

Summer Chinook Salmon in the Entiat River: Genetic Analysis of Hatchery and Natural Origin Adults Spawning in the Wild

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Prepared by:

Christian Smith and Brice Adams
U. S. Fish and Wildlife Service
Abernathy Fish Technology Center
1440 Abernathy Creek Road
Longview, WA 98632

In cooperation with:

William Gale and Matt Cooper
U.S. Fish and Wildlife Service
Mid-Columbia River Fishery Resource Office
7501 Icicle Creek Road
Leavenworth, WA 98826

ABSTRACT

In evaluating potential impacts of a summer Chinook salmon (*Oncorhynchus tshawytscha*) program at Entiat National Fish Hatchery, it would be helpful to know whether the population of naturally spawning summer Chinook salmon in the Entiat River is typical of this lineage or if they represent a divergent endemic population. Specifically we wanted to know whether naturally spawning summer Chinook salmon in the Entiat River are distinct from the large number of spawners straying into the Entiat River basin from nearby summer Chinook hatchery programs in the Methow, Wenatchee and Okanogan basins. We analyzed 13 microsatellite markers in 272 samples of Entiat River summer Chinook salmon taken during carcass surveys between 2000 and 2010. These were compared to hatchery and wild samples from other summer and fall Chinook salmon populations and to broodstock samples from the recently established summer Chinook salmon program at Entiat NFH. We found no evidence of genetic divergence between adipose-clipped and unclipped summer Chinook salmon spawning in the Entiat River, and no evidence of temporal structure among collection years. We further found no evidence that these fish were more distinct from local hatchery populations of summer Chinook salmon, than were naturally spawning populations in the Wenatchee, Methow and Okanogan rivers.

INTRODUCTION

The Entiat River lies within the geographic boundaries of the Upper Columbia River Summer/Fall-run Chinook salmon (*Oncorhynchus tshawytscha*) Evolutionarily Significant Unit (ESU) which includes all late-returning Chinook salmon (summer runs and fall runs) from the main stem of the Columbia River and its tributaries (excluding the Snake River) between Chief Joseph and McNary dams (Waknitz *et al.* 1995). Salmon populations in this region were heavily impacted by mitigation efforts following construction of Grand Coulee Dam in 1939. All adult fish arriving at Rock Island Dam between 1939 and 1943 were taken to National Fish Hatcheries on the Methow or Wenatchee Rivers for artificial spawning, or fenced into reaches of the Entiat or Wenatchee River for natural spawning. Distinctions were made between spring run (those crossing Rock Island Dam before August 20) and late run (those crossing Rock Island Dam after August 20), but not between fall and summer run populations in the latter group. All extant summer and fall Chinook salmon populations above Rock Island Dam are thus the progeny of a mixture of lineages and populations which was created between 1939 and 1943. Between 1941 and 1976 an estimated 200×10^6 late-returning Chinook salmon juveniles were planted into the Upper Columbia River Region, and 8.6×10^6 were planted directly into the Entiat River (Waknitz *et al.* 1995). It is unknown whether summer Chinook salmon were endemic to the Entiat River prior to mitigation.

Presently, hatchery-origin and natural-origin summer Chinook salmon return to the Entiat River each year and spawn to produce an average of 150 redds (Hamstreet 2011). It is thought that approximately 1/3 of the summer Chinook salmon spawning in the Entiat River are strays from

mainstem Columbia River hatchery programs (Appleby et al. 2010), which resulted in the USFWS Columbia Basin Hatchery Review Team giving the Entiat River summer Chinook salmon population a biological significance rating of “low” (USFWS 2007).

The primary goal of the USFWS, Entiat National Fish Hatchery (ENFH) is to help the Bureau of Reclamation (BOR) satisfy their mitigation requirements by providing salmon for local and regional harvest. To help determine the future direction of ENFH the USFWS, BOR and other co-managers (Yakama Indian Nation, Colville Confederated Tribes (CCT), NOAA-Fisheries and, Washington Department of Fish and Wildlife (WDFW)) held a series of meetings to discuss the possible alternatives for future hatchery programs at ENFH (Appleby et al. 2010). The result of these discussions was the determination that the addition of a summer Chinook program at ENFH might best satisfy both the mitigation requirements of BOR and the concern for minimizing the impact of the hatchery production on listed stocks of spring Chinook salmon and steelhead trout in the Entiat River basin.

In considering the potential impacts of a summer Chinook salmon program at ENFH, it would be helpful to know whether the population of naturally spawning summer Chinook salmon in the Entiat River is typical of this lineage or if they represent a divergent endemic population.

