176	148	324
2013	471*	9	462	0	276	186	462
2014	455*	2	453	0	200	253	453
2015	258*	1	257	0	169	88	257
2016	423	0	423	0	239	184	423
2017	290	0	290	0	169	121	290
2018	94	0	94	0	49	45	94
Carcass	8	3	5	1	1	3	4
2019	254	2	252	0	195	57	252
2020	819	7	812	3	364	445	809
Carcass	60	14	46	46	0	0	0
Total	3827	43	3784	52	2046	1686	3732

Genetic parentage analysis

To estimate the number of salmon returning to Fall Creek Dam in 2016 – 2020 that were offspring of previously sampled salmon, we compared multilocus microsatellite and circadian

clock genotypes of putative offspring to the genotypes of putative parents. The putative parents include those reintroduced above Fall Creek Dam in 2011 – 2017 (Table 1; Figure 2).

The genotype comparisons were first conducted within the maximum-likelihood framework of the parentage assignment program CERVUS Version 3.07 (Kalinowski *et al.* 2007). Parent(s)-offspring assignments were made using a strict 95% confidence criterion, a minimum of eight markers, an assumed parent sampling rate of 95%, and a maximum of one mismatch between parent-offspring pairs (maximum of two mismatches for parent-offspring trios). Parentage assignments from CERVUS were then verified using the combined PLS-FL algorithm implemented in COLONY Version 2.0.6.8 (Jones and Wang 2010). The second analysis was conducted because CERVUS's likelihood-based parentage assignment method requires an accurate estimate of the number of parents contributing to a cohort (Harrison *et al.* 2013) and unknown parental contributions could impact assignment accuracy. Moreover, simulation studies have suggested that COLONY's assignment protocol is the most accurate of three alternate pedigree reconstruction methods (Harrison *et al.* 2013). COLONY was run using the following parameters: medium run length, polygamous male and female setting, allele dropout and miscalled allele rate prior of 1% per locus, and an assumed 95% rate of sampled parents. We also estimated the allele miscall rate and dropout rate in our dataset using COLONY (Wang 2018) (Table A2).

An automated, reproducible procedure for choosing the best consensus genetic pedigree from the outputs of CERVUS and COLONY was generated for UWR spring Chinook salmon projects. This approach was first implemented in the North Santiam River (O'Malley *et al.* 2023a) and has since been used in the McKenzie River (O'Malley *et al.* 2023b) and South Santiam River (O'Malley *et al.* 2024). Note, this approach uses an updated version of COLONY (Version 2.0.6.6).

To assess if, and subsequently how, the new procedure may affect parentage assignments, we re-analyzed samples from O'Malley and Bohn (2018) along with those from 2016 – 2020. Except for three instances, assignments were identical between the two analyses. As a result, we used the new automated, reproducible procedure to generate a genetic pedigree for samples collected in 2011 – 2020.

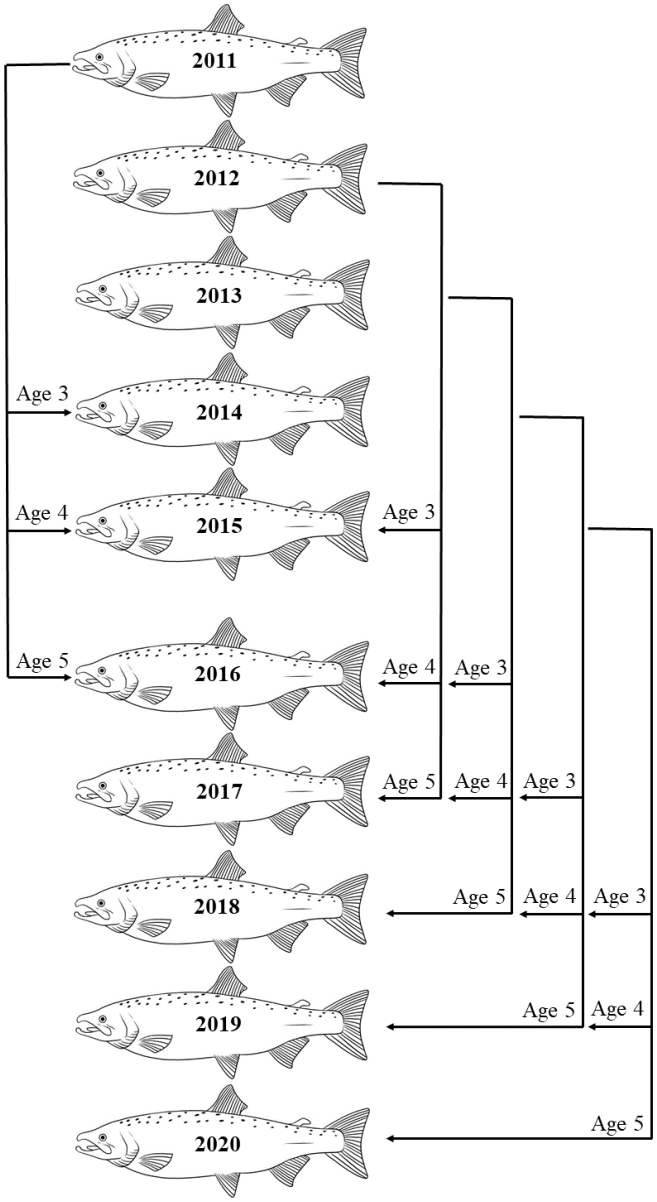


Figure 2. Framework used to reconstruct parent(s) – offspring relationships for spring Chinook salmon sampled in Fall Creek, Oregon. Throughout the report, we present results focused on candidate parents sampled from 2011 – 2017 and potential offspring sampled from 2014 – 2020. Results are based on assignments of potential age-3, age-4, and age-5 offspring to candidate parents.

Parentage assignments and inferred age at maturity

For each assigned offspring, we subtracted the return year (e.g., 2016) by each parent(s) release year (e.g., 2013) to infer age at maturity (e.g., age-3) and determined the age structure of adult returns from 2011 – 2020. Note, because our candidate parents include salmon that returned in

2011 – 2017, we were able to identify age-3, age-4, and age-5 offspring in 2014 – 2020.

Fitness of reintroduced salmon

The TLF of Chinook salmon reintroduced above Fall Creek Dam in 2011 – 2015 was estimated from the number of adult offspring identified in subsequent years. For instance, the TLF of Chinook salmon in 2011 was estimated from salmon sampled in 2014 – 2016. We also estimated partial TLF for salmon reintroduced above Fall Creek Dam in 2016 based on age-3 and age-4 offspring and partial TLF for salmon reintroduced in 2017 based on age-3 offspring (Figure 2).

Predictors of fitness

We applied generalized linear models (GLMs) and generalized linear mixed models (GLMMs) to identify predictors of TLF of salmon released above Fall Creek Dam from 2011 – 2015. These two statistical approaches were used to evaluate predictors of TLF within each year and across years, respectively. Specifically, we attempted to fit five individual GLMs to the data from each release year and a single GLMM using data across release years. For the GLMs, we considered the influence of *sex*, *release day*, *length*, *release group density*, *release group sex ratio*, and three interaction terms, *sex*release day*, *sex*release group density*, and *sex*release group sex ratio* in the full model. *Release day* is the Julian day that individuals were released above Fall Creek Dam and was modeled as a continuous predictor. Release group is defined as the individuals released at a single location during a single day. *Release group density* is the number of individuals in a release group. *Release group sex ratio* is the ratio of males to females in a release group and was log-transformed before being included as a predictor. *Release location* was not used as a predictor, as most salmon were released at one location, except a small portion of returning adults in 2011. For the GLMM across years we considered two additional fixed predictors of interest for TLF: *total number of fish released annually* and *annual sex ratio*. Differences across years and release groups were accounted for in the GLMM as random effects.

Our modeling approach primarily followed the recommendations of Zuur *et al.* (2009) and Bolker (2015). For each of the annual GLMs, we conducted an exploratory data analysis to identify the distribution and link function that provided the best fit to the data, calculated the variance inflation factors of main effects to examine multicollinearity among predictors, and determined if non-linear effects of *release day* or *release group density* improved the model. After exploratory data analysis, we fit the full model using the negative binomial distribution and

log link function using the *glm.nb* function from the *MASS* package in *R*. We conducted model selection based on both likelihood ratio tests for each predictor and the backward stepwise selection using Wald tests for significant effects of each predictor. After a final model was selected, we conducted model validation using rootograms and QQ-plots of randomized quantile residuals from the *COUNTREG* package in *R*. We also examined models for goodness of fit, overdispersion and influence of outliers using residuals simulated by the *DHARMa* package in *R*. Effect plots of significant predictors retained in the final model were generated using the *effects* package in *R* and conditioned on the typical values of all other significant predictors in the final model. For the GLMM evaluating TLF across years, we used a similar approach with the following changes to the model fitting, selection, and validation. All models were fit using the *glmmTMB* function of the *glmmTMP* package in *R*. To identify the random effects structure with the best fit (i.e., lowest AIC), we varied predictors treated as random effects in a fully saturated model. After refitting the random effects structure, model selection of fixed effects was conducted using the same approach as the annual GLMs. We validated the final model using simulated residuals from the *DHARMa* package in *R*.

Cohort replacement rate

Cohort replacement rate (CRR), or "the number of future spawners produced by a spawner" (Botsford and Brittnacher 1998), was estimated for salmon reintroduced above Fall Creek Dam in 2011 – 2015. For instance, we calculated CRR_{total} as the total number of age-3, age-4, and age-5 adult offspring assigned to salmon reintroduced above Fall Creek Dam in 2011 divided by the total number of salmon reintroduced that year. We also report both a female-only CRR_F and a male-only CRR_M. For example, CRR_F is the total number of age-3, age-4, and age-5 female adult offspring produced by females reintroduced above Fall Creek Dam divided by the total number of females above the dam.

Effective number of breeders

The effective number of breeders (N_b) among salmon reintroduced above Fall Creek Dam in 2011 – 2015 was estimated using the linkage disequilibrium (LD) method, as implemented in the program NEESTIMATOR v2.1 (Waples and Do 2008; Do *et al.* 2014). This method examines patterns of LD (nonrandom allelic associations, which are suggestive of common ancestry) among offspring of a cohort. N_b was calculated using the genotypes of all offspring assigned to

each parental cohort from 2011 – 2015. We excluded singletons and generated 95% confidence intervals using a jackknife re-sampling method (Waples and Do 2008). For each year, we calculated the ratio of N_b to the number of candidate parents (N_{cand}). N_{cand} potentially underrepresents the total number of salmon in the parental cohort above the dam due to incomplete sampling or incomplete genotyping. Note, the previous report (O'Malley and Bohn 2018) used an earlier software version (NEESTIMATOR v2.0) and excluded all alleles with a minor allele frequency below 0.004.

Release day in 2011 – 2020

We used a non-parametric Wilcoxon rank sum test to evaluate shifts in the distribution of release day above Fall Creek Dam within and among years from 2011 – 2020. We also compared male and female spring Chinook salmon release day within years and compared release day within each sex among years. Wilcoxon rank sum tests were performed in base *R*.

Circadian clock genes, microsatellite markers, and release day within years

Sufficient sample sizes (n >20) of late (released August 1 and after) spring Chinook salmon were only available in some years. As a result, intra-annual genetic variation was only evaluated in 2011 (O'Malley and Bohn 2018) and 2016 (Table A3). We estimated pairwise F_{ST} (Weir and Cockerham 1984) based on variation at each of the four circadian clock genes to test for potentially adaptive genetic differentiation and used a permutation test with 5000 iterations to assess statistical significance (Genetix Version 4.02; Belkhir *et al.* 2004). In addition, we estimated pairwise F_{ST} based on variation at the 11 microsatellite markers to test for neutral genetic differentiation between the early- and late-released groups and used a permutation test to assess statistical significance.

Circadian clock genes, microsatellite markers, and sex within years

To test for differences between male and female spring Chinook salmon within each year, we estimated pairwise F_{ST} (Weir and Cockerham 1984) based on variation at the four circadian clock genes (i.e., potentially adaptive differentiation) as well as based on variation at the 11 microsatellite markers (i.e., neutral differentiation). We used a permutation test with 5000 iterations to assess statistical significance (Genetix Version 4.02; Belkhir *et al.* 2004).

Circadian clock genes, microsatellite markers, and release day among years

Given the observed shifts in release day from 2011 – 2020, we adjusted our early-release day to June 1 and tested for genetic differences across years. First, we estimated pairwise F_{ST} (Weir and Cockerham 1984) based on variation at each of the four circadian clock genes to test for potentially adaptive genetic differentiation and used a permutation test with 5000 iterations to assess statistical significance (Genetix Version 4.02; Belkhir *et al.* 2004). Next, we estimated pairwise F_{ST} based on variation at 11 microsatellite markers to test for neutral genetic differentiation among these early-released salmon across years and used a permutation test to assess statistical significance.

Analysis of circadian clock gene variation in juveniles exiting Fall Creek Reservoir

In 2016, juvenile spring Chinook salmon were sampled for genetic analysis (n = 88) and measured (e.g., fork length) prior to exiting the Fall Creek Reservoir. We compared the genotypes of the outmigrants to the genotypes of returning adults in 2018 – 2020. Of the 43 outmigrants successfully genotyped, none matched the returning adults. As a result, we were unable to evaluate the age, size, and timing of juvenile outmigrants relative to the age and return timing of adults.

RESULTS

Parentage assignments and inferred age at maturity

O'Malley and Bohn (2018) determined that 10% (46/453) of the salmon returning in 2014 assigned as age-3 offspring of salmon reintroduced above Fall Creek Dam in 2011. In addition, 87% (224/257) of the salmon that returned in 2015 were assigned to previously reintroduced salmon. Of the 224 assigned salmon that returned in 2015, 113 were identified as age-3 (assigned to parents from 2012; 50%), 111 as age-4 (assigned to parents from 2011; 50%). Note, these assignments are likely underestimates since potential parents that returned in 2009 and 2010 were not sampled.

Here, we attempted to assign 1872 potential offspring to 2573 candidate parents and found that 1804 adult salmon returning in 2016 – 2020 assigned to 420 parents sampled in 2011 – 2017 for an overall assignment rate of 96% (1804/1872) (Table 2). Of the tissue samples collected from 2016 – 2020, only 26 failed to genotype at eight or more genetic markers (Table 1). Most of these failed samples were collected from carcasses in 2018 (n = 3) and 2020 (n = 14). Of the

samples successfully genotyped at eight or more genetic markers from 2016 – 2020, we identified 50 sets of identical genotypes. The majority were from carcass samples collected in 2020 (n = 46) which matched individuals reintroduced earlier that year. Three sets of identical genotypes from 2020 were sampled on the same day. The remaining sample was a carcass from 2018 that matched an individual reintroduced earlier that year.

2016 – Results from genetic parentage analysis revealed 92% (391/423) of the adult salmon that returned in 2016 assigned to previously sampled salmon (Table 3c). Out of the 391 assigned salmon, 49 were identified as age-3 (assigned to parents from 2013; 12%), 335 as age-4 (assigned to parents from 2012; 86%), and seven as age-5 (assigned to parents from 2011; 2%; Figure 3; Table 4).

2017 – Results from genetic parentage analysis revealed 94% (272/290) of the adult salmon that returned in 2017 assigned to previously sampled salmon (Table 3d). Out of the 272 assigned salmon, 28 were identified as age-3 (assigned to parents from 2014; 10%), 208 as age-4 (assigned to parents from 2013; 77%), and 36 as age-5 (assigned to parents from 2012; 13%; Figure 3; Table 4).

2018 – Results from genetic parentage analysis revealed 95% (93/98) of the adult salmon that returned in 2018 assigned to previously sampled salmon (Table 3e). Out of the 93 assigned salmon, 23 were identified as age-3 (assigned to parents from 2015; 25%), 59 as age-4 (assigned to parents from 2014; 63%), and 11 as age-5 (assigned to parents from 2013; 12%; Figure 3; Table 4). Of the four individuals sampled as carcasses one assigned to a 2014 parent pair.

