Genetic Evaluation of Juvenile Chinook Salmon in the Entiat River

FINAL REPORT

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Summary

Differentiation between runs of subyearling Chinook rearing within the Entiat River during summer months has not been possible despite management needs and study objectives. We used genetic run assignments of 1,013 subyearling Chinook out-migrants from two years to determine the accuracy of run assignments made using catch-frequency patterns. Additional genetic run assignments were performed on 542 juveniles collected during summer and winter rearing periods to assess spatial and temporal distribution by run type. Finally, all samples were analyzed for the potential of hybridization between run types. Genetic assignment to run had an associated assignment probability of 1.0 for both spring and summer Chinook and run assignments using catch-frequency patterns were on average 68% correct when compared to genotype based assignments. During most outmigration months, a significant difference in median fish length was found between juveniles of each run, and length differences increased through the outmigration period. The proportion of subyearling spring Chinook encountered during summer rearing periods was positively correlated with upstream distance from the mouth of the Entiat River. We detected hybridization between spring and summer Chinook in both rotary screw trap (3.2%) and mark recapture sampling (1.5%). Hybrid Chinook were found throughout the summer and winter rearing areas and throughout the subyearling emigration period ranging in size from 56 – 106 mm fork-length. Given the variable outmigration timing of ESA-listed spring Chinook and the presence of hybridization between runs within the Entiat River, we recommend additional genetic analysis to explore the possibility of increasing the accuracy of run assignments through the incorporation of length data and assessing the amount of annual variability associated with hybrid juveniles.

Introduction

Accurate assessments of population performance (smolt-to-adult return (SAR), smolt-per-redd, emigrant-per-red, etc.) serve as key underpinnings to evaluate the recovery of ESA-listed salmonids. Assessments can become complicated when targeted species exhibit a high degree of plasticity in life-history expression or when phenotypically similar runs of the same

species exist. Complications of this type increase uncertainty surrounding the management of the species but also present opportunities for adaptive learning. Such an opportunity has presented itself to monitoring programs within a tributary to the Upper Columbia River where both ESA-listed and non-listed runs of Chinook Salmon (hereafter; "Chinook") show considerable overlap in juvenile life-history expression.

Two distinct lineages of Chinook salmon (*Oncorhynchus tshawytscha*) exist within the Upper Columbia and Snake River Basin: spring-run and summer/fall-run (Waples et al. 2004). These two lineages are highly genetically divergent from one another, which reflects historical isolation and patterns of recolonization. The two lineages differ in many life history traits, notably juvenile rearing and migratory behavior. Spring-run ('stream-type') Chinook salmon typically spend one full year rearing in their natal stream before emigrating downstream to the ocean. Spring Chinook generally return to their natal stream to spawn earlier in the year and typically spawn in headwater reaches further upstream with colder water temperatures (Healey 1991). Summer/fall-run ('ocean type') Chinook salmon typically rear in their natal streams for only a few months before emigrating downstream to the ocean for rearing. Summer/fall-run fish typically return to natal streams later in the year and generally spawn further downstream in warmer waters (Healy 1991). Like many salmonids, Chinook salmon life history is not fixed, and migration timing, maturation timing, spawning location, etc., varies within lineages and within different watersheds. For example, summer Chinook Salmon in the upper Columbia River Basin can exhibit one of three distinct freshwater life histories; (age-0) ocean-reared juveniles that emigrate shortly after egg emergence and overwinter in the ocean, (age-1) stream-reared juveniles that remain to overwinter in their natal stream prior to emigration to the ocean in the spring, and (age-1) reservoir-reared juveniles that emigrate from their natal stream following egg emergence but remain to overwinter in a reservoir prior to ocean entry (Healy 1991). Some river systems support both spring- and summer/fall-run Chinook, whereas others support predominantly one lineage. Anthropogenic activities such as translocations of adult Chinook and hatchery supplementation have altered the natural distribution of the different lineages throughout the Columbia River Basin.

The Entiat River, a tributary to the Columbia River on the eastern slope of the Cascade Mountains in Washington, supports populations of both ESA-listed spring and non-listed

summer Chinook. The US Fish & Wildlife Service (USFWS) Mid-Columbia Fish and Wildlife Conservation Office (MCFWCO) has monitored salmonid populations within the Entiat River since 1994. As operator of the Entiat National Fish Hatchery (NFH), the USFWS is especially interested in understanding the impacts of hatchery production upon naturally produced salmonids. Annual spawning ground surveys have documented redd counts, spawn timing, and distribution throughout the Entiat River basin. Juvenile population monitoring has predominantly been performed through the operation of a rotary-screw trap (RST) in the lower Entiat River since 2003. RST monitoring efforts have provided estimates of productivity (egg-to-emigrant survival, recruits-per-spawner, etc.), out-migrant abundance, and smolt-to-adult return (SAR) rates for naturally produced spring Chinook.

The 2008 Federal Columbia River Power System Biological Opinion identified the Entiat River basin as an Intensively Monitored Watershed (IMW; RPA 57.1) and in 2010 a formal IMW study was implemented by the Integrated Status and Effectiveness Monitoring Program (ISEMP; BPA project number 2003-017-00). The Entiat IMW study is a watershed-scale restoration effort combined with effectiveness monitoring intended to maximize detection of fish responses to restoration efforts. The role of the MCFWCO in support of the IMW study was to enhance our existing adult and juvenile monitoring efforts through the development and implementation of mark-recapture (MR) surveys to support seasonal, life-stage specific assessments (abundance, growth, survival, and movement) of juvenile spring Chinook and steelhead (*O. mykiss*).

In the Entiat River, differentiation between yearling and subyearling Chinook encountered at the RST in the spring and early summer months is based on differences in body length and all yearlings are classified as spring Chinook. To partition between subyearling run types encountered later in the season, daily catch rates are evaluated to determine the date at which the catch-frequency is lowest (hereafter; “date-nadir”) between runs. All subyearling Chinook captured after the date-nadir are considered spring Chinook while preceding captures are classified as summer Chinook (for further discussion see Grote and Desgroseillier 2016). The date-nadir method has allowed for differentiation between run types of subyearling Chinook but lacks the flexibility to account for expected variation in outmigration timing. Similarly, the

inability to classify subyearling Chinook run type during summer period MR surveys has limited the Entiat IMW study's assessments of abundance, growth, survival, and movement patterns.

Genetic assignment tools provide an alternative method for classifying fish of unknown origin to lineage, population, or run type. The two major run types of Chinook in the upper Columbia River (spring and summer/fall) are easily distinguishable based on every class of genetic marker type examined to date (e.g., Waples et al. 2004; Seeb et al. 2007), including the current single nucleotide polymorphism (SNP) baseline (Matala et al. 2011). Nonetheless, prior to applying genetic assignment tools in a new system, it is prudent to 1) ensure that populations in that system are represented in the baseline, and 2) use simulations and /or fish of known origin to test assignment accuracy in that particular system (e.g., Smith et al. 2005). Our objectives for this project were to:

(i) add Entiat River spring Chinook to published versions of the current SNP baseline and assess the utility of genetic assignments to accurately differentiate between run types of juvenile Chinook.

(ii) test the appropriateness of the date-nadir method currently utilized for assigning run to juvenile Chinook at the RST using genetic assignment data.

(iii) assess the composition of rearing juvenile Chinook encountered during MR surveys by run type.

(iv) assess the likelihood of accurately identifying hybridization between spring and summer run Chinook.

Methods

Sample Collection

Tissue samples from adult summer Chinook were collected from a subset of Entiat NFH broodstock in 2011 (n=31), 2012 (n=31), and 2013 (n=32). Samples from juvenile Chinook were collected both at the RST and from a number of different rearing locations within the Entiat River over the span of several years (Figure 1). Captured juveniles were anesthetized in a water bath with a measured amount of tricaine (MS-222) and buffered with sodium bicarbonate. All

individuals were measured to the nearest millimeter (mm) of fork-length, weighed to the nearest tenth of a gram and assessed for the presence of a mark from prior capture. Unmarked individuals ≥ 50 mm fork-length were marked with Passive Integrated Transponder (PIT) tags. PIT tagging of juvenile fish followed guidelines set forth by Pacific State Marine Fisheries Commission PIT Tag Information System (PTAGIS). Fish were tagged using a disinfected hollow needle to insert the PIT tag into the abdominal cavity. Genetic material was collected from a subset of captured individuals ≥ 50 mm fork-length (sample rate of 1:10) by taking a small clip of tissue from the ventral fin which was stored in 95% non-denatured ethanol until DNA extraction. After handling, all individuals were allowed a full recovery prior to release near their respective origin of capture.

