APPENDIX H

Genetic Diversity of Natural Chiwawa River Spring Chinook Salmon, 2007.

Assessing the Genetic Diversity of Natural Chiwawa River Spring Chinook Salmon and Evaluating the Effectiveness of its Supportive Hatchery Supplementation Program

Developed for

Chelan County PUD

and the

Habitat Conservation Plan's Hatchery Committee

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March 30, 2007

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Executive Summary

The main objective of this study was to determine the potential impacts of the Chiwawa River Supplementation Program on natural spring Chinook in the upper Wenatchee system. We did this by investigating population differentiation between temporally replicated Chiwawa River natural and hatchery samples from the Wenatchee River watershed using microsatellite DNA allele frequencies and the statistical assignment of individual fish to specific populations. Additionally, to assess the genetic effect of the hatchery program, we investigated the relationship between census and effective population sizes using collections obtained before and after the supplementation program. In this summary, we briefly describe the salient results contained within this report; however, each "Task" within the Results/Discussion section below contains extended coverage for each topic along with an expanded interpretation of each result.

Overall, we observed substantial genetic diversity within collections, with heterozygosities equal to roughly 80%, over thirteen microsatellite markers. Microsatellite allele frequencies among temporally replicated collections from the same population (i.e., location) were variable, resulting in significant genetic differentiation among these collections. However, these difference are likely the result of salmon life history in this area, as four-year-old Chinook comprise a majority of returns each year. That is, the genetic tests are detecting the differences of contributing parents from each cohort, rather than a hatchery effect.

Analysis of Chiwawa River Collections

To assess the multiple competing hypotheses regarding population differentiation within and among Chiwawa River collections, we found it necessary to organized the Chiwawa genetic data into three data sets: (1) fish origin (hatchery versus natural), (2) spawning location (hatchery broodstock versus in-river (natural) spawners), and (3) four "treatment" groups (1. hatchery-origin hatchery broodstock, 2. hatchery-origin natural spawner, 3. natural-origin natural spawner, and 4. natural-origin hatchery broodstock). We conducted separate analyses using each of the three data sets, with each analysis

touching on some aspect of the components necessary to move through the Conceptual Process outlined by Murdoch and Peven (2005).

Origin Dataset – We report that allele frequencies within and between natural- and hatchery-origin collections are significantly different, but there does not appear to be a robust signal indicating that the recent natural-origin collections have diverged greatly from the pre- or early post-supplementation collections. Genetic drift will occur in all populations, but does not appear to be a major factor affecting allele frequencies within the Chiwawa collections.

Spawning Location Dataset – There are significant allele frequency differences within and between hatchery broodstock and natural spawner collections. However, in recent years the allele frequency differences between the hatchery broodstock and natural spawner collections have declined. Furthermore, based on linkage disequilibrium, there is a genetic signal that is consistent with increasing homogenization of allele frequencies within hatchery broodstock collections, but a similar homogenization within the natural spawner collection is not apparent. These data suggest that there exists consistent year-to-year variation in allele frequencies among hatchery and natural spawning collections, but there is a trend toward homogenization of the allele frequencies of the natural- and hatchery-origin fish that compose the hatchery broodstock.

Four Treatment dataset – Although there are signals of allelic differentiation among Chiwawa River collections, there are no robust signs that these collections are substantially different from each other. We used two different analyses to measure the degree of genetic variation that exists among individuals and collections within the Chiwawa River. First, we conducted a principal component analysis using all Chiwawa samples with complete genotypes (i.e., no missing alleles from any locus). Although the first two principal component axes account for only 10.5% of the total molecular variance, a substantially greater portion of that variance is among individual fish, regardless of their identity, rather than among hatchery and natural collections. The

variances in principal component scores among individuals are 11 and 13 times greater than the variance in scores among collections.

Secondly, using an Analysis of Molecular Variance (AMOVA), we were able to determine how best to group populations, with "best" being defined as that grouping that accounts for the greatest proportion of among group (i.e., population) variance. Furthermore, by partitioning molecular variance into different hierarchical components, we are able to determine what level accounts for the majority of the molecular variance. The AMOVA results clearly show that nearly all molecular variation, no matter how the data are organized, resides within a collection. The percentage of total molecular variance occurring within collections ranged from 99.68% to 99.74%. These results indicate that the significant differences among collections of Chiwawa fish account for less than one percent of the total molecular variance, and these differences cannot be attributed to fish origin or spawning location.

Effective Population Size (N_e)

The contemporary estimate of N_e calculated using genetic data combined for Chiwawa natural-origin spawners (NOS) and hatchery-origin spawners (HOS) Chinook is N_e=386.8, which is slightly larger than the pre-hatchery N_e we estimated using demographic data from 1989 – 1992. Additionally, the N_e /N ratio calculated using 386.8 for N_e and the arithmetic mean yearly census of NOS and HOS Chinook from 1989 – 2005 for N is 0.40. These results suggest the N_e has not declined during the period of Chiwawa Hatchery Supplementation Program operation.

Analysis Of Upper Wenatchee Tributary Collections

We compared genetic data for spring Chinook collected from the major spawning aggregates of the Wenatchee River. We observed significant differences in allele frequencies among temporally replicated collections within populations, and among populations within the upper Wenatchee. However, these differences account for a very small portion of the overall molecular variance, and these populations overall are very similar to each other. Of all the populations within the Wenatchee River, the White River

appears to be the most distinct. Yet, this distinction is more a matter of detail than of large significance, as the median F_{ST} between White River collections and all other collections (except the Little Wenatchee collection; see Results/Discussion) is less than 1.5% among population variance. We consider the implications of these results in the Conclusion section that follows the Results/Discussion section. Additionally, there is no evidence that the Chiwawa River Supplementation Program has changed the allele frequencies in the Nason Creek and White River populations, despite the presence of hatchery-origin fish in both these systems.

Introduction

Murdoch and Peven (2005) outlined 10 objectives to assess the impact (positive or negative) of hatchery operations mitigating the operation of Rock Island Dam. Two objectives relate to monitoring the genetic integrity of populations:

Objective 3: Determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery program. Additionally, determine if hatchery programs have caused changes in phenotypic characteristics of natural populations.

Objective 5: Determine if the stray rate of hatchery fish is below the acceptable levels to maintain genetic variation between stocks.

This study addresses Objective 3 (above), and documents analyses and results WDFW completed for populations of spring Chinook (*Oncorhynchus tshawytscha*) in the Wenatchee River watershed. This study was not intended to specifically address Objective 5 (above); however, genetic data provide results relevant to Objective 5. The critical component of Objective 3 is to determine if hatchery supplementation has effected change. Furthermore, change in this context means altering census size and/or genetic marker allele frequencies; we did not attempt to measure changes in fitness. Perhaps a more meaningful rewording of Objective 3 is, "Did the hatchery supplementation program succeed at increasing the census size of a target population while leaving genetic integrity intact?" In order to evaluate cause and effect of hatchery supplementation, we surveyed and compared genetic variation in samples collected before and after potential effects from the Chiwawa Hatchery Supplementation Program. Samples were acquired from the primary spawning aggregates in the upper Wenatchee River watershed: Nason Creek, Little Wenatchee River, White River, and Chiwawa River. Hatchery samples were acquired from programs that could potentially affect genetic composition of Wenatchee stocks, the integrated Chiwawa River stock (local stock), Leavenworth National Fish Hatchery spring Chinook (Carson Stock – non local), and Entiat NFH (Carson Stock – non local). Additionally, the genetic markers used were the Genetic Analysis of Pacific Salmonids (GAPS) (Seeb et al. in review) standardized

microsatellites, so all data from the Wenatchee study will be available for inclusion in the GAPS Chinook coastwide microsatellite baseline.

History of Artificial Propagation

Artificial propagation in the upper Columbia River began in 1899 when hatcheries were constructed on the Wenatchee and Methow rivers (Mullan 1987). These initial operations were small, with the Tumwater Hatchery on the Wenatchee River releasing several hundred thousand fry, and the Methow River hatchery producing few Chinook salmon before it was closed in 1913 (Craig and Suomela 1941, Nelson and Bodle 1990). The Leavenworth State Hatchery operated in the Wenatchee River Basin between 1913 and 1931 using eggs from non-native stocks (Willamette River spring-run and lower Columbia Chinook hatchery fall-run). These early attempts at hatchery production were largely unsuccessful for spring-run Chinook (WDF 1934). Between 1931 and 1939, no Chinook salmon hatcheries were in operation above Rock Island Dam (Rkm 730).

In 1938, the last salmon was allowed to pass upstream through the uncompleted Grand Coulee Dam (Rkm 959). To mitigate the loss of habitat, adult Chinook salmon were trapped, under the auspices of the Grand Coulee Fish Maintenance Project (GCFMP), at Rock Island Dam beginning in May 1939, and relocated into three of the remaining accessible tributaries to the upper Columbia River: the Wenatchee, Entiat, and Methow Rivers. GCFMP transfers continued through the autumn of 1943. Spring- and summer/fall-run fish were differentiated at Rock Island Dam based on a 9 July cutoff date for Chinook arrivals at Rock Island Dam (Fish and Hanavan 1948). Spring-run adults collected at Rock Island Dam (pre 9 July fish) were either transported to Nason Creek on the Wenatchee River to spawn naturally (1939-43), or to the newly constructed Leavenworth NFH (1940) for holding and subsequent spawning (1940-43). Eggs were incubated on site or transferred to the Entiat NFH (1941) and Winthrop NFH (1941). In 1944 spring-run adults were allowed to freely pass Rock Island Dam. The GCFMP did not differentiate among late-run stocks (post 9 July fish) passing Rock Island Dam. Late-run offspring reared at the Leavenworth NFH, Entiat NFH, and Winthrop NFHs were an

amalgamation of summer and fall upper Columbia River populations (Fish and Hanavan 1948). Late-run fish were transplanted into the upper and lower Wenatchee, Methow, and Entiat Rivers.

After 1943, the Winthrop NFH continued to use local spring-run Chinook for hatchery production, while the other NFHs largely focused on summer-run Chinook salmon. Renewed emphasis on spring run production in the mid-1970s saw the inclusion of local and non-local eggs (Carson NFH stock, Klickitat River stock, and Cowlitz River stock) to the NFHs. In the early 1980s, imports of non-native eggs were reduced significantly, and thereafter the Leavenworth, Entiat, and Winthrop NFHs have relied on adults returning to their facilities for their egg needs (Chapman et al. 1995). Regarding late-run Chinook, due to the variety of methods employed to collect broodstock at dams, hatcheries, or the result of juvenile introductions into various areas, Chinook populations and runs (i.e., summer and fall) have been mixed considerably in the upper Columbia system over the past five decades (reviewed in Chapman et al. 1994).

Washington Department of Fish and Wildlife (WDFW) operates two facilities producing spring-run Chinook, the Methow Fish Hatchery (MFH) owned by Douglas County PUD that began operation in 1992 and Eastbank Fish Hatchery (EFH) owned by Chelan County PUD that began operation in 1989. Both programs were designed to implement supplementation (supportive breeding) programs for naturally spawning populations on the Methow and Wenatchee Rivers, respectively (Chapman et al. 1995). As part of the Rock Island Mitigation Agreement between Chelan County Public Utility District and the fishery management parties (RISPA 1989), a supplementation (supportive breeding) program was initiated in 1989 on the Chiwawa River to mitigate smolt mortality resulting from the operation of Rock Island Hydroelectric Project. EFH uses broodstock collected at a weir on the Chiwawa River, although in recent years hatchery fish have been collected at Tumwater Dam. Similarly, the MFHC uses returning adults collected at weirs on the Methow River and its tributaries, the Twisp and Chewuch Rivers (Chapman et al. 1995; Bugert 1998). Although low run size and trap efficiency has resulted in most broodstock being collected from the hatchery outfall or in some years Wells Dam,

progeny produced from these programs are reared at and released from satellite sites on the tributaries where the adults were collected. Numerous other facilities have reared spring-run Chinook salmon on an intermittent basis.

Previous Genetic Studies – Population differentiation

Waples et al. (1991a) examined 21 polymorphic allozyme loci in samples from 44 populations of Chinook salmon in the Columbia River Basin. These authors reported three major clusters of Columbia River Basin Chinook salmon: 1) Snake River spring- and summer-run Chinook salmon, and mid and upper Columbia River spring-run Chinook salmon, 2) Willamette River spring-run Chinook salmon, 3) mid and upper Columbia River fall- and summer-run Chinook salmon, Snake River fall-run Chinook salmon, and lower Columbia River fall- and spring-run Chinook salmon. Utter et al. (1995) examined allele frequency variability at 36 allozyme loci in samples of 16 upper Columbia River Chinook populations. Utter et al. (1995) indicated that spring-run populations were distinct from summer- and fall-run populations, where the average genetic distance between spring-run and late-run Chinook were about eight times the average of genetic distances between samples within each group. Additionally, allele frequency differences among spring-run populations were considerably greater than that among summer- and fall-run populations in the upper Columbia River. Utter et al. (1995) also reported hatchery populations of spring-run Chinook salmon were genetically distinct from natural spring-run populations, but hatchery populations of fall-run Chinook salmon were not genetically distinct from natural fall-run populations.

As part of an evaluation of the relative reproductive success for the Chiwawa River supplementation program, Murdoch et al. (2006), used eleven microsatellite loci to assess population differentiation among spring Chinook salmon population samples in the upper Wenatchee River. Murdoch et al. (2006) reported a >99% accuracy of correctly identifying spring-run and fall-run Chinook from the Wenatchee River. They also reported slight, but significantly different genetic variation among wild spring populations and between wild and hatchery stocks. Yet, since the spring-run populations

are genetically similar, identifying individuals genetically from the upper tributaries of the Wenatchee River was difficult. This result is exemplified in their individual assignment results, where < 8% of spring-run individuals, hatchery or wild, were correctly assigned using their criterion of an LOD (log of odds) score greater than 2. Murdoch et al. (2006) also reported contemporary natural spring Chinook show heterozygote deficit and low linkage disequilibrium (LD), while contemporary hatchery spring Chinook show heterozygote excess and high LD.

Williamson et al. (submitted) have continued the work of Murdoch et al. (2006) by analyzing Chiwawa River demographic data from 1989 – 2005 to estimate the proportions of recruits that were produced by Chinook with hatchery or wild origin. In an "ideal" population, the genetic size (i.e., effective size or N_e) and the census size are equal; however various demographic factors such as unequal sex ratios and variance in reproductive success among individuals reduces the genetic size below the census size. It is generally thought that the genetic size is approximately 10-33% the census size (Bartley et al. 1992; RS Waples pers. comm.), although values have been reported outside this range (Araki et al. 2007; Arden and Kapuscinski 2003; Heath et al. 2002). Despite being difficult to estimate, the effective population size in many respects is a more important parameter to know than census size, because N_e determines how genetic diversity is distributed within populations and how the forces of evolution (i.e., forces that change genetic diversity over time) will affect the genetic variation present.

Williamson et al. (submitted) used demographic data to 1) investigate the effect of unequal sex ratio on genetic diversity, 2) investigate the effect of variation in reproductive success on genetic diversity, 3) investigate the effect of fluctuations in population size on genetic diversity, and 4) estimate the effective population size, using the inbreeding method (Ryman and Laikre 1991). Most importantly, they use demographic data from 1989 – 2000 to assess the impact of the Chiwawa Hatchery Supplementation Program on the effective population size of natural-origin Chiwawa River spring Chinook. They estimate that the N_e of naturally spawning Chiwawa Chinook (i.e., both hatchery- and wild-origin fish on the spawning grounds) from 1989 –

1992 was N_e = 2683 and in 1997 – 2000 was N_e = 989. They compare spawning ground N_e to estimates calculated from combined broodstock and naturally spawning Chinook demographic data. The combined inbreeding N_e estimate from 1989 – 1992 was N_e = 147 and in 1997 – 2000 was N_e = 490. Williamson et al. (submitted) argue that since the combined N_e estimate is lower than the naturally spawning estimate, the supplementation program has had a negative impact on the Chiwawa River N_e.

Williamson et al. (submitted) also present genetic data for Chinook recovered on spawning grounds in upper Wenatchee River tributaries in 2004 and 2005. These genetic data are derived from the Murdoch et al. (2006) study. They compare samples collected from Chiwawa River (i.e., hatchery and wild), White River, Nason Creek, and Leavenworth Hatchery. Additionally, they include a 1994 Chiwawa River wild smolt sample for comparison with the 2004 brood year. Williamson et al. (submitted) report statistically significant genetic differentiation among Chiwawa River, White River and Nason Creek. Additionally, they report that the 1994 and 2004 Chiwawa River wild samples are not statistically different, but the 2004 Chiwawa wild and hatchery collections are statistically different.