Specifically we wish to know whether naturally spawning summer Chinook salmon in the Entiat River are distinct from the large number of spawners straying into the Entiat River basin from nearby summer Chinook hatchery programs in the Methow, Wenatchee and Okanogan basins.

Previous genetic analyses of summer Chinook salmon from the Upper Columbia River have

revealed a lack of divergence not only among collections from different rivers, but also among summer and fall Chinook salmon (e.g., Utter *et al.* 1995; Waples *et al.* 2004; Narum *et al.* 2010). A recent study of population structure among summer Chinook salmon included samples from the Entiat River and found that they clustered with other Upper Columbia River summer and fall run collections, and reported low values of genetic divergence (F_{ST}) between collections from the Entiat, Wenatchee, and Okanogan basins (Kassler *et al.* 2011).

Here we report results of a genetic analysis of population structure among hatchery-origin and wild-origin summer Chinook salmon spawning naturally in the Entiat River. Our objectives were to 1) test for genetic divergence between hatchery-origin and wild-origin samples of naturally spawning summer Chinook salmon from the Entiat River, and 2) characterize patterns of divergence between summer Chinook salmon from the Entiat River and summer Chinook salmon from adjacent hatchery and wild populations.

METHODS AND MATERIALS

The USFWS Mid-Columbia River Fisheries Resource Office (MCRFRO) conducted monitoring of spring and summer Chinook salmon populations in the Entiat River from 1994 thru the present following methods described by Hamstreet (2011). Surveys included collection of genetic samples (either scales on scale-cards or fin clips in ethanol) from carcasses of adult summer Chinook salmon. Where possible (i.e. where carcasses were not too degraded), each fish was identified as “adipose-clipped” (suggesting hatchery-origin) or “unclipped” (suggesting

wild-origin) based on the absence or presence of the adipose fin. For the present work we analyzed samples collected between 2000 and 2010 (Table 1).

DNA was extracted from a small ($\sim 2\text{mm}^2$) piece of each sample using a DNAeasy-96 Tissue Kit (QIAGEN). The polymerase chain reaction (PCR) was used to amplify 13 microsatellite loci (Appendix 1) from each DNA sample. Loci were amplified in 10 μl reaction volumes consisting of 5.0 μl 2x QIAGEN Multiplex PCR Master Mix (final concentration of 3 mM MgCl_2), 2.0 μl of extracted DNA. Primer concentrations and annealing temperatures for each PCR multiplex are listed in Appendix 2. Liquid handling was performed using a JANUS Automated Workstation (Perkin Elmer). PCR products were size-fractionated using an AB3130 DNA Sequencer (Applied Biosystems), and raw microsatellite data (electropherograms) were analyzed using GENEMAPPER 4.0. Amplified products were binned into alleles used in the standardized coastwide Chinook salmon baseline.

All genotypes were scored by two independent readers. Following completion of the data collection, 10% of all samples were re-analyzed in order to estimate genotyping error rates. Individual samples for which more than three loci (23%) failed were removed from the data set. The Microsoft Excel add-in Microsatellite Toolkit (Park 2001) was used to scan the dataset for individuals with identical genotypes.

Table 1. Samples analyzed with 13 microsatellite markers. The number of samples processed is listed under “Attempted”. The numbers of samples successfully analyzed ($\geq 10/13$ loci successfully genotyped) were categorized as follows: adipose-clipped (H), unclipped (W), and unknown (U). Cells with two numbers separated by a “/” indicate the number successfully analyzed and the number remaining after duplicate genotypes, spring run individuals and a fall tule Chinook salmon individual were removed (see RESULTS).

Description	Year	Sample type	Attempted	Successful			Percent
			H	W	U		
Entiat River SUS carcass	2000	scale	82	18/10	26/23	6/2	42.7%
Entiat River SUS carcass	2002	scale	70	0	29	0	41.4%
Entiat River SUS carcass	2004	scale	70	8	28/27	6	58.6%
Entiat River SUS carcass	2006	scale	70	1	36/35	1	52.9%
Entiat River SUS carcass	2008	fin clip	79	14	31/28	4	58.2%
Entiat River SUS carcass	2009	fin clip	80	15/14	36	2	65.0%
Entiat River SUS carcass	2010	fin clip	73	8	24	0	45.2%
ENFH SUS broodstock	2009	fin clip	88	88			100.0%
ENFH SUS broodstock	2010	fin clip	100	99			99.0%
Total			712	242	202	15	

Genetic assignment tests were used as a final quality control measure for our samples. This was done to identify spring run samples inadvertently included in the collections prior to using those collections to characterize summer run. The conditional maximum likelihood was used to estimate mixture proportions (Millar 1987) and the probability of each genotype was calculated following the method of Rannala & Mountain (1997) for each population in the GAPS baseline with the program ONCOR (Kalinowski *et al.* 2007) Probabilities of assignment to reporting groups (in this case Regional Groups identified by Seeb *et al.* (2007) were calculated by summing probabilities over populations.