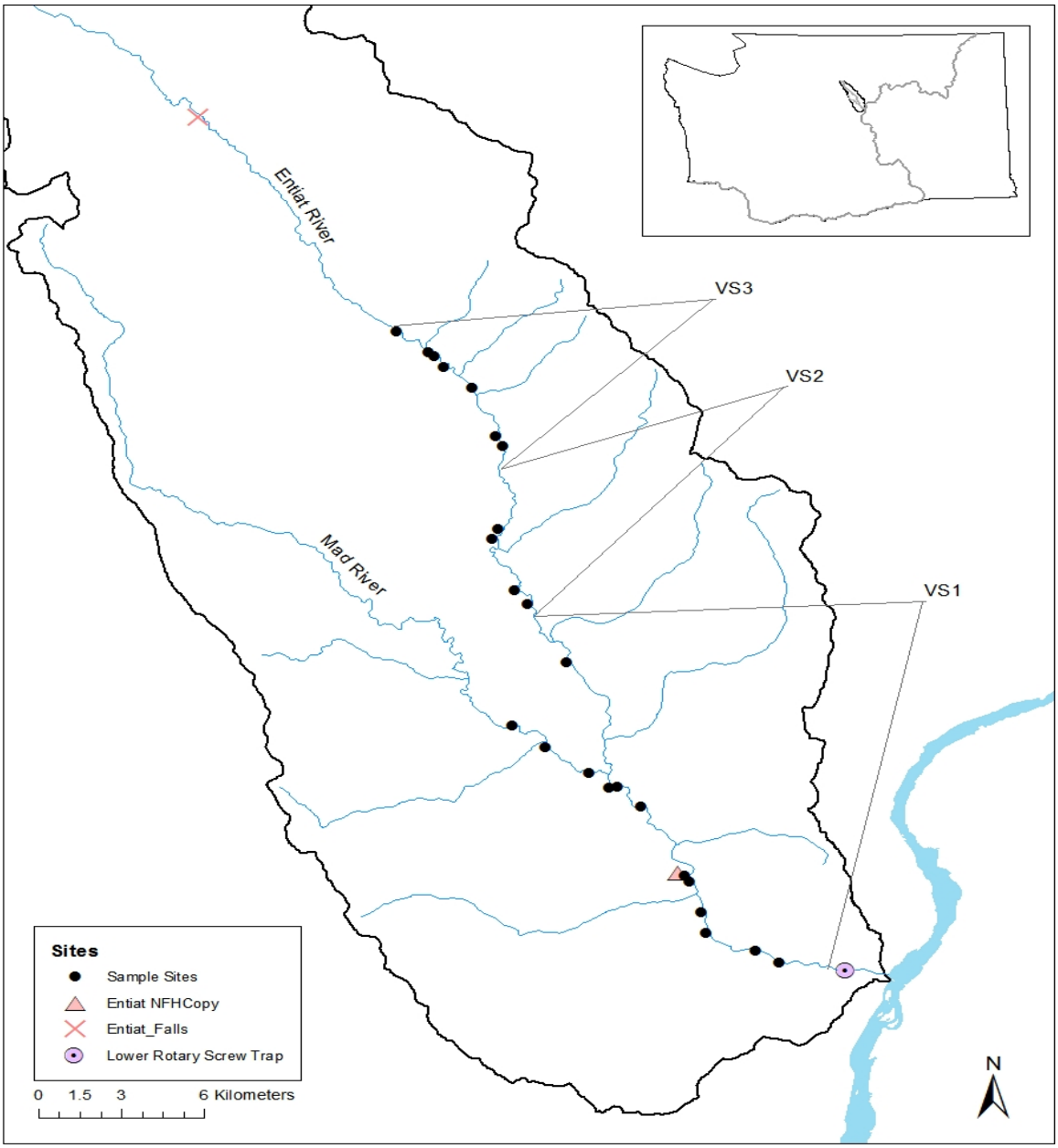


Figure 1. Study site, the Entiat River, WA. Individual geomorphic valley segments (VS) denoted by blue lines.

In 2009, tissue samples were collected from two sampling intervals at the RST; July 8–August 24 (n=97), and September 27–November 16 (n=82; Figure 2). In 2011, RST tissue samples were collected continuously July 18–November 19 (n=834; Figure 3).

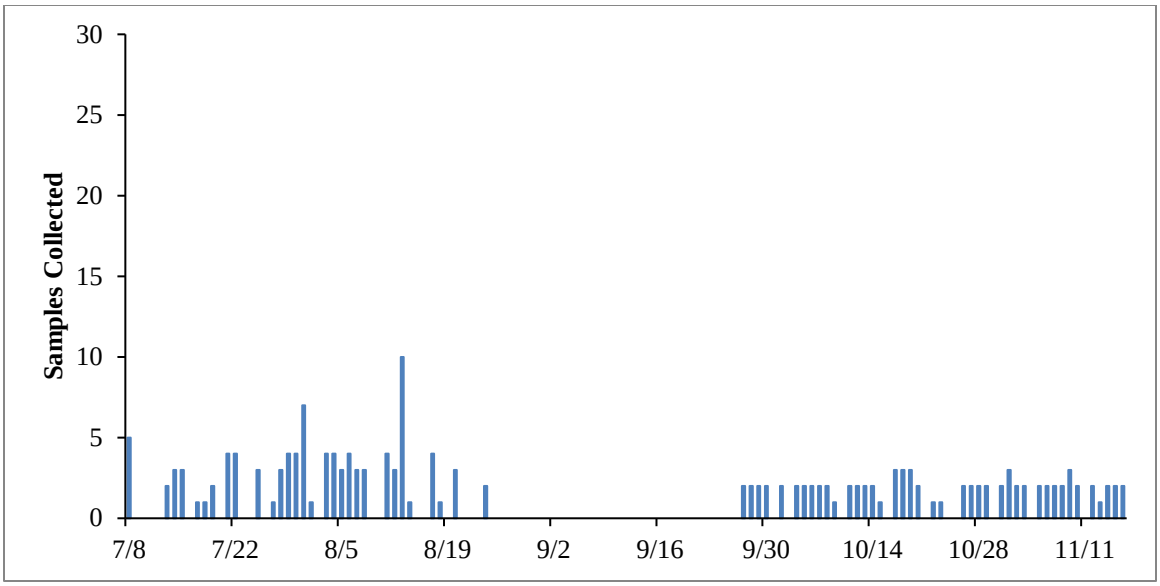


Figure 2. Tissue samples collected from subyearling Chinook at the rotary-screw trap during 2009, no samples were collected August 25–September 26.

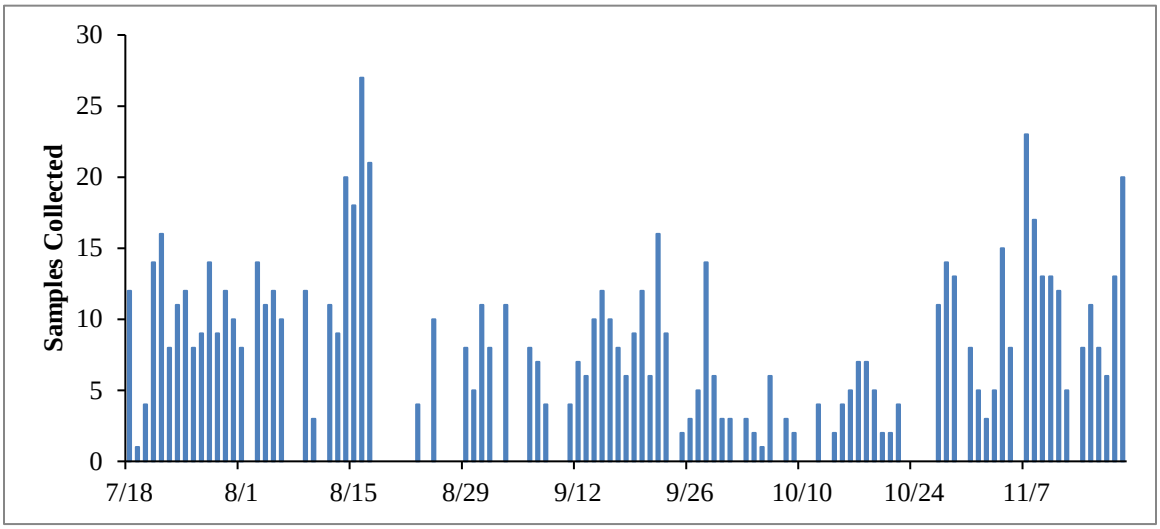


Figure 3. Tissue samples collected from subyearling Chinook at the rotary-screw trap during 2011.

Tissue samples were collected from 542 individuals captured during MR sampling in the summer (August) of 2012 (n=144), 2013 (n=145), and 2014 (n=116; Figure 4) and the winter (March) of 2013 (n=56) and 2014 (n=81; Figure 5). MR sample sites were grouped into three distinct geomorphic valley segments in the Entiat River and one in the lower Mad River (Figure 1).

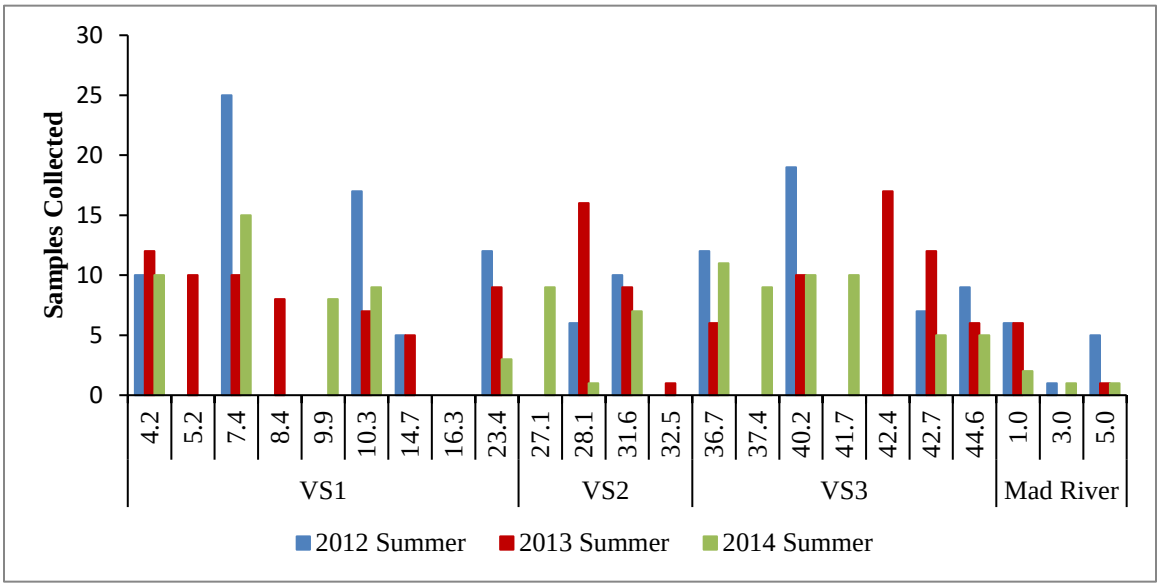


Figure 4. Tissue samples collected from subyearling Chinook during summer MR sampling 2012-2014 by valley segment (VS) and river kilometer.