Study Objectives

This study investigated within and among population genetic diversity to assess the effect of the Chiwawa Hatchery's supplemental program on the natural Chiwawa River spring Chinook population. Differences among temporal population samples, the census size, heterozygosity, and allelic diversity were documented. We investigated population differentiation between the Chiwawa River natural and hatchery samples, and among all temporally replicated samples from the Wenatchee River watershed using microsatellite DNA allele frequencies and the statistical assignment of individual fish to specific populations. To assess the genetic effect of the hatchery program, correlation between census and effective population sizes were investigated using temporally replicated samples obtained before and after the supplementation program operation. To address the hypotheses associated with Objective 3 in Murdock and Peven (2005) we developed

eleven specific "Tasks" (Blankenship and Murdoch 2006), to which we analyzed specific genetic data. We present the results from these analyses specific to each individual Task.

Methods and Materials

Tissue collection and DNA extraction

We analyzed thirty-two population collections of adult spring Chinook salmon (*Oncorhynchus tshawytscha*) obtained from the Wenatchee River between 1989 and 2006 (Table 1). Nine collections of natural Chinook adults from the Chiwawa River (n=501), and nine collections of Chiwawa Hatchery Chinook (n=595) were collected at a weir located in the lower Chiwawa River. The 1993 and 1994 Chiwawa Hatchery samples are smolt samples from the 1991 and 1992 hatchery brood years, respectively. Additional samples were collected from upper Wenatchee River tributaries, White River, Little Wenatchee River, and Nason Creek. Six collections of natural White River Chinook (n=179), one collection from the Little Wenatchee (n=19), and six collections from Nason Creek (n=268) were obtained. Single collections were obtained for Chinook spawning in the mainstem Wenatchee River and Leavenworth National Fish Hatchery. An additional out-of-basin collection from Entiat River was also included in the analysis. Samples collected in 1992 or earlier are scale samples. All other samples were either fin clips or operculum punches, stored immediately in ethanol after collection. DNA was extracted from stored tissue using Nucleospin 96 Tissue following the manufacturer's standard protocol (Macherey-Nagel, Easton, PA, U.S.A.).

Laboratory analysis

We performed polymerase chain reaction (PCR) amplification on each fish sample using the 13 fluorescently end-labeled microsatellite marker loci standardized as part of the GAPS project (Seeb et al. in review). GAPS genetic loci are: *Ogo*2, *Ogo*4 (Olsen et al. 1998); *Oki*100 (unpublished); *Omm*1080 (Rexroad et al. 2001); *Ots*201b (unpublished); *Ots*208b, *Ots*211, *Ots*212, and *Ots*213 (Grieg et al. 2003); *Ots*3M, *Ots*9 (Banks et al.

1999); *Ots*G474 (Williamson et al. 2002); *Ssa*408 (Cairney et al. 2000). PCR reaction volumes were 10 µL, and contained 1 µL 10x PCR buffer (Promega), 1.0 µL MgCl2 (1.5 mM final) (Promega), 0.2 µL 10 mM dNTP mix (Promega), and 0.1 units/mL Taq DNA polymerase (Promega). Loci were amplified as part of multiplexed sets, so primer molarities and annealing temperatures varied. Multiplex one had an annealing temperature of 50°C, and used 0.37 Molar (M) *Oki*100, 0.35 M *Ots*201b, and 0.20 M *Ots*208b, and 0.20 M *Ssa*408. Multiplex two had an annealing temperature of 63°C, and used 0.10 M *Ogo*2, and 0.25 M of a non-GAPS locus (*Ssa* 197). Multiplex three had an annealing temperature of 56°C, and used 0.18 M *Ogo*4, 0.18 M *Ots*213, and 0.16 M *Ots*G474. Multiplex four had an annealing temperature of 53°C, and used 0.26 M *Omm*1080, and 0.12 M *Ots*3M. Multiplex five had an annealing temperature of 60°C, and used 0.30 M *Ots*212, 0.20 M *Ots*211, and 0.10 M *Ots*9. Thermal cycling was conducted on either a PTC200 thermal cycler (MJ Research) or GeneAmp 9700 (Applied Biosystems) as follows: 95°C (2 min); 30 cycles of 95°C for 30 sec., 30 sec. annealing, and 72°C for 30 sec.; a final 72°C extension and then a 10°C hold. PCR products were visualized by electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.7 (Applied Biosystems). Standardization of genetic data to GAPS allele standards was conducted following Seeb et al. (in review).

Genetic data analysis

Assessing within population genetic diversity - Heterozygosity measurements are reported using Nei's (1987) unbiased gene diversity formula (i.e., expected heterozygosity) and Hedrick's (1983) formula for observed heterozygosity. Both tests are implemented using the microsatellite toolkit (Park 2001). We used GENEPOP version 3.4 (Raymond and Rousset 1995) to assess Hardy-Weinberg equilibrium (HWE), where deviations from the neutral expectation of random associations among alleles are calculated using a Markov chain method (5000 iterations in this study) to obtain unbiased estimates of Fisher's exact test. Global estimates of F_{IS} according to Weir and Cockerham (1984) were calculated using GENEPOP version 3.4. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENEPOP version 3.4.

Linkage results for population collections are reported as the proportion of pairwise (locus by locus) tests that are significant (alpha = 0.01). Linkage disequilibrium is considered statistically significant if more than 5% of the pairwise tests based on permutation are significant for a collection.

Within- and among-population genetic differentiation – The temporal stability of allele frequencies within populations, and pairwise differences in allele frequencies among populations were assessed using several different procedures. First, we tested for differences in allele frequencies among populations defined in Table 1 using a randomization chi-square test implemented in GENEPOP version 3.4 (Raymond and Rousset 1995). This procedure tests for differences between pairs of populations where alleles are randomized between the populations (i.e., genic test). The null hypothesis for this test is that the allele frequency distributions between two populations are the same. A low p-value should be interpreted as the allele frequency distributions being compared are unlikely to be samples drawn from the same underlying distribution.

Second, to graphically describe allele frequency differences among populations we conducted a nonmetric multidimensional scaling analysis using allele-sharing distance matrices from two different data sets. Pairwise allele-sharing distances are calculated as 1 – (mean over all loci of the sums of the minima of the relative frequencies of each allele common to a pair of populations). To calculate the allele-sharing distances for each pair of populations we used PowerMarker v3.25 (Liu and Muse 2005). Nonmetric multidimensional scaling is a technique designed to construct an n-dimensional "map" of populations, given a set of pairwise distances between populations (Manly 1986). The output from this analysis is a set of coordinates along n-axes, with the coordinates specific to the number of n-dimensions selected. To simplify our analysis we selected a 2-dimensional analysis to represent the relative positions of each population in a typical bivariate plot. The goodness of fit between the original allele-sharing distances and the pairwise distances between all populations along the 2-dimensional plot is measured by a "stress" statistic. Kruskal (in Rohlf 2002) developed a five-tier guide for evaluating stress levels, ranging from a perfect fit (stress=0) to a poor fit (stress=0.40). We

conducted the nonmetric multidimensional scaling analysis for one data set containing Chiwawa natural- and hatchery-origin collections, and another data set containing Chiwawa broodstock and in-river spawner collections. We used the mdscale module in MATLAB R2006b (The Mathworks 2006) to generate the nonmetric multidimensional scaling coordinates.

We examined the geographic and temporal structure of populations in the upper Wenatchee (Chiwawa River, Nason Creek, and White River, only) using a series of analyses of molecular variance (AMOVAs). Here, we defined an AMOVA as an analysis of variance of allele frequencies, as originally designed by Cockerham (1969), but implemented in Arlequin v2.1 (Schneider et al. 2000). These analyses permit populations to be aggregated into groups, and molecular variance is then partitioned into within collections, among collections, but within groups, and among group components. With this approach, we were able to determine how best to group populations, with "best" being defined as that grouping that accounts for the greatest proportion of among group variance. Furthermore, by partitioning molecular variance into three different hierarchical components, we are able to determine what level accounts for the majority of the molecular variance.

Finally, we explored the partitioning of molecular variance between among-individuals and among-populations using a principal component analysis and multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984). Principal component analysis is a data-reduction technique whereby the correlation structure among variables can be used to combine variables into a series of multivariate components, with each original variable receiving a weighted value for each component based on its correlation with that component. Here, we used a program written by Warheit in MATLAB R2006b (The Mathworks 2006) that treats each allele for each locus as a single variable (13 loci = 26 alleles or variables), and these 26 "variables" were arranged into 26 components, with each component accounting for a decreasing amount of molecular variance. Estimates of F_{ST} were calculated using GENETIX version 4.05 (Belkhir et al.1996). To determine if the F_{ST} estimates were

statistically different from random (i.e., no structure), 1000 permutations were implemented in GENETIX version 4.05 (Belkhir et al.1996).

Effective population size (N_e) – Estimates of the effective population size were obtained using two methods, a multi-collection temporal method (Waples 1990), and a single-collection method (Waples 2006) using linkage disequilibrium data. The temporal method assumes that cohorts are used, but we did not decompose the collection year samples into their respective cohorts using age data. Therefore, N_e estimates that pertain to individual year classes of breeders are not valid; however the harmonic mean over all samples will estimate the contemporary N_e. Comparing samples from years *i* and *j*, Waples' (1990) temporal method estimates the effective number of breeders ($\hat{N}_{b(i,j)}$) according to:

$$\hat{N}_{b(i,j)} = \frac{b}{2(\hat{F} - 1/\hat{S}_{i,j})}$$

The standardized variance in allele frequency ($\hat{F}$) is calculated according to Pollack (1983). The parameter b is calculated analytically from age structure information and the number of years between samples (Tajima 1992). The age-at-maturity information required to calculate b was obtained from Murdoch et al. (2006) for this analysis. They observed for Chiwawa Hatchery Chinook that 8.6% matured at age 2, 4% at age 3, 87% at age 4, and 0.4% at age 5. For Chiwawa natural Chinook, Murdoch et al. (2006) observed that 1.8% matured at age 3, 81.6% at age 4, and 16.7% at age 5. The harmonic mean of sample sizes from years *i* and *j* is $\tilde{S}_{i,j}$. Over all pairwise comparisons the harmonic mean of all $\hat{N}_{b(i,j)}$ is $\tilde{N}_b$, the contemporary estimate of the effective population size (N_e). SALMONNb (Waples et al. 2007) was used to calculate $\tilde{N}_b$. As suggested by authors, alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

The method of Waples (2006) uses linkage disequilibrium (i.e., mean squared correlation of allele frequencies at different gene loci) as a means of estimating effective population size (N_e) from a single sample. While this method is biased in some cases where N_e /N

ratio is less the 0.1 and the sample size is less than the true N_e, it has been shown to produce comparable results to the temporal method. Burrows' delta method is used to estimate LD, and a bias corrected estimate of N_e is calculated after eliminating alleles with frequency less than 0.05. This test was implemented using LDN_e (Do and Waples unpublished). In age-structured species, N_e estimates based on LD are best interpreted as the effective number of breeders (N_b) that produced the sample (Waples 2006). N_b should be multiplied by the mean generation length (i.e., 4 in this case) to obtain an overall estimate of N_e based on an N_b estimate. We analyzed collections categorized by spawning location (i.e., hatchery broodstock or in-river) and did not analyze collections categorized by origin (i.e., hatchery or natural). Waples' (2006) method estimates N_e from observed LD, therefore the corresponding N_e estimates for the hatchery collections would be low and the estimates for the natural collections would be high. Yet, since the supplementation program is integrated, and hatchery fish can spawn naturally, we feel it inappropriate to analyze the hatchery and natural samples as if they were separate, which would essentially partition all the LD into the hatchery samples.

Each collection has an N_b estimate and an associated confidence interval. If the confidence interval includes infinity, it means that sampling error accounts for all the LD observed (i.e., empirical LD is less than expected LD). The usual interpretation is that there is no evidence for any disequilibrium caused by genetic drift in a finite number of parents. Since the LD method estimates the number of breeders that contributed to the sample being analyzed, in order to calculate an N_e /N ratio, the appropriate census size must be used. The census size used to derive a ratio was the estimate four years prior to the collection analyzed using LD, which assumed a strict four-year-old lifecycle, although the observed proportion of four-year-olds was approximately 85% each year. The census numbers (Table 2) used to calculate the ratios for Chiwawa broodstock and in-river spawners were combined NOS (natural-origin spawners) and HOS (hatchery-origin spawners) census estimates.

Individual assignment – A population baseline file was constructed containing all 1704 individual Chinook from 34 population collections (Table 1; Chiwawa origin data set

plus all samples from other populations). All individuals in the baseline had geneotypes that included nine or more loci. Individual Chinook were assigned to their most likely population of origin based on the partial Bayesian criteria of Rannala and Mountain (1997), using a "jack-knife" procedure, where each individual to be assigned was removed from the baseline prior to the calculation of population likelihoods. This procedure was implemented in a program written by Warheit in MATLAB R2006b (The Mathworks 2006). Two assignment criteria were used, 1) the population with the largest posterior probability for an individual was the "most-likely" population of origin (i.e., all individuals assigned to a collection), and 2) an assignment was consider valid only if the posterior probability was greater than or equal to 0.9. Please note that while the analysis used 34 population collections to assign Rannala and Mountain likelihoods for each individual, these likelihoods were aggregated based on "population" (i.e., Chiwawa, Nason, White, and so on) and posterior probabilities were calculated for population location, rather than individual collections.

Results and Discussion

In this section we combine our presentation and interpretations of the genetic analyses. Additionally, this section will be organized based on the task list presented in the study plan. Overall conclusions are provided following this section.

Task 1: Determine trend in census size for Chiwawa River spring Chinook.

Census data from 1989 – 2005 are provided in Table 2 for the Chiwawa Hatchery broodstock and spring Chinook present in the Chiwawa River. The demographic data for naturally spawning Chinook are based on redd sampling and carcass surveys, while broodstock data are based on Chiwawa hatchery records. As the supplementation program is integrated by design, we also present the proportion of natural-origin broodstock (pNOB) incorporated into the hatchery, in addition to the number of natural-origin (NOS) and hatchery-origin (HOS) spawners present in Chiwawa River. The

census size fluctuated yearly, and a general reduction in census size was observed in the mid to late 1990's. This trend was apparent in both the broodstock and in the river. The arithmetic mean census size from 1989 – 2005 for the Chiwawa Hatchery (i.e., broodstock) was N=87.5 per year. The arithmetic mean census size from 1989 – 2005 for the Chiwawa River (i.e., NOS and HOS combined) was N=961.9 per year. For collection years when adult Chiwawa hatchery-origin fish would have been absent in the Chiwawa River (1989 – 1992), the arithmetic mean of natural Chiwawa Chinook census size is N=962.7. We will use this number as the baseline census size to assess if census size has changed. We used two different values for the contemporary census size in the Chiwawa River, NOS only and NOS + HOS. Additionally, we used collection years 2002 – 2005 for the contemporary NOS and HOS estimates, as these are the most recent data and the number of years included for estimation is the same as the pre-hatchery estimate above (i.e., four years). For NOS only, the arithmetic mean census size from 2002 – 2005 was N=536.0. For total census size (i.e., NOS and HOS combined), the arithmetic mean census size from 2002 – 2005 was N=1324.0. For the demographic data presented here, the contemporary census size is larger than the census estimate derived from the years prior to hatchery operation.

Task 2: Document the observed genetic diversity.

Genetic Diversity Categorized By Origin

For Chiwawa River collections categorized by origin (Table 1A), substantial genetic diversity was observed, with heterozygosity estimates over all loci, having a mean of 0.80. Genetic diversity was consistent with expected Hardy-Weinberg random mating genotypic proportions for ten of the eighteen collections. Eight of the nine Chiwawa natural collections were consistent with HWE, and two of nine Chiwawa Hatchery collections were consistent with HWE. F_{IS} is observed to be slight for all Chiwawa population collections, suggesting individuals within collections do not show excessive homozygosity.

The deviations from HWE observed were generally associated with hatchery collections. The two smolt collections (i.e., 1993 and 1994) showed significant deviations from HWE, which may be a function of non-random hatchery practices involving the contributing natural-origin parental broodstocks (i.e., 1991 and 1992 cohort). Deviations from HWE in the remaining hatchery collections may be the result of few individuals being represented in the broodstock (see below).