2019 – Results from genetic parentage analysis revealed 97% (245/252) of the adult salmon that returned in 2019 assigned to previously sampled salmon (Table 3f). Out of the 245 assigned salmon, 163 were identified as age-3 (assigned to parents from 2016; 67%), 74 as age-4 (assigned to parents from 2015; 30%), and eight as age-5 (assigned to parents from 2014; 3%; Figure 3; Table 4).

2020 – Results from genetic parentage analysis revealed 99% (803/809) of the adult salmon that returned in 2020 assigned to previously sampled salmon (Table 3g). Out of the 803 assigned salmon, five were identified as age-3 (assigned to parents from 2017; 1%), 798 as age-4

(assigned to parents from 2016; 99%), and zero as age-5 (assigned to parents from 2015; 0%; Figure 3; Table 4).

Table 2. Adult Chinook salmon returning in 2014 – 2020 that assigned to salmon reintroduced above Fall Creek Dam in 2011 – 2017. $n_{potential\ offspring}$ is the number of reintroduced salmon genotyped and retained after quality filtering. $n_{assigned}$ is the number of salmon and % Assigned is the percentage of salmon that assigned to salmon reintroduced above the dam. Note, 2016 is the first year when all potential parents were sampled (i.e., 2011 – 2013). Assignments for the 2014 and 2015 adult returns were first reported in O'Malley and Bohn (2018).

Overall			
Year	**$n_{potential\ offspring}$**	**$n_{assigned}$**	**% Assigned**
2014	453	46	10%
2015	257	224	87%
2016	423	391	92%
2017	290	272	94%
2018	98	93	95%
2019	252	245	97%
2020	809	803	99%

Table 3. Summary of adult offspring assigned to Chinook salmon above Fall Creek Dam in (a) 2014, (b) 2015, (c) 2016, (d) 2017, (e) 2018, (f) 2019, and (g) 2020. The total number of putative offspring and parents considered in the parentage analysis are indicated (N) and the number assigned to a parental pair (P), or to a female (F) or male (M) parent only. Assignments for the 2014 and 2015 adult returns were first reported in O'Malley and Bohn (2018). Note, in 2018, four potential offspring were sampled as carcasses.

(a)

Adult Offspring	**Parents**										
2014	2009			2010			2011 (N = 364)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
453	Not sampled			Not sampled			23	13	10	46	10%

(b)

Adult Offspring	**Parents**										
2015	2010			2011 (N = 364)			2012 (N = 324)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
257	Not sampled			102	8	1	108	4	1	224	87%

(c)

Adult Offspring	**Parents**										
2016	2011 (N = 364)			2012 (N = 324)			2013 (N = 462)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
423	5	2	-	316	19	-	39	9	1	391	92%

(d)

Adult Offspring	**Parents**										
2017	2012 (N = 324)			2013 (N = 462)			2014 (N = 453)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
290	33	3	-	152	45	11	25	3	-	272	94%

(e)

Adult Offspring	**Parents**										
2018	2013 (N = 462)			2014 (N = 453)			2015 (N = 257)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
98	10	1	-	54	1	4	23	-	-	93	95%

(f)

Adult Offspring	**Parents**										
2019	2014 (N = 453)			2015 (N = 257)			2016 (N = 423)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
252	8	-	-	74	-	-	159	4	-	245	97%

(g)

Adult Offspring	**Parents**										
2020	2015 (N = 257)			2016 (N = 423)			2017 (N = 290)			**Assigned (%)**	
N	P	F	M	P	F	M	P	F	M		
809	-	-	-	757	40	1	3	2	-	803	99%

Table 4. Inferred age at maturity for spring Chinook salmon returning in 2014 – 2020 with number (n) and percent (% Assigned) assigned for each age class. Age at maturity for the 2014 and 2015 adult returns were first reported in O'Malley and Bohn (2018) and do not include age-4 and age-5, and age-5 adult offspring, respectfully (*).

Year	**$n_{offspring\ assigned}$**	**Age at Return**	**n**	**% Assigned**
2014*	46	3	46	100%
		4	-	-
		5	-	-
2015*	224	3	113	50%
		4	111	50%
		5	-	-
2016	391	3	49	12%
		4	335	86%
		5	7	2%
2017	272	3	28	10%
		4	208	77%
		5	36	13%
2018	93	3	23	25%
		4	59	63%
		5	11	12%

2019	245	3	163	67%
		4	74	30%
		5	8	3%
2020	803	3	5	1%
		4	798	99%
		5	0	0%

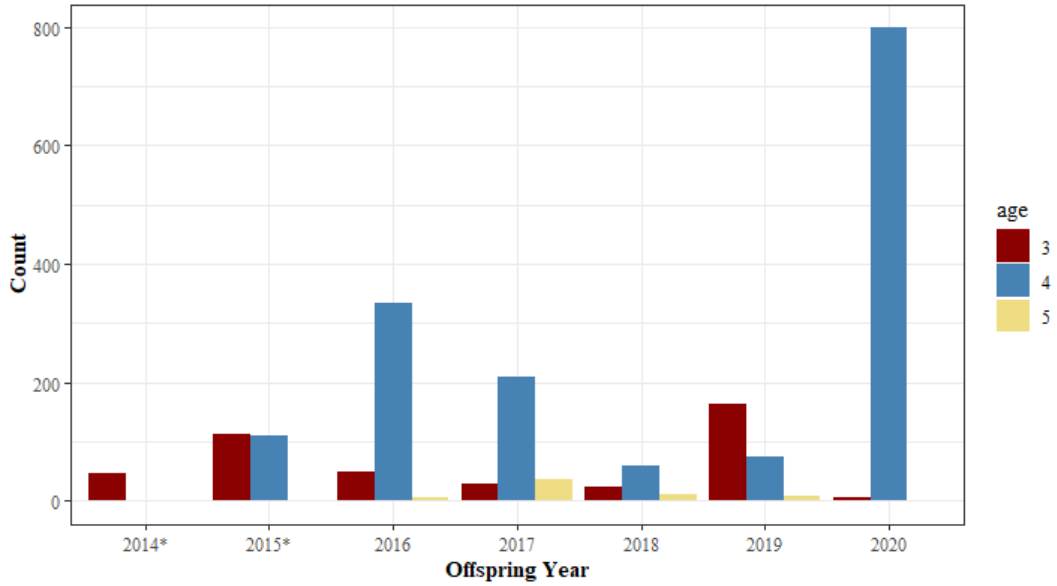


Figure 3. Inferred age at maturity for salmon sampled in Fall Creek from 2014 – 2020. Age at maturity for the 2014 and 2015 adult returns were first reported in O'Malley and Bohn (2018) and do not include age-4 and age-5, and age-5 adult offspring, respectfully (*).

Total Lifetime Fitness

2011 – As estimated from age-3, age-4, and age-5 offspring, the mean TLF for Chinook salmon above Fall Creek Dam during 2011 was 0.81 ± 3.51 SD (n = 364; range = 0 – 40) with 3% (12/364) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 0.98 ± 4.55 SD (n = 156; range = 0 – 40) whereas mean fitness of males was 0.68 ± 2.46 SD (n = 208; range = 0 – 22) (Table 6).

2012 – As estimated from age-3, age-4, and age-5 offspring, the mean TLF for Chinook salmon above Fall Creek Dam during 2012 was 2.90 ± 7.39 SD (n = 324; range = 0 – 54) with 25% (80/324) of the salmon producing one or more offspring (Table 5). Mean fitness of females was

3.26 ± 7.99 SD (n = 148; range = 0 – 44) whereas mean fitness of males was 2.60 ± 6.86 SD (n = 176; range = 0 – 54) (Table 6).

2013 – As estimated from age-3, age-4, and age-5 offspring, the mean TLF for Chinook salmon above Fall Creek Dam during 2013 was 1.02 ± 3.81 SD (n = 462; range = 0 – 28) with 12% (55/462) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 1.38 ± 4.68 SD (n = 186; range = 0 – 26) whereas mean fitness of males was 0.77 ± 3.08 SD (n = 276; range = 0 – 28) (Table 6).

2014 – As estimated from age-3, age-4, and age-5 offspring, the mean TLF for Chinook salmon above Fall Creek Dam during 2014 was 0.40 ± 1.56 SD (n = 453; range = 0 – 15) with 11% (49/453) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 0.36 ± 1.53 SD (n = 253; range = 0 – 15) whereas mean fitness of males was 0.45 ± 1.59 SD (n = 200; range = 0 – 14) (Table 6).

2015 – As estimated from age-3, age-4, and age-5 offspring, the mean TLF for Chinook salmon above Fall Creek Dam during 2015 was 0.75 ± 3.01 SD (n = 257; range = 0 – 24) with 15% (38/257) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 1.10 ± 4.11 SD (n = 88; range = 0 – 24) whereas mean fitness of males was 0.57 ± 2.22 SD (n = 169; range = 0 – 20) (Table 6).

2016 – As estimated from age-3 and age-4 offspring, the mean partial TLF for Chinook salmon above Fall Creek Dam during 2016 was 4.44 ± 8.79 SD (n = 423; range = 0 – 60) with 42% (179/423) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 5.22 ± 9.50 SD (n = 184; range = 0 – 60) whereas mean fitness of males was 3.84 ± 8.17 SD (n = 239; range = 0 – 56) (Table 6). Mean partial TLF for salmon above Fall Creek Dam was notably high (Figure 4).

2017 – As estimated from age-3 offspring, the mean partial TLF for Chinook salmon above Fall Creek Dam during 2017 was 0.03 ± 0.18 SD (n = 290; range = 0 – 2) with 2% (7/290) of the salmon producing one or more offspring (Table 5). Mean fitness of females was 0.04 ± 0.24 SD (n = 121; range = 0 – 2) whereas mean fitness of males was 0.02 ± 0.13 SD (n = 169; range = 0 – 1) (Table 6).

Table 5. Mean TLF for the number (N) of Chinook salmon reintroduced above Fall Creek Dam in 2011 – 2015, standard deviation (SD), and range. Note, 2016 and 2017 estimates do not include potential age-5, and age-4 and age-5 offspring, respectively (*).

Year	N	Mean TLF	SD	Range
2011	364	0.81	3.51	0 - 40
2012	324	2.90	7.39	0 - 54
2013	462	1.02	3.81	0 - 28
2014	453	0.40	1.56	0 - 15
2015	257	0.75	3.01	0 - 24
2016*	423	4.44	8.79	0 - 60
2017*	290	0.03	0.18	0 - 2

Table 6. Mean TLF for the number (n) of female and male Chinook salmon reintroduced above Fall Creek Dam in 2011 – 2015, standard deviation (SD), and range for each year. Note, 2016 and 2017 estimates do not include potential age-5, and age-4 and age-5 offspring, respectively (*).

Year	Sex	n	Mean TLF	SD	Range
2011	F	156	0.98	4.55	0 - 40
	M	208	0.68	2.46	0 - 22
2012	F	148	3.26	7.99	0 - 44
	M	176	2.60	6.86	0 - 54
2013	F	186	1.38	4.68	0 - 26
	M	276	0.77	3.08	0 - 28
2014	F	253	0.36	1.53	0 - 15
	M	200	0.45	1.59	0 - 14
2015	F	88	1.10	4.11	0 - 24
	M	169	0.57	2.22	0 - 20
2016*	F	184	5.22	9.50	0 - 60
	M	239	3.84	8.17	0 - 56
2017*	F	121	0.04	0.24	0 - 2
	M	169	0.02	0.13	0 - 1

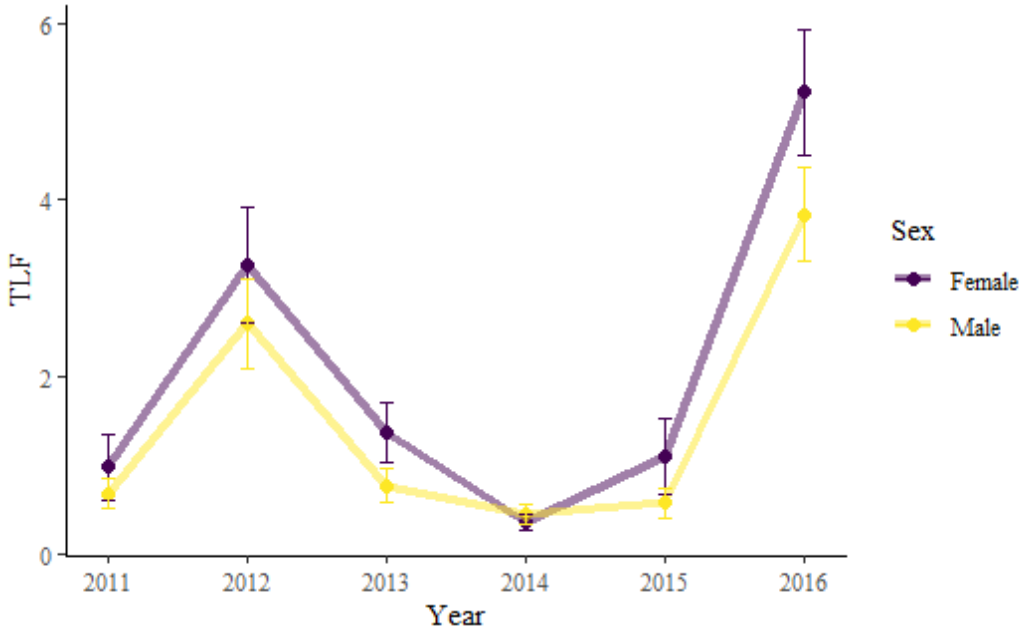


Figure 4. Mean TLF and standard error for Chinook salmon reintroduced above Fall Creek Dam from 2011 – 2015 by sex. Note, 2016 estimates do not include potential age-5 offspring.

Predictors of Total Lifetime Fitness

2011 – We fit the full model with *sex, release day, length, release group density, release group sex ratio,* and three interaction terms, *sex*release day, sex*release group density, and sex*release group sex ratio.* After model selection, *length, release group density,* and *release group sex ratio* were retained as predictors. Model results indicated *length* and *release group density* were significant positive predictors of TLF, in that TLF increased with increasing *length* of fish and increasing *release group density* (Table 7).

2012 – We fit the full model with *sex, release day, length, release group density, release group sex ratio,* and three interaction terms, *sex*release day, sex*release group density, and sex*release group sex ratio.* After model selection, no predictors were retained by either likelihood ratio tests of individual predictors or backward selection using Wald tests (Table 7).

2013 – There was strong collinearity between *length* and *sex*, and we were unable to parse the effect of *sex* from the effects of *length*. Therefore, we fit the model twice, once with *length* removed and with *sex, release day, release group density, release group sex ratio,* and three interaction terms, *sex*release day, sex*release group density, and sex*release group sex ratio,* and another with *sex* removed, and with *release day, release group density,* and *release group*

sex ratio. After model selection, no predictors were retained by either likelihood ratio tests of individual predictors or backward selection using Wald tests for either model (Table 7).

2014 – *Length* was not included in the model because it was not recorded for nearly half (207/453) of the individuals. We fit the model with *sex, release day, release group density, release group sex ratio,* and three interaction terms, *sex*release day, sex*release group density, and sex*release group sex ratio.* After model selection, *release day* was the only retained predictor. Model results indicated *release day* was a significant negative predictor of TLF, in that TLF decreased with increasing Julian day (Table 7).

2015 – There was strong collinearity between *length* and *sex*, and we were unable to parse the effect of *sex* from the effects of *length*. Therefore, we fit the model twice, once with *length* removed and with *sex, release day, release group density, release group sex ratio,* and three interaction terms, *sex*release day, sex*release group density, and sex*release group sex ratio*, and another with *sex* removed, and with *release day, release group density,* and *release group sex ratio*. After model selection, *length* was the only retained predictor. Model results indicated *length* was a positive predictor of TLF, in that TLF increased with increasing *length* of fish (Table 7).

Table 7. Results of generalized linear models examining the influence of *sex*, *release day*, *length*, *release group density*, *release group sex ratio*, *sex*release day*, *sex*release group density*, and *sex*release group sex ratio* on the total lifetime fitness of Chinook salmon above Fall Creek Dam in 2011 – 2015. Estimated effect (β) and standard error (SE) of each predictor on the link (log) scale for predictors that were retained in the final model are presented below. Two approaches were used for significance testing: Wald tests (Wald p-value) which identify predictors associated with TLF, and likelihood ratio tests (LRT p-value) which identify predictors associated with model fit. Estimated effects on the response scale (TLF) are presented in Supplemental Figure 1.