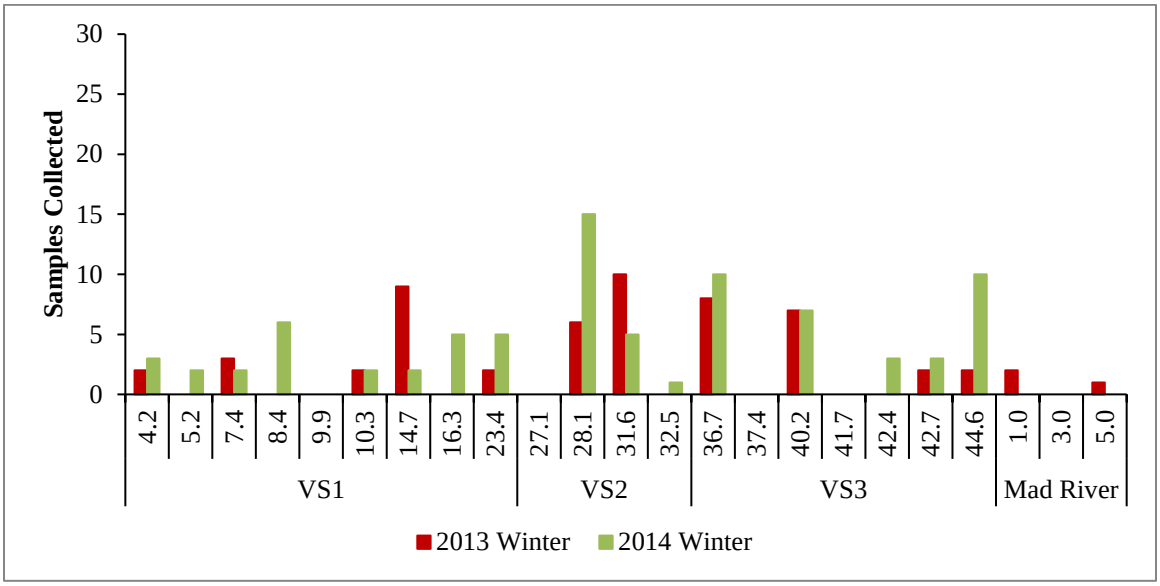


Figure 5. Tissue samples collected from yearling Chinook during winter period MR sampling 2013 and 2014 by valley segment (VS) and river kilometer.

Lab and Genetic Baseline Analysis

DNA was extracted from all fin clips using Qiagen DNeasy 96 blood and tissue extraction kits (QIAGEN Inc.) following the manufacturer's protocol. We genotyped each individual at 96 single nucleotide polymorphism (SNP) markers. We utilized the same markers used by Matala et al. (2011) to develop the Columbia River SNP baseline for Chinook salmon. Thus, allowing comparisons between this study and previously generated genotypic data. All samples were pre-amplified at the 96 loci following the protocol detailed by Smith et al. (2011) to reduce genotyping failure and error rates. The resulting pre-amplified product was then diluted 1:20 with deionized water before further processing. The 96 SNP makers were processed using TaqMan® SNP Genotyping Assays (Life Technology, Inc.) on a Fluidigm® EP-1™ System with 96.96 Dynamic Arrays following the manufacturer's protocol (Fluidigm Corporation). Multi-locus genotypes of each individual fish were visualized and scored using Fluidigm® SNP Genotyping Analysis software and were confirmed by two researchers.

Analyses for this project were conducted using an existing baseline dataset (Matala et al. 2011) which has been augmented over time by various agency laboratories working on Columbia Basin Chinook. Baseline data were downloaded from the FishGen.net website (www.fishgen.net). We compiled available genotype data from 40 Columbia River spring and summer/fall Chinook collections and added the Entiat NFH summer Chinook samples to the baseline (see Appendix Table 1 for a list of the baseline populations). We tested each baseline population for departures from Hardy-Weinberg equilibrium (HWE) using exact tests implemented in the program GENEPOP v4.1 (Raymond and Rousset 1995). Significance values were adjusted for multiple tests using sequential Bonferroni corrections (Rice 1989). We examined genetic variation among baseline collections in two ways. First we estimated the level of genetic variation among each population pair (pairwise F_{ST}) using the program GENEPOP. We also conducted a discriminant analysis of principal components (DAPC) for our baseline dataset using the *adegenet* package (Jombart 2008) for the R statistical environment. DAPC is similar to principal components analysis (PCA) but unlike PCA, which maximizes the total variation in the dataset, DAPC maximizes the variation among different groups or clusters and minimizes variation within groups (Jombart et al. 2010).

We performed leave-one-out assignment tests of our baseline dataset using methods described by Rannala and Mountain (1997) implemented in the program ONCOR (Kalinowski et al. 2007) in order to assess our accuracy for assigning fish to their population and reporting group of origin (hereafter; "run"). In this analysis, each individual in the baseline was removed and treated as an unknown origin individual. Allele frequencies were then recalculated without that individual and the unknown individual was assigned to its most likely population and run. Population and run assignments were then compared to collection locations to determine baseline assignment accuracy. In this case, populations were organized into three runs; Lower Columbia River, spring, and summer/fall. A list of populations within each run can be found in Appendix Table 1. To further assess baseline run assignment accuracy, we removed the Entiat River spring and summer Chinook collections from the baseline dataset (resulting in a reduced baseline with 39 populations) and assigned individuals from these two collections to their most likely run. Assignment probabilities for these individuals were calculated following the methods of Rannala and Mountain (1997).

Genetic Assignment of Run Classification

We used the baseline described above to assign juvenile Chinook collected at the RST and during mark-recapture surveys to run type. We did not assign individuals to their population of origin due to the low expected success rate based on results of the leave-one-out tests (see below). Genetic assignments and the associated probability calculations were conducted using ONCOR. Genetic run assignments were then compared to assignments made using the date-nadir method at the RST. We first assessed the number of correct assignments by year for both spring and summer Chinook. Next we determined the proportion of spring and summer Chinook based on genetic assignment by month (July-November) for the 2009 and 2011 collections. We then tested for differences in the monthly proportion of spring Chinook captured at the RST between years before testing for correlation between capture month and proportion of spring Chinook. For juvenile Chinook encountered during MR sampling, we first assessed between year variability in run assignments at locations where sampling was consistently performed during each occasion and then tested for correlation between run proportion and river kilometer (RKM) for each capture season. For both RST and MR individuals, we assessed differences in fork-length between run types. For RST fish we assessed monthly differences in fork-length while

differences were assessed by capture period (i.e., winter vs. summer) for MR fish. Finally, we tested for correlation between fork-length and capture month for RST fish and fork-length and RKM for MR fish. All statistical analysis were performed using the SigmaPlot statistical package (Systat Software, San Jose, CA).

Determination of Hybridization

Because there is temporal and spatial overlap in spring and summer Chinook spawning in the Entiat River, the possibility for introgression (i.e., 'hybridization') between the two lineages exists. We first tested our ability to accurately identify hybrids by using the program HYBRIDLAB (Nielsen et al. 2006) to simulate hybrid individuals. We used empirical genotype data from Entiat River spring and summer Chinook to simulate 100 F1 hybrids, 100 F2 hybrids, 100 backcross hybrids with spring (BC_Spring) and 100 backcross hybrids with summer Chinook (BC_Summer). We then analyzed parental type and hybrid individuals with the program NEWHYBRIDS (Anderson and Thompson 2002) to determine the probability that each individual was a spring, summer, F1, F2, or backcross hybrid Chinook. We conducted 10 separate NEWHYBRIDS runs of our simulated dataset, each run with 50,000 burn-in iterations followed by 100,000 data collection iterations.

We then ran two additional NEWHYBRIDS analyses using the parameters described above; one for fish captured by the RST and one for fish captured during MR sampling. Datasets for these analyses included baseline Entiat River spring and summer Chinook as well as the juvenile Chinook from the RST and MR collections. Based on simulation results (see below) individuals with a probability of 0.9 or greater of being a spring Chinook were classified as 'pure' spring, individuals with a probability of 0.9 or greater of being a summer Chinook were classified as a 'pure' summer run. Based on the results of our simulations (see below), rather than trying to classify individuals as F1, F2, BC_Spring, or BC_Summer, we took the sum of the probabilities for each of the four hybrid classes and if that value was 0.9 or greater, an individual was classified as a hybrid. We compared the hybrid status of each individual with the collection information to determine if there were any associations between hybrid status and collection/outmigration date for the RST fish or capture location for the MR fish.

Results

Genetic Baseline Analysis

Of the 96 SNP loci we ran, six loci were dropped from analysis because they did not amplify well either in a single population, or across all populations. Baseline samples that were missing genotypes at 14 or more loci were also dropped from the analysis. The baseline dataset used for analysis for this project consisted of 41 total populations with two lower Columbia populations, 33 spring run populations, and six summer/fall run populations. The number of samples representing each population ranged from 22–161 (Appendix Table 1). Following Bonferroni corrections, we observed several loci in collections that did not conform to HWE expectations including: three in Sandy River, one in Spring Creek NFH, one in Klickitat hatchery, one in Klickitat wild, one in Methow River spring, one in Deschutes River summer, and one in Entiat NFH. All departures from HWE expectations were due to a heterozygote deficit.

Pairwise F_{ST} values ranged widely. For example, the comparison between the Lower Yakima and Hanford Reach populations was 0.001 and the comparison between Camas Creek and Spring Creek NFH was 0.514 (Figure 6). Pairwise F_{ST} values among the different runs (i.e., spring vs. summer) tended to be at least an order of magnitude greater than comparisons between populations within each run (Figure 6). For example, the lowest pairwise F_{ST} we observed among spring and summer/fall populations was 0.208. The DAPC plot of the baseline dataset showed three main clusters of individuals (Figure 7). The first principal component on the plot (x-axis) separated the spring Chinook collections from all other collections and the second principal component on the plot (y-axis) separated the summer/fall Chinook collections from the lower Columbia collections. Within the different clusters on the plot, there tended to be considerable overlap among samples from different populations, particularly within the cluster of spring Chinook populations. Entiat NFH summer Chinook broodstock was a new addition to the Columbia River baseline and all samples from this collection clustered with the other summer/fall-run collections (Figure 7).

The percentage of samples assigned to the population they were collected from were highly variable and ranged from 8.9–98.4% for the Lochsa River and Spring Creek NFH, respectively (Figure 8). Assignments to runs were much higher and ranged from 92.5% for the Sandy River to 100% for 35 of the baseline populations (Figure 8). When we removed the Entiat River spring and summer Chinook collections from the baseline dataset and treated them as unknown, all of the samples were assigned to their run with probability of 1.0. These results along with the results of leave-one-out assignment tests suggest high accuracy for identifying individuals as either spring or summer Chinook.