Additionally, linkage disequilibrium (LD) was also common for Chiwawa hatchery-origin collections and minimal for Chiwawa natural-origin collections. The random association of alleles between loci (i.e., linkage equilibrium) is expected under ideal conditions. LD is observed when particular genotypes are encountered more than expected by chance. Laboratory artifacts (e.g. null alleles) or physical linkage of loci on the same chromosome can cause LD, but the LD we observed was not associated with certain locus combinations, which you would expect if either artifacts or physical linkage were the cause of LD. LD was observed for seven of the nine hatchery-origin collections. As with the deviations from HWE, the high LD in the 1993 and 1994 hatchery-origin collections may be a result of non-random hatchery practices. The substantial LD observed in the hatchery-origin adult collections (collection years 2000, 2001, 2004, and 2006) might be the result of small parental broodstock sizes contributing to those returning adults. During the mid 1990's, the Chiwawa broodstock size was low, with zero individuals collected in 1995 and 1999; so fewer individuals would be contributing to the hatchery adult returns than the natural. This idea is corroborated by the lower LD observed for the 2005 hatchery-origin collection, which had a contributing parental broodstock size in 2001 (i.e., the major contributing parental generation) approximately eight times as large as the previous few collection years (Table 2). LD reappears in the 2006 Chiwawa hatchery-origin collection, which had a contributing parental broodstock size (i.e., for the most-part, the 2002 hatchery brood year) five times lower (Table 2) than that of the 2005 collection.

While seven of nine hatchery-origin collections showed significant LD, only one natural origin collection showed LD, and for this collection, only 10% of the loci-pairs were in

disequilibrium (Table 1). The fact that LD predominated in the hatchery samples, suggests that variance in reproductive success (i.e., overrepresentation of particular parents) is higher in the hatchery-origin than in natural-origin collections.

Genetic Diversity Categorized By Spawning Location

For upper Wenatchee River collections categorized by spawning location (Table 1B), substantial genetic diversity was observed, with heterozygosity estimates over all loci, having a mean of 0.79 and ranging from a low of 0.69 (1993 White River) to 0.85 (1993 Little Wenatchee). Genetic diversity was consistent with HWE for nineteen of twenty-nine population collections. For the collections that departed from HWE, seven were from the Chiwawa River, one was from Leavenworth Hatchery, one was the Wenatchee mainstem collection of hatchery-origin – naturally spawning fish, and one was from the White River. F_{IS} is observed to be slight for all population collections except the 1993 White River collection (10% heterozygote deficit) (Table 1B). Collections deviating with HWE generally correlated with collections having high LD. Twelve population collections showed a proportion of pairwise linkage disequilibrium tests (across all loci) greater than 5% (Table 1B), eight of which were Chiwawa collections.

Starting in 1996, spawning location collections are composed of both natural- and hatchery-origin samples. The LD seen in the later spawning location collections may be caused by an admixing effect (i.e., mixing two populations), where random mating has not had the chance to freely associate alleles into genotypes. Interestingly, there appears to be a trend of reducing LD through time within the broodstock collections (Table 1B), which suggests that a “homogenizing” effect is taking place within the Chiwawa River. This observation is discussed more fully in Task 3 below.

<u>Task 3:</u> Test for population differentiation among collections within the Chiwawa River and associated supplementation program.

Introduction

Task 3 was designed to address two hypotheses listed as part of Objective 3 in Murdoch and Peven (2005):

- Ho: Allele frequency $_{\text{Hatchery}}$ = Allele frequency $_{\text{Naturally produced}}$ = Allele frequency $_{\text{Donor pop}}$.
- Ho: Genetic distance between subpopulations $_{\text{Year x}}$ = Genetic distance between subpopulations $_{\text{Year y}}$

Murdoch and Peven (2005) proposed these two hypotheses to help evaluate the Chiwawa supplementation program through the "Conceptual Process" (Figure 5 in Murdoch and Peven 2005; repeated here as Figure 1). There are two components to the first hypothesis, which must be considered separately. The first component involves comparisons between natural-origin populations in the Chiwawa to determine if there have been changes in allele frequencies or genetic distances, through time starting with the donor population. Documenting a change does not necessarily indicate that the supplementation program has directly affected the natural origin fish, as additional tests would be necessary to support that hypothesis. The intent of the second component is to determine if the hatchery produced populations have the same genetic composition as the naturally produced populations.

Although on the surface these two components and their associated comparisons may appear simple, from a hypothesis-testing perspective the analyses are complicated by the fact that natural-origin fish may have had hatchery-origin parents, and hatchery-origin fish may have had natural-origin parents. As such, we organized the Chiwawa genetic data into three data sets: (1) fish origin (hatchery versus natural), (2) spawning location (hatchery broodstock versus in-river (natural) spawners), and (3) four "treatment" groups (1. hatchery-origin hatchery broodstock, 2. hatchery-origin natural spawner, 3. natural-origin natural spawner, and 4. natural-origin hatchery broodstock). We conducted separate analyses using each of the three data sets, with each analysis touching on some aspect of the components necessary to move through the Conceptual Process (Figure 1).

Hatchery- Versus Natural-Origin

We address the following questions with the origin data set:

1. Are there changes in allele frequencies and allele sharing distances in the natural-origin collections from pre-supplementation to today?
2. Are there changes in allele frequencies and allele sharing distances in the hatchery-origin collections from early supplementation to today?
3. Are there significant differences in allele frequencies and large allele sharing distances between hatchery- and natural-origin adults from a collection year, and has this pattern changed through time?

Genic Differentiation Tests – We explicitly tested the hypothesis of no significant differentiation within natural- or hatchery-origin collections from the Chiwawa River using a randomization chi-square test. We show the results for the pairwise comparisons among natural-origin collections from the Chiwawa River populations in the first block of the second page of Table 3. Ten of the 36 (28%) pairwise comparisons have highly significant allele frequency differences, while only 12 of the 36 comparisons (33%) showed no significant differences. Eight of these 12 comparisons involved the 1996 collection, which included only eight samples and therefore provided little power to differentiate allele frequencies. If we exclude the 1996 collection, only 14% of the pairwise comparisons showed no significant differences, and here all but one of these comparisons involved the 1989 collection. The 1989 collection appeared to be the least differentiated collection in the natural-origin data set in that all pairwise comparisons were either not significant, or only mildly significant at the nominal critical value. No comparisons involving the 1989 collection were significant using a Bonferroni-corrected critical value, and 1989 is the only natural-origin collection in our data set that can be classified as "pre-supplementation."

We can interpret these results to indicate that although there appears to be significant year-to-year differences in allele frequencies among post-supplementation collections, the allele frequencies between each post-supplementation collection and the 1989 pre-supplementation collection are not greatly different. However, the level of differentiation

does increase from the early post-supplementation years to the more recent years (2001, 2004-2006), although the statistical level of this significance never exceeds the Bonferroni-corrected critical value. Finally, sample sizes were also small for the 1989 collection (n = 36) and we cannot eliminate a reduction in power as a contributing factor for the lack of significance for these tests.

As with the hatchery-origin collections, most pairwise comparisons of allele frequencies between hatchery-origin samples were significant (Table 3, first page, upper block). Out of the 36 pairwise comparisons, all but three are significant at some level, and most comparisons are highly significant. Similar to the natural-origin analysis, the non-significant results were limited to comparisons involving the 1996, which included only eight samples.

As a result of this analysis *we reject the hypothesis that there was no significant differentiation among natural- or hatchery-origin collections from the Chiwawa River*. Furthermore, the allele frequencies of the hatchery-origin collections are significantly different from those of natural-origin collections (Table 3, first page, second block). For those fish collected in the same year, allele frequencies are significantly different between hatchery- and natural-origin collections, although in 2005 the level of significance was below the Bonferroni critical value (Table 3). The next step is to examine the pattern of allelic differentiation to discover first if there is a trend among the data, and second, if this trend suggests that the allele frequency differences among Chiwawa River natural-origin fish collections has been affected by the hatchery-origin fish.

Allele-sharing and Nonmetric Multidimensional Scaling – We constructed a pairwise allele-sharing distance matrix for all hatchery- and natural-origin collections from the Chiwawa River and subjected this matrix to a nonmetric multidimensional scaling analysis, restricting the analysis to two dimensions (Figure 2). The stress statistic for this analysis is 0.09, a value Kruskal (in Rohlf 2002) listed as a good to excellent fit between the actual allele-sharing distances and the Euclidean (straight-line) distances in the plot.

In other words, Figure 2 is a good visual representation of the allele sharing distance matrix; collections with a high percentage of alleles shared will be closer to each other than collections with a lower percentage of alleles shared.

With the exception of the two outlier years (1996 and 1998) the Chiwawa natural-origin collections form a tight cluster indicating an overall common set of shared alleles among these collections. Even if we ignore the 1996 and 1998 hatchery-origin collections, there appears to be a greater variance in shared alleles among the Chiwawa hatchery-origin collections than the natural-origin collections (Figure 2). In fact, the median percentage of alleles shared among the Chiwawa natural-origin collections is 76% compared with 69% alleles shared among the Chiwawa hatchery-origin collections.

Also, there appears to be a convergence in allele sharing distances (i.e., a decrease in allele frequency differences) between the hatchery- and natural-origin fish from the late 1980s/early 1990s to 2006. The series of red arrows in Figure 2 represent the progression of change in hatchery-origin allele sharing distances from 1996 (first adult hatchery origin fish in our analysis) to 2006 and this progression is decidedly in the direction of the natural-origin cluster. However, the most recent natural-origin collections (2001, 2004-2006) appear to have pulled closer to the hatchery-origin collections, compared with the 1989 natural-origin collection (note the close proximity of the 2000 and 1989 natural-origin collections). Nevertheless, the cluster of natural-origin collections adjacent to the hatchery-origin collections in Figure 2 also includes the 1993 natural-origin collection. Qualitatively, it appears that the initial hatchery-origin and natural-origin collections were more different from each other in terms of the percentage of shared alleles than are the most recent hatchery- and natural-origin collections. This may have been a result of a non-random sample of natural-origin fish that was used as broodstock in the initial years of the supplementation program (see discussion in Task 2 concerning deviations from HWE and linkage disequilibrium).

That being said, we do need to emphasize that Figure 2 is dominated by five outlier collections (two each from the 1996 and 1998 collections, and the 1994 smolt collection).

The 1996 and 1998 collections are characterized by small samples sizes, and the 1994 smolt collection has nearly all pairs of loci in linkage disequilibrium (Table 1). If we eliminate these five outlier groups, both the hatchery- and natural-origin collections form a relatively tight cluster. Excluding the five outliers, the median percentage of shared alleles among all pairwise combinations of Chiwawa hatchery versus Chiwawa natural collections is 76%. This compares with a median pairwise percentage of 79% among only Chiwawa natural-origin collections. That is, there are nearly as many alleles shared between the hatchery-origin and natural-origin collections as there are among the natural-origin collections themselves. There is also a narrowing of differences between natural- and hatchery-origin fish from the same collection years from 1993 (76% shared alleles) through 2006 (83% shared alleles).

If allelic differentiation among collections is a function of genetic drift, we would expect a positive correlation between the number of years between two collections and the allele sharing distance. That is, if genetic drift is the primary cause of allele frequency differences between two collections, the greater the number of years between the two collections the larger the allele-sharing distance. For both the natural- and hatchery-origin collections we examined the relationship between the number of years between a pair of collections and the collections' allele-sharing distance (Figure 3). Although the relationship between time interval and allele distance appears to be a positive function in the natural collections, the slope of the regression line is 0.0017, and is not significantly different from zero. Furthermore, the correlation coefficient (r^2) equals 0.1068, which means that the time interval between collections accounts for only 10% of the pairwise differences in allelic distance. The hatchery-origin collections do show a significantly positive slope (0.0037; $p = 0.0254$) and a regression coefficient nearly three times greater than that for the natural-origin collections. However, the correlation coefficient is still relatively small ($r^2 = 0.3290$), indicating that the time interval between collections accounts for one-third of the pairwise differences in allelic distance. The results suggest that if genetic drift is a factor in allelic differentiation between collections, it is only a minor factor, and appears to have affected the hatchery-origin collections more than the natural-origin collections.

If four-year-old fish dominate each collection year, we would expect a closer relationship among collections that are spaced at intervals of four years. The average percentage of alleles shared between two natural-origin collections that are separated by four years or a multiple of four years is 81%, compared with 78% for natural-origin collections separated by years that are not divisible by four. Likewise, for hatchery-origin collections the average percentage of alleles shared is 80% and 75% for collections separated by years divisible and not divisible by four, respectively. Although the percent differences described above are relatively small, they are consistent with the idea that allelic differences between collections are a function of year-to-year variability among different cohorts of four year-old fish.

Summary – The allele frequencies within and between natural- and hatchery-origin collections are significantly different, but there does not appear to be a robust signal indicating that the recent natural-origin collections have diverged greatly from the pre- or early post-supplementation collections. Genetic drift will occur in all populations, but does not appear to be a major factor with the Chiwawa collections. We propose that the differences among collections are a function of differences in allele frequencies among cohorts of the four year-old fish that dominate each collection.

Hatchery Broodstock Versus Natural (In-River) Spawners

We address the following questions with the spawner data set:

1. Are there changes in allele frequencies and allele sharing distances in the natural spawning collections from pre-supplementation to today?
2. Are there changes in allele frequencies and allele sharing distances in the hatchery broodstock collections from early supplementation to today?
3. Are there significant differences in allele frequencies and large allele sharing distances between hatchery and natural spawning adults from a collection year, and has this pattern changed through time?

Genic Differentiation Tests – For the most part there are significant differences in allele frequencies among collections for both the hatchery broodstock and natural spawners (Table 4), and these differences are consistent with the origin data set (Table 3). There are four collection years with paired samples (2001, 2004-2006) where we can compare allele frequency differences between the hatchery broodstock and natural spawners, within the same year. The 2001 hatchery broodstock and natural spawner collections have significantly different allele frequencies, but the level of significance decreased from 2001 to 2004, and become non-significant in 2005 and 2006 (Table 4). This indicates that by 2005, the hatchery broodstock and natural spawners collections were effectively sampling from the same population of fish. Additionally, the percentage of alleles shared between the hatchery broodstock and the natural spawners increased from 76% in 2001 to 86% in 2006 (allele sharing distance matrix, not shown). From this analysis, we conclude that although there are year-to-year differences in allele frequencies within the natural and hatchery spawner collections, *there appears to be a convergence of allele frequencies within collection-year, between the natural and hatchery spawner populations*.

Linkage Disequilibrium – Linkage disequilibrium is the correlation of alleles between two loci, and can occur for several reasons. If two loci are physically linked on the same chromosome, than alleles from each of these loci should be correlated. However, linkage between two loci can occur as a result of population bottlenecks, small population sizes, and natural selection. If any of these conditions had occurred or were occurring within the Chiwawa River system, we would expect to find substantial linkage disequilibrium in many or perhaps all Chiwawa collections. However, many Chiwawa collections, especially the natural-origin collections, do not show linkage disequilibrium (Table 1), and it would appear that the linkage disequilibrium within certain Chiwawa collections is not a function of the processes listed above. Linkage disequilibrium can also result if the collection is composed of an admixture. That is, if two or more reproductively isolated populations are combined into a single collection, the collection will show linkage disequilibrium. Each broodstock and natural spawning collection is composed of natural- and hatchery-origin fish. If these hatchery- and natural-origin fish are drawn from the

same population, the spawning collections should not show substantial linkage disequilibrium. However, if the hatchery- and natural-origin fish are from different populations (i.e., full hatchery – natural integration has not been achieved), the spawning collections should show substantial linkage disequilibrium.

There are only three Chiwawa spawning collections that are not composed of both hatchery- and natural-origin samples: 1989 (natural-origin, natural spawner), 1993 (natural-origin, hatchery broodstock), and 2001 (natural-origin, natural spawner). Of the 10 spawning collections with both hatchery- and natural-origin fish, seven show significant linkage disequilibrium. Two of the three collections that did not show linkage disequilibrium are the 1996 and 1998 hatchery broodstock collections, which are composed of only seven natural- and six hatchery-origin fish, and two natural- and 19 hatchery-origin fish, respectively. Within the hatchery broodstock collections with linkage disequilibrium, the percent of loci pairs showing linkage decreased from 32% in 2000 to 13% in 2001 and 2004, to only 1% and 5% in 2005 and 2006, respectively (Table 1). If the homogenization of allele frequencies of natural- and hatchery-origin fish was increasing from 2000 to 2006, we would expect a decrease in linkage disequilibrium among the broodstock collections. This is what occurred within the hatchery broodstock collections, but did not occur within the natural spawner collections, where the percent of loci pairs showing linkage was 18% in 2004, 6% in 2005, and 10% in 2006 (Table 1). Furthermore, the 2001 natural spawner collection, with no hatchery-origin component showed linkage disequilibrium with 9% of loci pairs.