In addition to data collected at AFTC, genotype data from the Genetic Analysis of Pacific Salmon (GAPS) Consortium baseline and the State of Washington (Kassler *et al.* 2011; Table 2) were incorporated into the population structure analysis below.

Population structure among summer Chinook salmon in the Entiat River

An initial test of divergence among years and among adipose-clipped and unclipped samples was done using an analysis of molecular variance (Excoffier *et al.* 1992). For this analysis, we only included collection years in which ten or more samples were available from both adipose-clipped and unclipped fish (2000, 2008, and 2009). Divergence among years was tested using a null distribution of 2×10^4 permutations in which samples were shuffled among years, and divergence between adipose-clipped and unclipped fish was tested using a null distribution of the same size in which individuals were shuffled between these categories with the program ARLEQUIN (Excoffier *et al.* 2005).

Table 2. Collections of summer and fall Chinook salmon used for population structure analysis. The hatchery-origin or wild-origin status of each collection (H/W) is listed, along with sample sizes (N). Estimates of expected heterozygosity (H_e), observed heterozygosity (H_o), numbers of loci exhibiting departures from Hardy-Weinberg equilibrium (HWE) before / after corrections for multiple tests, numbers of loci pairs exhibiting genotypic disequilibrium (GD) before / after corrections for multiple tests, and average allelic richness (A_R) are given for each collection.

Number	Description	H/W	N	Source [†]	H_e	H_o	HWE	GD	A_R
1	Entiat River Carcass*	H	55	1	0.860	0.861	1/0	17/4	12.9
2	Entiat River Carcass*	W	202	1	0.856	0.838	0/0	1/1	12.6
3	Entiat River Carcass*	U	15	1	0.833	0.810	0/0	2/0	12.1
4	Entiat NFH broodstock	H	88	1	0.868	0.870	2/0	9/1	12.9
5	Entiat NFH broodstock	H	99	1	0.864	0.870	3/1	31/14	12.4
6	Eastbank Hatchery	H	207	2	0.860	0.865	1/0	1/1	12.6
7	Methow River	H	44	2	0.852	0.855	2/0	12/2	12.7
8	Methow River	W	282	2	0.855	0.851	1/0	7/3	12.5
9	Okanogan River	H	174	2	0.859	0.868	6/3	30/10	12.6
10	Okanogan River	W	351	2	0.859	0.857	0/0	9/1	12.6
11	Wells Hatchery	H	254	2	0.867	0.867	2/0	8/1	12.6
12	Wenatchee River	H	153	2	0.856	0.841	7/4	19/2	12.4
13	Wenatchee River	W	466	2	0.851	0.850	1/0	13/4	12.5
14	Hanford Reach	W	204	3	0.875	0.865	1/0	5/1	13.3
15	Lyons Ferry Hatchery	H	184	3	0.864	0.853	2/1	10/2	12.4
16	Priest Rapids Hatchery	H	81	3	0.872	0.859	1/1	2/1	13.0
17	Umatilla Hatchery	H	189	3	0.870	0.872	2/0	18/5	13.1

*The Entiat River Carcass collections from Table 1 were pooled into three collections for the population structure analysis.

[†]1 = present study, 2 = Kassler *et al.* 2011, 3 = GAPS baseline.

Pairwise tests of divergence among groups were performed on pools of collections (divergence among return years was tested by pooling adipose-clipped and unclipped fish within each year, divergence between adipose-clipped and unclipped fish was done by pooling across years). The log likelihood ratio statistic (G test) was used to test for allele frequency differences between each pair of collections with the program GENEPOP (Rousset 2008). Settings for Markov chain were: dememorization number = 10^4 , number of batches = 10^3 , and iterations per batch = 5×10^3 . Pairwise estimates of F_{ST} (Weir and Cockerham 1984) were calculated for each pair of collections. A null distribution was generated by permuting individuals among collections to generate 10^4 replicate data sets, and significance of each estimate was assessed by comparing the observed statistic to the null distribution (Belkhir *et al.* 2004). For all tests of divergence $\alpha = 0.05$.