Year	Predictor	$\boldsymbol{\beta}$	SE	Wald p-value	LRT p-value
2011					
	(Intercept)	-8.318	2.171		
	length	0.099	0.028	**0.000**	**0.004**
	release group density	0.016	0.008	**0.041**	**0.052**
	release group sex ratio	0.192	0.312	0.537	0.589
2012					
	(Intercept)	0.904	3.802		

2013					
	(Intercept)	-3.034	5.620		
2014					
	(Intercept)	5.076	2.960		
	release day	-0.040	0.020	**0.043**	**0.051**
2015					
	(Intercept)	-6.302	2.306		
	length	0.081	0.032	**0.011**	**0.014**

Mixed Model (2011 – 2015) – Though excluded from some individual year analyses due to missing data, *length* was included in the cross-year model. We did not find evidence of strong multicollinearity among the evaluated predictors of TLF and included all predictors at the start of model selection. *Release location* was not included in the model as almost all fish over the selected years were released at one location. Therefore, we used a random effects structure that included random intercepts for both *release year* and *release group*. After model selection of fixed effects, only one predictor was included in the final model, *length* (Table 8). The best random effects structure according to AIC included random intercept for *release year*. Model results indicated length was a significant positive predictor of TLF, in that TLF increased with increasing *length* of fish. The association between *length* and TLF are presented on the response scale (TLF) in Supplemental Figure 2.

Table 8. Results of generalized linear mixed model examining the influence of *sex*, *release day*, *length*, *release group density*, *release group sex ratio*, total number of fish released annually, annual *sex ratio*, *sex*release group density*, *sex*release group sex ratio* and *sex*annual sex ratio* on the total lifetime fitness of spring Chinook salmon above Fall Creek in 2011 – 2015. *Release year* was included as a random effect. Estimated effect (β) and standard error (SE) of each fixed predictor on the link (log) scale for predictors that were retained in the final model are presented below. Two approaches were used for significance testing: Wald tests (Wald p-value) which identify predictors associated with TLF, and likelihood ratio tests (LRT p-value) which identify predictors associated with model fit. Estimated variance (σ2) and standard deviation (SD) are presented for random effects. Significant p-values are in bold. Estimated effects on the response scale (TLF) are presented in Supplemental Figure 2.

Fixed effect	$\boldsymbol{\beta}$	**SE**	**Wald p-value**	**LRT p-value**
(Intercept)	-4.247	1.102		
length	0.056	0.014	**0.000**	**0.000**
Random effect	$\boldsymbol{\sigma^2}$	**SD**		
release year	0.396	0.629		

Cohort Replacement Rate

Total cohort replacement rate (CRR_{total}) was less than one in most years from 2011 – 2015 indicating that the reintroduced population above Fall Creek Dam is not replacing itself (Table 9). However, in 2012, CRR_{total} was highest at 1.49. The minimum CRR_{total} (0.21) was observed in 2014. Females were the limiting sex in all years except in 2014 when the sex ratio was female biased with 0.8 males for every female fish. Consistent with CRR_{total}, the maximum CRR_F (1.34) was observed in 2012 and minimum CRR_F (0.14) was observed in 2014. Similarly for males, maximum CRR_M (1.55) was observed in 2012 and minimum CRR_M (0.26) was observed in 2014.

2011 – During 2014 – 2016, 164 salmon returned to Fall Creek and were determined to be offspring of salmon above Fall Creek Dam in 2011 (N = 364) for a CRR_{total} of 0.45. Male and female replacement rates were CRR_M of 0.34 and CRR_F of 0.48, respectively (Table 9).

2012 – During 2015 – 2017, 484 salmon returned to Fall Creek and were determined to be offspring of salmon above Fall Creek Dam in 2012 (N = 324) for a CRR_{total} of 1.49. Male and female replacement rates were CRR_M of 1.55 and CRR_F of 1.34, respectively (Table 9).

2013 – During 2016 – 2018, 268 salmon returned to Fall Creek and were determined to be offspring of salmon above Fall Creek Dam in 2013 (N = 462) for a CRR_{total} of 0.58. Male and female replacement rates were CRR_M of 0.49 and CRR_F of 0.50, respectively (Table 9).

2014 – During 2017 – 2019, 95 salmon returned to Fall Creek and were determined to be offspring of salmon above Fall Creek Dam in 2014 (N = 453) for the lowest CRR_{total} of 0.21. Male and female replacement rates were CRR_M of 0.26 and CRR_F of 0.14, respectively (Table 9).

2015 – During 2018 – 2020, 97 salmon returned to Fall Creek and were determined to be offspring of salmon above Fall Creek Dam in 2015 (N = 257) for a CRR_{total} of 0.38. Male and female replacement rates were CRR_M of 0.28 and CRR_F of 0.57, respectively (Table 9).

Table 9. Cohort Replacement Rate (CRR) per parent year. CRR_{total} was estimated by determining the number of offspring successfully assigned to at least one parent in a given year ($n_{offspring}$) and dividing it by the number of candidate parents ($n_{candidate}$) in that year. We also present CRR_F: the number of female adult offspring successfully assigned to at least one female

parent in a given year, divided by the number of female parents ($n_{cand\ female}$) in that year. CRR_M is the equivalent value for males.

Parent Year	$n_{offspring}$	$n_{candidate}$	$n_{cand\ female}$	$n_{cand\ male}$	Sex Ratio (M:F)	CRR_{total}	CRR_M	CRR_F
2011	164	364	156	208	1.3 : 1.0	0.45	0.34	0.48
2012	484	324	148	176	1.2 : 1.0	1.49	1.55	1.34
2013	268	462	186	276	1.5 : 1.0	0.58	0.49	0.50
2014	95	453	253	200	1.0 : 1.3	0.21	0.26	0.14
2015	97	257	88	169	1.9 : 1.0	0.38	0.28	0.57

Effective Number of Breeders

The effective number of breeders (N_b) ranged from 25.1 in 2015 to 50.0 in 2012 (Table 10). The ratio of N_b to the number of candidate parents ranged from 0.07 in 2014 to 0.15 in 2012. N_b was lower than the number of candidate parents with one or more offspring in the pedigree ($n_{success}$) in all years.

Table 10. Effective number of breeders (N_b) per parent year, with upper and lower 95% confidence intervals (CI) based on jack-knife, as estimated by NeEstimator v2.1. Number of candidate parents (n_{cand}) is the number of successfully genotyped salmon above Fall Creek Dam. $n_{success}$ is the number of candidate parents with one or more offspring in the pedigree. $n_{offspring}$ is the number of salmon assigned to candidate parents above the dam that parent year. The N_b:n_{cand} ratio is provided for each year.

Year	n_{cand}	n_{sucess}	$n_{offspring}$	N_b	CI_{lower}	CI_{upper}	N_b:N_{cand}
2011	364	66	164	37.2	31.1	44.6	0.10
2012	324	86	484	50.0	45.7	54.6	0.15
2013	462	55	268	38.4	34.2	43.0	0.08
2014	453	49	95	31.9	25.4	40.5	0.07
2015	257	38	97	25.1	19.8	31.8	0.10

Release day in 2011 – 2020

O'Malley and Bohn (2018) reported differences in release day between all years assessed 2011 – 2015 (Wilcoxon rank sum test, $p < 0.0001$), except for 2013 vs. 2014, when the median release day was May 28 and June 2, respectively (Table A4). Here, we found that Chinook salmon release day was similar in 2016, 2018, and 2020 (Table A4), while all other cross-year comparisons to 2011 – 2020 were significantly different (Wilcoxon rank sum test, $p < 0.05$).

O'Malley and Bohn (2018) also reported that male and female spring Chinook salmon were released above Fall Creek Dam at a similar time in 2011 and 2012. In 2013, 2014, and 2015, median release day for male spring Chinook salmon was five, two, and seven days later, respectively, than the median release day for females in the same year (Figure 5). However, 2013 was the only significant comparison between males and females (Wilcoxon rank sum test, $p = 0.01$). Here, we found that in 2016 – 2020, male spring Chinook were released earlier than females in all years, except 2019. For instance, in 2016, 2017, 2018 and 2020, median release day for male salmon was six, seven, four, and three days earlier, respectively, than the median release day for female salmon in the same year (Figure 5; Table A4). However, the only significant comparisons were 2016 and 2018 (Wilcoxon rank sum test, $p < 0.01$ and $p < 0.05$). In 2019, the median release day for male salmon was six days later than for females (Wilcoxon rank sum test, $p < 0.0001$).

Intra-annual variation in circadian clock genes, microsatellite markers, and release day

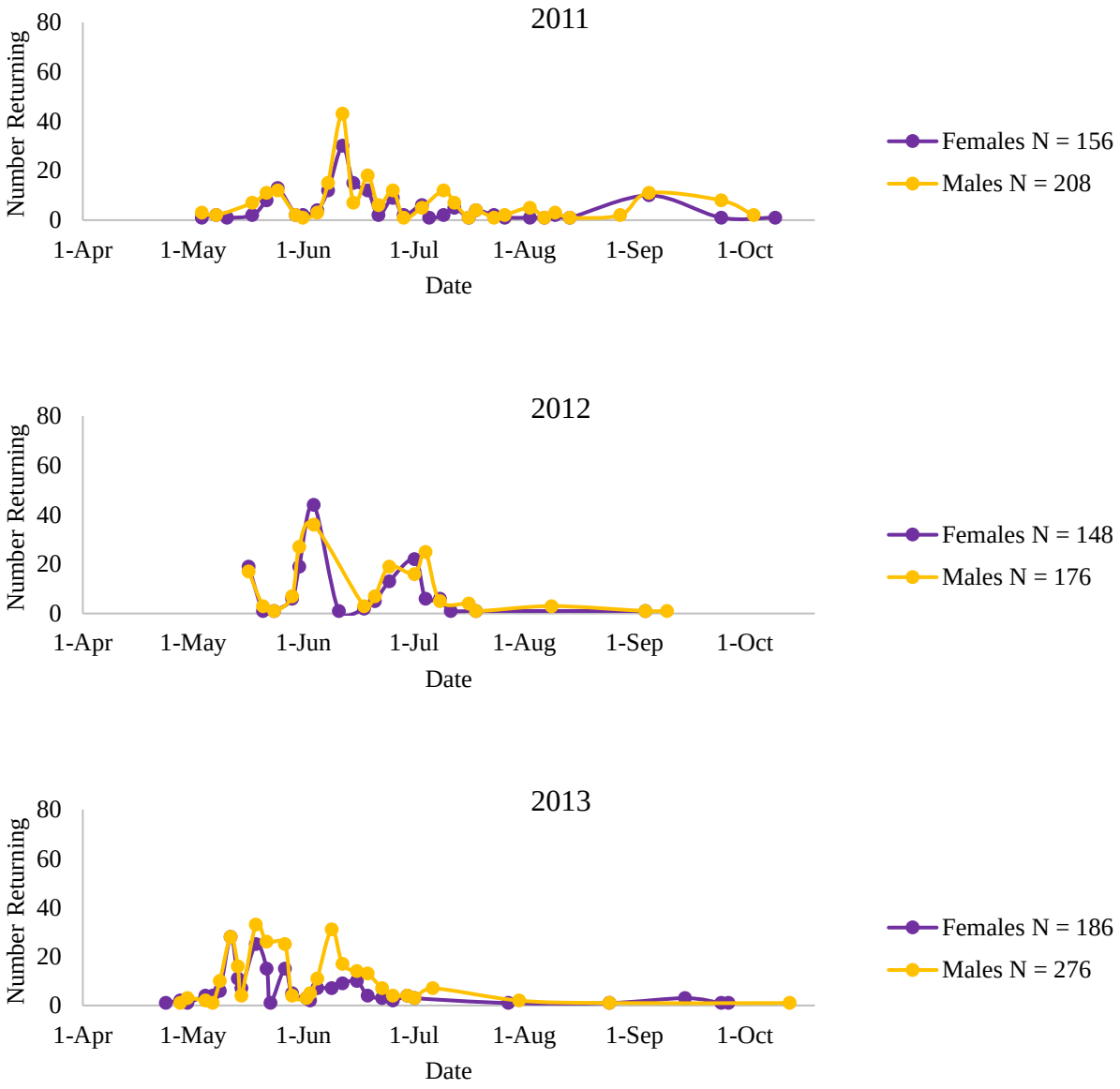
As stated in the methods section, sufficient sample sizes to compare early- and late-released spring Chinook salmon were limited in 2011 – 2020. O'Malley and Bohn (2018) reported that early and late spring Chinook salmon in 2011 were not significantly differentiated based on variation at the four circadian clock genes (F_{ST} range: -0.004 to 0.004) (Supplemental Table 1), and the 11 neutral microsatellites ($F_{ST} = 0.001$). Similarly, we found no evidence for significant genetic differentiation between early and late spring Chinook salmon in 2016 based on variation at the four circadian clock genes (F_{ST} range: -0.002 to 0.004) (Supplemental Table 1), and the 11 neutral microsatellite markers ($F_{ST} = 0.002$).

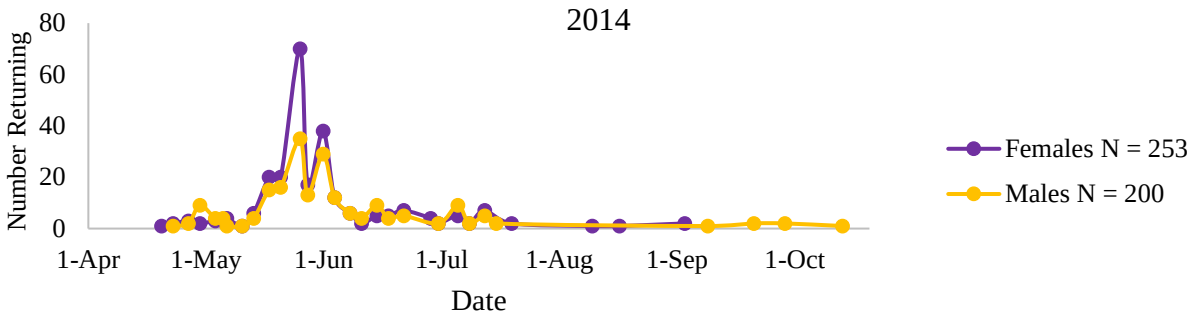
Intra-annual variation in circadian clock genes, microsatellite markers, and sex

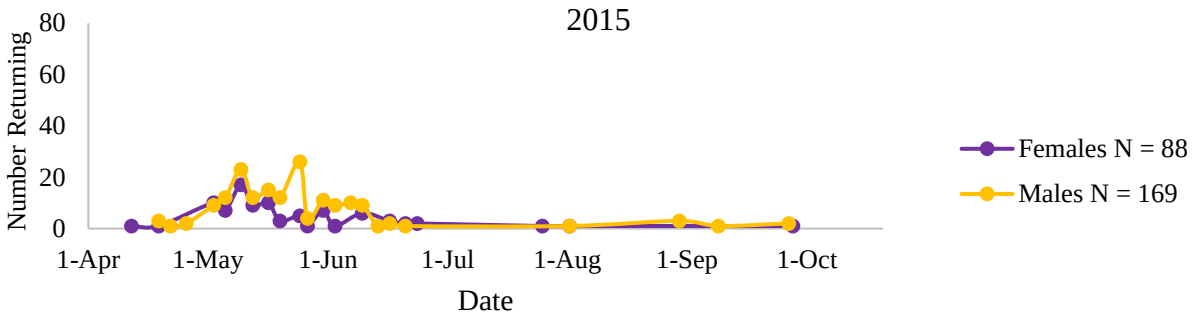
O'Malley and Bohn (2018) reported significant genetic differences between the sexes based on variation at *Omy1009UW* in 2013 and 2015, and *Ots515NWFSC* in 2015. However, the degree of genetic differentiation was similar to that observed for the 11 neutral microsatellite markers and therefore did not provide evidence for potentially adaptive differentiation.