There is no correlation between percent of loci pairs showing linkage disequilibrium and percent of broodstock composed of hatchery-origin fish ($r^2 = 0.0045$). Furthermore, the natural spawner and hatchery broodstock collections were each composed of roughly the same average percentage of hatchery-origin fish (57% and 53%, respectively). If the decrease in linkage disequilibrium among the hatchery broodstock collections from 2000 to 2006 was a result of a homogenization of allele frequencies of natural- and hatchery-origin fish in the broodstock, the same degree of homogenization did not occur within the

natural spawner collections. This would occur if natural- and hatchery-origin fish spawning within the river remain segregated, either by habitat or by fish behavior.

Summary – As with the origin data set, there are significant allele frequency differences within and between hatchery broodstock and natural spawner collections. However, in recent years the allele frequency differences between the hatchery broodstock and natural spawner collections has declined. Furthermore, based on linkage disequilibrium, there is a genetic signal that is consistent with increasing homogenization of allele frequencies within hatchery broodstock collections, but a similar homogenization within the natural spawner collection is not apparent. These data suggest that there exists consistent year-to-year variation in allele frequencies among hatchery and natural spawning collections, but there is a trend toward homogenization of the allele frequencies of the natural- and hatchery-origin fish that compose the hatchery broodstock.

Four Treatment Groups

Analyses of genetic differences between hatchery (broodstock) and natural spawner collections is confounded by the fact that each these two groups are composed of fish of natural- and hatchery-origin. To understand the effects of hatchery supplementation on *natural-origin fish that spawn naturally*, we needed to divide the Chiwawa data set into four mutually exclusive groups: (1) hatchery-origin hatchery broodstock, (2) hatchery-origin natural spawner, (3) natural-origin hatchery broodstock, and (4) natural-origin natural spawner, with each group consisting of multiple collection years, for a total of 25 different groups.

Allele-sharing and Nonmetric Multidimensional Scaling –As with previous analyses discussed above, we constructed a pairwise allele-sharing distance matrix for all collections from each of these treatment groups and subjected this matrix to a nonmetric multidimensional scaling analysis, restricting the analysis to two dimensions. Figure 4 shows that five outlier groups dominate the allele-sharing distances within this data set. These outlier groups are also present in Figure 2, as discussed above, and Figure 2 and 4 resemble each other because the same fish are included in each analysis. The difference

between Figures 2 and 4 is that in Figure 4 the fish are grouped into collection year and the four treatment groups, rather than collection year and two treatment groups (hatchery- versus natural-origin).

Figure 4 does not provide useful resolution of the groups within the polygon, because the outlier groups dominate the allele sharing distances. We removed the five outlier groups from Figure 4, recalculated the allele sharing distances and subjected this new matrix to a multidimensional scaling analysis (Figure 5). Figure 5 shows separation among the 2001, 2004-2006 collections, but this separation does not necessarily indicate that within-year collections are more similar to each other than any collection is to a collection from another year. For example, the 2006 natural-origin natural spawner and the 2005 natural-origin hatchery broodstock collections share 81% alleles, while the 2006 natural-origin natural spawner and 2006 hatchery-origin hatchery broodstock collections share 75% alleles. There does not appear to be any discernable pattern of change in allele-sharing distance among the collections relevant to pre- or post-supplementation. Although the 1989 pre-supplementation natural-origin collection appears distinct (Figure 5), the 1993 natural-origin hatchery broodstock collection appears quite similar to the 2005 and 2006 natural-origin collections (Figure 5). The 1993 natural-origin hatchery broodstock collection, although not technically pre-supplementation, is composed of fish whose ancestry cannot be traced to any Chiwawa hatchery fish. Therefore, there is no clear pattern of allele sharing change from pre-supplementation to recent collections.

There does appear to be some change in the average percentage of alleles shared within the 2001 to 2006 collections, with an increase from 74% in 2001 and 2004 to 78% and 79% in 2005 and 2006, respectively. The results provided by this analysis are consistent with the results presented in the origin and spawner data sets. That is, there are allele frequency and allele sharing differences among the collections, but analyses do not strongly suggest that these differences are a function of the supplementation program. Furthermore, there is also a weak signal that the hatchery and natural collections within the most recent years are more similar to each other than in the previous years.

Overall Genetic Variance – Although there are signals of allelic differentiation among Chiwawa River collections, there are no robust signs that these collections are substantially different from each other. We used two different analyses to measure the degree of genetic variation that exists among individuals and collections within the Chiwawa River. First, we conducted a principal component analysis using all Chiwawa samples with complete genotypes (i.e., no missing alleles from any locus). Although the first two principal component axes account for only 10.5% of the total molecular variance, a substantially greater portion of that variance is among individual fish, regardless of their identity, rather than among hatchery and natural collections (Figure 6). The variances in principal component scores among individuals are 11 and 13 times greater than the variance in scores among collections, along the first and second axes, respectively.

Second, we conducted a series of analyses of molecular variance (AMOVA) to ascertain the percentage of molecular variance that could be attributed to differences among collections. We organized these analyses to test also for differences in the hierarchical structure of the data. That is, we tested for differences among collections using the following framework:

- No organizational structure – all 25 origin-spawner collections considered separately
- Origin-spawner collections organized into 10 collection year groups
- Origin-spawner collections organized into 2 breeding location groups (hatchery versus natural)
- Origin-spawner collections organized into 2 origin groups (hatchery versus natural)
- Origin-spawner collections organized into the 4 origin-spawner groups

It is clear from this analysis that nearly all molecular variation, no matter how the data are organized, resides within a collection (Table 5). The percentage of total molecular variance occurring within collections ranged from 99.68% to 99.74%. The among group variance component was limited to less than 0.26% and in all organizational structures,

except "no structure," the among group percentage was not significantly greater than zero. Furthermore, none of the organizational structures provided better resolution than "no structure" in terms of accounting for molecular variance within the data set. *These results indicate that if there are significant differences among collections of Chiwawa fish, these differences account for less than one percent of the total molecular variance, and these differences cannot be attributed to fish origin or spawning location.*

Summary and Conclusions

We reject the null hypothesis that the allele frequencies of the hatchery collections equal the allele frequencies of the natural collections, which equals the allele frequency of the donor population. Furthermore, because the allele-sharing distances are not consistent within and among collections years, we also reject the second stated hypothesis discussed above. However, there is an extremely small amount of genetic variance that can be attributed to among collection differences. The allelic differentiation that does exist among collections does not appear to be a function of fish origin, spawning location, genetic drift, or collection year. Figure 5 and related statistics does suggest that hatchery and natural collections in 2005 and 2006 are more similar to each other than previous years' collections, and this would be expected in a successful integrated hatchery supplementation program.

Since each of these collection years are generally composed of four-year-old fish, the differentiation among these collections for the most part is differentiation among specific cohorts. The slightly greater percentage of alleles shared among collections that are separated in time by multiples of four years, compared with collections that are not separated in time as such, suggests that cohort differences may be the most important factor accounting for differences in allele frequencies among collections.

<u>Task 4:</u> Develop a model of genetic drift.

See Task 3

Task 5: Analyze spring Chinook population samples from the Chiwawa River and Chiwawa Hatchery from multiple generations.

See Task 3

Task 6: Analyze among population differences for upper Wenatchee spring Chinook.

Supplementation of the Chiwawa River spring Chinook population may affect populations within the Wenatchee River watershed other than the Chiwawa River stock. If the stray rate for Chiwawa hatchery-origin fish is greater than that for natural-origin fish, an increase in gene flow from the Chiwawa population into other populations may result. If this gene flow is high enough, Chiwawa River fish may alter the genetic structure of these other populations. Records from field observations indicate that hatchery-origin fish are present in all major spawning aggregates (A.R Murdoch, unpublished data), and these fish are successfully reproducing (Blankenship et al 2006). The intent of this task is to investigate if there have been changes to the genetic structure of the spring Chinook stocks within upper Wenatchee tributaries during the past 15-20 years, and if changes have occurred, are they a function of the Chiwawa River Supplementation Program? Therefore, we ask the following two questions:

1. Are allele frequencies within populations in the upper Wenatchee stable through time? That is, is there significant allelic differentiation among collections within upper Wenatchee populations?
2. Are the recent collections from the upper Wenatchee populations more similar to the Chiwawa population than earlier collections from the same populations?

For this task we analyzed natural spawning collections from the White River (natural-origin), Little Wenatchee River (natural-origin), Nason Creek (natural-origin), and

Wenatchee mainstem (hatchery-origin), and hatchery collections from Leavenworth NFH and Entiat River NFH (Table 1). We also included in the analysis the natural- and hatchery-origin collections from the Chiwawa River. There are no repeated collections from Leavenworth, Entiat, Little Wenatchee, and Wenatchee mainstem (Table 1), so for many of the analyses we have limited our discussion to the Chiwawa River, White River, and Nason Creek collections. Furthermore, genetic structure of the Little Wenatchee collection, which consisted of only 19 samples, was unexpectedly quite different from the other collections. For example, the F_{ST} statistic measures the percent of total molecular variation that can be attributed to differences between populations. The median F_{ST} for all pairwise combinations of collections from all populations, except Little Wenatchee (33 populations, 528 individual F_{ST} statistics) equals 0.010 (1%), with a range of 0.000 to 0.037 (Table 6). The median F_{ST} for the Little Wenatchee paired with all other collections (33 individual F_{ST} statistics) equals 0.106 (10.6%), with a range of 0.074 to 0.121. The ten-fold increase in the F_{ST} statistic indicates that either the Little Wenatchee spring Chinook is unique among the upper Wenatchee River stocks, or this 1993 collection is somehow aberrant. Therefore, we exclude the Little Wenatchee collection from many other analyses.

Population Differentiation – Table 3 provides the levels of significance for all pairwise genic differentiation tests. Most between-collection comparisons are highly significant, with no pattern of increasing or decreasing differentiation with time, and no differences when comparisons are made with Chiwawa hatchery- versus Chiwawa natural-origin fish. For example, excluding the outlier 1996 and 1998 Chiwawa hatchery- and natural-origin collections, Nason Creek showed highly significant allele frequency differences between the Chiwawa hatchery- and natural-origin collections at 100% and 86% of the comparisons, respectively. The same comparisons with the White River produced 100% and 93% highly significant allele frequency comparisons, respectively. Allele frequencies between Nason Creek and White River were likewise differentiated from each other.

The collection allele frequencies within the upper Wenatchee system are significantly different, and these differences do not appear to change as a function of time (Table 3). Nason Creek shows greater within-population year-to-year variation in allele frequencies than does the White River, with 47% of the pairwise comparisons showing highly significant differences, compared with only 13% for the White River. However, the 2005 and 2006 collections from the White River appear to be somewhat more differentiated from not only each other, but from the earlier collections from the White River.

Despite the high degree of temporal and spatial structure suggested by the genic differentiation tests, as described above for within-Chiwawa analysis (Task 3), most of the genetic variation within this data set occurs within populations, rather than between populations (Table 6). The F_{ST} values for most population comparisons are between 0.01 and 0.02, indicating 1% to 2% among-population variance, with the remaining 98% to 99% variance occurring within populations. The White River shows the highest median F_{ST} among the natural-origin collections, equal to 0.014, compared with 0.009 for both the Nason Creek and Chiwawa natural-origin collections. The median F_{ST} for the Chiwawa hatchery-origin collections (0.012) was higher than that for the Chiwawa natural-origin collections.

Table 7 summarizes the information from the F_{ST} analyses, under five different temporal and spatial scenarios. Under all scenarios, over 99% of the molecular variance is within populations. There is significantly greater spatial structure among populations ("Origin") in 2005 and 2006 than from 1989 to 1996. That is, there appears to be more spatial structure among the Chiwawa hatchery-origin, Chiwawa natural-origin, White River, and Nason Creek now, than in 1989 to 1996, despite the potential homogenizing and cumulative effect of hatchery strays. However, we stress that the amount of molecular variance associated with the among population differences, despite being significantly greater than 0.00%, is limited to only 0.43%.

Allele-sharing and Nonmetric Multidimensional Scaling – As in the Chiwawa River data discussed above, we constructed an allele-sharing distance matrix and then subjected

that matrix to a multidimensional scaling analysis (Figure 7). Consistent with all previously discussed multidimensional scaling analyses, the 1996 and 1998 adult, and the 1994 smolt collections are outliers. There is clear separation between the White River collections and all other natural-origin and Chiwawa hatchery-origin collections, indicating that there are more alleles shared among the Nason Creek and Chiwawa collections, than with the White River collections. Furthermore, there is a slight separation between the Chiwawa natural-origin natural spawner collections and Nason Creek collections, suggesting different groups of shared alleles between these populations. There is more variation in the allele-sharing distances among collections involved with the Chiwawa hatchery (origin or broodstock) than any of the natural-origin collections, even if we exclude the 1994, 1996, and 1998 collections. This suggests that there is more year-to-year variation in the composition of hatchery-origin and hatchery broodstock than within natural-origin populations throughout the upper Wenatchee. All Wenatchee mainstem fish are hatchery-origin, and if these fish are from the Chiwawa Supplementation Program (rather than from Leavenworth), it is not unexpected that this collection would be plotted within the Chiwawa polygon (Figure 7).

Assignment of Individual to Populations – Finally, we conducted individual assignment tests whereby we assigned each individual fish to a population, based on a procedure developed by Rannala and Mountain (1997) (Table 8 and 9). Individual fish may be correctly assigned to the population from which they were collected, or incorrectly assigned to a different population. Incorrect assignments may occur if the fish is an actual migrant (i.e., source population different from population where collected), or because the genotype for that fish matches more closely with a population different from its source. If there are many individuals from a population incorrectly assigned to populations other than its source population, that original population is either unreal (i.e., an admixture), or there is considerable gene flow between that population and other populations. Furthermore, in assigning individuals to populations, we can either accept the assignment with the highest probability, regardless of how low that probability may be, or we can establish a more stringent criterion, such as to not accept an assignment unless the posterior probability is equal to or greater than 0.90. This value is roughly

equal to having the likelihood of the most-likely population equal to 10 times that of the second most-likely population.

We provide a summary of the assignments in Tables 8 and 9. On average, nearly 50% of the fish are assigned incorrectly if we accept all assignments (Table 8), but the incorrect assignment rate drops to roughly 10% when we accept only those assignments with probabilities greater than 0.90. However, with this more stringent criterion, nearly 64% of the fish go unassigned. These results indicate that the allele frequency distributions for these populations are very similar, and it would be very difficult to assign an individual fish of unknown origin to the correct population. If all fish are assigned, there is a 50% chance, overall, of a correct assignment. If you accept only those assignment with the 0.90 criterion, nearly two-thirds of the fish would be unassigned, but there is a 90% chance of correctly assigning those fish that are indeed assigned.

Of all the populations in the data set, there are fewer errors associated with assigning fish to the White River. If all fish are assigned (Table 8), 72% of those fish assigned to the White River, are actually from the White River (115 fish out of a total of 159 fish assigned to the White River). This compares to a rate of only 52% and 53% for Nason Creek and Chiwawa natural-origin, respectively, and 60% for the Chiwawa hatchery-origin collections. With the 0.90 criterion (Table 9), 89% of the fish assigned to the White River, are actually from the White River, compared with 70% and 65% for Nason Creek and Chiwawa natural origin, respectively, and 81% for the Chiwawa hatchery origin.

When all fish are assigned, most of the incorrectly assigned fish from Nason Creek and White River are assigned to Chiwawa River, at roughly equal frequencies to the hatchery- and natural-origin populations. Incorrectly assigned fish to other populations occur at a slightly higher rate in Nason Creek than in the White River. However, when only those fish meeting the 0.90 criterion are assigned (Table 9), incorrectly assigned fish from Nason Creek are distributed among White and Chiwawa Rivers, as well as Leavenworth NFH, and the Entiat NFH. Mis-assignment to the Chiwawa hatchery-origin was the

highest among the Nason Creek collections, equal to nearly 14%. This contrasts with the White River where mis-assignments do not exceed 7% anywhere, and there is a roughly even distribution of mis-assignments among Nason Creek and Chiwawa River collections.