All of the methods described above were based on groups of individuals and thus made the assumption that collection data were biologically meaningful (i.e. that when a fish was sampled or whether or not it had an adipose fin was informative regarding population structure). In contrast, the model developed by Pritchard *et al.* (2000; Falush *et al.* 2003) and implemented in the program STRUCTURE is based on individual genotypes and does not require the above assumption. We ran STRUCTURE for k (the number of populations) from one thru ten, with ten replicate runs per value of k. For each replicate, the MCMC was run for 15×10^4 iterations, with the first 5×10^4 being used as a burn-in. Posterior probabilities of the model at each value of k were assessed following Pritchard *et al.* (2000).

Divergence between summer Chinook salmon from the Entiat River and summer and fall Chinook salmon from adjacent rivers.

In order to compare adipose-clipped and unclipped summer Chinook from the Entiat River to summer and fall Chinook salmon from other rivers, these categories were pooled across years (Table 2, top two rows). Expected (H_e) and observed (H_o) heterozygosity were calculated for each collection using the program GDA (Lewis and Zaykin 2001). Testing for genotypic ratios that departed from Hardy-Weinberg Equilibrium (HWE) was conducted using Fisher's exact tests in GENEPOP. The log likelihood ratio statistic was used to test for genotypic disequilibrium (composite linkage disequilibrium; Weir 1979) between each pair of loci in each collection. Genetic diversity within each collection was measured as allelic richness (AR), the number of alleles observed per collection, corrected via rarefaction for unequal numbers of fish per collection. Allelic richness values for each collection were averaged across loci.

Correspondence analysis was performed using the program GENETIX in order to facilitate visual evaluation of divergence among summer and fall Chinook salmon from the collections listed in Table 2. Allele frequency heterogeneity tests and F_{ST} significance tests were performed on these collections exactly as described above.

Results and Discussion

Data evaluation

Whether a sample was from a live fish or a carcass appears to have been a greater predictor of genotyping success than whether fin tissue or scales were taken (Table 1). Our genotyping success rate was lower for carcass samples (41 – 65%) than for fresh samples (99-100%).

Among carcass samples our mean genotyping success rate was modestly higher for fin tissue (56%) than for scales (49%). The thirteen microsatellites examined here exhibited a total of 420 alleles in the summer and fall Chinook salmon collections listed in Table 2, with between 9 and 53 alleles per locus. In summer Chinook salmon samples from the Entiat River, we observed 361 alleles with between 7 and 48 alleles per locus (Appendix 1). Our QA/QC revealed a total of 4 scored conflicts out of 803 genotypes analyzed twice, resulting in a rate of 5.0×10^{-3} .

Assuming errors were equally likely to occur in the first and second runs, this gives an estimated error rate of 2.5×10^{-3} .

A scan for duplicate genotypes revealed five pairs and a single trio of individuals with identical genotypes. Each group of identical genotypes was from a single collection year. The probability of two fish having identical genotypes is exceedingly small, and such results generally reflect sample collection error (two clips taken from the same fish, or cross-contamination) or genotyping error (e.g., PCR contamination or file handling error). We deleted all but the first instance of each genotype, thus removing seven individuals.

Table 3. Results of genetic assignment tests. The genetic reporting groups used were described by Seeb et al. (2007).

Genetic Reporting Group	Number of fish	Mean assignment probability	Standard deviation
Lower Columbia River fall	1	1.000	NA
Upper Columbia River spring	8	0.971	0.066
Snake River fall	1	0.719	NA
Snake River spring/summer	5	0.905	0.096
Upper Columbia River summer/fall	271	0.992	0.038

Genetic assignment tests resulted in most (~95%) of the carcass samples being assigned to Upper Columbia River summer/fall. Two samples were assigned to other fall runs (Lower Columbia River fall and Snake River fall), eight were assigned to Upper Columbia River spring, and five were assigned to Snake River spring/summer (Table 3). When assignment tests were performed on the Entiat NFH broodstock samples, 185 assigned to Upper Columbia River summer/fall and 2 assigned to Snake River fall (results not shown). Upper Columbia River summer/fall run are very distinct from Upper Columbia Spring run and from Snake River spring/summer (Utter et al. 1995; Waples et al. 2004; Narum et al. 2010), making mis-assignment extremely unlikely. The high probability of the assignment of the one individual to Lower Columbia River fall (1.000) gives us good confidence in this result also. Genetic divergence between Upper Columbia River summer/fall and Snake River fall is lower than divergence between either of these and Upper Columbia River spring or Snake River spring/summer (Waples et al. 2004; Narum et al. 2010), so the probability of mis-assignment between the former two is relatively greater. Moreover, given the relatively low probability score for the individual assigned to Snake River fall (0.719) and the fact that Entiat NFH broodstock fish occasionally assign as Snake River fall, we decided not to omit the individual assigned as Snake River fall run. Thus a total of 14 individuals were removed from the data set based on the genetic assignment tests.