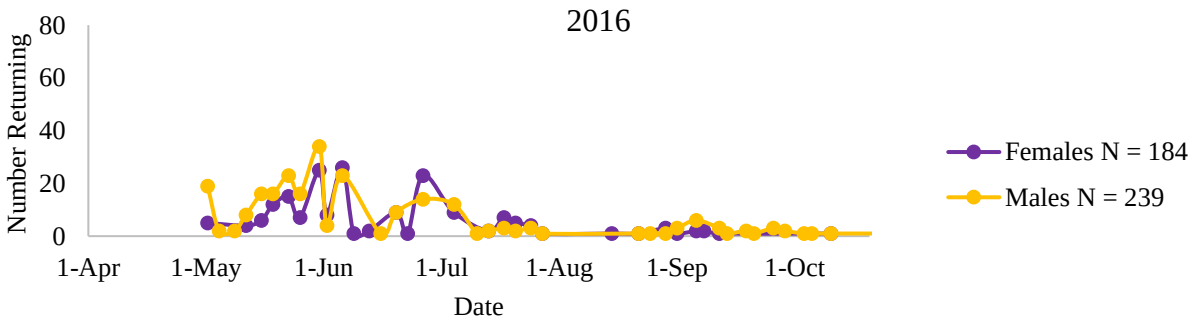
Similarly, we found evidence for significant differentiation between males and females based on variation at *Omy1009UW* in two years of the five years examined (2016 – 2020). In 2016, males and females were differentiated ($F_{ST} = 0.004$) (Supplemental Table 2) as well as in 2019 ($F_{ST} =$

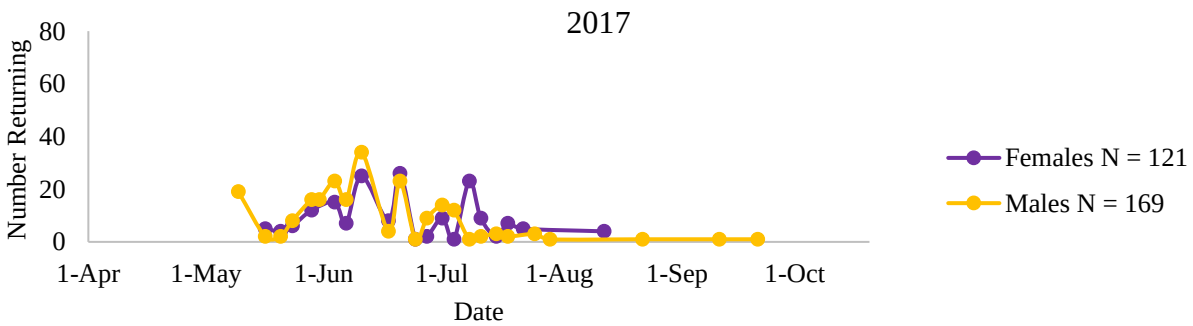
0.018), when the magnitude of differentiation was twice that based on the 11 neutral microsatellite markers (F_{ST} = 0.009).




2014
Number Returning
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date
Females N = 253
Males N = 200


2015
Number Returning
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date
Females N = 88
Males N = 169


2016
Number Returning
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date
Females N = 184
Males N = 239


2017
Number Returning
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date
Females N = 121
Males N = 169

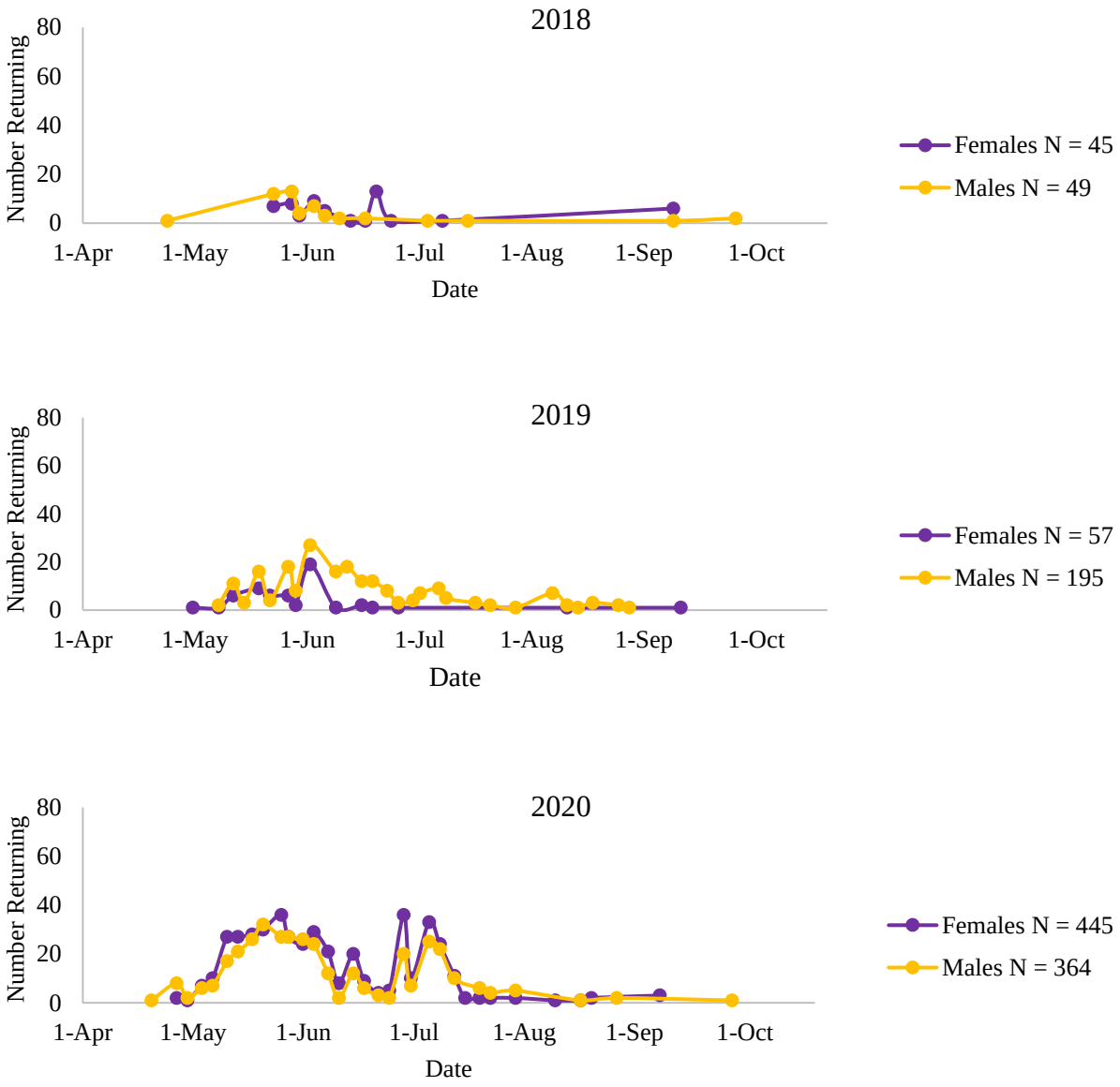


Figure 5. Distributions of release times for female and male adult spring Chinook above Fall Creek Dam in 2011 – 2020. Sample sizes are reported for each sex within each year.

Inter-annual variation in circadian clock genes, microsatellite markers, and release day

Across almost all years (2011 – 2020), we found evidence for significant inter-annual differentiation among early (released before June 1) spring Chinook salmon based on variation at *Ots515NWFSC* (Table 11a) and *Omy1009UW* (Table 11b). The magnitude of differentiation was similar to that for the 11 neutral microsatellite markers (Table 11e) with five notable exceptions for *Ots515NWFSC*; 2011 vs. 2012, 2015, 2016, 2017 and 2020 (F_{ST} range = 0.034 – 0.042; Table

11a). In addition, we found evidence for significant differentiation between early-released salmon in 2017 and most years based on variation at *OmyFbxw11* (F_{ST} range = 0.030 – 0.079; Table 11c). The comparison between 2011 vs 2012 was also notable at *OmyFbxw11* (F_{ST} = 0.033; Table 11c).

Table 11. Tests for inter-annual differentiation among early released (before June 1) spring Chinook salmon based on variation at (a) *Ots515NWFSC*, (b) *Omy1009UW*, (c) *OmyFbxw11*, (d) *OtsClock1b*, and (e) 11 microsatellite markers. Significant F_{ST} estimates after correcting for multiple comparisons following the method of Benjamini and Hochberg (1995) are indicated in **bold-faced** type.

(a) *Ots515NWFSC*

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
2011	-	**0.037**	**0.008**	**0.006**	**0.040**	**0.042**	**0.036**	0.012	0.005	**0.034**
2012		-	**0.009**	**0.016**	**0.006**	0.003	-0.003	**0.009**	**0.015**	**0.010**
2013			-	**0.005**	**0.019**	**0.016**	**0.009**	0.005	0.002	**0.019**
2014				-	**0.018**	**0.021**	**0.013**	0.005	**0.004**	**0.014**
2015					-	0.001	**0.007**	**0.009**	**0.015**	**0.008**
2016						-	0.006	**0.012**	**0.015**	**0.006**
2017							-	0.003	**0.014**	**0.015**
2018								-	0.002	**0.009**
2019									-	**0.014**
2020										-

(b) *Omy1009UW*

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
2011	-	**0.022**	**0.021**	**0.020**	**0.012**	**0.024**	**0.026**	**0.020**	**0.012**	**0.026**
2012		-	**0.007**	**0.010**	**0.015**	**0.009**	**0.014**	**0.014**	**0.017**	**0.010**
2013			-	**0.002**	**0.013**	**0.010**	**0.006**	**0.007**	**0.010**	**0.011**
2014				-	**0.011**	**0.010**	**0.005**	**0.003**	**0.009**	**0.012**
2015					-	**0.007**	**0.014**	**0.011**	0.002	**0.012**
2016						-	**0.009**	**0.015**	**0.009**	**0.003**
2017							-	**0.007**	**0.010**	**0.010**
2018								-	**0.006**	**0.019**
2019									-	**0.010**
2020										-

(c) *OmyFbxw11*

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
2011	-	**0.033**	0.003	**0.020**	-0.001	-0.003	0.004	0.004	-0.001	-0.004
2012		-	0.011	-0.001	**0.013**	**0.017**	**0.079**	0.008	**0.025**	**0.024**
2013			-	0.005	0.003	0.004	**0.025**	0.006	0.009	0.004
2014				-	0.005	**0.010**	**0.070**	0.000	**0.014**	**0.017**

2015					-	-0.002	**0.030**	-0.004	-0.002	0.003
2016						-	**0.022**	-0.001	-0.002	0.001
2017							-	0.038	**0.026**	0.006
2018								-	-0.003	0.008
2019									-	0.003
2020										-

(d) *OtsClock1b*

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
2011	-	0.002	-0.004	0.003	-0.004	-0.005	0.001	0.006	-0.005	-0.001
2012		-	0.000	-0.003	-0.001	0.005	-0.007	-0.007	0.000	0.018
2013			-	0.001	-0.002	-0.001	-0.001	0.001	-0.004	0.005
2014				-	0.000	0.005	-0.004	-0.004	0.001	**0.017**
2015					-	-0.001	-0.002	0.000	-0.004	0.006
2016						-	0.003	0.007	-0.003	0.001
2017							-	-0.007	0.000	0.017
2018								-	0.003	0.023
2019									-	0.003
2020										-

(e) Neutral markers

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
2011	-	**0.018**	**0.014**	**0.011**	**0.011**	**0.016**	**0.016**	**0.012**	**0.012**	**0.015**
2012		-	**0.012**	**0.014**	**0.008**	**0.007**	**0.019**	**0.018**	**0.014**	**0.011**
2013			-	**0.005**	**0.012**	**0.010**	**0.007**	**0.008**	**0.012**	**0.010**
2014				-	**0.010**	**0.012**	**0.009**	**0.004**	**0.010**	**0.013**
2015					-	**0.006**	**0.020**	**0.011**	**0.008**	**0.009**
2016						-	**0.014**	**0.012**	**0.009**	**0.003**
2017							-	**0.008**	**0.013**	**0.013**
2018								-	**0.006**	**0.012**
2019									-	**0.010**
2020										-

Intra-annual and inter-annual variation in fork length

O'Malley and Bohn (2018) reported that male spring Chinook salmon reintroduced above Fall Creek Dam in 2013 and 2015 were significantly shorter than females released above the dam in the same respective year (Wilcoxon rank sum tests, $p < 0.0001$ for both comparisons). Furthermore, males released in 2013 and 2015 were also significantly shorter than males released in 2011, 2012, and 2014 (Wilcoxon rank sum tests, $p < 0.0001$ for all comparisons). Female fork

lengths were more consistent across years, but females reintroduced in 2013 were significantly shorter than females reintroduced in all other years from 2011 – 2015 (Wilcoxon rank sum tests, $p < 0.0001$ for all comparisons). The inter-annual differences in male fork lengths may be explained by age at return. For instance, the genetic parentage analysis results indicated that a large number of age-3 males returned in 2015, and these males were significantly shorter than age-4 males (Wilcoxon rank sum test, $p < 0.0001$).

Here, we report that male spring Chinook salmon released above Fall Creek Dam in 2016 – 2020 were significantly shorter than females released above the dam in the same respective year (Figure 6; Wilcoxon rank sum tests, $p < 0.0001$), except for 2020. Across years, the average size of males fluctuated with some years significantly shorter or longer than others (Figure 6). In 2019, for instance, males were significantly shorter than males released above Fall Creek Dam in all other years (2011 – 2020). In 2020, male spring Chinook salmon were significantly longer than males in all other years, except for the 2014 returns (Wilcoxon rank sum tests, $p < 0.0001$).

This inter-annual difference in male fork lengths may be explained by the age at which male spring Chinook returned to Fall Creek Dam. For instance, results from the genetic parentage analysis indicate that a disproportionately large number of age-3 fish returned to the dam in 2019, on average four times as many as in previous years, and of those, the majority were males (Table 4; Figure 7f). Similarly, many age-4 fish returned in 2020, however, with a more equal sex ratio, with 0.8 male fish per female age-4 fish (Figure 7g). Across all years, the age-3 males were significantly shorter than age-4 males (Figure 7; Wilcoxon rank sum test, $p < 0.0001$).

Female fork lengths were more consistent across years, though females returning in 2020 were significantly longer than females in most years, except 2014, 2018, and 2019 (Wilcoxon rank sum test, $p < 0.0001$). Notably, 99% (798/803) of the females that returned and were released above the dam in 2020 were age-4.