Summary and Conclusions – There is little geographic or temporal structure among populations within the upper Wenatchee systems. Among population molecular variance is limited to 1% or less. The little variance that can be attributed to among populations indicates that the White River is more differentiated from the Chiwawa and Nason populations than these populations are from each other. Furthermore, although we cannot rule out a hatchery effect on the Nason Creek and White River populations, there is no indication there has been any temporal changes in allele frequencies within these populations that can be attributed directly to the Chiwawa River Supplementation Program. In fact, Table 7 weakly suggests that there is more differentiation among these populations now, than there was before or at the early stages of Chiwawa supplementation.

Therefore, returning to our two original questions, there are significant differences in allele frequencies among collections within populations, and among populations within the upper Wenatchee spring Chinook stocks. However, these differences account for a very small portion of the overall molecular variance, and these populations overall are very similar to each other. There is no evidence that the Chiwawa River Supplementation Program has changed the allele frequencies in the Nason Creek and White River populations, despite the presence of hatchery-origin fish in both these systems. Finally, of all the populations within the Wenatchee River, the White River appears to be the most distinct. Yet, this distinction is more a matter of detail than of large significance, as the median F_{ST} between White River collections and all other collections (except the Little Wenatchee) is less than 1.5% among population variance.

Task 7: Calculate the inbreeding effective population size using demographic data for each sample year, and document the ratio of census to effective size.

This analysis was completed by Williamson et al. (submitted).

Task 8: Calculate LD N_b using genetic data for each sample year, and document the ratio of census to effective size.

We report N_e estimated for the Chiwawa River collections based on the bias correction method of Waples (2006) implemented in LDNe (Do and Waples unpublished). N_e estimates based on LD are best interpreted as the effective number of breeders (N_b) that produced the sample (Waples 2006).

For collections categorized by spawning location (i.e., hatchery broodstock or natural), estimates of N_b are shown in Table 10. Considering the hatchery broodstock, N_b estimates range from 30.4 (1996) to 274.3 (2005). To obtain N_e /N ratios, the N_b estimate is multiplied by four (i.e., mean generation length) and divided by the total in river (i.e., NOS [natural-origin spawners] plus HOS [hatchery-origin spawners]) census data from four years prior (i.e., major cohort; see Table 2). The observed N_e /N ratios for the broodstock collections range from 11% to 54% of the census estimate, excluding the 2000 collection which is 106%. A ratio greater than one is possible under special circumstances, and certain artificial mating schemes within hatcheries can inflate N_e above N; yet, it is unknown if this is the case for this collection. While no direct comparisons are possible, the N_b estimates reported by Williamson et al. (submitted) for Chiwawa broodstock collections from 2000 – 2003 are similar in magnitude to our estimates. For Chiwawa natural spawner collections, the N_b estimates range from 5.2 (1989) to 231.5 (2005), with observed N_e /N ratios of 22% - 48% of the census estimate.

<u>Task 9:</u> Calculate N_b using the temporal method for multiple samples from the same location.

Estimates of effective number of breeders (N_b) derived from Waples' (1990) temporal method are shown in Tables 11-13. Eight collection years were used for the Chiwawa broodstock collections (Table 11). The harmonic mean of all pairwise estimates of N_b ($\tilde{N}_b$) was 269.4. This estimate is the contemporary N_e for Chiwawa broodstock collections. For the five collection years of Chiwawa in-river spawners (Table 12), the estimated $\tilde{N}_b$ = 224.2. This estimate is the contemporary N_e for Chiwawa River natural spawner collections. Since the Chiwawa Supplementation Program is integrated by design, we also performed another estimation of N_e using composite hatchery and natural samples. There are paired samples from 2004-2006. We combined genetic data for hatchery (HOS) and natural (NOS) origin fish from 2004 – 2006 to create a single Chiwawa River natural spawner sample for each year. The three composite samples from 2004 – 2006 were then analyzed using the temporal method (Table 13), resulting in a $\tilde{N}_b$ = 386.8. This estimate is the contemporary N_e for Chiwawa River.

Williamson et al. (submitted) estimated N_e using Waples' (1990) temporal method for Chinook captured in 2004 and 2005, and used age data to decompose brood years into consecutive cohorts from 2000 – 2003. They report for Chiwawa broodstock a $\tilde{N}_b$ = 50.4. This estimate is not similar to our Chiwawa broodstock estimate. However, if we analyze the hatchery-origin Chinook only, our estimate is $\tilde{N}_b$ = 80.1 for collection years 1989 – 2006 (data not shown). Williamson et al. (submitted) report for Chiwawa naturally spawning Chinook a $\tilde{N}_b$ = 242.7, which is slightly higher than our estimate for in-river spawners from 1989 – 2006, but lower than our estimate from combined NOS and HOS Chinook from 2004 – 2006 collection years.

<u>Task 10:</u> Use available data and the Ryman-Laikre and Wang-Ryman models to determine the expected change of N_e for natural spring Chinook salmon in the Wenatchee River due to hatchery operation.

N_e is generally thought to be between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992; RS Waples pers. comm.). We used this range to generate an estimate of N_e for Chiwawa natural spawners prior to hatchery operation. For brood years 1989 – 1992, the arithmetic mean census size was N=962.7 (Table 2), resulting in an estimated N_e ranging from 96.3 – 317.7. The contemporary estimate of N_e calculated using genetic data for the Chiwawa in-river spawners is N_e=224.2 (Table 12), falling in the middle of the pre-hatchery range. The N_e /N ratio calculated using 224.2 and the arithmetic census of NOS Chinook from 1989 – 2005 is 0.42. A more appropriate contemporary N_e to compare with the pre-hatchery estimate (i.e., 96.3 – 317.7) is the combined NOS and HOS estimate from natural spawners, since the supplementation program is integrated. As discussed above, the contemporary estimate of N_e calculated using genetic data for Chiwawa NOS and HOS Chinook is N_e=386.8 (Table 13), which is slightly larger than the pre-hatchery range, suggesting the N_e has not declined during the period of hatchery operation. The N_e /N ratio calculated using 386.8 and the arithmetic census of NOS and HOS Chinook from 1989 – 2005 is 0.40. These results suggest the Chiwawa Hatchery Supplementation Program has not resulted in a smaller N_e for the natural spawners from the Chiwawa River.

Williamson et al. (submitted) argued that since their combined (i.e., broodstock and natural) N_e estimate was lower than the naturally spawning estimate, the supplementation program likely had a negative impact on the Chiwawa River N_e. We disagree with this interpretation of these data. Since the natural spawning component is mixed hatchery and natural ancestry, the N_e estimates from natural spawning data are the results that bear on possible hatchery impacts. The census data show the population declined in the mid 1990's and rebounded by 2000 (Table 2). This trend is reflected in the N_e results, as shown above, and Williamson et al. (submitted) clearly show in their Table 4 the N_e was lower in 2000 (N_e = 989) than it was in 1992 (N_e = 2683). Yet, the important comparison

they make in our view was the natural spawning N_e versus the natural only component N_e (i.e., hypothetically excluding hatchery program). Williamson et al. (submitted) report the 1989 – 1992 N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) was essentially the same as the natural only component estimate, 2683 and 2776, respectively. This result is not surprising since no HOS fish were present between 1989 – 1992. They also report that the 1997 – 2000 N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) was N_e =989, while the natural-origin estimate of N_e in 1997 – 2000 was N_e = 629. Since the natural-origin estimate of 629 is lower than 989, the N_e estimate from all in-river spawners, we argue that their analysis of demographic data show the N_e estimated from naturally spawning Chinook (i.e., NOS and HOS integrated) is larger only if the hatchery Chinook in the river are ignored.

Task 11: Use individual assignment methods to determine the power of self-assignment for upper Wenatchee River tributaries.

See "Assignment of Individual to Populations" in Task 6

Conclusions

Has the Chiwawa Hatchery Supplementation Program succeeded at increasing the census size of the target population while leaving genetic integrity intact? This is an important question, as hatcheries can impact natural populations by reducing overall genetic diversity (Ryman and Laikre 1991), reducing the fitness of the natural populations through relaxation of selection or inadvertent positive selection of traits advantageous in the hatchery (Ford 2002; Lynch and O'Hely 2001), and by reducing the reproductive success of natural populations (McLean et al. 2003). The census data presented here show that the current natural spawning census size is similar to the pre-supplementation census size. Despite large numbers of hatchery-origin fish on the Chiwawa River spawning grounds, the genetic diversity of the natural-origin collections appear unaffected by the supplementation program; heterozygosities are high, and contemporary N_e is similar (perhaps slightly higher) than pre-supplementation N_e. We did find

significant year-to-year differences in allele frequencies in both the origin and spawner datasets, but these differences do not appear to be related to fish origin, spawning area, or genetic drift. However, we do suggest that cohort differences may be the most important factor accounting for differences in allele frequencies among collections.

The main objective of this study was to determine the potential impacts of the hatchery program on natural spring Chinook in the upper Wenatchee system. We did this by analyzing temporally replicated collections from the Chiwawa River, and by comparing genetic diversity prior to the presumed effect of the Chiwawa Hatchery Supplementation Program, with contemporary collections. We report that the genetic diversity present in the Chiwawa River is unchanged (allowing for differences among cohorts) from 1989 – 2006, and the contemporary estimate of the effective population size (N_e) using genetic data is approximately the same as the N_e estimate extrapolated from 1989 – 1992 census data (i.e., pre-hatchery collection years). We observed substantial genetic diversity, with heterozygosities ~80% over thirteen microsatellite markers. Yet, temporal variation in allele frequencies was the norm among temporal collections from the same populations (i.e., location). The genetic differentiation of replicated collections from the same population is likely the result of salmon life history in this area, as four-year-old Chinook comprise a majority of returns each year. The genetic tests are detecting the differences of contributing parents for each cohort. An important point related to the temporal variation, is that the hatchery broodstock is composed in part of the natural origin Chinook from the Chiwawa River. When we compared the genetic data (within a collection year) for Chinook brought into the hatchery as broodstock with the Chinook that remained in the river (years 2001, 2004 – 2006), there was a trend of decreasing statistical differences in allele frequencies from 2001 to 2004, and no differences were detected for 2005 and 2006. While the replicated collections may have detectable differences in allele frequencies, those differences reflect actual differences in cohorts, not the result of hatchery operations, and the hatchery broodstock collection method captures the differences in returning Chiwawa River spring adults each year. We conclude from these results that the genetic diversity of natural spring Chiwawa Chinook has been maintained during the Chiwawa Hatchery Supplementation Program.

We observe slight, but statistically significant population differentiation between Chiwawa River, White River, and Nason Creek collections. Murdoch et al (2006) and Williamson et al. (submitted) also observed population differentiation between Chiwawa River, White River, and Nason Creek collections. Yet, 99.3% of the genetic variation observed was within samples, very little variance could be attributed to population differences (i.e., population structure). The AMOVA analysis and poor individual assignment results suggest the occurrence of gene flow among Wenatchee River locations or a very recent divergence of these groups. While Murdoch et al. 2006 did not perform an AMOVA analysis, their F_{ST} results provide comparable data to our among-population results. Murdoch et al. 2006 report F_{ST} ranging from 2%-3% for pairwise comparisons between of Chiwawa, White, and Nason River collections. Since F_{ST} is an estimate of among-sample variance, these results also imply a majority of the genetic variance (i.e., 97%-98%) resides within collections. To provide further context for the magnitude of these variance estimates, we present the among-group data from Murdoch et al. 2006 comparing summer-run and spring-run Chinook from the Wenatchee River. They report that approximately 91% of observed genetic variance is within-collection for comparisons between collections of summer- and spring-run Chinook. Ultimately, the information provided by this and other reports will be incorporated into the management process for Wenatchee River Chinook. However, we would like to emphasize that the application of these genetic data to management is more about the goals related to the distribution of genetic diversity in the future than specific data values reported. If Chinook are collected at Tumwater Dam instead of within the upper Wenatchee River tributaries, a vast majority of the genetic variation present in the basin would be captured, although any differences among tributaries would be mixed. Alternatively, management policies could be crafted to promote and maintain the among-group genetic diversity that genetic studies consistently observe to be non-zero within the Wenatchee River.

We agree with Murdoch et al. (2006) that it appears hatchery Chinook are not contributing to reproduction in proportion to their abundance. Additionally, if the total census size (i.e., NOS and HOS combined) within the Chiwawa River does not continue

to increase, genetic diversity may decline within this system, given the smaller N_e within the hatchery-origin collections compared with the natural-origin collections.

Acknowledgements

We would like to thank Denise Hawkins, Craig Busack, and Cheryl Dean for helpful comments regarding this project. This project was funded by Chelan County PUD and the Washington State General Fund.

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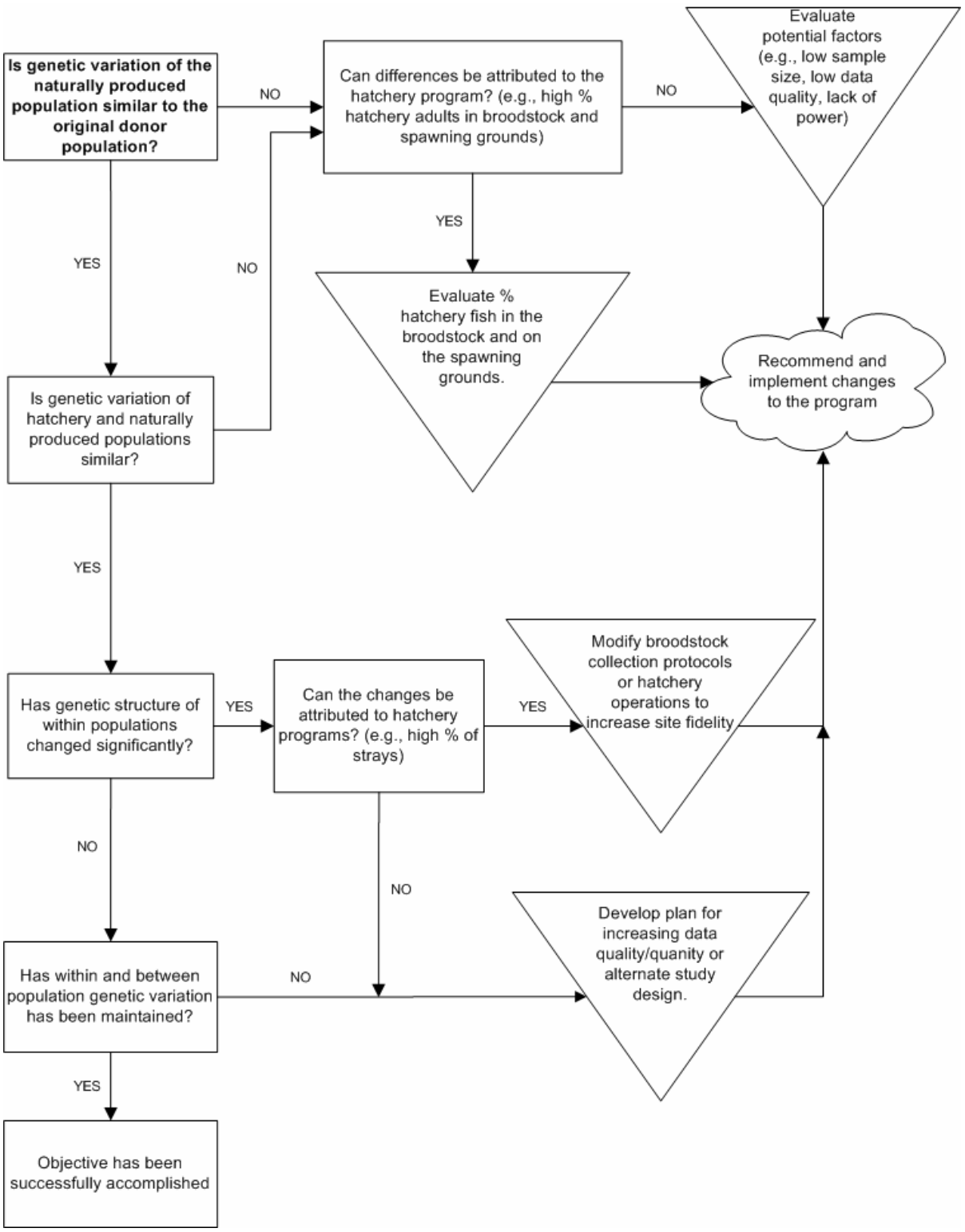

Figure 1. Conceptual process for evaluating potential changes in genetic variation in the Chiwawa naturally produced populations as a result of the supplementation hatchery programs (From Murdoch and Peven 2005).