Population structure among summer Chinook salmon in the Entiat River

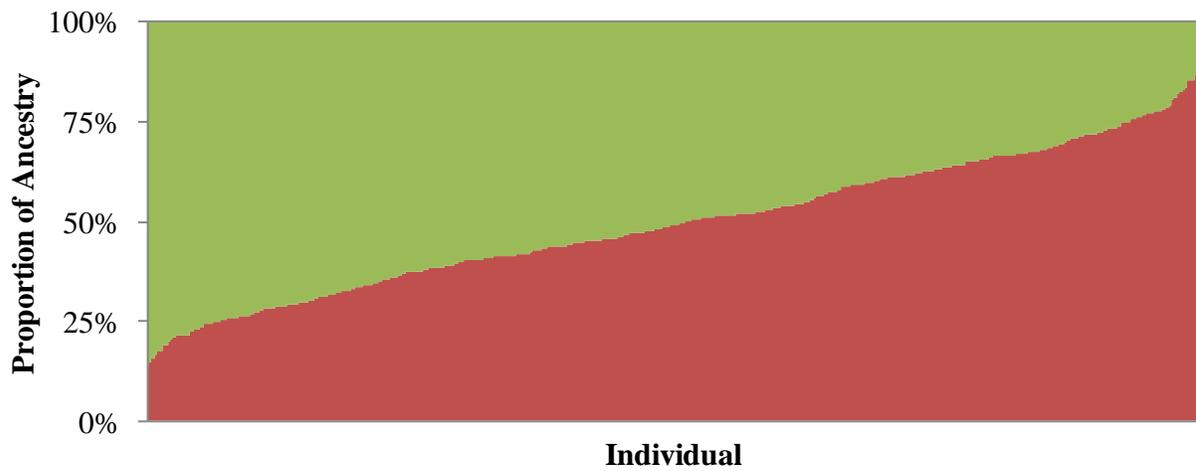
Analysis of molecular variance for the three years of samples (2000, 2008, and 2009) revealed that the proportion of genetic variance observed among years in the Entiat River summer Chinook salmon collections was small (0.13%) and non-significant ($p = 0.576$). Similarly, the

proportion of variance explained by separating these samples among adipose-clipped and unclipped groups explained a small (0.00%) and non-significant ($p = 0.757$) proportion of the observed variance. While these results indicate that our data revealed no population structure, the low sample sizes prohibit strong inferences.

Tests of allele frequency heterogeneity and F_{ST} significance among Entiat River summer Chinook salmon collections were performed on pooled samples in order to increase sample sizes. When samples were pooled into seven collection years (i.e. clipped and un-clipped fish from each year pooled) none of the 21 pairwise F_{ST} values were significant (p-values ranged from 0.202 – 0.972). One test of allele frequency heterogeneity was significant at $\alpha=0.05$, (2006 vs. 2010; $p = 0.026$), but was not significant after application of a standard Bonferroni correction for multiple comparisons ($\alpha=0.05/21= 0.002$). P-values for other pairwise tests ranged from 0.061 – 0.951. When samples were pooled across years into adipose-clipped and unclipped categories, F_{ST} was 0.000 ($p = 0.556$) and the heterogeneity test was non-significant ($p=0.928$). Tests of allele frequency heterogeneity and F_{ST} significance are two of the most reliable tools available for using genetic data to identify population structure (see Waples and Gaggiotti 2006 for a discussion). Our observation of a single marginally-significant heterogeneity test and no significant F_{ST} values suggest that population structure was not detectable with the present data.

Results of the STRUCTURE analysis also did not indicate population structure among the Entiat River summer Chinook salmon samples. Posterior probabilities for increasing values for k (where k = the number of putative populations) did not show a trend of increase or decrease for

Figure 1. Results of STRUCTURE analysis of Entiat River summer Chinook salmon samples with the number of populations (k) set to two. Each vertical line represents an individual fish, and the proportion of each color in that vertical line indicates the proportion the respective lineage. Individuals were sorted in order of increasing proportion of ancestry in the second (red) lineage.



values from 1-10. Even under the simplest scenario for population structure ($k=2$), most individuals were assigned about half of their proportion of ancestry (Q) in each of the two populations (mean value of $Q = 0.495$, stdev = 0.167; Figure 1), and no individuals had all ($\geq 90\%$) of their ancestry assigned to a single lineage. For values of k greater than two, similar patterns were observed, with each individual having some proportion ancestry assigned to each lineage. This is the result expected under the STRUCTURE model in the case that the value of k being set in the model is greater than the true value of k . As was the case for the population-based metrics, the individual based model implemented in STRUCTURE failed to reveal population structure among Entiat River summer Chinook salmon.