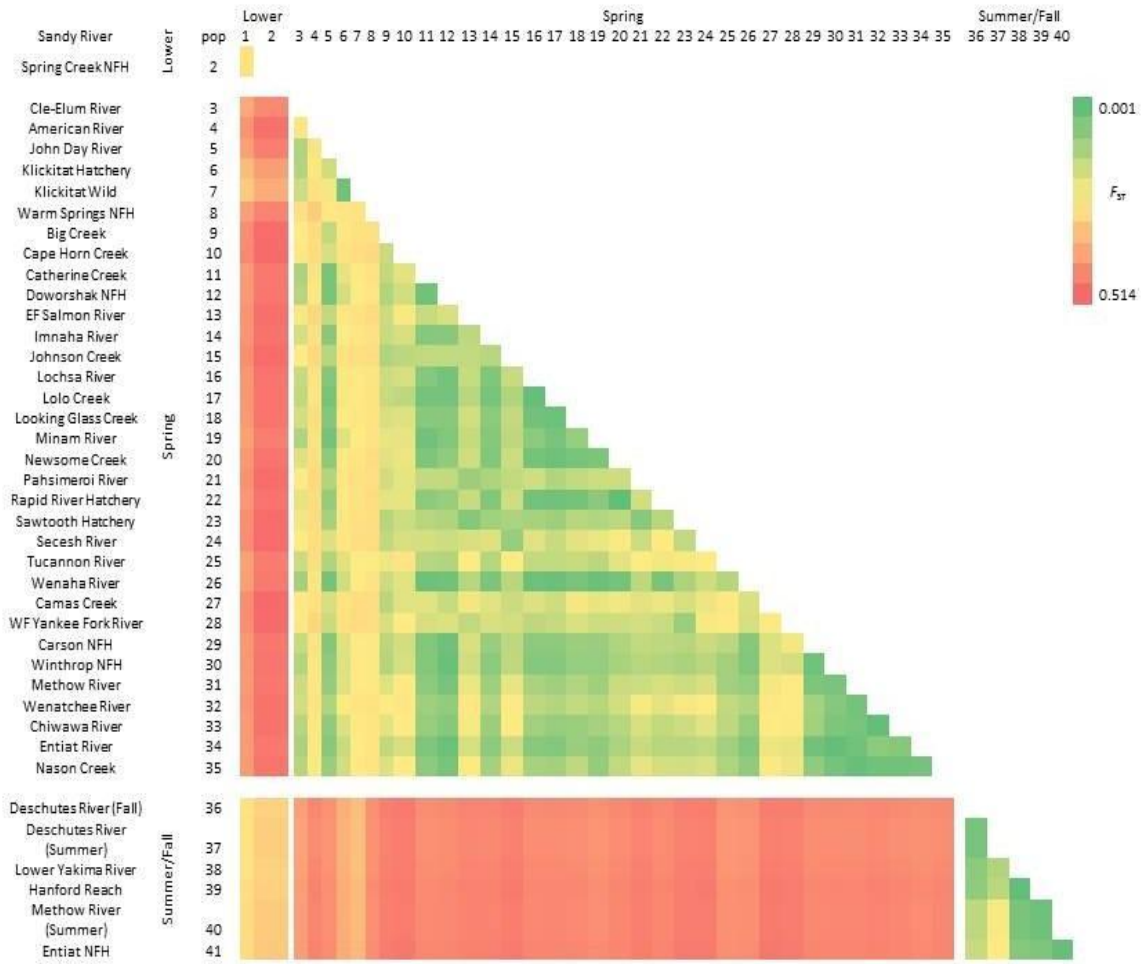


Figure 6. Heat map of pairwise F_{ST} values for 41 Chinook populations used as a genetic assignment baseline. Green cells denote lower pairwise F_{ST} estimates (and greater genetic similarity) among populations, orange and red cells denote greater estimates (and greater genetic divergence).

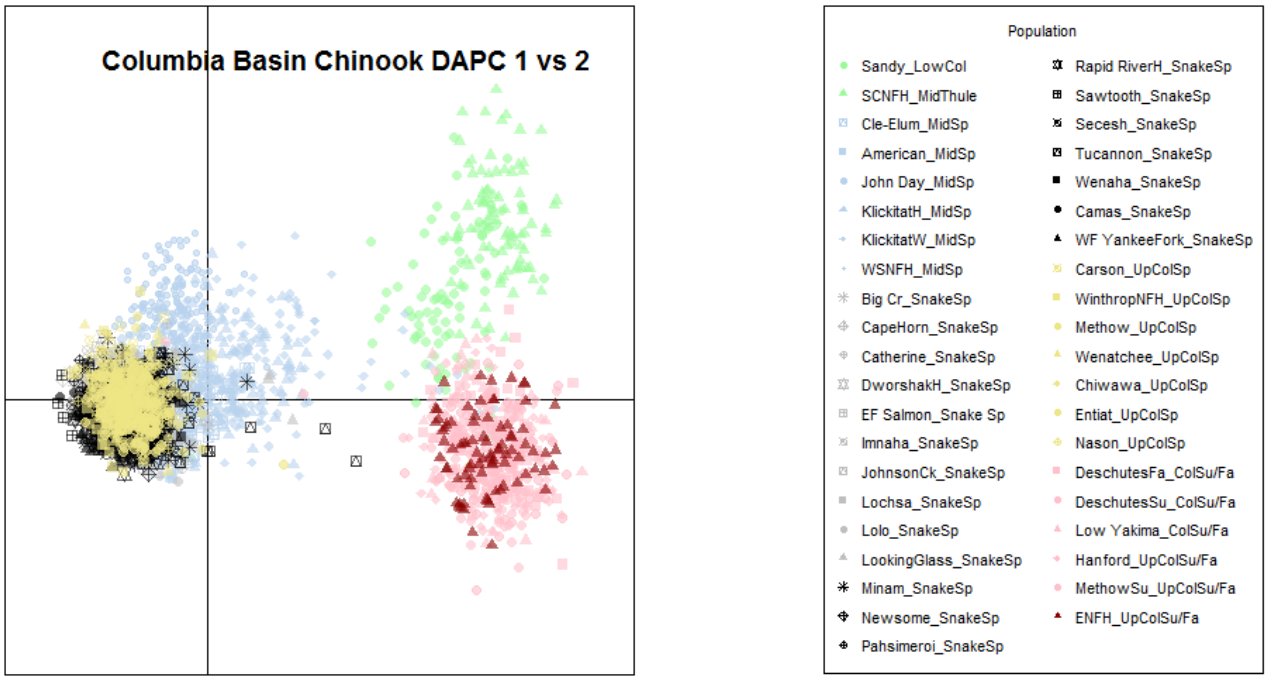


Figure 7. Discriminant analysis of principal components (DAPC) for the Chinook baseline used in this study. Each point on the plot represents a sampled fish and colors and shapes denote populations and run types.

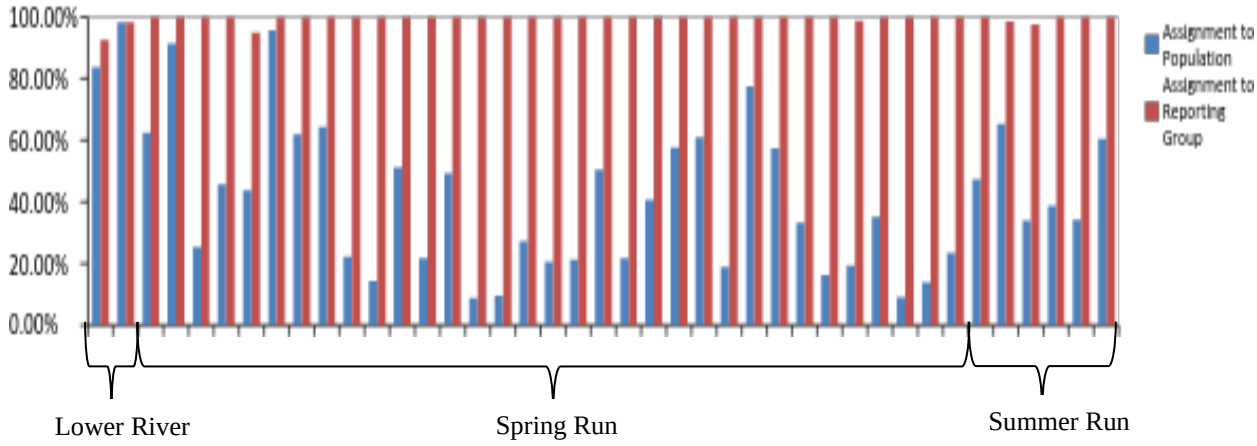


Figure 8. Assignment success rates for 41 Chinook populations during leave-one-out tests of the baseline dataset. The blue bars represent the proportion of samples from each population that were assigned to the population they were collected from and the red bars represent the proportion of samples from each population that were assigned to the run that they were collected from.

Genetic Assignment of Run Classification from Rotary Screw Trap Sampling

In 2009, the RST collection included 58 (32%) samples genetically assigned as spring and 121 (68%) samples assigned as summer/fall Chinook. In 2011, the RST collection included 434 (52%) samples assigned as spring and 400 (48%) samples assigned as summer/fall Chinook. Assignment probabilities ranged from 0.70–1.0; however, only three samples had assignment probabilities less than 1.0.

Comparisons of in-season run assignments using the date-nadir method to genotype based assignments varied between years. When compared to genotype assignments, overall the date-nadir method assigned 82.5% (80/97) and 61.8% (335/542) of summer Chinook correctly for 2009 and 2011, respectively (Table 2). The date-nadir method assigned 50.0% (82/41) and 77.7% (226/291) of spring Chinook correctly when compared to genotype assignment for 2009 and 2011, respectively. Success rates for in-season run assignments were highest for summer Chinook during July of 2009 (94%) and lowest during July of 2011 (43%; Table 1). Monthly proportions of spring Chinook collected during 2009 did not differ significantly ($P=<0.05$) from 2011 (Mann-Whitney test; $T=22.0$, $P=0.31$). The proportion of spring Chinook captured in the RST increased from summer through fall months. When pooled between years, a significant, positive correlation between RST sampling month and the proportion of spring Chinook encountered was evident (Spearman; $r_s=0.689$, $P=0.025$).