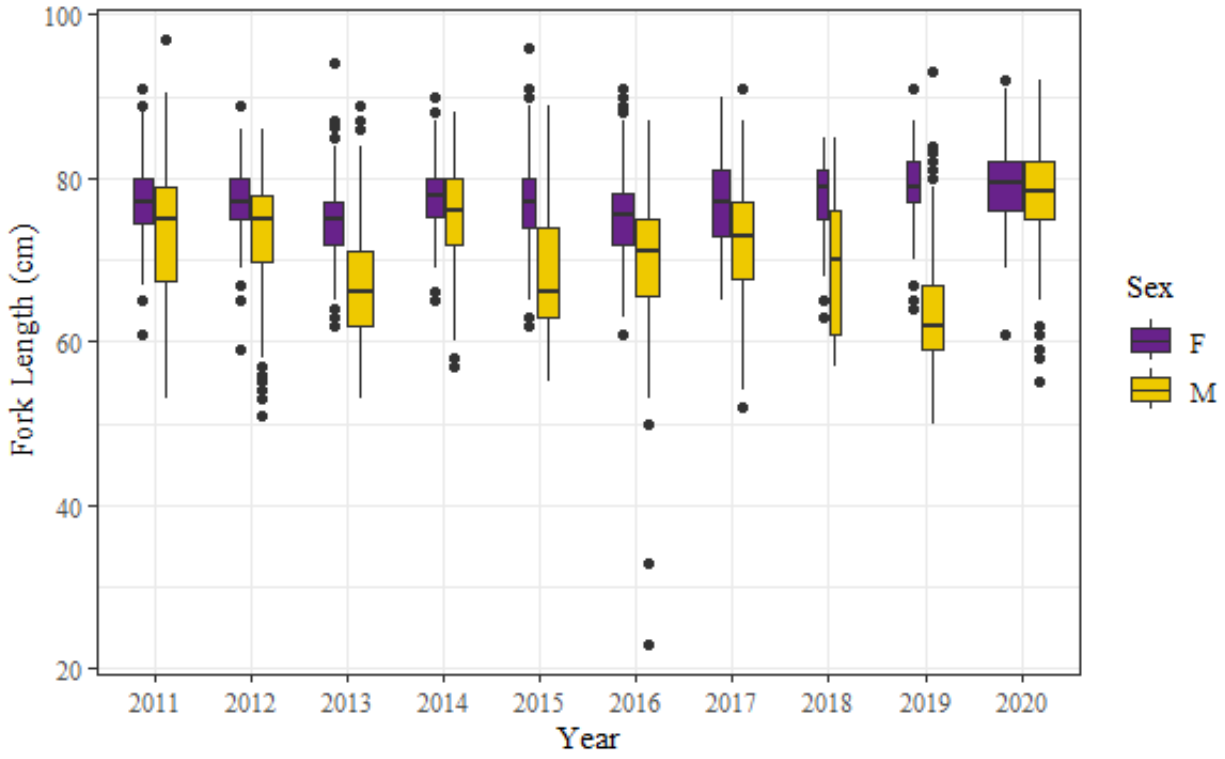
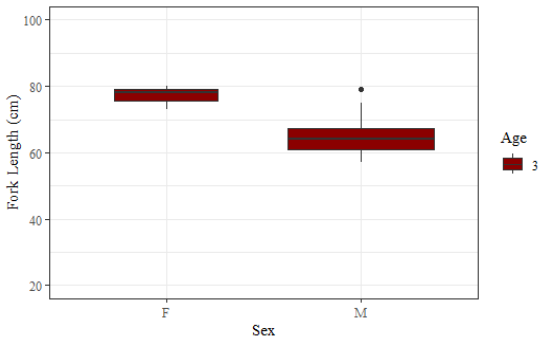
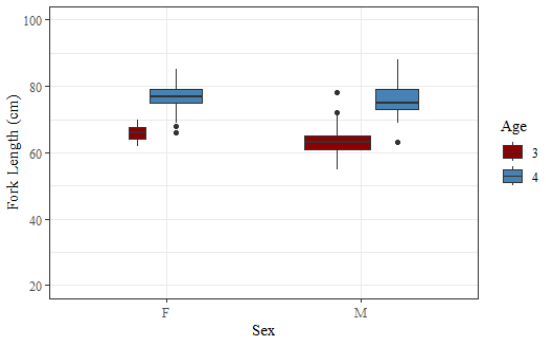


Figure 6. Differences in fork length among female (F) and male (M) spring Chinook salmon released above Fall Creek Dam in 2011 – 2020. Box widths scaled to sample size for each group.

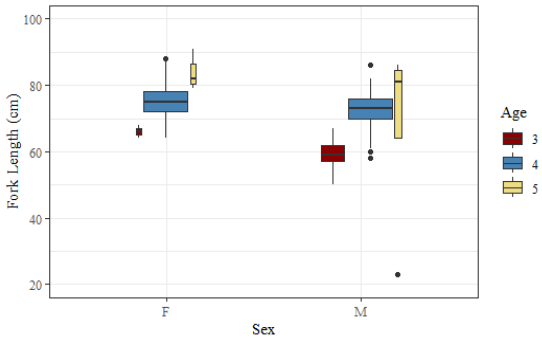
(a) 2014



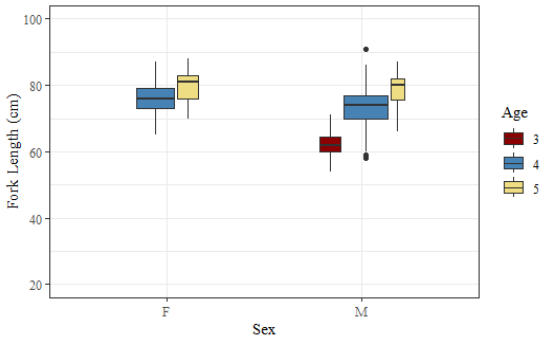
(b) 2015



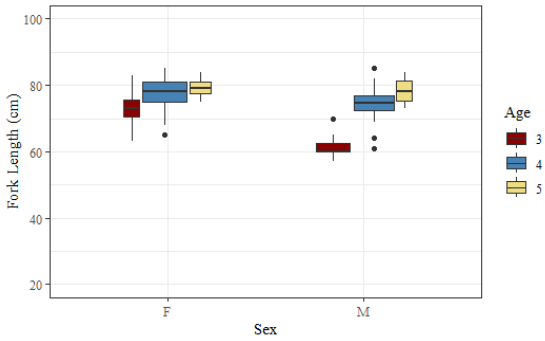
(c) 2016



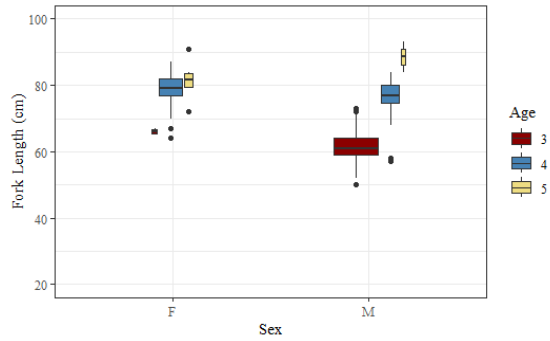
(d) 2017



(e) 2018



(f) 2019



(g) 2020

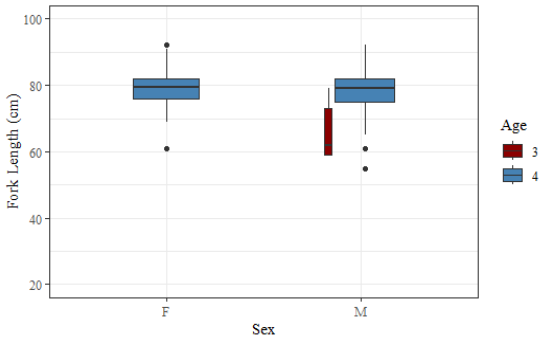


Figure 7. Differences in fork length and age-class among the 2014 – 2020 spring Chinook salmon that assigned as offspring of salmon released above Fall Creek Dam in 2011 – 2017. Fork lengths of females (F) and males (M) returning at age-3, age-4, and age-5 in (a) 2014, (b) 2015, (c) 2016, (d) 2017, (e) 2018, (f) 2019, and (g) 2020. Box widths scaled to sample size for each group.

DISCUSSION

O'Malley and Bohn (2018) conducted an initial genetic parentage analysis of spring Chinook salmon reintroduced above Fall Creek Dam that focused on the 2011 – 2015 adult returns. We extended the analysis by incorporating the 2016 – 2020 adult returns. Here, we summarize the primary findings for the 2011 – 2020 adult returns to Fall Creek Dam and compare the results to those for spring Chinook salmon reintroduced above dams in the South Santiam River (O'Malley et al. 2024), North Santiam River (O'Malley et al. 2023), and McKenzie River (O'Malley et al. 2023).

1. From 2011 to 2017, the average number of salmon that were released per year above Fall Creek Dam was 367. In 2018, a new Adult Fish Collection Facility became fully operational and only 98 adult salmon were sampled at the facility and released above the dam. In 2019 and 2020, the number of salmon sampled at the facility and reintroduced above the dam increased to 252 and 809, respectively.

2. In 2014, 10% (46/453) of salmon reintroduced above Fall Creek Dam assigned to salmon previously reintroduced above the dam. In 2015, 87% (224/257) assigned to salmon previously released above the dam. It is important to note that potential parents returning in 2009 and 2010 were not sampled so these assignments are likely underestimates. In 2016, the first year all potential parents (2011 – 2013) were sampled, the assignment rate increased to 92% (391/423) and subsequently increased every year thereafter with an assignment rate of 99% (803/809) in 2020.

3. The inferred age structure based on the genetic parentage analysis results indicates that most salmon return to Fall Creek Dam at age-3 and age-4, with few age-5 salmon. Our results differ from the estimated age structure based on scale data collected from Fall

Creek Chinook salmon in 2014 and 2015, which indicated predominately age-4 and age-5 returns (e.g., 4% age-3, 67% age-4, and 26% age-5; C. Sharpe, ODFW).

4. Above Fall Creek Dam in 2011 – 2015, the sex ratio was male biased in all years except 2014 when the sex ratio was slightly female biased.

5. Above Fall Creek Dam in 2011 – 2015, mean TLF ranged from a low of 0.40 ± 1.56 SD (n = 453; range 0 – 15) in 2014 to a high of 2.90 ± 7.39 SD (n = 324; range 0 – 54) in 2012. The same pattern was also observed in the North Santiam River with the lowest mean TLF in 2014 and highest mean TLF in 2012. Similarly, in the South Santiam River, the lowest mean TLF was observed in 2014 while the highest mean TLF was observed in 2013 followed closely by 2012. In contrast, the lowest mean TLF in the South Fork McKenzie was observed in 2015 and the highest mean TLF was observed in 2011.

6. Above Fall Creek Dam in 2011 – 2015, mean female TLF was greater than mean male TLF in all years except 2014, the only year the sex ratio was slightly female biased. In the North Santiam River from 2011 – 2015, mean HOR female TLF was greater than male TLF in all years, except 2012, the only year the sex ratio was female biased. In the South Santiam River from 2011 – 2015, mean NOR female TLF was greater than male TLF in all years except 2014 when mean TLF was nearly equal. The sex ratio was male biased in all years except 2014 when it was equal.

   A similar pattern in mean TLF and sex ratio was not evident in the South Fork McKenzie River from 2011 – 2015. Mean NOR female TLF was less than mean NOR male TLF in all years except 2012 and 2015 when female mean TLF was greater and when mean TLF was equal, respectively. In contrast, mean HOR female TLF was greater than male in 2011 – 2013 while mean HOR male TLF was greater in 2014 and 2015. The overall sex ratio (i.e., NOR and HOR) was male biased in 2011 and 2012, and female biased in 2013 – 2015.

7. Notably, in 2016, the mean partial TLF was high for salmon above Fall Creek Dam, 4.44 ± 8.79 SD (n = 423; range 0 – 60). Correspondingly, in the South Santiam River in 2016, the mean partial TLF for salmon above Foster Dam was 1.05 ± 2.80 SD (n = 256; range 0 – 18). The previous highest mean TLF estimate for salmon in this river was 0.29 ± 1.27 SD (n = 918; range 0 – 15) in 2013. Similarly, in the North Santiam River, an exceptionally large number of salmon (n = 1174) released above Detroit Dam in 2019 and 2020 were assigned to 2016 parents. In contrast, the mean partial TLF for salmon returning to the South Fork McKenzie River was 0.36 ± 0.80 in 2016 which was not notably higher than in other years.

8. Statistical power to detect and delineate significant relationships between TLF of Chinook salmon released above Fall Creek Dam and various predictors was limited in the annual GLMs given the many independent model selection/hypotheses when the data are separated out by year. Consequently, these results are subject to a multiple comparisons problem and may point to spurious associations between TLF and some predictors, especially for those with marginal effect sizes and significance. Therefore, the significance of predictors within each year should be approached with caution.

9. Based on the annual GLMs, *length* was associated with TLF in two (2011 and 2015) of the five years. Broadly, TLF increased as *length* increased. In 2011, we also found that TLF increased with increasing *release group density* whereas in 2014 TLF decreased with later *release day*.

10. Based on the GLMM that collectively considered TLF of salmon above Fall Creek Dam in 2011 – 2015, we found that *length* was a significant positive predictor of TLF, in that TLF increased with increasing *length* of fish (Supplemental Figure 2).

11. CRR_{total} was less than one in most years from 2011 – 2015 indicating that the reintroduced population above Fall Creek Dam is not replacing itself (Table 9). However, in 2012, CRR_{total} was 1.49 indicating replacement had been met. The minimum CRR_{total} (0.21) was observed in 2014. Consistent with CRR_{total}, the maximum CRR_F (1.34) was observed in 2012 and minimum CRR_F (0.14) was observed in 2014. Similarly for males,

maximum CRR_M (1.55) was observed in 2012 and minimum CRR_M (0.26) was observed in 2014.

In the North Santiam River, the maximum CRR_{total} was also observed in 2012 (0.67) while in the South Santiam River the maximum CRR_{total} was observed in 2013 (0.16) followed by 2012 (0.14). The minimum CRR_{total} in the North and South Santiam Rivers was observed in 2014 (0.07 and 0.04, respectively). In the South Fork McKenzie, the maximum CRR_{total} was observed in 2011 (0.39) while the minimum CRR_{total} was observed in 2015 (0.09) followed by 2014 (0.11).

12. Above Fall Creek Dam from 2011 – 2015, the fluctuation in N_b reflected the fluctuation in the number of candidate parents with one or more offspring ($n_{success}$). However, these two estimates did not relate to the number of candidates parents ($n_{\boldsymbol{cand}}$) released above Fall Creek Dam. For instance, as $n_{\boldsymbol{cand}}$ increased, $n_{success}$ and N_b generally decreased, except in 2015. This is reflected in N_b: N_{cand} ratio which was lowest in 2014 (0.07) when the second greatest number of salmon (n = 453) were reintroduced above Fall Creek Dam.

13. From 2011 – 2015, the date of first release for salmon above Fall Creek Dam shifted earlier each year from May 5 (2011) to April 13 (2015). From 2016 – 2020, the date of first release for salmon reintroduced above the dam did not follow the same trend as the first five years, with releases variously early May or late April over the years.

14. From 2011 – 2015, there were significant differences in release day between all years assessed except for 2013 vs. 2014, when the median release day was May 28 and June 2, respectively. Here, we found that Chinook salmon release day was similar in 2016, 2018, and 2020, while all other cross-year comparisons to 2011 – 2020 were significantly different.

15. From 2011 – 2020, the median release day of males and females was significantly different in four of the years. For instance, in 2013 and 2019, males were released

significantly later than females whereas in 2016 and 2018, males were released significantly earlier than females.

16. We compared early (released before June 1) Chinook salmon across years and found significant differences based on variation at two genetic markers which have previously been shown to be associated with return timing in Chinook salmon. For example, based on variation at *Ots515NWFSC* early released salmon in 2011 were significantly different from early released salmon in five years (2012, 2015, 2016, 2017, and 2020). Based on variation at *OmyFbxw11,* early released salmon in 2017 were significantly different from early released salmon in most years. These results provide evidence for potentially adaptive genetic differences since the magnitude of differentiation is substantially larger than that based on neutral microsatellite markers.

Reintroduction program

Results from the genetic parentage analysis indicate that most spring Chinook salmon released above Fall Creek Dam were offspring of salmon reintroduced above the dam in previous years. Productivity above the dam in 2011 – 2015 was highly variable across years, with replacement being met in 2012 (CRR_{total} = 1.49) in contrast to low productivity of reintroduced adults in 2014 (CRR_{total} = 0.21). It is important to note that productivity of adult Chinook salmon above dams on the North Santiam and South Santiam Rivers was also high in 2012 and low in 2014. This finding suggests that basin-wide and/or ocean conditions likely influenced productivity above the dams across these years. Interestingly, the observed trends in productivity in these three systems did not apply to Chinook salmon in the South Fork McKenzie River.

Release day

The notable shift of earlier spring Chinook salmon returns to Fall Creek Dam from 2011 – 2015 prompted further investigation to determine if there was a genetic basis. O'Malley and Bohn (2018) reported significant differences between early released Chinook salmon across those years based on variation at genetic markers previously shown to be associated with adult return timing. From 2016 – 2020, however, return timing varied among years and lacked a sustained shift to an earlier return timing. While we detected significant genetic differences between early

released salmon across years based on variation at the adult return timing markers, the shift to an earlier return timing did not persist across the year period.

