Genetic Analysis of Natural-origin Spring Chinook and Comparison to Spring Chinook from an Integrated Supplementation Program and Captive Broodstock Program in the Tucannon River

by

Todd W. Kassler and Cheryl A. Dean

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U.S. Department of Energy
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Portland, OR 97283-3621

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Washington Department of Fish and Wildlife, Molecular Genetics Laboratory
600 Capitol Way N, Olympia, WA 98501

Abstract

A collection of natural-origin spring Chinook from 1986 was compared to samples from two spawner groups (supplementation program and in-river spawners), and to collections of hatchery- and natural-origin from the Tucannon River. Samples from the captive brood program at the Tucannon River Hatchery were also compared. A microsatellite DNA analysis was conducted to determine if there have been any changes to the genetic diversity of spring Chinook in the Tucannon River. The measures of genetic diversity (heterozygosity and allelic richness) revealed similar levels within each spawner group and collection based on origin over time. Assessment of within population diversity indicates that the spawner groups and collections by origin have not undergone a loss of diversity and are not represented by family groups. We did detect that collections of the captive brood are not within Hardy-Weinberg proportions and have significant linkage disequilibrium as a possible result of using equal numbers of individuals from two brood years that are differentiated. The collection of captive brood progeny returns in 2008; however is within expected proportions and indicates there has not been a genetic change to the spawner group collection or collections by origin. The pairwise F_{ST} values identify the variation between any two groups is approximately 1.0% or less indicating the differences among the groups is small. Factorial correspondence analysis identifies similarity among collections that are separated by four years and represent the genetic differences among primary brood years and not genetic changes to the natural-origin collection from 1986. The combination of all the results demonstrates that the genetic diversity of spring Chinook in the Tucannon River has not significantly changed as a result of the supplementation or captive brood programs.

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Introduction

The Tucannon River is a tributary of the Snake River in southeastern Washington and returning salmon have to traverse up the Columbia River and Snake River past six hydroelectric dams. Because of the reduction in returning salmon due to the dams, a plan was developed (Lower Snake River Compensation Plan) to mitigate for the loss (USACE 1975). As part of the Lower Snake River Compensation Plan, funding was made available to Washington State to build or modify two facilities (Lyons Ferry Hatchery and Tucannon River Hatchery) to provide fish production to mitigate for impacts caused by the dams. In 1985, the spring Chinook supplementation program was initiated in the Tucannon River by capturing wild endemic adults and spawning them at the Tucannon River Hatchery. By 1989, the hatchery was integrating natural and hatchery-origin spring Chinook in the broodstock and both natural and hatchery-origin spring Chinook were naturally spawning in the river.

Spring Chinook in the Snake River basin (including the Tucannon River) were listed as “Endangered” in 1992 by the National Marine Fisheries Service (Bumgarner and Gallinat 2001). That status was changed to “Threatened” in 1995. Adult returns declined precipitously during the mid 1990s so a captive brood program was proposed by WDFW and the co-managers in addition to the supplementation program that had begun in 1985 (Bumgarner and Gallinat 2001).

The plans for the captive broodstock program were developed and in 1997 the program began. A portion of the returning hatchery and natural-origin adults to the supplementation program were spawned. Subsamples of those fry were separated for the captive brood program while the remaining fry were included with the supplementation program; therefore the adults for the production of fry for the captive brood program had offspring that were represented in both the captive brood and the supplementation programs. Production of fry for the captive brood program was done for six years from 1997-2002 brood years (BY). Beginning in 2000 the first captive brood fish were mature and were spawned with natural origin adult returns and other mature individuals from the captive brood program. The first batch of offspring from the captive brood were marked and then released in 2002 and continued until 2008. The first adult returns (three year olds) from the released captive brood offspring were in 2004. A complete description of the captive brood program development and the number of families used for each brood year is described in Gallinat et al. (2009).

The hatchery programs in the Tucannon River (supplementation and captive brood) are being conducted with the possibility that artificial propagation may have negative effects on the genetic profile of spring Chinook in the Tucannon River. The genetic effects could result in the fitness loss and lower reproductive success. A paper by Fraser (2008) addresses many of the possible genetic issues associated with a captive brood program and if genetic diversity can be conserved in natural-origin populations of salmonids.

This study uses a microsatellite DNA analysis to evaluate spring Chinook from three spawner groups (in-river spawners; supplementation spawners, and the captive brood program). Analysis of natural- and hatchery-origin are also used to determine the impacts of spawner group in addition to spawner origin. Analysis was conducted on collections from 1986, 1997 – 1998, and 2000 – 2008. The collection from 1986 was prior to the return of Chinook that were produced by the supplementation program and is therefore a collection of the wild endemic stock. This analysis provided a measure of the genetic diversity of Tucannon River spring Chinook prior to the supplementation program and evaluation of genetic changes over 12 years including the time of the captive brood program in the Tucannon River.

Materials and Methods

Collections

A total of 2,545 samples were analyzed at 14 microsatellite loci (13 coastwide GAPS loci plus *Ssa-197*). Samples were identified as hatchery or natural-origin and collected from in-river (natural- and hatchery-origin Chinook spawn together naturally) and the supplementation program (natural- and hatchery-origin Chinook are used) from 1997– 1998 and 2000 – 2008. Marking (i.e., adipose fin clip, visible implant elastomer) and tagging with coded-wire tags (CWT) made it possible to positively identify each hatchery-origin Chinook. Chinook that were unmarked were considered to be natural-origin; however they could have been Tucannon River hatchery fish that had lost their tags or were unmarked hatchery strays. Samples were also collected from the captive brood program that included three groups of samples: adults for production of the captive brood; captive brood; and offspring of the captive brood that returned as adults. The adults used for the production of the captive brood were sampled from 1997 – 2001. Two year old Chinook from 2002 were also used for production of the captive brood; however there were only four fish identified for this collection and therefore not included in our analyses. Captive brood samples were sampled as adults when they were being spawned from 2000 – 2006 and offspring of the captive brood were collected in 2008 when they returned. A collection of natural-origin Chinook from 1986 was also included in the analyses for comparison.