Table 1. Comparison of in-season run assignments using the date-nadir method to run assignments based on genotype for Entiat River subyearling Chinook captured at the rotary-screw trap in 2009 and 2011. N/A denotes a month that runs were not assigned.

Collection Period	Summer Run			Spring Run		
	Date-nadir Assignment	Genetic Assignment	True Assignment	Date-nadir Assignment	Genetic Assignment	True Assignment
Jul-09	47	44	93.6%	N/A	N/A	N/A
Aug-09[a]	50	36	72.0%	N/A	N/A	N/A
Sept-09[a]	N/A	N/A	N/A	8	1	12.5%
Oct-09	N/A	N/A	N/A	45	14	31.1%
Nov-09	N/A	N/A	N/A	29	26	89.7%
2009 Total	**97**	**80**	**82.5%**	**82**	**41**	**50.0%**
Jul-11	140	60	42.9%	N/A	N/A	N/A
Aug-11	214	167	78.0%	N/A	N/A	N/A
Sept-11	182	105	57.7%	4	2	50.0%
Oct-11	6	3	50.0%	102	73	71.6%
Nov-11	N/A	N/A	N/A	185	151	81.6%
2011 Total	**542**	**335**	**61.8%**	**291**	**226**	**77.7%**

[a] Sampling did not occur between August 25th and September 26th.

Fork-lengths of individuals captured at the RST were compared for each collection month to determine if a significant difference existed between run types based on genetic assignments. In 2009, fork-lengths differed significantly between summer and spring Chinook in August, October, and November (Figure 9; Table 2). In July and September of 2009, low sample size precluded fork-length analysis. In 2011, all months were tested and showed a significant difference between the fork-lengths of summer and spring Chinook (Figure 10; Table 2).

Table 2. Results of Mann-Whitney tests for differences in monthly fork-lengths of subyearling summer and spring Chinook sampled at the Entiat River RST during 2009 and 2011.

Sample Period	Summer-run				Spring-run				Test Results	
	n	Median	25%	75%	n	Median	25%	75%	T	P
Jul-09[a]	44	71.0	66.0	82.0	3	79.0	51.0	84.0	N/A	N/A
Aug-09	36	70.5	67.0	78.8	14	85.5	81.8	91.3	545.5	<0.001
Sep-09[a]	7	69.0	65.0	74.0	1	N/A	N/A	N/A	N/A	N/A
Oct-09	31	75.0	71.0	80.0	14	87.5	83.0	94.0	492.5	<0.001
Nov-09	3	73.0	70.0	78.0	26	87.5	82.8	94.3	10.0	0.013
Jul-11	60	60.0	56.0	65.0	80	64.0	59.0	70.8	3573.5	0.006
Aug-11	67	65.0	60.0	73.0	47	76.0	70.0	85.0	6965.0	<0.001
Sep-11	107	69.0	64.0	76.0	79	92.0	87.0	98.0	11216.5	<0.001
Oct-11	32	75.5	69.0	80.0	76	90.5	86.0	97.0	756.0	<0.001
Nov-11	34	84.0	77.0	90.0	144	89.5	84.3	94.0	2177.5	<0.001

[a] low sample size prohibited use of Mann-Whitney test.

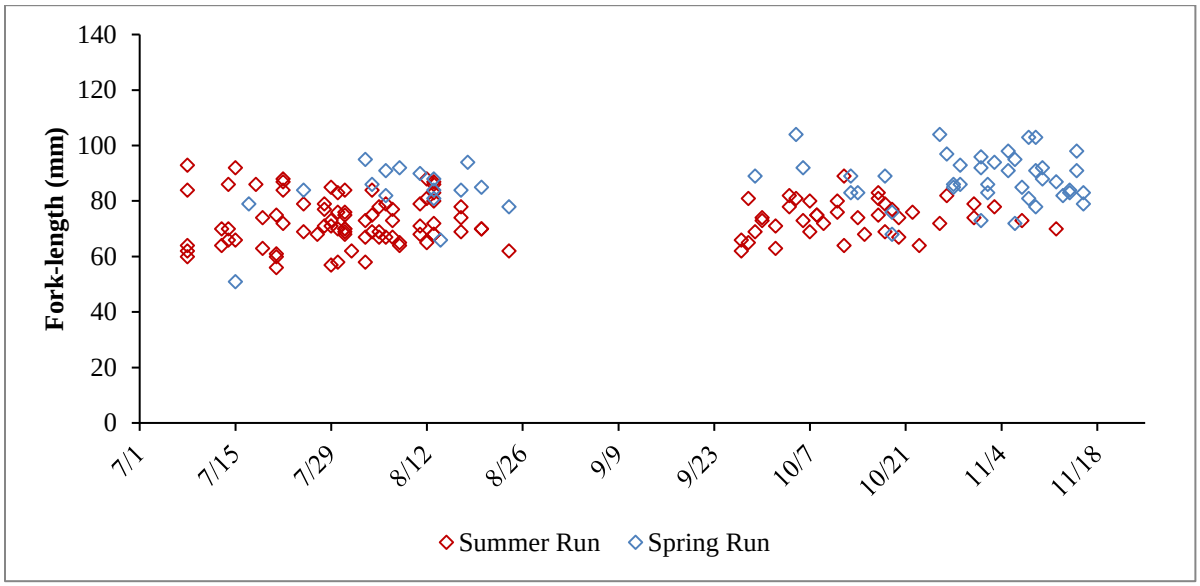


Figure 9. Genetic assignments for subyearling Entiat River Chinook based on size at outmigration in 2009.

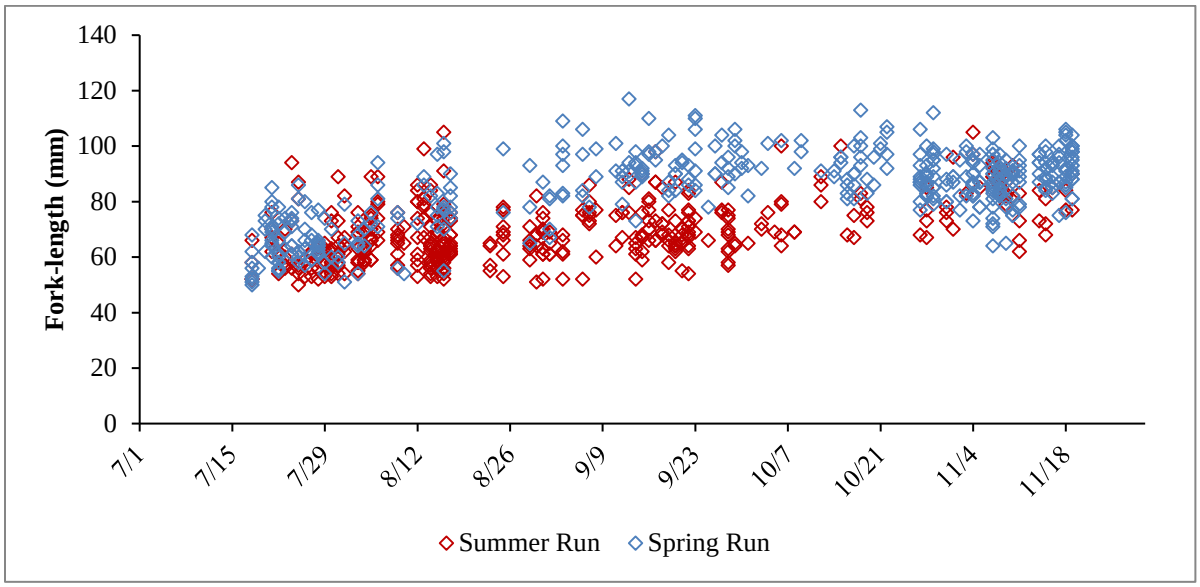


Figure 10. Genetic assignments for subyearling Entiat River Chinook based on size at outmigration in 2011.

Genetic Assignment of Run Classification from Mark Recapture Sampling

Overall, more spring Chinook were collected during MR sampling than summer Chinook although proportions varied between seasons and years. During the summer MR effort, 82.4%, 69.9% and 54.4% of all subyearling Chinook were assigned spring Chinook during 2012, 2013 and 2014, respectively, while the 2013 and 2014 winter period MR sampling resulted in 92.5% and 84.6% assignment to spring Chinook (Appendix Table 2). Probability values for genetic assignments of MR juveniles collected throughout the Entiat River basin were all 1.0. Genetic assignments to spring Chinook were consistently higher in valley segment 3 and the Mad River during both summer and winter MR sampling (Appendix Table 2). The proportion of spring Chinook assignments for Entiat River MR sites sampled consistently between years (n=10; Appendix Table 2), was not significantly different between years for summer (Kruskal-Wallis ANOVA; H=1.477, df=2, P=0.478) or winter sampling (Mann-Whitney test; T=125.0, P=0.096). Pooling years across sampling seasons resulted in a significant, positive correlation between RKM and the proportion of spring Chinook captured during the summer (Spearman; r_s=0.635, P=0.002; Figure 11); however, correlation during the winter period was weak and not significant (Spearman; r_s=-0.159, P=0.496; Figure 12).