Next steps

Genetic parentage analysis is an effective approach to 1) evaluate and monitor a Chinook salmon reintroduction program within a river and 2) identify trends in productivity across multiple reintroduction programs. For example, our estimates of population productivity across years were similar in three of the four reintroduction programs highlighting the importance of basin-wide or ocean conditions as well as variables associated with a specific river system.

There was a shift to earlier return timing to Fall Creek Dam in 2011 – 2015 that did not persist in 2016 – 2020. While we found evidence for potentially adaptive genetic differentiation among early released Chinook salmon, there was no evidence of strong directional selection across all years. Therefore, we recommend focusing solely on the use of genetic parentage analysis to evaluate and monitor productivity of spring Chinook salmon released above Fall Creek Dam in future investigations.

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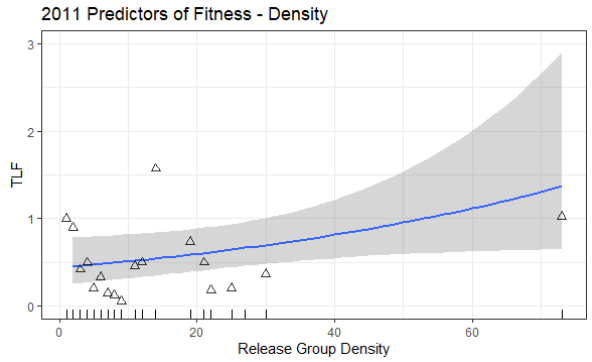
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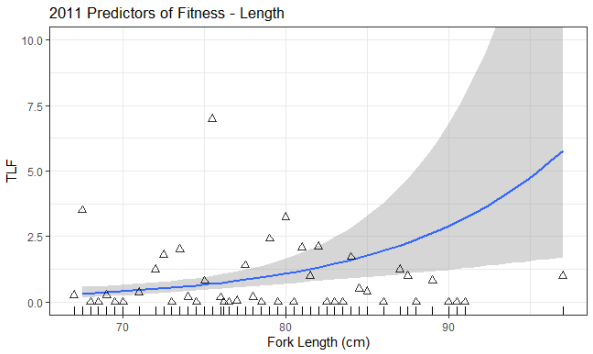
Williamson KS, JF Cordes, B May (2002) Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. *Molecular Ecology Notes* 2:17-19.

Supplemental Figures

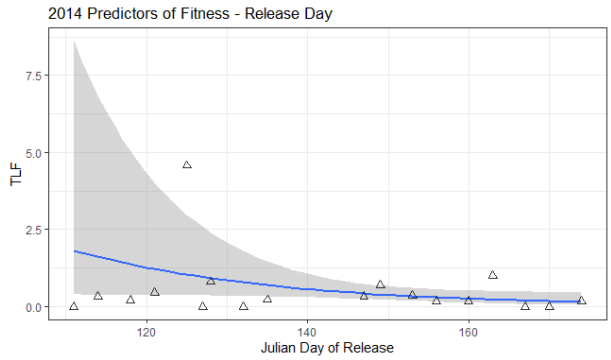
(a)



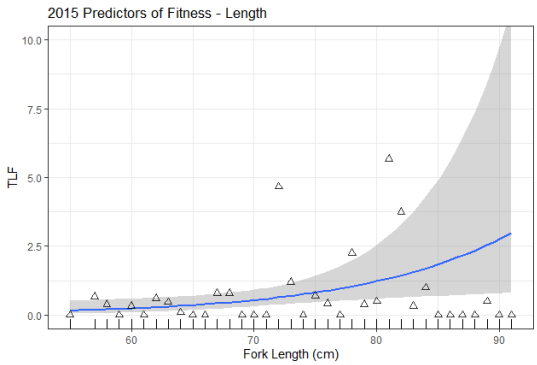
(b)



(c)



(d)



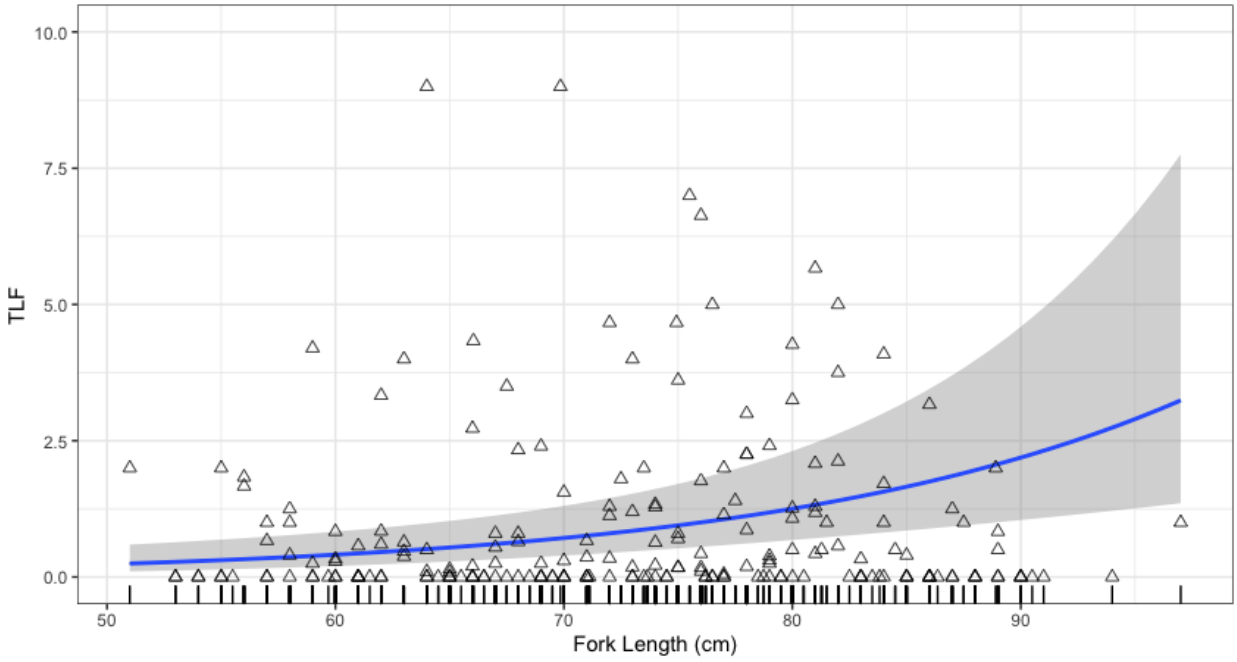
Supplemental Figure 1. Effect plots of significant predictors of TLF from the annual GLMs. Estimated effects of significant predictors are presented as lines with 95% confidence intervals. Estimated effects of predictors from final models with more than one predictor are conditioned on the mean value of other numeric predictors, or the weighted average of the within-level fitted values for categorical predictors. Empirical TLF averaged over each level of the predictor are presented as triangles for main effects. The rug plot at the bottom of each figure displays the levels of the predictors used to fit the model.

(a) Estimated effect of *release group density* on TLF in 2011. *Release group sex ratio* and *length* were also included in the final model.

(b) Estimated effect of *length* on TLF in 2011. *Release group density* and *release group sex ratio* were also included in the final model.

(c) Estimated effect of *release day* on TLF in 2014.

(d) Estimated effect of *length* on TLF in 2015.



Supplemental Figure 2. Estimated effect of *length* as a predictor of TLF from salmon reintroduced above Fall Creek Dam 2011 – 2015 (lines with 95% confidence intervals). Empirical TLF averaged over each level of the predictor are presented as triangles. The rug plot at the bottom of each figure displays the levels of the predictors used to fit the model.

Supplemental Information

Circadian clock genes and microsatellite marker variation

Across years (2016-2020), two of the four circadian clock genes and the 11 microsatellite markers showed significant deviations from Hardy-Weinberg proportions (HWP) and linkage equilibrium after correcting for multiple comparisons following Benjamini and Hochberg (1995) (Table A2). These deviations may be attributed to sampling of related individuals. None of the genetic markers showed consistent signs of null alleles.

Allelic diversity at the four circadian clock genes ranged from 3 – 77 alleles, with *Omy1009UW* showing the highest allelic diversity (Table A5 and A6). There were no major changes in allelic richness or heterozygosity at the four circadian clock genes across years.

The circadian clock genes and neutral microsatellite markers were included in the parentage analysis, resulting in a total of fifteen genetic markers with an average of 27 alleles per marker (Table A5). The non-exclusion probability for a candidate parental pair with all fifteen genetic markers was estimated to be 9.3×10^{-18} in Cervus (Kalinowski *et al*. 2007).

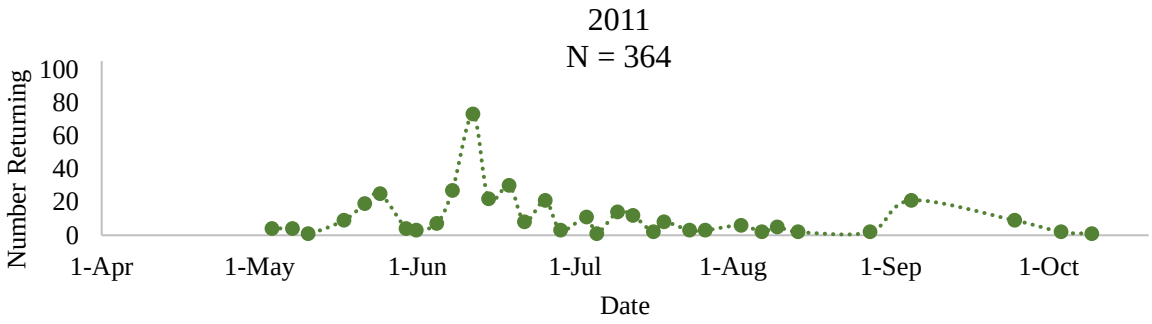
Supplemental Table 1. Test statistics for the measure of intra-annual genetic differentiation between early (released before July 1) and late (released August 1 and after) spring Chinook salmon above Fall Creek in 2011 and 2016. Significant F_{ST} estimates after correcting for multiple comparisons following the method of Benjamini and Hochberg (1995) are indicated in **bold-faced** type.

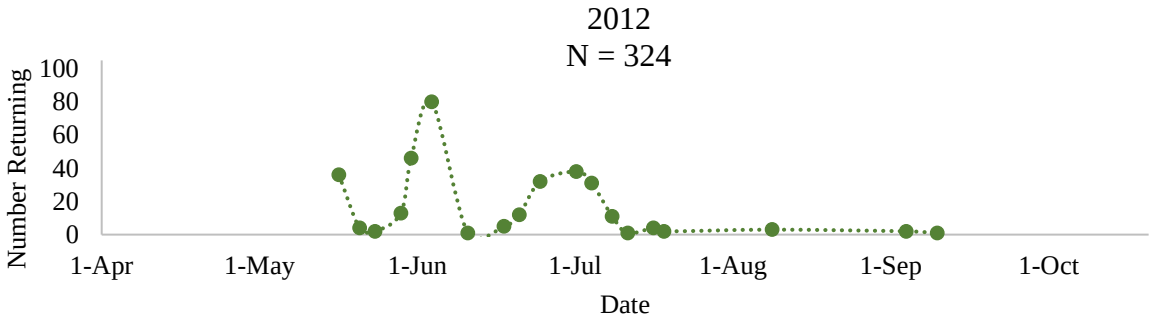
Marker	2011	2016
Ots515NWFWC	-0.002	-0.002
Omy1009UW	0.004	-0.001
OmyFbxw11	-0.004	-0.004
OtsClock1b	-0.001	0.004
Neutral microsatellite markers	0.001	0.002

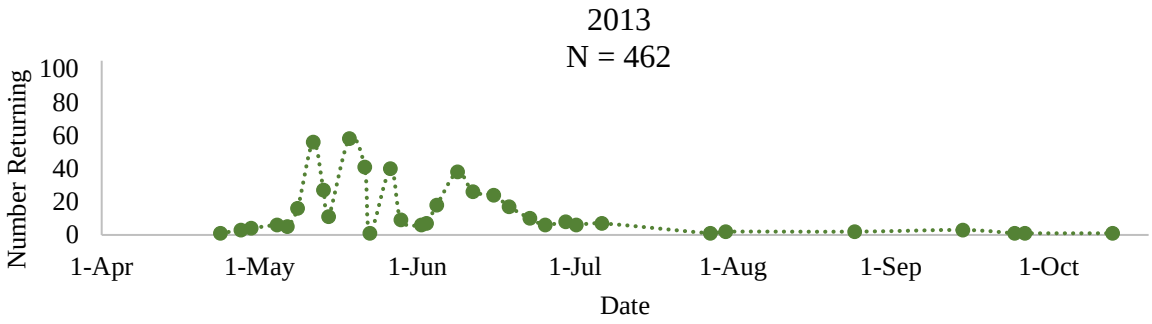
Supplemental Table 2. Test statistics for the measure of intra-annual genetic differentiation between male and female spring Chinook salmon released above Fall Creek Dam. Significant F_{ST} estimates after correcting for multiple comparisons following the method of Benjamini and Hochberg (1995) are indicated in **bold-faced** type.

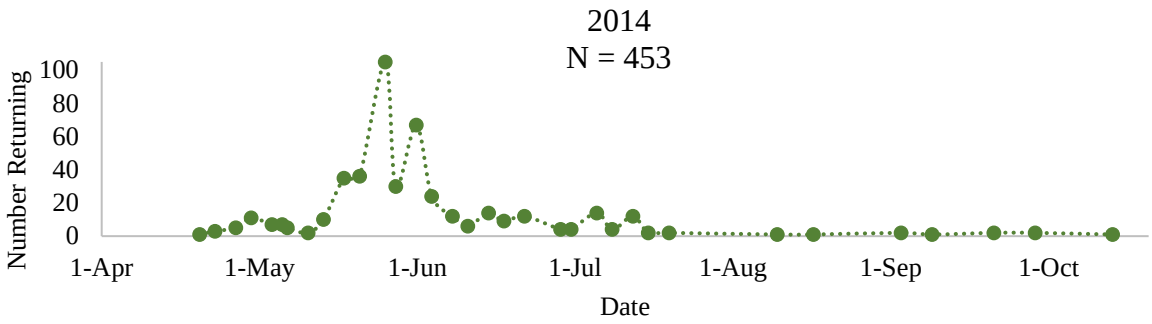
Marker	2016	2017	2018	2019	2020
Ots515NWFSC	0.000	-0.002	-0.002	0.002	0.001
Omy1009UW	**0.004**	0.001	0.007	**0.018**	0.001
OmyFbxw11	0.001	0.001	0.002	-0.004	0.001
OtsClock1b	-0.002	-0.002	-0.007	0.024	0.000
Neutral microsatellite markers	0.001	**0.002**	0.004	**0.009**	0.000