Divergence between summer Chinook salmon from the Entiat River and summer and fall Chinook salmon from adjacent rivers.

Diversity statistics (H_e , H_o , AR) did not indicate any more or less genetic diversity among Entiat River summer Chinook than among other summer and fall populations (Table 2). Genotypic disequilibrium was high in some populations, including the wild summer Chinook from Entiat River (17/78 pairs of loci at $\alpha=0.05$). This may indicate family structure (disproportionate representation of specific families across years), as is typical in small populations or populations composed largely of strays.

Correspondence analysis clustered both the adipose-clipped and unclipped Entiat River summer Chinook salmon very near one another, and near hatchery populations from the Methow,

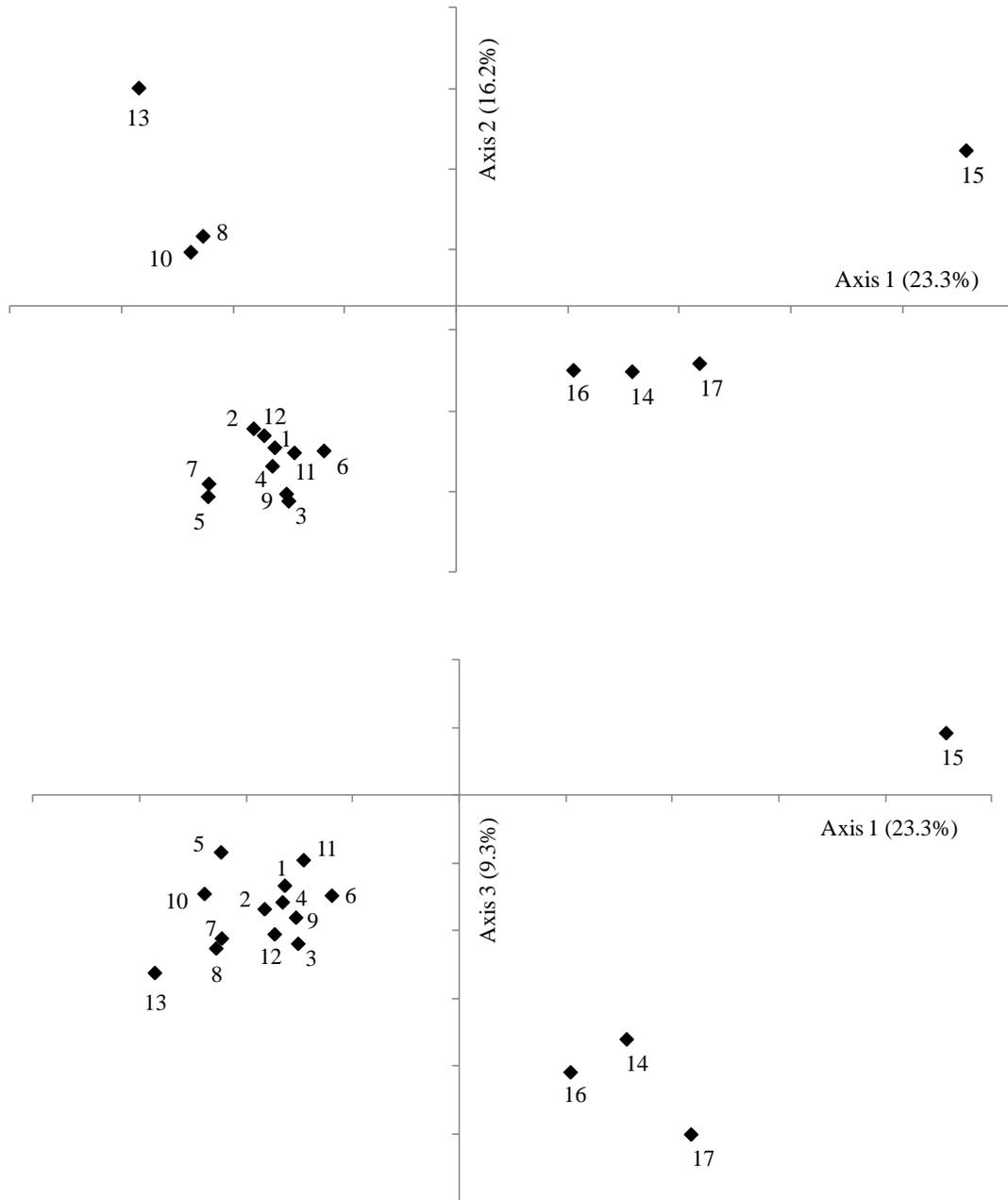


Figure 2. Correspondence analysis of the 17 collections of Chinook salmon listed in Table 2. The first axis separates the summer (1-13) from the fall collections (14-17), the second axis separates Methow (8), Okanogan (10) and Wenatchee (13) wild collections from the other summer collections. The second and third axes both separate Lyons Ferry Hatchery (15) from the other fall collections.