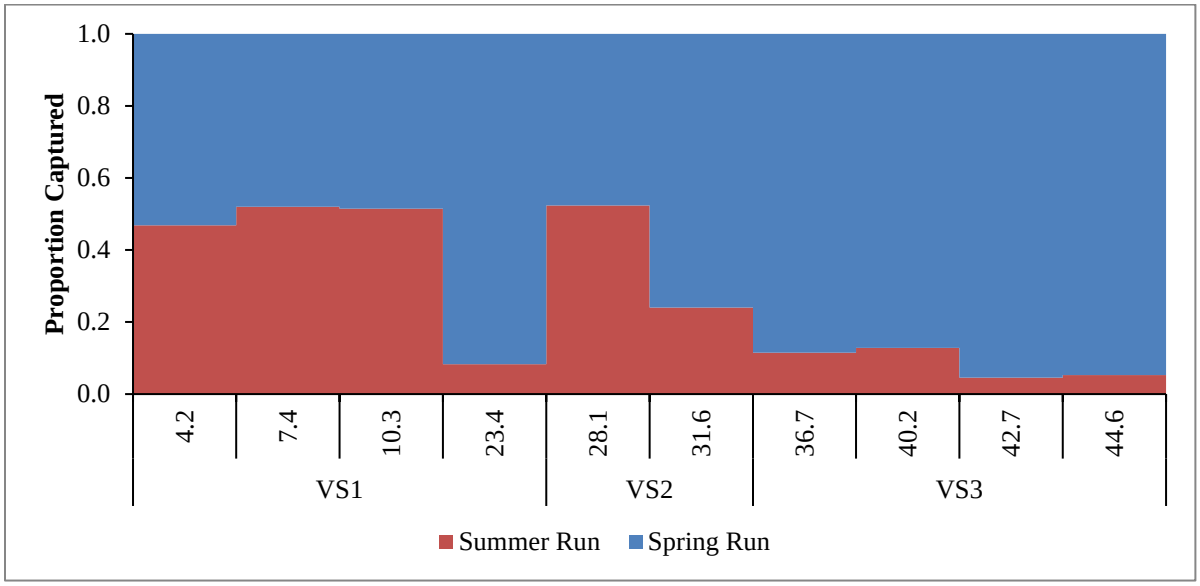


Figure 11. Genetic assignment of spring and summer Chinook during summer MR sampling in the Entiat River. Capture data pooled between years (2012, 2013, and 2014).

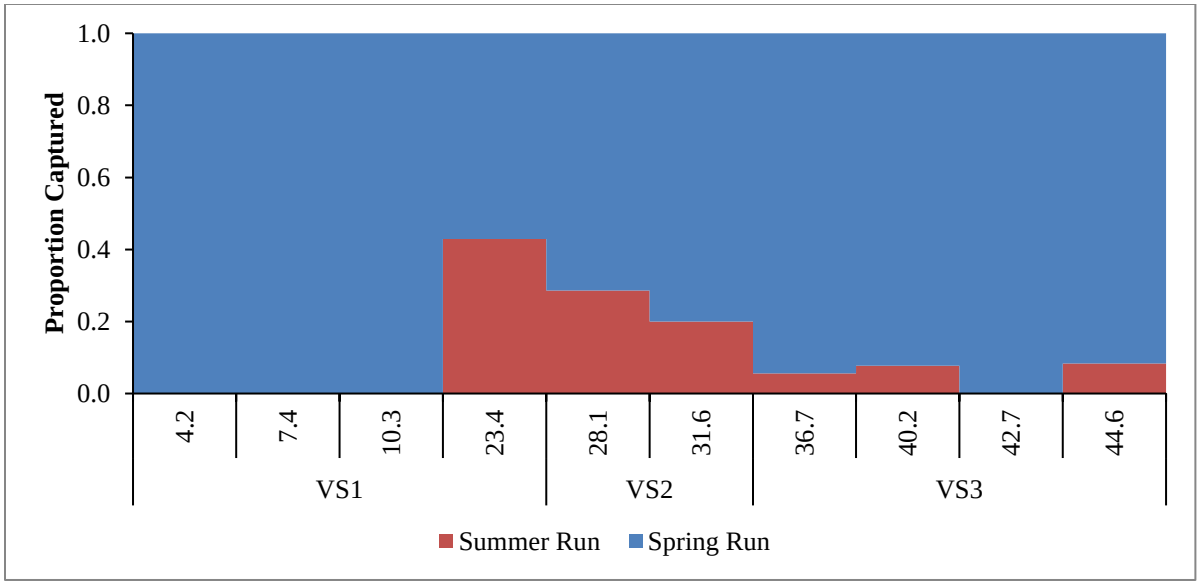


Figure 12. Genetic assignment of spring and summer Chinook during winter MR sampling in the Entiat River. Capture data pooled between years (2013 and 2014).

Similar to out-migrating subyearlings, fork-lengths of rearing summer and spring Chinook were significantly different during each sampling event regardless of season (Table 3). Between year comparison of spring Chinook fork-length by sampling period indicated a significant difference for the summer period (Kruskal-Wallis ANOVA; H=34.447, df=2, P=<0.001) with individuals encountered in 2014 being significantly larger (Dunn's multiple comparison; Q=3.714, P=<0.001) and individuals in 2012 significantly smaller (Dunn's multiple comparison; Q=5.785, P=<0.001). A significant, negative correlation was found between RKM and fork-length for subyearling Chinook sampled during the summer of 2012 (Spearman; r_s=-0.465, P=<0.001) and 2013 (Spearman; r_s=-0.253, P=0.016) but not for 2014 (Spearman; r_s=-0.013, P=0.907). Between year comparisons of spring Chinook fork-lengths from winter sampling did not differ significantly (Mann-Whitney test; T=1664.5, P=0.820) and a significant correlation between RKM and fork-length was not found when years were combined (Spearman; r_s=0.138, P=0.219). Summer Chinook fork-lengths were not significantly different between years during summer (Kruskal-Wallis ANOVA; H=5.842, df=2, P= 0.054) or winter sampling (Mann-Whitney test; T=29.5, P=0.999). No correlation was detected between RKM and fork-length for

either summer (Spearman; r_s=0.071, P=0.453) or winter sampling (Spearman; r_s=-0.143, P=0.615) when years were pooled by season.

Table 3. Results of Mann-Whitney tests for differences in fork-lengths between summer and spring Chinook sampled during summer and winter period MR surveys in the Entiat River.

Sample Period	Summer-run				Spring-run				Test Results	
	n	**Median**	**25%**	**75%**	**n**	**Median**	**25%**	**75%**	**T**	**P**
Summer 2012	30	60.0	54.8	69.3	91	73.0	66.0	80.0	939.5	<0.001
Summer 2013	34	60.0	57.0	67.0	91	78.0	72.0	90.0	927.5	<0.001
Summer 2014	49	66.0	57.5	76.0	88	85.0	75.3	93.0	2038.0	<0.001
Winter 2013	4	80.0	72.8	85.0	40	92.5	89.0	96.0	21.5	0.005
Winter 2014	10	81.0	76.8	83.5	41	92.0	89.5	95.5	78.5	<0.001

Determination of Hybridization

Based on our simulations, the mean probability that we correctly identified one of the parental strains (spring or summer) as a pure (non-hybrid) individual was 0.943 for summer and 0.973 for spring Chinook (Table 4). The simulated hybrid individuals generally had the highest probability of corresponding to the correct hybrid class, however the results for several simulated hybrids indicated that the greatest probability was not to the true hybrid class (e.g., a simulated F1 with a probability of 0.6 assigning as a backcross; Figure 13). The mean probability that simulated hybrid samples were identified as the true hybrid class ranged from 0.773 for F2 hybrids to 0.895 for BC_Spring (Table 4). Because assignments to the different hybrid classes were somewhat low compared to parental assignments, we also examined the mean probabilities that simulated hybrids fit into any of the four hybrid classes (e.g., 'P(Hybrid)' in Table 4). These probabilities represent the sum of the probabilities from the four different hybrid classes. These probabilities were higher and ranged from 0.959 for BC_Summer to 1.0 for F1 and F2 hybrids therefore, we focused on the probability that an individual was a hybrid rather than the specific hybrid class.

Table 4. Mean probability of assignment for spring, summer, and four hybrid classes of Entiat River Chinook (based on simulations). The P(Hybrid) column represents the sum of the four hybrid classes.

Genetic Class	Mean probability						
	Spring	**Summer**	**F1**	**F2**	**BC_Spring**	**BC_Summer**	**P(Hybrid)**
Spring	**0.943**	0.000	0.010	0.000	0.047	0.000	0.057
Summer	0.000	**0.973**	0.000	0.000	0.000	0.027	0.027
F1	0.000	0.000	**0.884**	0.062	0.026	0.028	1.000
F2	0.000	0.000	0.077	**0.773**	0.080	0.071	1.000
BC_Spring	0.024	0.000	0.027	0.054	**0.895**	0.000	0.976
BC_Summer	0.000	0.041	0.028	0.048	0.000	**0.884**	0.959