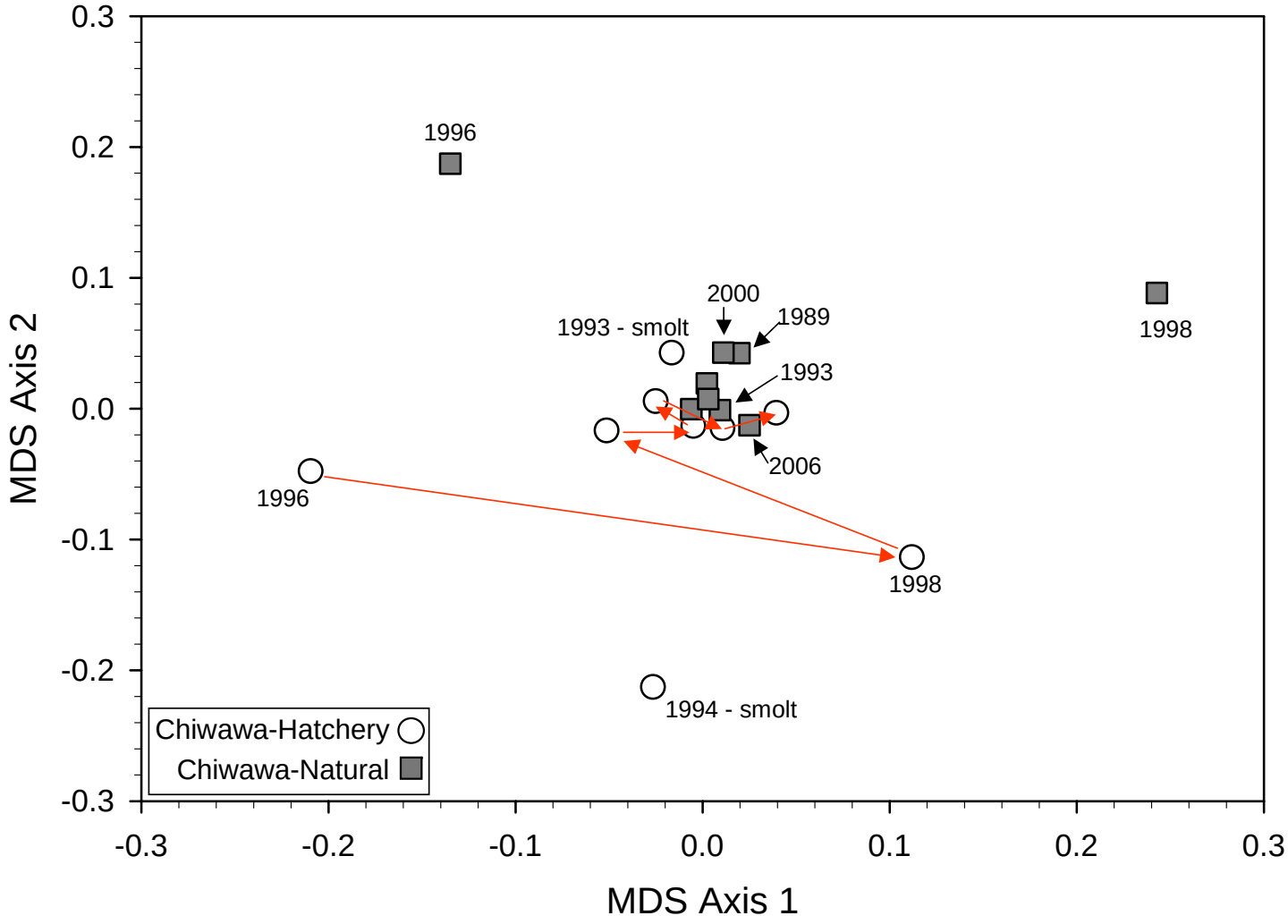

Figure 2. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by fish origin (i.e., hatchery versus natural). The red arrows connect consecutive hatchery-origin collections starting with the first adult collection (1996) and ending with the 2006 collection (see Table 1 for collection years).

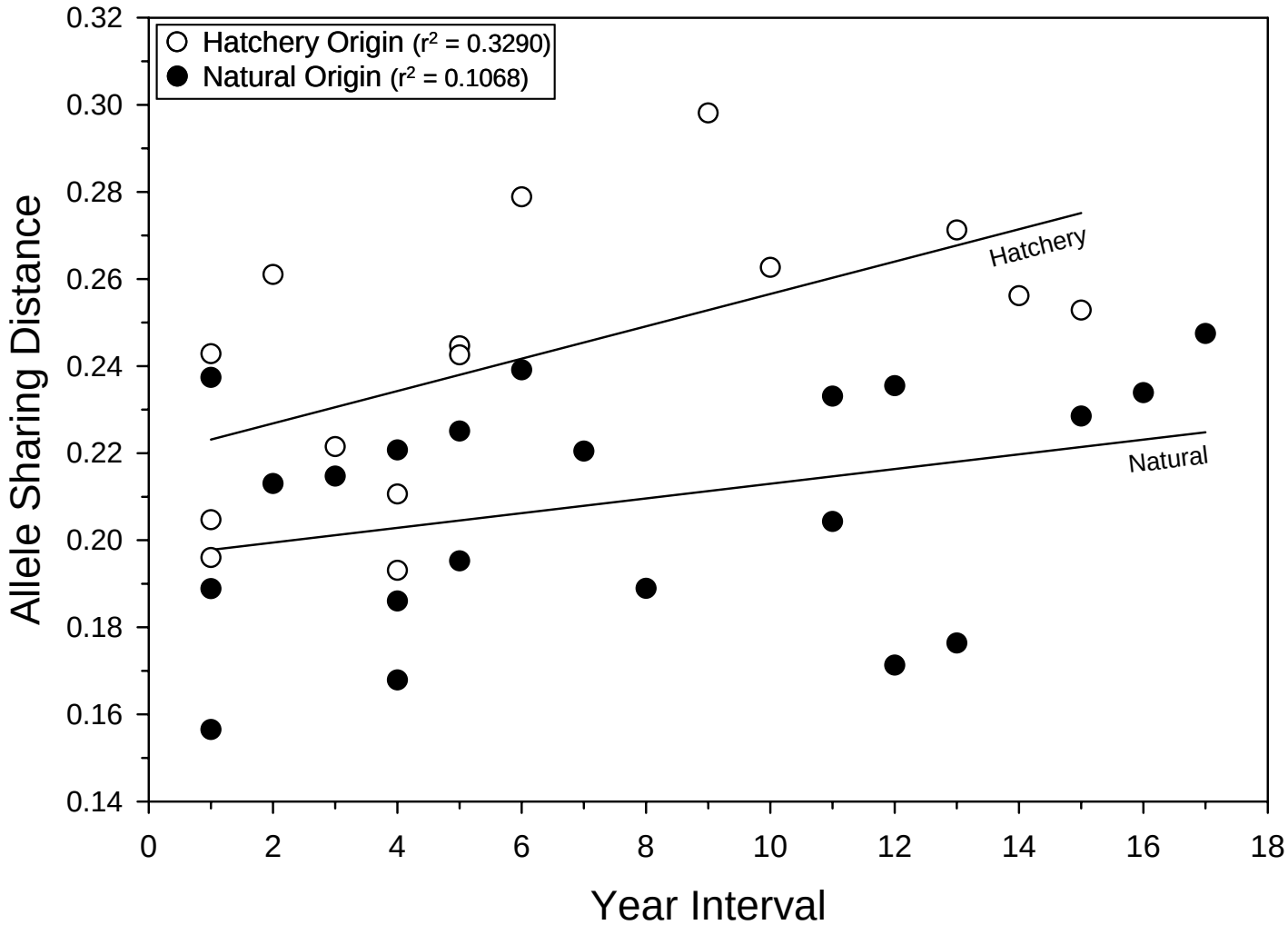

Figure 3. Relationships between the time interval in years and allele sharing distances, with each circle representing the pairwise relationship between two Chiwawa collections. Separate regression lines for the natural- and hatchery-origin collections. The slope for the natural-origin collection is not significantly different from zero (p=0.1483), while the slope for hatchery-origin collection is significantly greater than zero (p=0.0254) indicating a positive relationship between time interval and allele sharing distance.

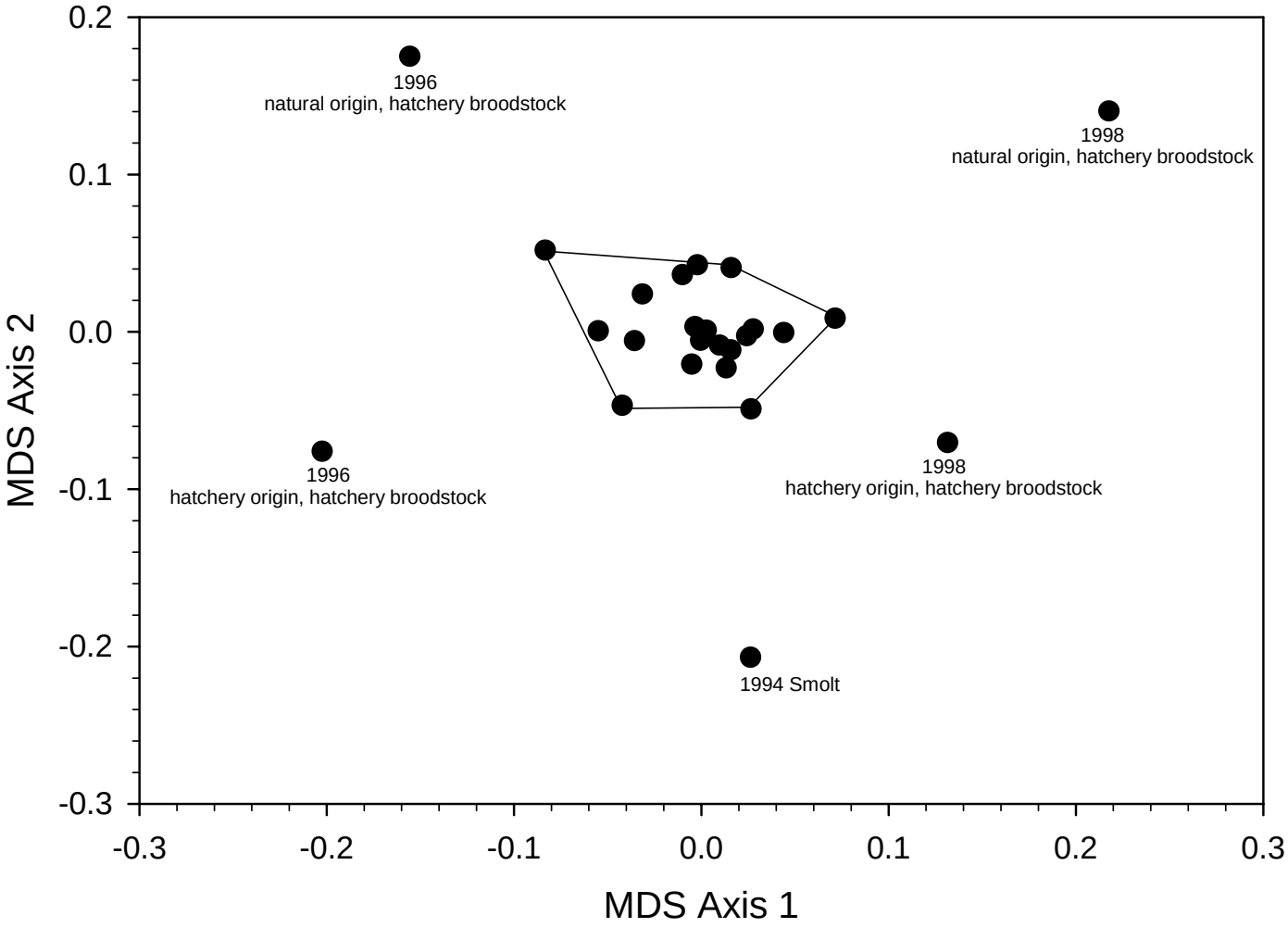

Figure 4. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa data set organized by four treatment groups, as discussed in the text. Each circle represents a single collection within each of the four treatment groups, and the polygon encloses all groups that are not outliers. Each outlier group is specifically labeled.

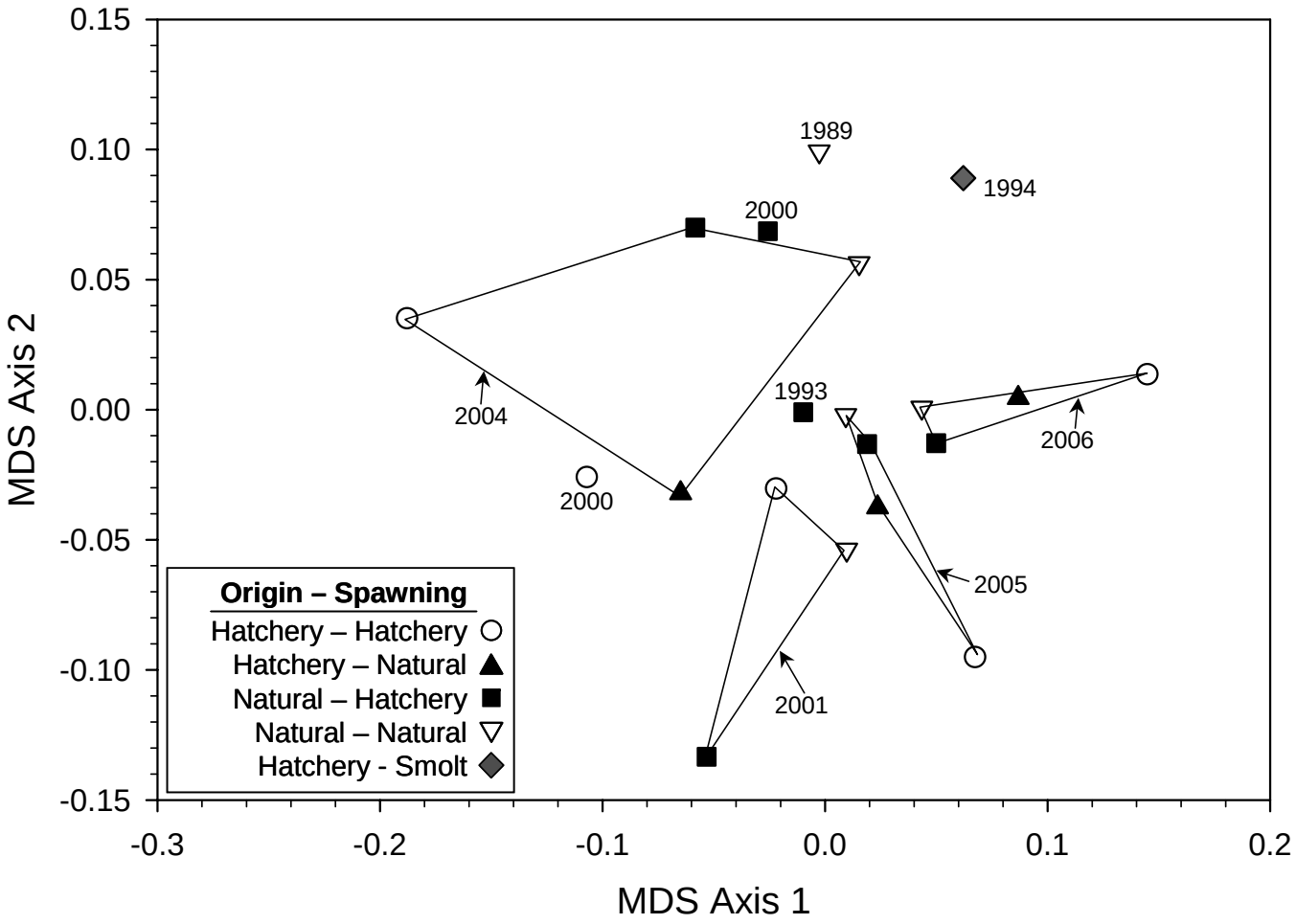

Figure 5. As in Figure 4, but allele-sharing distance matrix recalculated without the five outlier groups shown in Figure 4. Polygons group together treatment groups from the same collection year. Dates associated with symbols also refer to collection year. Collection years 2004-2006 included all four treatment groups, while collection year 2001 did not include a hatchery-origin natural spawner group. Legend is read as follows: Open circles refer to hatchery-origin hatchery spawner group, while filled box refers to natural-origin hatchery spawner group, and so on.