Wenatchee, and Okanogan rivers (Figure 2). The first axis of our correspondence analysis separated the summer populations from fall populations, similar to the primary distinction revealed by the neighbor-joining analysis reported by Kassler et al. (2011). The second axis revealed divergence within these two groups, and the third axis revealed further divergence among fall populations. The summer Chinook salmon populations identified as most divergent by this analysis were wild-origin fish from the Wenatchee, Methow and Okanogan Rivers.

Estimates of F_{ST} between Entiat River and other summer populations ranged from 0.000-0.004 (Table 4). If we ignore the Entiat River unknown samples (unknown whether or not they were adipose-clipped), which had a sample size of only 15, then the range of F_{ST} s between Entiat River and other fall populations was 0.000-0.002. Neither F_{ST} s nor allele frequency heterogeneity tests indicated significant ($\alpha=0.05$) divergence between collections from the Entiat River, the Methow River, or Okanogan River. Based only on the present data, we could thus not identify these three as different populations.

In conclusion, we found no evidence of genetic divergence between adipose-clipped and unclipped summer Chinook salmon spawning in the Entiat River, and no evidence of temporal structure among these fish between 2000 and 2010. We further found no evidence that these fish were more distinct from local hatchery populations of summer Chinook salmon, than were wild-spawning populations in the Wenatchee, Methow and Okanogan rivers. Similar to past genetic analyses of Chinook salmon within the Upper Columbia River Summer/Fall-run ESU, our results

Table 4. Pairwise estimates of divergence between collections of summer and fall Chinook salmon. Estimates of F_{ST} are given above diagonal, and p-values for pairwise tests of allele frequency heterogeneity are given below diagonal. Shaded cells indicate non-significant test results ($\alpha=0.05$). Tests with $\chi^2 = \infty$ are listed as HS (highly significant).

	Entiat R. clip	Entiat R. unclip	Entiat R. unk	Entiat NFH 2009	Entiat NFH 2010	Eastbank H	Methow R. H	Methow R. W	Okanogan R. H	Okanogan R. W	Wells H	Wenatchee R. H	Wenatchee R. W	Hanford Reach W	Lyons Ferry H	Priest Rapids H	Umatilla H
Entiat R. clip	-	0.000	0.004	0.000	0.002	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.004	0.015	0.001	0.004
Entiat R. unclip	0.928	-	0.003	0.001	0.002	0.001	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.006	0.019	0.003	0.007
Entiat R. unk	0.281	0.341	-	0.004	0.006	0.005	0.007	0.006	0.005	0.005	0.007	0.005	0.005	0.009	0.023	0.007	0.009
Entiat NFH 2009	0.760	0.013	0.587	-	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.006	0.018	0.003	0.006
Entiat NFH 2010	0.003	0.000	0.002	0.001	-	0.003	0.004	0.003	0.003	0.003	0.002	0.003	0.004	0.008	0.019	0.005	0.008
Eastbank H	0.116	0.007	0.153	0.002	HS	-	0.002	0.001	0.000	0.001	0.001	0.001	0.001	0.003	0.014	0.002	0.004
Methow R. H	0.770	0.182	0.062	0.171	0.000	0.455	-	0.001	0.003	0.001	0.003	0.001	0.002	0.010	0.025	0.007	0.011
Methow R. W	0.704	0.195	0.021	0.003	0.000	0.001	0.145	-	0.001	0.001	0.001	0.000	0.000	0.005	0.018	0.003	0.006
Okanogan R. H	0.633	0.000	0.018	0.003	0.000	0.588	0.037	0.000	-	0.001	0.001	0.001	0.001	0.004	0.017	0.002	0.005
Okanogan R. W	0.012	0.000	0.071	0.000	HS	0.007	0.155	0.000	0.000	-	0.002	0.001	0.002	0.006	0.019	0.004	0.007
Wells H	0.212	0.000	0.018	0.053	0.001	0.000	0.013	0.000	0.000	0.000	-	0.002	0.002	0.005	0.014	0.003	0.005
Wenatchee R. H	0.651	0.221	0.115	0.005	0.000	0.040	0.317	0.210	0.000	0.000	0.000	-	0.000	0.005	0.018	0.002	0.006
Wenatchee R. W	0.390	0.203	0.069	0.000	0.000	0.000	0.018	0.448	0.000	HS	HS	0.498	-	0.006	0.019	0.003	0.006
Hanford Reach W	0.000	HS	0.010	0.000	HS	HS	0.000	HS	0.000	HS	HS	0.000	HS	-	0.008	0.000	0.000
Lyons Ferry H	HS	HS	0.000	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	-	0.009	0.007
Priest Rapids H	0.216	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	HS	0.000	0.000	0.000	0.049	HS	-	0.000
Umatilla H	0.000	HS	0.009	0.000	HS	HS	0.000	HS	HS	HS	HS	HS	HS	0.005	HS	0.054	-