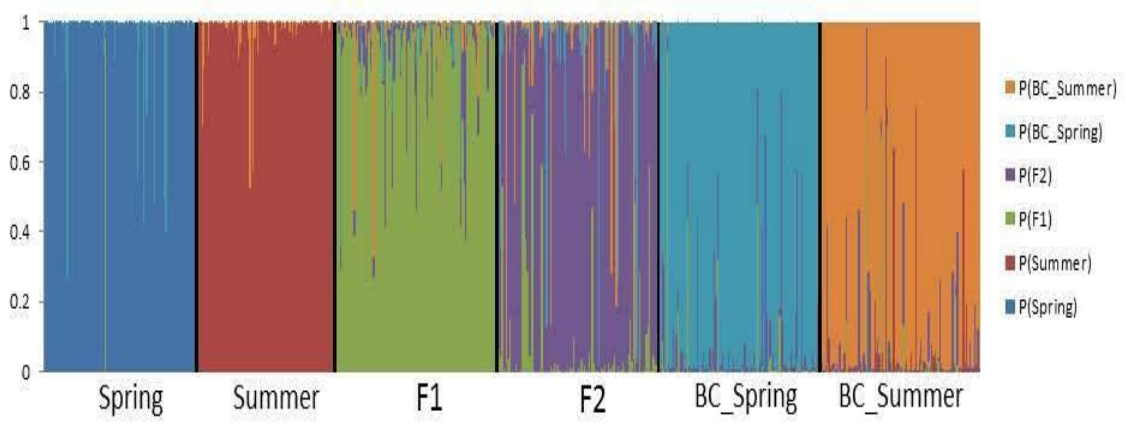


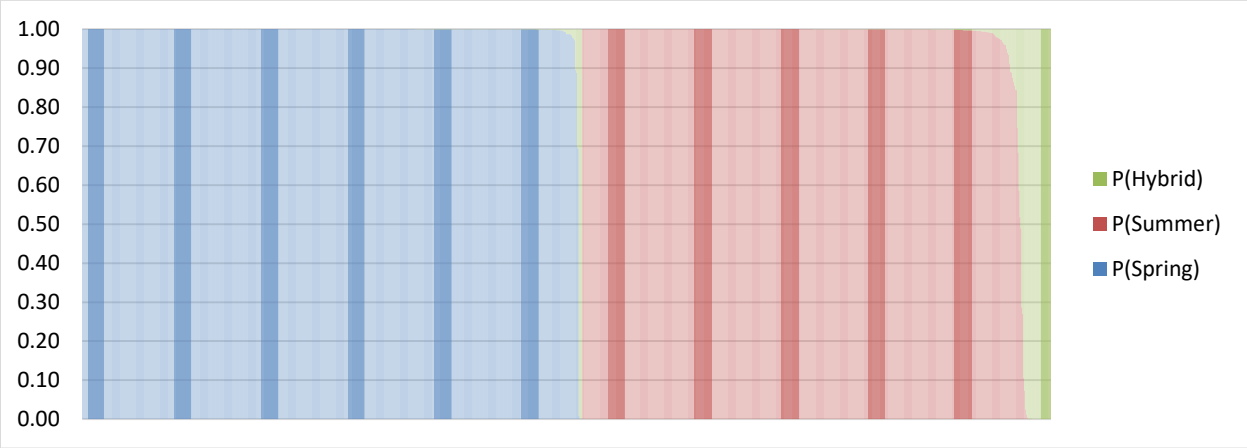
Figure 13. NEWHYBRIDS results for pure Entiat spring and summer Chinook plus 100 simulated hybrids from each of four categories: F1, F2, backcross with spring-run (BC_Spring) and backcross with summer-run (BC_Summer). Vertical bars represent an individual fish in the analysis and the shading corresponds to the probability an individual fits into that particular hybrid class.

Overall, we observed a low number of hybrid individuals in both the RST and MR samples. Genetic analysis of 1,013 subyearling Chinook captured at the RST indicated that, 447

individuals (44.1%) were pure spring Chinook, 515 (50.8%) were pure summer Chinook, 32 (3.2%) were hybrids, and 19 (1.9%) individuals did not fall into any category (i.e., probability < 0.9; Figure14). All but two of the hybrids were collected in 2011 and they were collected throughout the sample period (July – November). The RST hybrid individuals ranged in size from 51 to 106 mm fork-length (Appendix Table 3). Genetic analysis of the 542 juvenile Chinook captured during MR sampling indicated that 384 (70.8%) were pure spring Chinook, 130 (24.0%) were pure summer Chinook, eight (1.5%) were hybrids, and three (0.6%) did not fit into any category (Figure 14). Hybrid individuals were detected during each of the five MR sampling periods with exception to the summer period of 2014. Rates of hybridization were highest (3.7%) for individuals collected during the 2014 winter sampling period. Of the eight individuals identified as hybrids, four were collected in valley segment 1, one was collected in valley segment 2, three were collected in valley segment 3, and none were collected in the Mad River (Table 5).

Table 5. Collection information and hybrid probabilities for juvenile Chinook collected during mark-recapture surveys identified as hybrids 2012–2014.

Year	Season	VS	RKM	FRKL (mm)	P(Hybrid)
2012	Summer	1	10.26	69	0.994
2013	Winter	3	36.69	76	1.000
2013	Summer	1	4.20	78	1.000
2013	Summer	1	8.42	81	1.000
2013	Summer	3	40.24	65	1.000
2014	Winter	1	4.20	85	1.000
2014	Winter	2	31.56	76	1.000
2014	Winter	3	44.59	87	1.000



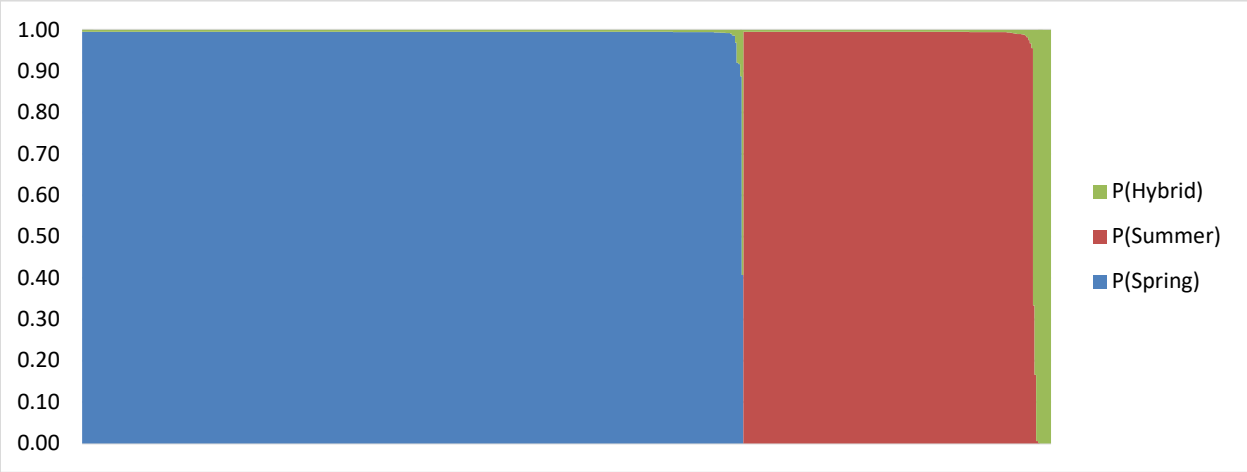


Figure 14. NEWHYRBIDS results for 2009 and 2011 Entiat River RST emigrating subyearling Chinook (top) and 2012-2014 mark-recapture juveniles (bottom). Vertical bars represent samples in the analysis and the shading corresponds to the probability the individual fits the six hybrid classes shown at right.

Discussion

Objective 1 - Genetic baseline analysis

The addition of Entiat NFH summer Chinook to the existing Columbia River genetic baseline improved our ability to differentiate between spring and summer run Chinook. Prior genetic analysis had reduced assignment to spring run type with roughly 70% of all assignments having a probability ≥ 0.9 (C. Smith, unpublished data). Following the addition of Entiat NFH summer Chinook and several other key Columbia and Snake river Chinook populations to the genetic baseline, assignment probability improved to 1.0 in greater than 99% of all assignments and allowed for the successful assessments of RST date-nadir run classification, spatiotemporal rearing distribution, and the presence of hybrid individuals among rearing and out-migrating juvenile Chinook. These results provide additional support for the deep genetic divergence between Spring and Summer Chinook and highlight the importance of a robust population baseline for accurate run-type assignments.

Objective 2 - Assessment of rotary-screw trap date-nadir run classifications

Genetic assignments indicate moderate to high variability in juvenile out-migration timing between years which was reflected in varying assignment accuracy using the date-nadir method. A positive correlation between capture month and the proportion of spring Chinook encountered at the RST provides some support for the use of the date-nadir method although the accuracy of this method remains a concern. In-season run assignments using the date-nadir method had the highest accuracy during the months of August (77%) and November (83%). The difference between proportions of spring Chinook out-migrants present during July of 2009 (6%) and 2011 (57%) was unexpected. A number of abiotic and biotic factors are known to impact the movement and out-migration timing of juvenile Chinook and further evaluation beyond the scope of our current evaluation is needed to fully understand these relationships.

Significant differences in fork-lengths between spring and summer Chinook found in most months during 2009 and 2011 may be helpful to reduce error associated with in-season run

assignments. In both 2009 and 2011, differences in fork-length between runs increased during the sampling season providing greater definition between run types. Overlap in fork-length between run types appears to diminish in the summer months; therefore, it may be beneficial to utilize this information in combination with the date-nadir method to improve upon run assignment accuracy. Additional genetic assignments and comparisons to run type determination based on the date-nadir method should be conducted to provide further evaluation of this method including the amount of inter-annual variability.

The need to differentiate between juvenile Chinook in systems where runs overlap spatially or temporally is not unique to the Entiat River. For example, morphological assessments performed at two lower Snake River dams to differentiate between ESA-listed spring and fall subyearling Chinook indicated 26% assignment accuracy for spring Chinook when compared with results from a genetic analysis (Tiffan et al. 2000). In the Sacramento and San Joaquin river basins, a length-at-date method that accounted for daily differences in fork-length was 49% accurate at assigning runs between four overlapping juvenile run types (Harvey et al. 2014). When compared to these published values, the date-nadir method performed considerably better, assigning spring Chinook accurately in 72% of all cases.

The operation of a rotary-screw trap in the lower Entiat River has provided estimates of ESA-listed spring Chinook freshwater productivity and abundance since 2003. Coupled with ongoing adult spawning surveys, these efforts represent critical baseline information necessary for assessing the recovery of this population. Currently the date-nadir method is the preferred option for differentiating between Chinook runs, though its use should be limited to a coarse-scale means of differentiation. We urge a careful consideration prior to utilizing these data in applications requiring greater accuracy. Genetic analysis has proven to be a reliable and accurate means to partition between out-migrant run types and would increase the accuracy of these estimates substantially. Differences in fork-length between spring and summer run out-migrants may also represent a means to increasing the accuracy of RST derived estimates in the future although additional genetic analysis would be required to better assess if these differences were consistent throughout a longer time period.

Objective 3 -Genetic assignment of run type for juvenile Chinook encountered during MR surveys

Genotype based run assignment for juvenile Chinook encountered during MR sampling consistently showed spring Chinook distribution during summer sampling was highest in valley segment 3, lowest in the Mad River, and was positively correlated with upstream RKM. The spatial distribution of subyearling spring Chinook captured in MR sampling was likely attributed to spawning distribution. In 2009-2012 nearly 70% of all spring Chinook spawned in and above valley segment 3, 29% in valley segment 2, less than 1% in the Mad River, and none spawned in valley segment 1 (G. S. Fraser, unpublished data). This finding is important for a number of reasons. First, based on these results, habitat actions seeking to benefit summer rearing life-stages of spring Chinook should target upriver reaches where higher proportions of spring Chinook are found. Additionally, past and present research, monitoring, and evaluation (RM&E) efforts focused on ESA-listed spring Chinook may not adequately account for possible impacts caused by high proportions of summer run Chinook rearing within the lower Entiat River during the summer months. This could conceivably impact seasonal estimates of growth, movement, survival, abundance, or affinity to specific habitat types or features. Finally, our findings support a downstream dispersal of spring Chinook following the summer rearing period and a more homogenous use of the Entiat River for overwinter rearing.