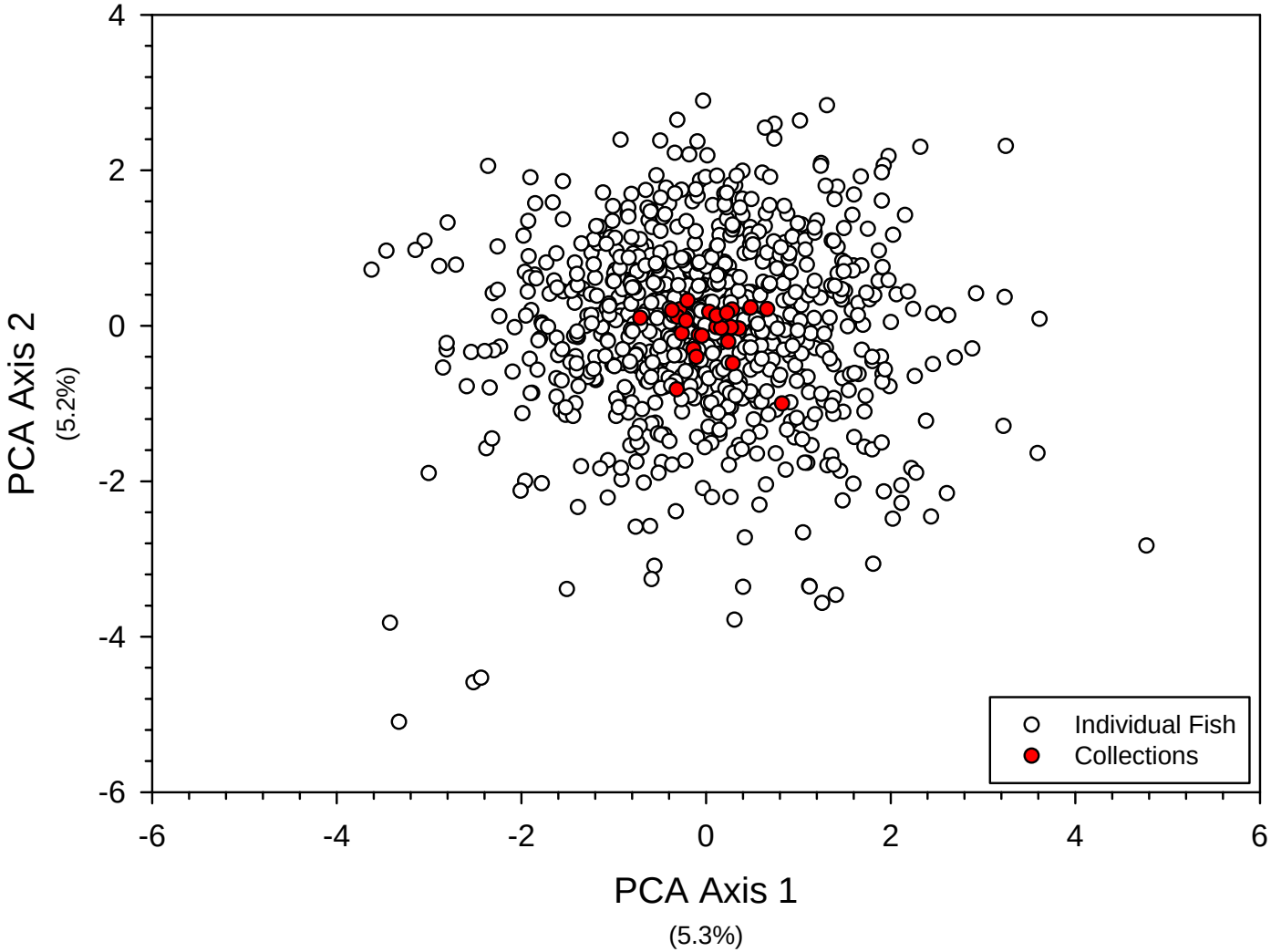

Figure 6. Principal component (PC) analysis of individual fish from the Chiwawa River. Only fish with complete microsatellite genotypes were included in the analysis (n = 757). Open circles are the PC scores for individual fish, and the filled circles are the centroids (bivariate means) for each of the 25 groups discussed in the text. PC axes 1 and 2 account for only 10.5% of the total molecular variance.

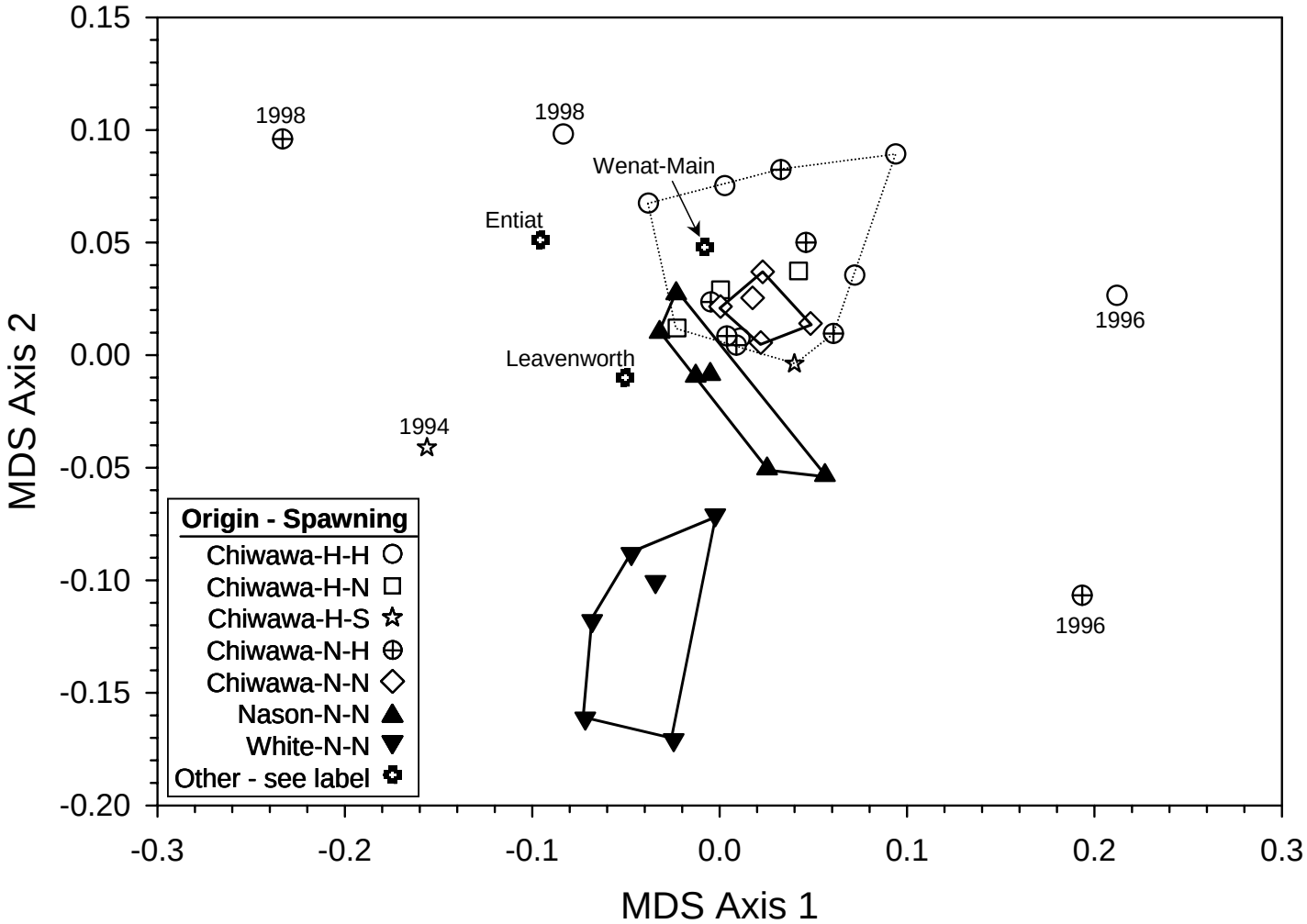

Figure 7. Multidimensional scaling plot from an allele-sharing distance matrix calculated from the Chiwawa origin data set and all other non-Chiwawa collections, except Little Wenatchee River. Legend is read with abbreviations beginning with origin and then spawning location. H=hatchery, N=natural, and S=smolts. Polygons with solid lines enclose the natural-origin natural spawner collections from each population (i.e., river). The polygon with the dotted lines enclose all Chiwawa collections, except for the five outlier collections, as discussed in text.

Table 1 Summary of within population genetic data. Chiwawa collection data are summarized in A) by origin of the sample (i.e., clipped vs. non-clipped). All collection data are summarized in B) by spawning location (i.e., hatchery broodstock or on spawning grounds). Hz is heterozygosity, HWE is the statistical significance of deviations from Hardy-Weinberg expectations (* = 0.05, ** = 0.01, and *** = 0.001), LD is the proportion of pairwise locus tests (across all populations) exhibiting linkage disequilibrium (bolded values are statistically significant), and the last column is mean number of alleles per locus.

Collection	Sample size	Gene Diversity	Observed Hz	HWE	F_{IS}	LD	Mean # Alleles
A) Origin							
1993 Chiwawa Hatchery	95	0.77	0.79	***	-0.02	**0.86**	14.00
1994 Chiwawa Hatchery	95	0.76	0.77	***	-0.01	**0.91**	11.38
1996 Chiwawa Hatchery	8	0.75	0.81	-	-0.01	0.00	8.23
1998 Chiwawa Hatchery	27	0.81	0.82	-	0.00	0.04	12.62
2000 Chiwawa Hatchery	43	0.75	0.78	***	-0.01	**0.19**	12.46
2001 Chiwawa Hatchery	69	0.77	0.80	***	-0.02	**0.14**	15.31
2004 Chiwawa Hatchery	72	0.77	0.77	***	0.01	**0.45**	15.92
2005 Chiwawa Hatchery	91	0.79	0.82	*	-0.03	**0.05**	16.15
2006 Chiwawa Hatchery	95	0.80	0.84	***	-0.05	**0.49**	15.85
1989 Chiwawa Natural	36	0.76	0.78	-	0.01	0.00	12.77
1993 Chiwawa Natural	62	0.78	0.81	-	-0.02	0.04	15.85
1996 Chiwawa Natural	8	0.72	0.78	-	-0.02	0.00	7.54
1998 Chiwawa Natural	10	0.78	0.84	-	0.00	0.00	8.23
2000 Chiwawa Natural	39	0.78	0.79	***	0.00	**0.10**	14.00
2001 Chiwawa Natural	75	0.78	0.80	-	-0.03	0.03	15.31
2004 Chiwawa Natural	85	0.78	0.77	-	0.02	0.01	15.77
2005 Chiwawa Natural	90	0.79	0.79	-	0.01	0.01	16.15
2006 Chiwawa Natural	96	0.80	0.81	-	-0.01	0.01	16.46

Table 1 Within population genetic data analysis summary continued.

Collection	Sample size	Gene Diversity	Observed Hz	HW	F_{IS}	LD	Mean # Alleles
B) Spawning Location							
1993 Chiwawa Broodstock	62	0.78	0.81	-	-0.02	0.00	15.85
1996 Chiwawa Broodstock	16	0.75	0.79	-	-0.02	0.00	10.92
1998 Chiwawa Broodstock	37	0.82	0.83	-	0.00	0.01	14.38
2000 Chiwawa Broodstock	82	0.78	0.78	***	0.00	**0.32**	15.62
2001 Chiwawa Broodstock	89	0.78	0.80	*	-0.02	**0.13**	15.77
2004 Chiwawa Broodstock	61	0.77	0.76	*	0.02	**0.13**	14.92
2005 Chiwawa Broodstock	75	0.79	0.78	*	0.02	0.01	15.85
2006 Chiwawa Broodstock	89	0.80	0.83	-	-0.03	**0.05**	16.46
1989 Chiwawa River	36	0.76	0.78	-	0.01	0.00	12.77
2001 Chiwawa River	55	0.78	0.80	-	-0.02	**0.09**	14.00
2004 Chiwawa River	96	0.78	0.78	*	0.01	**0.18**	17.23
2005 Chiwawa River	106	0.79	0.82	*	-0.02	**0.06**	16.69
2006 Chiwawa River	102	0.80	0.83	***	-0.03	**0.10**	16.77
1989 White River	48	0.75	0.75	-	0.01	0.01	12.85
1991 White River	19	0.76	0.76	-	0.03	0.00	10.92
1992 White River	22	0.75	0.79	-	-0.02	0.01	11.00
1993 White River	21	0.75	0.69	*	0.10	0.00	10.15
2005 White River	29	0.75	0.77	-	-0.01	0.03	12.23
2006 White River	40	0.76	0.76	-	0.01	0.04	13.38

Table 1 Within population genetic data analysis summary continued.

Collection	Sample size	Gene Diversity	Observed Hz	HW	F_{IS}	LD	Mean # Alleles
1993 Little Wenatchee R.	19	0.84	0.85	-	0.02	0.00	11.23
1993 Nason Creek	45	0.78	0.80	-	-0.01	0.01	13.77
2000 Nason Creek	51	0.76	0.78	-	-0.02	**0.13**	13.92
2001 Nason Creek	41	0.79	0.81	-	-0.01	**0.08**	14.23
2004 Nason Creek	38	0.76	0.76	-	0.02	0.03	13.23
2005 Nason Creek	45	0.78	0.82	-	-0.04	0.03	14.92
2006 Nason Creek	48	0.80	0.82	-	-0.01	0.00	15.77
2001 Wenatchee River	32	0.79	0.80	*	0.00	0.04	12.85
2000 Leavenworth NFH	73	0.80	0.82	*	-0.02	**0.15**	16.23
1997 Entiat NFH	37	0.81	0.83	-	-0.01	**0.06**	14.38

Table 2 Demographic data for Chiwawa Hatchery and Chiwawa natural spring Chinook salmon. BS is census size of hatchery broodstock, pNOB is the proportion of hatchery broodstock of natural origin, NOS is the census size of natural-origin spawners present in Chiwawa River, HOS is the census size of hatchery-origin spawners present in Chiwawa River, Total is NOS and HOS combined, and pNOS is the proportion of spawners present in Chiwawa River of natural origin.

	Hatchery		In River			
Brood Year	BS	pNOB	NOS	HOS	Total	pNOS
1989	28	1	1392	0	1392	1.00
1990	18	1	775	0	775	1.00
1991	32	1	585	0	585	1.00
1992	78	1	1099	0	1099	1.00
1993	94	1	677	491	1168	0.58
1994	11	0.64	190	90	280	0.68
1995	0	0	8	50	58	0.14
1996	18	0.44	131	51	182	0.72
1997	111	0.29	210	179	389	0.54
1998	47	0.28	134	45	178	0.75
1999	0	0	119	13	132	0.90
2000	30	0.3	378	310	688	0.55
2001	371	0.3	1280	2850	4130	0.31
2002	71	0.28	694	919	1613	0.43
2003	94	0.44	380	223	603	0.63
2004	215	0.39	820	788	1608	0.51
2005	270	0.33	250	1222	1472	0.17

Table 3 Levels of significance for pairwise tests of genic differentiation among all hatchery- and natural-origin collections used in this analysis. HS = highly significant (P < 0.000095; the Bonferroni corrected p-value for an alpha = 0.05); * = P < 0.05 (nominal critical value for most statistical test); - = P > 0.05 (not significant). A significant result between pairs of populations indicates that the allele frequencies between the pair are significantly different. Results are read by comparing the collections along the rows to collections along columns. The top block for each section is a symmetric matrix, as it compares collections within the same group.