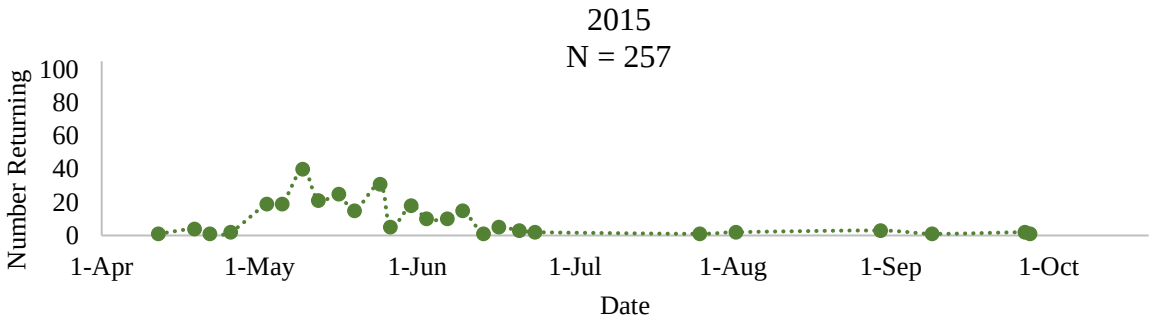
Appendix

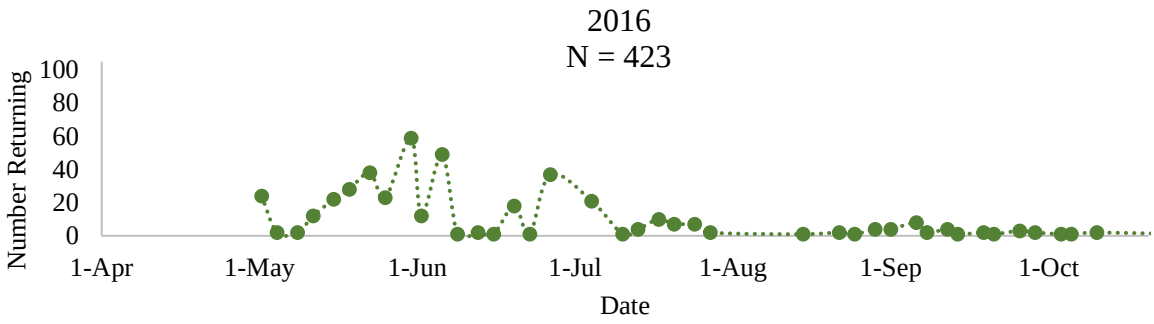

2011
N = 364
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date

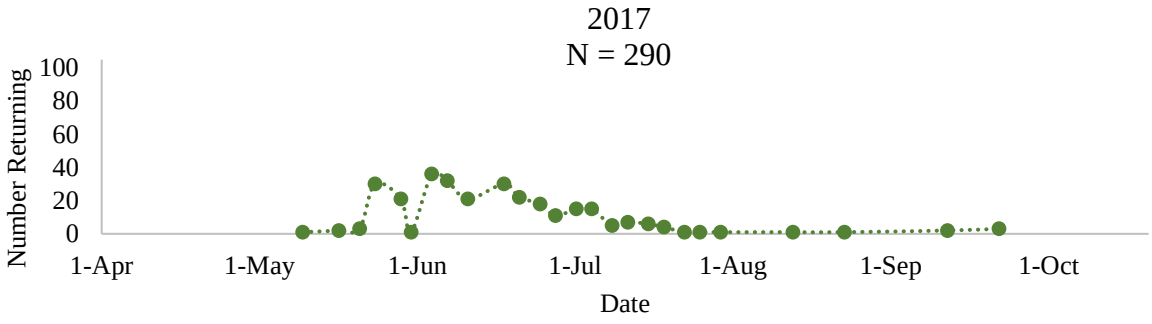

2012
N = 324
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date


2013
N = 462
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date


2014
N = 453
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date


2015
N = 257
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date


2016
N = 423
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date


2017
N = 290
Number Returning
100
80
60
40
20
0
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
Date

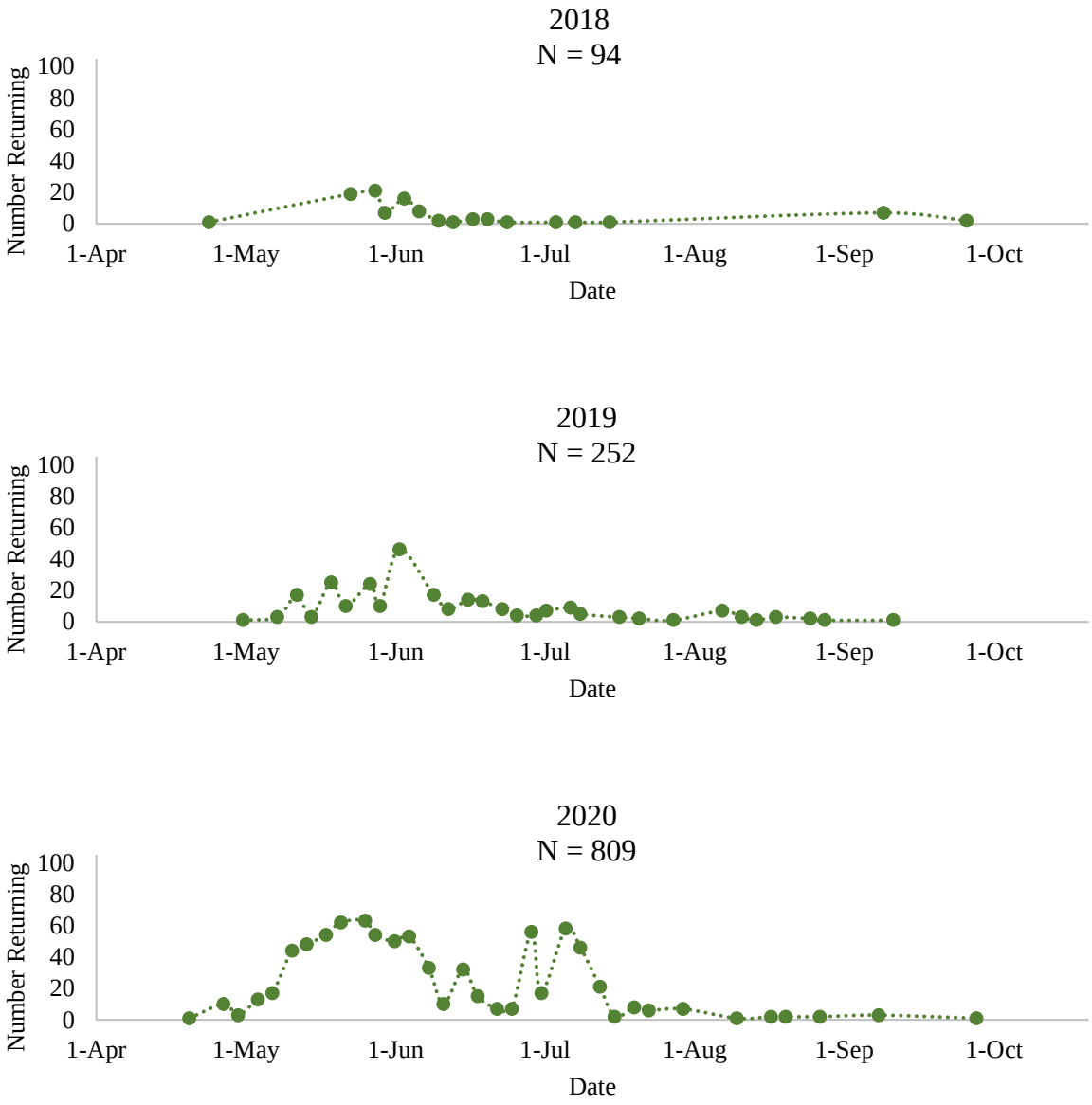


Figure A1. Release day of adult spring Chinook salmon above Fall Creek Dam in 2011 – 2020.

Table A1. Summary of release day, and number of female and male Chinook salmon reintroduced above Fall Creek Dam in 2011 – 2020 and included as candidate parents in our parentage analysis after filtering for missingness (genotyped at < 8 microsatellite markers) and removing duplicates.

Year	Release Day	Candidate Females	Candidate Males	Total Candidate Parents
2011	May 5	1	3	4

	May 9	2	2	4
	May 12	1	0	1
	May 19	2	7	9
	May 23	8	11	19
	May 26	13	12	25
	May 31	2	2	4
	Jun. 2	2	1	3
	Jun. 6	4	3	7
	Jun. 9	12	15	27
	Jun. 13	30	43	73
	Jun. 16	15	7	22
	Jun. 20	12	18	30
	Jun. 23	2	6	8
	Jun. 27	9	12	21
	Jun. 30	2	1	3
	Jul. 5	6	5	11
	Jul. 7	1	12	13
	Jul. 11	2	7	9
	Jul. 14	5	1	6
	Jul. 18	1	4	5
	Jul. 20	4	1	5
	Jul. 25	2	2	4
	Jul. 28	1	5	6
	Aug. 4	1	1	2
	Aug. 8	1	3	4
	Aug. 11	2	1	3
	Aug. 15	1	2	3
	Sep. 6	10	11	21
	Sep. 26	1	8	9
	Oct. 11	1	2	3
	Total	156	208	364
2012	May 17	19	17	36
	May 21	1	3	4
	May 24	1	1	2
	May 29	6	7	13
	May 31	19	27	46
	Jun. 4	44	36	80
	Jun. 11	1	0	1
	Jun. 18	2	3	5
	Jun. 21	5	7	12
	Jun. 25	13	19	32
	Jul. 2	22	16	38
	Jul. 5	6	25	31

	Jul. 9	6	5	11
	Jul. 12	1	0	1
	Jul. 17	0	4	4
	Jul. 19	1	1	2
	Aug. 9	0	3	3
	Sep. 4	1	1	2
	Sep. 10	0	1	1
	Total	148	176	324
2013	Apr. 25	1	0	1
	Apr. 29	2	1	3
	May 1	1	3	4
	May 6	4	2	6
	May 8	4	1	5
	May 10	6	10	16
	May 13	28	28	56
	May 15	11	16	27
	May 16	7	4	11
	May 20	25	33	58
	May 23	15	26	41
	May 24	1	0	1
	May 28	15	25	40
	May 30	5	4	9
	Jun. 30	3	3	6
	Jun. 4	2	5	7
	Jun. 6	7	11	18
	Jun. 10	7	31	38
	Jun. 13	9	17	26
	Jun. 17	10	14	24
	Jun. 20	4	13	17
	Jun. 24	3	7	10
	Jun. 27	2	4	6
	Jul. 1	4	4	8
	Jul. 3	3	3	6
	Jul. 8	0	7	7
	Jul. 29	1	0	1
	Aug. 1	0	2	2
	Aug. 26	1	1	2
	Sep. 16	3	0	3
	Sep. 26	1	0	1
	Sep. 28	1	0	1
	Oct. 15	0	1	1
	Total	186	276	462
2014	Apr. 21	1	0	1

	Apr. 24	2	1	3
	Apr. 28	3	2	5
	May 1	2	9	11
	May 5	3	4	7
	May 7	3	4	7
	May 8	4	1	5
	May 12	1	1	2
	May 15	6	4	10
	May 19	20	15	35
	May 22	20	16	36
	May 27	70	35	105
	May 29	17	13	30
	Jun. 2	38	29	67
	Jun. 5	12	12	24
	Jun. 9	6	6	12
	Jun. 12	2	4	6
	Jun. 16	5	9	14
	Jun. 19	5	4	9
	Jun. 23	7	5	12
	Jun. 30	4	0	4
	Jul. 2	2	2	4
	Jul. 7	5	9	14
	Jul. 10	2	2	4
	Jul. 14	7	5	12
	Jul. 17	0	2	2
	Jul. 21	2	0	2
	Aug. 11	1	0	1
	Aug. 18	1	0	1
	Sep. 4	2	0	2
	Sep. 10	0	1	1
	Sep. 22	0	2	2
	Sep. 30	0	2	2
	Oct. 15	0	1	1
	Total	253	200	453
2015	Apr. 13	1	0	1
	Apr. 20	1	3	4
	Apr. 23	0	1	1
	Apr. 27	0	2	2
	May 4	10	9	19
	May 7	7	12	19
	May 11	17	23	40
	May 14	9	12	21
	May 18	10	15	25

	May 21	3	12	15
	May 26	5	26	31
	May 28	1	4	5
	Jun. 1	7	11	18
	Jun. 4	1	9	10
	Jun. 8	0	10	10
	Jun. 11	6	9	15
	Jun. 15	0	1	1
	Jun. 18	3	2	5
	Jun. 22	2	1	3
	Jun. 25	2	0	2
	Jul. 27	1	0	1
	Aug. 3	1	1	2
	Aug. 31	0	3	3
	Sep. 10	0	1	1
	Sep. 28	0	2	2
	Sep. 29	1	0	1
	Total	88	169	257
2016	May 2	5	19	24
	May 5	0	2	2
	May 9	0	2	2
	May 12	4	8	12
	May 16	6	16	22
	May 19	12	16	28
	May 23	15	23	38
	May 26	7	16	23
	May 31	25	34	59
	Jun. 2	8	4	12
	Jun. 6	26	23	49
	Jun. 9	1	0	1
	Jun. 13	2	0	2
	Jun. 16	0	1	1
	Jun. 20	9	9	18
	Jun. 23	1	0	1
	Jun. 27	23	14	37
	Jul. 5	9	12	21
	Jul. 11	0	1	1
	Jul. 14	2	2	4
	Jul. 18	7	3	10
	Jul. 21	5	2	7
	Jul. 25	4	3	7
	Jul. 28	1	1	2
	Aug. 15	1	0	1

	Aug. 22	1	1	2
	Aug. 25	0	1	1
	Aug. 29	3	1	4
	Sep. 1	1	3	4
	Sep. 6	2	6	8
	Sep. 8	2	0	2
	Sep. 12	1	3	4
	Sep. 14	0	1	1
	Sep. 19	0	2	2
	Sep. 21	0	1	1
	Sep. 26	0	3	3
	Sep. 29	0	2	2
	Oct. 4	0	1	1
	Oct. 6	0	1	1
	Oct. 11	1	1	2
	Oct. 27	0	1	1
	Total	184	239	423
2017	May 11	0	1	1
	May 18	1	1	2
	May 22	1	2	3
	May 25	10	20	30
	May 30	7	14	21
	Jun. 1	0	1	1
	Jun. 5	14	22	36
	Jun. 8	13	19	32
	Jun. 12	10	11	21
	Jun. 19	16	14	30
	Jun. 22	8	14	22
	Jun. 26	9	9	18
	Jun. 29	4	7	11
	Jul. 3	8	7	15
	Jul. 6	8	7	15
	Jul. 10	4	1	5
	Jul. 13	3	4	7
	Jul. 17	2	4	6
	Jul. 20	1	3	4
	Jul. 24	1	0	1
	Jul. 27	0	1	1
	Jul. 31	0	1	1
	Aug. 14	1	0	1
	Aug. 24	0	1	1
	Sep. 13	0	2	2
	Sep. 23	0	3	3

	Total	121	169	290
2018	Apr. 25	0	1	1
	May 24	7	12	19
	May 29	8	13	21
	May 31	3	4	7
	Jun. 4	9	7	16
	Jun. 7	5	3	8
	Jun. 11	0	2	2
	Jun. 14	1	0	1
	Jun. 18	1	2	3
	Jun. 21	3	0	3
	Jun. 25	1	0	1
	Jul. 5	0	1	1
	Jul. 9	1	0	1
	Jul. 16	0	1	1
	Sep. 10	6	1	7
	Sep. 27	0	2	2
	Total	45	49	94
2019	May 2	1	0	1
	May 9	1	2	3
	May 13	6	11	17
	May 16	0	3	3
	May 20	9	16	25
	May 23	6	4	10
	May 28	6	18	24
	May 30	2	8	10
	Jun. 3	19	27	46
	Jun. 10	1	16	17
	Jun. 13	0	8	8
	Jun. 17	2	12	14
	Jun. 20	1	12	13
	Jun. 24	0	8	8
	Jun. 27	1	3	4
	Jul. 1	0	4	4
	Jul. 3	0	7	7
	Jul. 8	0	9	9
	Jul. 10	0	5	5
	Jul. 18	0	3	3
	Jul. 22	0	2	2
	Jul. 29	0	1	1
	Aug. 8	0	7	7
	Aug. 12	1	2	3
	Aug. 15	0	1	1

	Aug. 19	0	3	3
	Aug. 26	0	2	2
	Aug. 29	0	1	1
	Sep. 12	1	0	1
	Total	57	195	252
2020	Apr. 20	0	1	1
	Apr. 27	2	8	10
	Apr. 30	1	2	3
	May 4	7	6	13
	May 7	10	7	17
	May 11	27	17	44
	May 14	27	21	48
	May 18	28	26	54
	May 21	30	32	62
	May 26	36	27	63
	May 28	27	27	54
	Jun. 1	24	26	50
	Jun. 4	29	24	53
	Jun. 8	21	12	33
	Jun. 11	8	2	10
	Jun. 15	20	12	32
	Jun. 18	9	6	15
	Jun. 22	4	3	7
	Jun. 25	5	2	7
	Jun. 29	36	20	56
	Jul. 1	10	7	17
	Jul. 6	33	25	58
	Jul. 9	24	22	46
	Jul. 13	11	10	21
	Jul. 16	2	0	2
	Jul. 20	2	7	9
	Jul. 23	2	4	6
	Jul. 30	2	4	6
	Aug. 10	1	0	1
	Aug. 17	1	1	2
	Aug. 20	2	0	2
	Aug. 27	0	2	2
	Sep. 8	3	0	3
	Sep. 28	0	1	1
	Unknown	1	0	1
	Total	445	364	809

Table A2. Summary of variation and genotyping error rates of microsatellite markers for each year. PIC (Polymorphic information content), k (Number of alleles), NE-1P (probability of exclusion for first parent), NE-2P (probability of exclusion for second parent), NE-Pair (probability of exclusion for a parent pair), and HW (test of significance for a significant departure from Hardy Weinberg proportions) were calculated in GENEPOP (v4.7.5). ADO rate (allele dropout rate), and miscall rate were estimated in COLONY. Significance key: NS – not significant, * - significant at the 5% level, ** - significant at the 1% level, *** - significant at the 0.1% level.