The presence of yearling summer Chinook during winter MR sampling serves to further document plasticity within life-history expression. Genetic analysis indicated 12% of all juvenile Chinook sampled during March of 2013 and 2014 were summer Chinook that had overwintered in the Entiat River. Assuming no movement between valley segments prior to capture, the majority (56%) of summer Chinook appear to have overwintered in valley segment 2. Analysis of scale samples taken from post-spawn adult summer Chinook between 2006 and 2015 indicated that roughly 1% of returning adult summer Chinook overwintered in the Entiat River (Fraser and Hamstreet 2016). If our genetic analysis is representative of a longer-term trend, the low adult recruitment of this life history strategy suggests that either it is maladaptive or the majority of juveniles are exhibiting a precocious life-history strategy.

Comparisons of spring Chinook fork-lengths between annual summer sampling periods yielded significant differences. These were not unexpected as annual variation in water temperatures are known to impact emergence timing and subsequent growth opportunities (Quinn 2005). Similarly, the warmer water temperatures experienced in the lower Entiat River likely explained the negative correlations found between fork-length and upstream location during the summer rearing period. Significant differences were found in fork-lengths between spring and summer Chinook were noted during each sampling period and may be useful in determining future run assignments.

Objective 4 - Detection of hybrid juveniles

Simulation results showed that we cannot accurately identify the different hybrid classes (e.g., F1, F2, Backcross) with the current marker set. Advances in genetic technology have now made it possible to genotype tens of thousands of markers at relatively low costs and these advances have provided exciting new insights into hybridization (Hohenlohe et al. 2013). Perhaps future analyses utilizing new technology and larger maker sets will facilitate increased resolution for classifying hybrid individuals. We identified hybrid individuals in both the RST and MR collections. The presence of hybrid individuals in the out-migrant population indicated viability through juvenile life-stages. Hybridization between Chinook runs has been documented in other watersheds and in some cases appears to limit productivity. Smith and Engle (2011) estimated hybridization rates from 4.3–15% for juvenile fall Chinook emigrating from the White Salmon River, WA. However, the absence of hybrids in the adult spawning population suggested that hybrid juveniles represented a loss in overall population productivity. The implications of hybridization involving ESA listed Spring Chinook Salmon in the Entiat River are unclear. Since we only ran juvenile fish, it is unclear whether hybrids return as adults and potentially spawn. Future analysis of returning adults or carcasses may help to clarify the implications of hybridization.

In 2007, Entiat NFH transitioned from production of spring to summer Chinook. The last adult returns from the spring Chinook program occurred in 2010. Release numbers of Entiat

NFH-summer Chinook increased from 150,000 to 418,000 from 2011 to 2015. The first full production returns from the Entiat NFH summer Chinook program occurred in 2016 and the proportion of hatchery adults on the spawning grounds has been increasing (Fraser et al. 2018). Rates of hybridization may be expected to increase due to increases in Entiat NFH-summer Chinook returns. Hybridization levels detected in our study will provide baseline data to assess the potential impact of the Entiat NFH-summer Chinook program on ESA-listed spring Chinook in the Entiat River.

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Appendices

Appendix Table 1. Forty-one Columbia River Basin Chinook populations used for genetic assignment baseline. n=total number samples in the collection.

Population	Run	N
Sandy River	Lower River	67
Spring Creek NFH	Lower River	64
Cle-Elum River	Spring	56
American River	Spring	46
John Day River	Spring	55
Klickitat Hatchery	Spring	105
Klickitat Wild	Spring	158
Warm Springs NFH	Spring	161
Big Creek	Spring	71
Cape Horn Creek	Spring	59
Catherine Creek	Spring	63
Doworshak NFH	Spring	76
EF Salmon River	Spring	84
Imnaha River	Spring	78
Johnson Creek	Spring	85
Lochsa River	Spring	56
Lolo Creek	Spring	72
Looking Glass Creek	Spring	84
Minam River	Spring	63
Newsome Creek	Spring	80
Pahsimeroi River	Spring	77
Rapid River Hatchery	Spring	78
Sawtooth Hatchery	Spring	71
Secesh River	Spring	52
Tucannon River	Spring	54
Wenaha River	Spring	37
Camas Creek	Spring	40
WF Yankee Fork River	Spring	68
Carson NFH	Spring	78
Winthrop NFH	Spring	67
Methow River	Spring	83
Wenatchee River	Spring	68

Chiwawa River	Spring	22
Entiat River	Spring	86
Nason Creek	Spring	34
Deschutes River (Fall)	Summer/Fall	74
Deschutes River (Summer)	Summer/Fall	72
Lower Yakima River	Summer/Fall	44
Hanford Reach	Summer/Fall	75
Methow River (Summer)	Summer/Fall	76
Entiat NFH	Summer/Fall	33

Appendix Table 2. Genetic assignments to summer (SMR) or spring (SPG) juvenile Chinook collected during MR sampling by valley segment, RKM, season and year. N/A denotes samples were not collected or sampling did not occur.

Valley Segment	RKM	2012		2013				2014			
		Summer		Winter		Summer		Winter		Summer	
		SMR	SPG	SMR	SPG	SMR	SPG	SMR	SPG	SMR	SPG
VS1	4.2[a]	2	8	0	2	6	6	0	3	7	3
	5.2	N/A	N/A	N/A	N/A	4	6	0	2	N/A	N/A
	7.4[a]	6	19	0	3	6	4	0	2	14	1
	8.4	N/A	N/A	N/A	N/A	2	6	0	6	N/A	N/A
	9.9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	8	0
	10.3[a]	4	13	0	2	6	1	0	2	7	2
	14.7	1	3	0	9	0	5	1	1	N/A	N/A
	16.3	N/A	N/A	N/A	N/A	N/A	N/A	0	4	N/A	N/A
	23.4[a]	2	10	0	2	0	9	3	2	0	3
% of run		**22.1%**	**77.9%**	**0.0%**	**100%**	**39.3%**	**60.7%**	**15.4%**	**84.6%**	**80.0%**	**20.0%**
VS2	27.1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7	2
	28.1[a]	2	2	3	3	8	8	3	12	1	0
	31.6[a]	1	9	1	9	2	6	2	3	3	4
	32.5	N/A	N/A	N/A	N/A	0	1	0	1	N/A	N/A
% of run		**21.4%**	**78.6%**	**25.0%**	**75.0%**	**40.0%**	**60.0%**	**23.8%**	**76.2%**	**64.7%**	**35.3%**
VS3	36.7[a]	1	10	0	8	0	4	1	9	2	9
	37.4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	9
	40.2[a]	2	17	0	7	2	8	1	5	1	9
	41.7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	10
	42.4	N/A	N/A	N/A	N/A	4	11	0	2	N/A	N/A
	42.7[a]	0	5	0	2	0	12	0	3	1	4
	44.6[a]	1	7	0	2	0	6	1	9	0	5
% of run		**9.3%**	**90.7%**	**0.0%**	**100%**	**12.8%**	**87.2%**	**9.7%**	**90.3%**	**8.0%**	**92.0%**
Mad	1.0	0	5	0	2	2	4	N/A	N/A	1	1
	3.0	0	1	N/A	N/A	0	1	N/A	N/A	0	1
	5.0	0	5	N/A	N/A	N/A	N/A	N/A	N/A	0	1
% of run		**0.0**	**100**	**0.0**	**100**	**28.6**	**74.4**	**N/A**	**N/A**	**25.0**	**75.0**
Total % of run		**17.6%**	**82.4%**	**7.5%**	**92.5%**	**30.1%**	**69.6%**	**15.4%**	**84.6%**	**45.5%**	**54.5%**

[a] MR sampling was performed in each of the summer and winter periods.

Appendix Table 3. Collection information and hybrid class probabilities for subyearling Chinook collected from Entiat River RST in 2009 and 2011 identified as hybrids.

MCFWCO ID	AFTC ID	Date Collected	Fork-Length (mm)	P(Hybrid)
EtRSCS 09 100	1768-100	8/24/2009	78	1.000
ENFHSCS 09 167	1771-077	11/13/2009	82	1.000
11-12W-A-004	2590-004	7/18/2011	51	1.000
11-12W-A-006	2590-006	7/18/2011	62	1.000
11-12W-A-007	2590-007	7/18/2011	66	0.991
11-12W-A-014	2590-014	7/19/2011	56	1.000
11-12W-A-019	2590-019	7/20/2011	62	1.000
11-12W-A-022	2590-022	7/21/2011	80	1.000
11-12W-A-023	2590-023	7/21/2011	71	1.000
11-12W-A-033	2590-033	7/21/2011	78	0.999
11-12W-A-035	2590-035	7/22/2011	55	1.000
11-12W-A-043	2590-043	7/22/2011	74	1.000
11-12W-A-099	2590-099	7/27/2011	59	1.000
11-12W-A-100	2590-100	7/27/2011	59	1.000
11-12W-A-146	2591-046	8/1/2011	65	1.000
11-12W-A-160	2591-060	8/3/2011	64	1.000
11-12W-A-203	2592-003	8/9/2011	76	1.000
11-12W-A-280	2592-080	8/16/2011	78	1.000
11-12W-A-353	2593-053	8/31/2011	76	1.000
11-11W-F-003	2587-003	9/11/2011	91	1.000
11-11W-F-009	2594-009	9/12/2011	79	0.988
11-11W-F-036	2594-036	9/15/2011	90	1.000
11-11W-F-121	2595-021	9/26/2011	90	0.996
11-11W-F-182	2587-032	10/16/2011	83	1.000
11-11W-F-214	2588-014	10/27/2011	106	0.927
11-11W-F-236	2588-036	10/28/2011	83	1.000
11-11W-F-242	2588-042	10/29/2011	79	1.000
11-11W-F-244	2588-044	10/29/2011	85	1.000
11-11W-F-390	2589-090	11/15/2011	100	0.975
11-11W-F-402	2587-052	11/16/2011	94	1.000
11-11W-F-400	2589-100	11/16/2011	89	1.000
11-11W-F-430	2587-080	11/19/2011	81	1.000