		Chiwawa – Hatchery Origin								
		1993	**1994**	**1996**	**1998**	**2000**	**2001**	**2004**	**2005**	**2006**
Chiwawa – Hat. Origin	**1993**		HS	*	HS	HS	HS	HS	HS	HS
	1994	HS		HS	HS	HS	HS	HS	HS	HS
	1996	*	HS		*	-	*	-	-	*
	1998	HS	HS	*		HS	HS	HS	HS	HS
	2000	HS	HS	-	HS		HS	*	HS	HS
	2001	HS	HS	*	HS	HS		HS	*	HS
	2004	HS	HS	-	HS	*	HS		HS	HS
	2005	HS	HS	-	HS	HS	*	HS		HS
	2006	HS	HS	*	HS	HS	HS	HS	HS	
Chiwawa – Natural Origin	**1989**	HS	HS	-	HS	HS	*	HS	HS	HS
	1993	HS	HS	-	HS	HS	-	HS	*	HS
	1996	*	HS	-	*	-	-	-	-	-
	1998	HS	HS	-	-	HS	*	*	*	-
	2000	HS	HS	-	HS	HS	HS	*	HS	HS
	2001	HS	HS	-	HS	HS	HS	HS	*	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	*	HS	*	HS
	2006	HS	HS	-	*	HS	HS	HS	HS	HS
Nason	**1996**	HS	HS	-	HS	HS	HS	HS	HS	HS
	2000	HS	HS	*	HS	HS	HS	HS	HS	HS
	2001	HS	HS	-	HS	HS	HS	HS	HS	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	HS	HS	HS	HS
	2006	HS	HS	-	*	HS	HS	HS	HS	HS
White	**1989**	HS	HS	HS	HS	HS	HS	HS	HS	HS
	1991	HS	HS	-	HS	HS	HS	HS	HS	HS
	1992	HS	HS	*	HS	HS	HS	HS	HS	HS
	1993	HS	HS	*	HS	HS	HS	HS	HS	HS
	2005	HS	HS	-	HS	HS	HS	HS	HS	HS
	2006	HS	HS	HS	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	HS	HS	*	HS	HS	*	*	-	HS
	Leaven	HS	HS	*	HS	HS	HS	HS	HS	HS
	Entiat	HS	HS	*	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		Chiwawa – Natural Origin								
		1989	**1993**	**1996**	**1998**	**2000**	**2001**	**2004**	**2005**	**2006**
Chiwawa – Natural Origin	**1989**		-	-	-	-	*	*	*	*
	1993	-		-	*	*	*	HS	*	HS
	1996	-	-		-	-	-	-	-	-
	1998	-	*	-		*	*	HS	*	*
	2000	-	*	-	*		HS	-	HS	HS
	2001	*	*	-	*	HS		HS	*	HS
	2004	*	HS	-	HS	-	HS		HS	HS
	2005	*	*	-	*	HS	*	HS		*
	2006	*	HS	-	*	HS	HS	HS	*	
Nason	**1996**	*	*	-	*	*	HS	HS	HS	HS
	2000	HS	HS	HS	HS	HS	HS	HS	HS	HS
	2001	HS	*	-	*	HS	HS	HS	HS	HS
	2004	HS	HS	-	HS	HS	HS	HS	HS	HS
	2005	*	*	-	*	HS	HS	HS	HS	HS
	2006	HS	HS	-	-	HS	HS	HS	HS	HS
White	**1989**	HS	HS	*	HS	HS	HS	HS	HS	HS
	1991	HS	HS	*	-	HS	HS	HS	HS	HS
	1992	HS	HS	-	*	HS	HS	HS	HS	HS
	1993	HS	*	-	*	HS	HS	HS	HS	HS
	2005	HS	*	*	*	HS	HS	HS	*	HS
	2006	HS	HS	*	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	*	-	-	-	*	*	HS	*	*
	Leaven	HS	HS	*	*	HS	HS	HS	HS	HS
	Entiat	HS	HS	*	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		Nason					
		1996	**2000**	**2001**	**2004**	**2005**	**2006**
Nason	**1996**		HS	-	HS	-	*
	2000	HS		HS	HS	HS	HS
	2001	-	HS		*	-	*
	2004	HS	HS	*		*	HS
	2005	-	HS	-	*		-
	2006	*	HS	*	HS	-	
White	**1989**	HS	HS	HS	HS	HS	HS
	1991	*	HS	HS	HS	*	*
	1992	HS	HS	HS	HS	HS	HS
	1993	*	HS	HS	HS	HS	HS
	2005	*	HS	HS	HS	HS	HS
	2006	HS	HS	HS	HS	HS	HS
Other	**Wen-M**	HS	HS	HS	HS	*	HS
	Leaven	HS	HS	HS	HS	HS	HS
	Entiat	HS	HS	HS	HS	HS	HS

Table 3 (con't)

		White						Other		
		1989	**1991**	**1992**	**1993**	**2005**	**2006**	**Wen-M 2001**	**Leaven 2000**	**Entiat 1997**
White	**1989**		-	*	-	HS	HS	HS	HS	HS
	1991	-		-	-	*	*	*	HS	HS
	1992	*	-		-	*	*	HS	HS	HS
	1993	-	-	-		*	*	HS	HS	HS
	2005	HS	*	*	*		*	HS	HS	HS
	2006	HS	*	*	*	*		HS	HS	HS
Other	**Wen-M**	HS	*	HS	HS	HS	HS		HS	HS
	Leaven	HS	HS	HS	HS	HS	HS	HS		HS
	Entiat	HS	HS	HS	HS	HS	HS	HS	HS	

Table 4 Probabilities (above diagonal) and levels of significance (below diagonal) for pairwise tests of genic differentiation among all Chiwawa hatchery broodstock and Chiwawa natural spawner collections used in this analysis. HS = highly significant (P < 0.000476; the Bonferroni corrected p-value for an alpha = 0.05); * = P < 0.05 (nominal critical value for most statistical test); - = P > 0.05 (considered not significant). A significant result between pairs of populations indicates that the allele frequencies between the pair are significantly different. Pairwise comparisons between the hatchery broodstock and natural spawner collections from 2001, 2004, 2005, and 2006, respectively, are highlighted.

		Smolt		Hatchery Broodstock								Natural Spawners				
		1993	1994	1993	1996	1998	2000	2001	2004	2005	2006	1989	2001	2004	2005	2006
Smolt	1993		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	1994	HS		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Hatchery Broodstock	1993	HS	HS		0.9155	0.0000	0.0073	0.3647	0.0003	0.0694	0.0000	0.2220	0.0039	0.0008	0.0095	0.0000
	1996	HS	HS	-		0.0151	0.8388	0.0452	0.4916	0.3189	0.0716	0.5591	0.0759	0.8101	0.2364	0.0786
	1998	HS	HS	HS	*		0.0000	0.0000	0.0000	0.0000	0.0043	0.0000	0.0000	0.0000	0.0000	0.0005
	2000	HS	HS	*	-	HS		0.0000	0.4720	0.0000	0.0000	0.0036	0.0000	0.0712	0.0000	0.0000
	2001	HS	HS	-	*	HS	HS		0.0000	0.0059	0.0000	0.0003	0.0000	0.0000	0.0126	0.0000
	2004	HS	HS	*	-	HS	-	HS		0.0000	0.0000	0.0001	0.0000	0.0012	0.0000	0.0000
	2005	HS	HS	-	-	HS	HS	*	HS		0.0005	0.0024	0.0137	0.0025	0.7782	0.0018
	2006	HS	HS	HS	-	*	HS	HS	HS	*		0.0000	0.0000	0.0000	0.0000	0.5770
Natural Spawners	1989	HS	HS	-	-	HS	*	*	HS	*	HS		0.0023	0.0317	0.0000	0.0003
	2001	HS	HS	*	-	HS	HS	HS	HS	*	HS	*		0.0000	0.2641	0.0000
	2004	HS	HS	*	-	HS	-	HS	*	*	HS	*	HS		0.0000	0.0000
	2005	HS	HS	*	-	HS	HS	*	HS	-	HS	HS	-	HS		0.0000
	2006	HS	HS	HS	-	*	HS	HS	HS	*	-	*	HS	HS	HS	

Table 5 Analysis of molecular variance (AMOVA) for the Chiwawa collections, showing the partition of molecular variance into (1) within collections, (2) among collections but within group, and (3) among group components. Each column in the table represents a separate analysis testing for differences under a different spatial or temporal hypothesis. The different analyses are grouped together in a single table for comparisons. The values within the table are percentages and the parenthetical values are P-values, or probabilities, associated with that percentage. P-values greater than 0.05 indicate that the percentage is not significantly different from zero. For example, when collections are organized by hatchery- versus natural-origin ("Origin" – fourth column), 0.11% of the molecular variance is attributed to among group (i.e., hatchery- versus natural-origin), which is not significantly different from zero. No collections (first column) indicates no organization or grouping among all collections, and the among-group percentage is equal to the F_{ST} for the entire data set.

	No Structure	Collection Year	Spawning Location	Origin	Origin-Spawning Location
Among Groups	0.26 (0.00)	0.20 (0.43)	0.05 (0.48)	0.11 (0.15)	0.11 (0.06)
Among collections - Within groups	-	0.08 (0.003)	0.24 (0.00)	0.21 (0.00)	0.18 (0.06)
Within collections	99.74 (0.00)	99.72 (0.00)	99.71 (0.00)	99.68 (0.00)	99.71 (0.00)

Table 6 F_{ST} values for all pairwise combinations of populations. Each F_{ST} is the median value for all pairwise combinations of collections within each population (the number of collections within each population is shown parenthetically next to each population name on each row). For example, the F_{ST} for the Chiwawa hatchery versus the White River (0.019) is the median value of 54 pairwise comparisons. The bold values along the center diagonal are the median F_{ST} values within each collection. For those populations with only one collection, the diagonal value was set at 0.000.

	Chiwawa-Hatchery	Chiwawa-Natural	Entiat	Leaven-worth	Nason	Wenatchee-main	White	Little Wenatchee
Chiwawa-Hatchery (9)	**0.013**	0.008	0.016	0.012	0.011	0.005	0.019	0.111
Chiwawa-Natural (9)		**0.003**	0.012	0.011	0.007	0.003	0.014	0.105
Entiat (1)			**0.000**	0.005	0.010	0.008	0.019	0.078
Leavenworth (1)				**0.000**	0.007	0.008	0.014	0.092
Nason (6)					**0.006**	0.008	0.015	0.099
Wenatchee-main (1)						**0.000**	0.012	0.098
White (6)							**0.005**	0.113
Little Wenatchee (1)								**0.000**

Table 7 As in Table 5, except data includes Chiwawa hatchery- and natural-origin, Nason Creek, and White River collections

	All Years	All Years	1989-1996	2005-2006	2005-2006
	No Structure	Origin	Origin	Origin	Collection Year
Among Groups	0.28 (0.00)	0.33 (0.00)	-0.07 (0.67)	0.43 (0.01)	-0.06 (0.57)
Among Collections - Within groups	-	0.04 (0.00)	0.22 (0.00)	0.25 (0.00)	0.64 (0.00)
Within Collections	99.72	99.63	99.85	99.32	99.41

Table 8 Individual assignment results reported are the numbers of individuals assigned to each population using the partial Bayesian criteria of Rannala and Mountain (1997) and a "jack-knife" procedure (see Methods). The population with the highest posterior probability is considered the stock of origin (i.e., no unassigned individuals). Individuals from each population are assigned to specific populations (along rows). Bold values indicate correct assignment back to population of origin. Individuals assigned to a population are read down columns. For example, of the 595 individuals from Chiwawa hatchery origin, 134 individuals were assigned to Chiwawa natural origin (reading across). Of the 511 individuals assigned to Chiwawa natural origin (reading down), 60 were from Nason Creek.

Population	**Total**	**Unassigned**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1) Chiwawa Hatchery	595	0	**371**	134	2	16	0	45	15	12
2) Chiwawa Natural	501	0	156	**269**	4	5	0	42	9	16
3) Entiat	37	0	4	5	**13**	8	0	6	1	0
4) Leavenworth	73	0	9	8	3	**33**	0	17	0	3
5) Little Wenatchee	19	0	0	0	0	0	**19**	0	0	0
6) Nason	268	0	49	60	5	11	0	**131**	1	11
7) Wenatchee Mainstem	32	0	12	9	0	1	0	2	**6**	2
8) White	179	0	22	26	0	2	0	13	1	**115**
TOTAL	1704	0	623	511	27	76	19	256	33	159

Table 9 As in Table 8, except the posterior probability from the partial Bayesian criteria of Rannala and Mountain (1997) must be 0.90 or greater, to be assigned to a population. Those individuals with posterior probabilities less than 0.90 are unassigned.

Aggregate	**Total**	**Unassigned**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1) Chiwawa Hatchery	595	332	**214**	31	1	4	0	10	3	0
2) Chiwawa Natural	501	375	30	**82**	0	1	0	5	2	6
3) Entiat	37	24	1	1	**5**	4	0	2	0	0
4) Leavenworth	73	51	0	1	1	**19**	0	1	0	0
5) Little Wenatchee	19	2	0	0	0	0	**17**	0	0	0
6) Nason	268	188	11	6	2	5	0	**53**	0	3
7) Wenatchee Mainstem	32	23	4	3	0	0	0	0	**2**	0
8) White	179	92	4	3	0	1	0	5	1	**73**
TOTAL	1704	1087	264	127	9	34	17	76	8	82

Table 10 Estimates of N_e based on bias correction method of Waples (2006) implemented in LDNe (Do and Waples unpublished). Collections are categorized by spawning location. Sample size is the harmonic mean of the sample size, 95% CI is the confidence interval calculated using Waples' (2006) equation 12, and Major Cohort assumes that each collection is 100% four-year-olds.

	Sample size	Estimated N_b	95% CI	Major Cohort	Census	N_e/N
1993 Chiwawa Broodstock	58.4	103.1	77.0 - 149.7	1989	1392	0.30
1996 Chiwawa Broodstock	15.5	30.4	19.6 - 58.1	1992	1099	0.11
1998 Chiwawa Broodstock	33.4	37.7	29.8 - 49.7	1994	280	0.54
2000 Chiwawa Broodstock	77.8	48.4	41.4 - 57.2	1996	182	1.06
2001 Chiwawa Broodstock	80.4	49.6	42.2 - 59.2	1997	389	0.51
2004 Chiwawa Broodstock	56.6	48.1	39.0 - 60.9	2000	688	0.28
2005 Chiwawa Broodstock	73	274.3	148.9 - 1131.8	2001	4130	0.27
2006 Chiwawa Broodstock	88.4	198.3	136.1 - 340.5	2002	1613	0.49
1989 Chiwawa River	26.6	5.2	3.9 - 6.3	1985		
2001 Chiwawa River	46.7	38.6	31.0 - 49.3	1997	389	0.40
2004 Chiwawa River	88.5	82.6	67.3 - 104.4	2000	688	0.48
2005 Chiwawa River	104.2	231.5	161.8 - 382.7	2001	4130	0.22
2006 Chiwawa River	101.1	107.3	87.2 - 136	2002	1613	0.27

Table 11 Summary of output from program SALMONNb and data for eight Chiwawa broodstock collections from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1993	1996	1998	2000	2001	2004	2005	2006
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):								
1993	-	24.5	42.5	66.4	67.2	57.2	64.6	70.3
1996	82	-	21.2	25.8	26.0	24.4	25.6	26.4
1998	80	81	-	46.7	47.2	42.0	45.8	48.4
2000	80	82	84	-	78.6	65.2	75.1	82.7
2001	73	77	81	76	-	66.0	76.2	84.2
2004	77	81	75	76	78	-	63.5	69.0
2005	71	75	82	73	73	69	-	80.0
2006	81	80	84	75	74	75	72	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):								
1993	-	-742.7	406.9	1240.8	-5432.0	829.8	808.9	729.0
1996	22491.2	-	110.4	-1786.5	765.9	162.8	824.7	382.7
1998	10910.4	67299.1	-	101.8	237.1	69.6	307.0	140.0
2000	6910.0	742895.8	19122.7	-	490.6	1498.2	706.9	201.6
2001	49318.3	21402.8	9754.2	6126.6	-	307.8	82.0	362.5
2004	8338.4	257267.7	24283.0	145043.4	7095.7	-	269.7	140.1
2005	31511.8	22242.5	10015.8	6596.6	114931.1	8240.4	-	599.6
2006	6223.8	43935.2	73518.7	10152.5	5885.3	12827.0	6370.8	-

$\tilde{N}_b = 269.4$

Table 12 Summary of output from program SALMONNb and data for five Chiwawa in-river spawner collections from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1989	2001	2004	2005	2006
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):					
1989	-	33.3	40.2	41.7	42.2
2001	72	-	60.5	63.9	63.3
2004	72	77	-	95.3	94.0
2005	69	72	75	-	102.5
2006	76	76	77	78	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):					
1989	-	118.4	299.0	143.3	165.3
2001	40378.8	-	181.7	-1537.3	153.5
2004	10455.2	7265.5	-	387.1	329.4
2005	20923.6	68660.6	5040.7	-	356.8
2006	16227.2	8886.9	3802.0	4522.8	-

$\tilde{N}_b = 224.2$

Table 13 Summary of output from program SALMONNb and data for three brood years that combined Chiwawa natural- and hatchery-origin samples from Wenatchee River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $\hat{N}_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	2004	2005	2006
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):			
2004	-	162	164.3
2005	77	-	188.2
2006	76	75	-
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):			
2004	-	611.3	210.8
2005	9351.5	-	727.5
2006	14965.5	8673.9	-

$\tilde{N}_b$ = 386.8