revealed very little distinction among populations from different rivers (e.g., Utter *et al.* 1995; Waples *et al.* 2004; Narum *et al.* 2010; Kassler *et al.* 2011). The homogeneity of these populations is thought to reflect the history of confinements, translocation and cultural activities performed following the construction of Grand Coulee Dam (Utter *et al.* 1995). Whether or not a distinct population of summer Chinook salmon once existed in the Entiat River is not known, but our data provide no evidence that one exists presently. The ability of a population to respond to natural selection in a local environment is reduced as the number of strays to that population increases. Therefore, it is not only unsurprising that we did not find a distinct locally-adapted population, but it is unlikely that one could arise given the high proportion of strays (~30%; USFWS 2007) on the spawning grounds.

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DATA AVAILABILITY

Data for Entiat River collections are available from the authors upon request. Data from the GAPS baseline and The State of Washington may be obtained from the original respective sources.

LITERATURE CITED

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Appendix 1. Thirteen microsatellite loci used to analyze summer Chinook salmon from the Entiat River. Number of alleles indicates the number observed in Entiat River samples, not the entire baseline.

Locus	Primer Sequence (5' to 3')	Alleles	Citation
<i>Ots201b</i>	F- CAGGGCGTGACAATTATGC R- TGGACATCTGTGCGTTGC	42	unpublished
<i>Ots208b</i>	F- GGATGAACTGCAGCTTGTTATG R- GGCAATCACATACTTCAACTTCC	48	(Greig <i>et al.</i> 2003)
<i>Ots211</i>	F - TAGGTTACTGCTTCCGTCAATG R - GAGAGGTGGTAGGATTTGCAG	30	(Greig <i>et al.</i> 2003)
<i>Ots212</i>	F- TCTTTCCCTGTTCTCGCTTC R- CCGATGAAGAGCAGAAGAGAC	27	(Greig <i>et al.</i> 2003)
<i>Ogo4</i>	F- GTCGTCACTGGCATCAGCTA R- GAGTGGAGATGCAGCCAAAG	15	(Olsen <i>et al.</i> 1998)
<i>Ogo2</i>	F- ACATCGCACACCATAAGCAT R- GTTTCTTCGACTGTTTCTCTGTGTTGAG	17	(Olsen <i>et al.</i> 1998)
<i>Ots3M</i>	F- TGTCACTCACACTCTTTCAGGAG R- GAGAGTGCTGTCCAAAGGTGA	13	(Banks <i>et al.</i> 1999)
<i>Ots213</i>	F- CCCTACTCATGTCTCTATTTGGTG R- AGCCAAGGCATTTCTAAGTGAC	37	(Greig <i>et al.</i> 2003)
<i>Omm1080</i>	F- GAGACTGACACGGGTATTGA R- GTTATGTTGTCATGCCTAGGG	47	(Rexroad <i>et al.</i> 2001)
<i>Ssa408UOS</i>	F- AATGGATTACGGGTACGTTAGACA R- CTCTTGTGCAGGTTCTTCATCTGT	26	(Cairney <i>et al.</i> 2000)
<i>Ots9</i>	F- ATCAGGGAAAGCTTTGGAGA R- CCCTCTGTTACAGCTAGCA	7	(Banks <i>et al.</i> 1999)
<i>OtsG474</i>	F- TTAGCTTTGGACATTTTATCACAC R- CCAGAGCAGGGACCAGAAC	12	(Williamson <i>et al.</i> 2002)
<i>Oki100</i>	F- CCAGCACTCTCACTATTT R- CCAGAGTAGTCATCTCTG	40	unpublished