Offspring Year	Marker	PIC	k	NE-1P	NE-2P	NE-Pair	HW	ADO Rate	Miscall Rate
2016	*Ots515NWFSC*	0.825	13	0.473	0.307	0.132	***	0.0000	0.0036
2016	*Omy1009UW*	0.947	56	0.185	0.102	0.018	**	0.0000	0.0064
2016	*OmyFbxw11*	0.153	3	0.987	0.919	0.850	NS	0.0000	0.0000
2016	*OtsClock1b*	0.379	3	0.874	0.808	0.712	NS	0.0000	0.0000
2016	*Ogo2*	0.848	13	0.432	0.274	0.111	***	0.0000	0.0000
2016	*Ogo4*	0.601	9	0.752	0.594	0.417	**	0.0000	0.0032
2016	*Ots201*	0.904	18	0.306	0.180	0.053	***	0.0000	0.0000
2016	*Ots211*	0.891	20	0.333	0.200	0.061	***	0.0000	0.0033
2016	*Ots212*	0.865	15	0.401	0.249	0.095	***	0.0000	0.0032
2016	*Ots215*	0.929	28	0.237	0.135	0.031	***	0.0000	0.0024
2016	*OtsG249*	0.931	27	0.231	0.131	0.029	***	0.0000	0.0059
2016	*OtsG311*	0.948	38	0.181	0.100	0.017	***	0.0000	0.0158
2016	*OtsG409*	0.939	40	0.210	0.118	0.024	***	0.0000	0.0063
2016	*OtsG474*	0.714	10	0.638	0.456	0.259	*	0.0000	0.0000
2016	*Ssa408*	0.929	25	0.240	0.136	0.031	***	0.0000	0.0032
2017	*Ots515NWFSC*	0.866	14	0.394	0.244	0.090	***	0.0000	0.0033
2017	*Omy1009UW*	0.949	50	0.180	0.099	0.017	***	0.0000	0.0091
2017	*OmyFbxw11*	0.187	3	0.980	0.902	0.824	NS	0.0000	0.0023
2017	*OtsClock1b*	0.375	2	0.875	0.813	0.719	NS	0.0000	0.0000
2017	*Ogo2*	0.797	12	0.525	0.352	0.172	***	0.0000	0.0091
2017	*Ogo4*	0.545	8	0.800	0.649	0.479	NS	0.0000	0.0039
2017	*Ots201*	0.866	18	0.389	0.241	0.860	***	0.0000	0.0081
2017	*Ots211*	0.894	19	0.329	0.197	0.061	***	0.0000	0.0082
2017	*Ots212*	0.879	16	0.368	0.225	0.079	***	0.0000	0.0032
2017	*Ots215*	0.937	27	0.214	0.120	0.025	***	0.0000	0.0015
2017	*OtsG249*	0.934	28	0.225	0.127	0.027	***	0.0000	0.0088
2017	*OtsG311*	0.949	41	0.179	0.098	0.017	***	0.0000	0.0201
2017	*OtsG409*	0.947	35	0.185	0.105	0.180	***	0.0000	0.0092
2017	*OtsG474*	0.754	11	0.594	0.416	0.231	***	0.0000	0.0021
2017	*Ssa408*	0.927	24	0.243	0.139	0.032	***	0.0000	0.0066
2018	*Ots515NWFSC*	0.829	12	0.467	0.302	0.131	NS	0.0000	0.0000
2018	*Omy1009UW*	0.952	39	0.169	0.093	0.015	***	0.0000	0.0033
2018	*OmyFbxw11*	0.127	3	0.991	0.933	0.875	NS	0.0000	0.0049

2018	*OtsClock1b*	0.375	2	0.875	0.813	0.719	NS	0.0000	0.0002
2018	*Ogo2*	0.799	10	0.522	0.349	0.171	*	0.0000	0.0000
2018	*Ogo4*	0.611	7	0.744	0.584	0.404	NS	0.0000	0.0025
2018	*Ots201*	0.899	17	0.319	0.189	0.058	***	0.0000	0.0000
2018	*Ots211*	0.858	16	0.403	0.251	0.090	NS	0.0000	0.0000
2018	*Ots212*	0.866	12	0.396	0.245	0.093	NS	0.0000	0.0000
2018	*Ots215*	0.925	20	0.251	0.144	0.035	**	0.0000	0.0066
2018	*OtsG249*	0.927	23	0.243	0.138	0.032	***	0.0000	0.0085
2018	*OtsG311*	0.947	34	0.185	0.102	0.018	***	0.0000	0.0122
2018	*OtsG409*	0.931	28	0.232	0.132	0.029	**	0.0000	0.0000
2018	*OtsG474*	0.733	8	0.625	0.447	0.265	***	0.0000	0.0000
2018	*Ssa408*	0.907	20	0.297	0.175	0.049	**	0.0000	0.0039
2019	*Ots515NWFSC*	0.812	12	0.496	0.327	0.149	***	0.0000	0.0000
2019	*Omy1009UW*	0.948	44	0.182	0.100	0.018	***	0.0000	0.0033
2019	*OmyFbxw11*	0.136	3	0.990	0.929	0.867	NS	0.0000	0.0000
2019	*OtsClock1b*	0.378	3	0.876	0.809	0.713	*	0.0000	0.0000
2019	*Ogo2*	0.817	11	0.489	0.321	0.146	NS	0.0000	0.0018
2019	*Ogo4*	0.614	11	0.740	0.578	0.396	***	0.0000	0.0056
2019	*Ots201*	0.880	18	0.356	0.216	0.069	***	0.0000	0.0015
2019	*Ots211*	0.881	18	0.355	0.216	0.070	*	0.0000	0.0000
2019	*Ots212*	0.879	16	0.369	0.225	0.079	NS	0.0000	0.0029
2019	*Ots215*	0.936	23	0.218	0.122	0.026	***	0.0000	0.0032
2019	*OtsG249*	0.928	28	0.239	0.136	0.031	NS	0.0000	0.0113
2019	*OtsG311*	0.952	40	0.170	0.093	0.015	***	0.0000	0.0094
2019	*OtsG409*	0.947	37	0.186	0.103	0.018	***	0.0000	0.0057
2019	*OtsG474*	0.718	10	0.634	0.452	0.255	NS	0.0000	0.0000
2019	*Ssa408*	0.924	23	0.252	0.144	0.035	**	0.0000	0.0029
2020	*Ots515NWFSC*	0.820	12	0.481	0.313	0.136	***	0.0000	0.0013
2020	*Omy1009UW*	0.938	47	0.211	0.119	0.024	***	0.0000	0.0028
2020	*OmyFbxw11*	0.164	3	0.985	0.913	0.839	*	0.0000	0.0001
2020	*OtsClock1b*	0.367	3	0.883	0.816	0.723	NS	0.0000	0.0000
2020	*Ogo2*	0.843	12	0.441	0.281	0.116	***	0.0000	0.0000
2020	*Ogo4*	0.581	9	0.769	0.615	0.441	***	0.0000	0.0017
2020	*Ots201*	0.903	17	0.309	0.183	0.054	***	0.0000	0.0000
2020	*Ots211*	0.898	19	0.319	0.190	0.057	***	0.0000	0.0015
2020	*Ots212*	0.856	13	0.419	0.263	0.105	***	0.0000	0.0001
2020	*Ots215*	0.929	28	0.238	0.135	0.031	***	0.0000	0.0038
2020	*OtsG249*	0.925	28	0.250	0.143	0.034	***	0.0000	0.0089
2020	*OtsG311*	0.946	36	0.188	0.104	0.019	***	0.0000	0.0035
2020	*OtsG409*	0.934	35	0.222	0.125	0.027	***	0.0000	0.0037
2020	*OtsG474*	0.735	10	0.614	0.434	0.242	***	0.0000	0.0000
2020	*Ssa408*	0.927	25	0.244	0.139	0.032	***	0.0000	0.0005

Table A3. Summary of spring Chinook salmon included in the genetic parentage and/or circadian clock gene analysis. Early refers to adult salmon that were released above the dam prior to July 1, Middle refers to salmon that were released between July 1 – 31, and Late refers to salmon that were released above the dam on August 1 or after that date. M designates males while F designates females.

Year	Total	Early			Middle			Late		
		All	M	F	All	M	F	All	M	F
2011	364	260	143	117	54	32	22	50	33	17
2012	324	231	120	111	87	51	36	6	5	1
2013	462	430	258	172	22	14	8	10	4	6
2014	453	405	174	231	38	20	18	10	6	4
2015	257	247	162	85	1	0	1	9	7	2
2016	423	331	187	144	52	24	28	40	28	12
2017	290	228	135	93	55	28	27	7	6	1
2018	94	82	44	38	3	1	2	9	3	6
2019	252	203	148	55	31	31	0	18	16	2
2020	808	632	281	351	165	79	86	11	4	7

Table A4. Date of first release and median release day for male and female spring Chinook salmon above Fall Creek Dam in 2011 – 2020.

Year	Day of first release	Median release day	Males		Females	
			Day of first release	Median release day	Day of first release	Median release day
2011	May 5	June 24	May 5	June 16	May 5	June 16
2012	May 17	June 3	May 17	June 4	May 17	June 4
2013	April 25	May 26	April 29	May 28	April 25	May 23
2014	April 21	May 25	April 24	May 29	April 21	May 27
2015	April 13	May 16	April 20	May 21	April 13	May 14
2016	May 2	June 2	May 5	May 31	May 2	June 6
2017	May 11	June 12	May 11	June 12	May 18	June 19
2018	April 25	May 31	April 25	May 31	May 24	June 4
2019	May 2	June 3	May 9	June 3	May 2	May 28

2020	April 20	June 1	April 20	June 1	April 27	June 4

Table A5. Descriptive statistics for the 15 genetic markers used in the parentage analysis. K (number of alleles), PIC (polymorphic information content), NE-1P (probability of exclusion for first parent), NE-2P (probability of exclusion for second parent), and NE-Pair (probability of exclusion for a parent pair).

Marker	**k**	**n**	**Ho**	**He**	**PIC**	**NE-1p**	**NE-2p**	**NE-pp**
Circadian clock genes								
Ots515NWFSC	16	3706	0.854	0.849	0.854	0.458	0.295	0.124
Omy1009UW	77	3710	0.963	0.959	0.963	0.152	0.083	0.012
OmyFbxw11	4	3727	0.145	0.147	0.145	0.989	0.925	0.861
OtsClock1b	3	2723	0.509	0.501	0.509	0.874	0.809	0.713
Neutral microsatellite markers								
Ogo2	14	3709	0.849	0.847	0.849	0.465	0.301	0.13
Ogo4	14	3728	0.671	0.673	0.671	0.738	0.576	0.394
Ots201	21	3727	0.905	0.911	0.905	0.306	0.18	0.052
Ots211	23	3708	0.910	0.909	0.91	0.308	0.182	0.052
Ots212	22	3725	0.900	0.887	0.9	0.373	0.228	0.081
Ots215	34	3716	0.944	0.943	0.944	0.207	0.116	0.023
OtsG249	32	3720	0.943	0.94	0.943	0.215	0.121	0.025
OtsG311	48	3712	0.949	0.96	0.949	0.149	0.081	0.012
OtsG409	54	3705	0.944	0.948	0.944	0.189	0.105	0.019
OtsG474	11	3719	0.767	0.77	0.767	0.608	0.428	0.236
Ssa408	29	3714	0.942	0.935	0.942	0.232	0.131	0.029

Table A6. Observed and expected heterozygosity for the 15 genetic markers used in the circadian clock gene and parentage analysis. H_O = observed heterozygosity and H_E = expected heterozygosity.

	2011		**2012**		**2013**		**2014**		**2015**	
Marker	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**
Circadian clock genes										
Ots515NWFSC	0.832	0.810	0.858	0.847	0.855	0.846	0.876	0.850	0.891	0.841
Omy1009UW	0.970	0.950	0.969	0.953	0.974	0.957	0.980	0.962	0.941	0.948
OmyFbxw11	0.159	0.154	0.096	0.092	0.145	0.138	0.088	0.089	0.167	0.170
OtsClock1b	0.522	0.507	0.461	0.507	0.448	0.502	0.571	0.501	0.490	0.502
Neutral microsatellite markers										
Ogo2	0.892	0.853	0.758	0.816	0.817	0.840	0.807	0.819	0.874	0.846
Ogo4	0.684	0.699	0.651	0.700	0.667	0.669	0.722	0.669	0.662	0.700
Ots201	0.909	0.908	0.932	0.912	0.885	0.892	0.921	0.900	0.957	0.916
Ots211	0.904	0.895	0.857	0.905	0.909	0.902	0.894	0.904	0.906	0.910

Ots212	0.912	0.892	0.944	0.882	0.909	0.874	0.892	0.876	0.887	0.875
Ots215	0.948	0.935	0.960	0.928	0.932	0.934	0.958	0.938	0.945	0.935
OtsG249	0.914	0.921	0.969	0.940	0.944	0.938	0.958	0.936	0.910	0.930
OtsG311	0.953	0.947	0.978	0.956	0.926	0.959	0.969	0.958	0.953	0.948
OtsG409	0.959	0.939	0.931	0.938	0.908	0.946	0.947	0.947	0.957	0.941
OtsG474	0.726	0.755	0.790	0.742	0.770	0.771	0.795	0.798	0.770	0.740
Ssa408	0.959	0.926	0.910	0.920	0.941	0.931	0.938	0.927	0.957	0.927

	2016		**2017**		**2018**		**2019**		**2020**	
Marker	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**	**H_O**	**H_E**
Circadian clock genes										
Ots515NWFSC	0.886	0.841	0.893	0.878	0.796	0.846	0.819	0.831	0.825	0.837
Omy1009UW	0.943	0.949	0.955	0.951	0.957	0.954	0.964	0.950	0.964	0.941
OmyFbxw11	0.152	0.160	0.186	0.200	0.117	0.131	0.151	0.142	0.164	0.172
OtsClock1b	0.497	0.502	0.576	0.499	0.581	0.500	0.568	0.497	0.483	0.484
Neutral microsatellite markers										
Ogo2	0.863	0.863	0.859	0.820	0.840	0.822	0.865	0.836	0.886	0.858
Ogo4	0.660	0.661	0.631	0.606	0.670	0.669	0.706	0.670	0.660	0.644
Ots201	0.936	0.911	0.824	0.877	0.872	0.906	0.877	0.889	0.905	0.909
Ots211	0.912	0.899	0.948	0.902	0.915	0.869	0.937	0.890	0.922	0.905
Ots212	0.915	0.877	0.855	0.889	0.883	0.879	0.893	0.889	0.895	0.870
Ots215	0.929	0.933	0.931	0.940	0.957	0.929	0.944	0.940	0.944	0.933
OtsG249	0.941	0.935	0.910	0.937	0.936	0.931	0.956	0.932	0.957	0.929
OtsG311	0.953	0.950	0.955	0.951	0.947	0.949	0.952	0.954	0.933	0.948
OtsG409	0.938	0.942	0.962	0.949	0.968	0.934	0.980	0.949	0.940	0.938
OtsG474	0.811	0.745	0.807	0.785	0.755	0.770	0.798	0.748	0.711	0.766
Ssa408	0.938	0.933	0.959	0.931	0.926	0.913	0.925	0.928	0.948	0.931