The sample sizes for each of the collections and analyses that were conducted are shown in Table 1. A breakdown showing the number of individuals for each collection year and brood year is shown in Table 2.

Laboratory Analyses

Genomic DNA was extracted by digesting a small piece of fin tissue using the NucleoSpin® 96 Tissue kit (Macherey-Nagel Bethlehem, PA, USA) following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL. Descriptions of the loci assessed in this study and the annealing temperature for each locus are given in Table 3. PCR reactions were run with a simple thermal profile consisting of: denaturation at 95^{o}C for 3 min, denaturation at 95^{o}C for 15 sec, anneal for 30 sec at the appropriate temperature for each locus (Table 2), extension at 72^{o}C for 1 min, repeat cycle (steps 2-4), final extension at 72^{o}C for 10 minutes. PCR products were then run through the ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GENEMAPPER 3.7 software. Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et al. 2007).

Statistical Analyses

Allele frequencies were calculated using CONVERT (version 1.3; Glaubitz 2003). Tests for Hardy-Weinberg proportions for each locus and over all loci within each subpopulation were performed using GENEPOP (version 3.4; Raymond and Rousset 1995). Statistical significance of the Hardy-Weinberg proportions was evaluated using a Bonferroni correction of p-values (Rice 1989). Linkage disequilibrium was compared between each pair of loci for each collection using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the linkage disequilibrium analysis was evaluated using a Bonferroni correction of p-values (Rice 1989). The Bonferroni correction is a procedure that is employed to minimize Type I errors (declaring a significant difference due to chance) by dividing the 0.05 significance level by the total number of tests being conducted. Values that are significant after correction can then be evaluated based on their true significance and not by chance alone.

Observed and expected heterozygosity was computed for each subpopulation using GDA (Lewis and Zaykin 2001). Allelic richness (Weir and Cockerham 1984) was computed for each subpopulation with FSTAT (version 2.9.3.2;

Goudet 2001). Pairwise F_{ST} estimates were computed to examine population structure using GENETIX (version 4.03, Belkhir et al. 2001). These estimates use allelic and genotypic frequency data to assess differences between pairs of populations being analyzed.

Within a group, the coefficient of identity was calculated between each pair of samples in all collections using Queller and Goodnight (1989) estimator of relatedness in the program IDENTIX v.1.1 (Belkhir et al. 2002). Using this measure of relatedness, a value of 0.45 is expected for a full-sibling relationship (individuals sharing the same mother and father) between two individuals.

GENETIX (version 4.03, Belkhir et al. 2001) was used for a factorial correspondence analysis and a graphical representation of the genetic variation among all individual samples in multi-dimensional space. Genotypic data for an individual sample is transformed into a value and plotted. The multi-dimensional data space represents all the individual values. Each axis (three-dimensional in this case) is derived from the individual values that correspond to percent of total chi-square distance, with chi-square measuring the association between individual genotypes (weighted by the collection centroid when "sur populations" is selected for the analysis) and allele frequencies.

Grouping of Samples for Statistical Analyses

Eight different groupings of samples were analyzed to determine if there were genetic differences among hatchery or natural-origin samples, in-river or supplementation samples, and the captive brood. Analyses were first run on all samples from each collection year to determine if there were any significant differences among the collection years. Analyses were then run to compare the adults that were used to produce the captive brood, the captive brood and the returning offspring of the captive brood. Next we analyzed the temporal spawner groups of the in-river and supplementation collections. The adults used for production of the captive brood were included in the analysis of the supplementation spawners because some of their offspring were included in this group. The number of individuals from each brood year varied across the temporal collection years so we conducted analyses by dividing the in-river and supplementation samples into collections identified by brood year. Individual samples were then divided into their spawner origin (hatchery or natural-origin) for analysis. The samples were further divided into groupings of supplementation/hatchery-origin, supplementation/natural-origin, in-river/hatchery-origin, and in-river/natural-origin to be analyzed by collection year. The number of individuals for brood year was too small for analysis using brood year so we only conducted analyses using collection year.

Lastly, we compared the adults used for production of the captive brood to their offspring (the captive brood) with the individuals that were used in the supplementation program and their offspring. This analysis was conducted to determine if there were any genetic differences that resulted between the captive brood and the supplementation group and their offspring (some were full siblings). The captive brood were held and raised until they matured while their offspring in the supplementation program were released to the wild to migrate to the ocean and return when they matured. The adults that produced the captive brood were compared to the adults used for production of the supplementation program in each year. The adults of the captive brood were compared to their offspring and the adults used in the supplementation program were compared to their returning offspring (identified as hatchery-origin). Lastly, the captive brood samples were compared to the offspring produced by the supplementation program (including siblings of the captive brood). These samples were analyzed using their brood year because we wanted to have direct comparison of parent to offspring collections.

Results

Samples with genotypes for 10 or more loci were included in the analysis. Individual fish samples identified as strays (or unknown origin) by presence of adipose clip and no CWT, DIPs (dead in pond), and PSM (pre-spawn mortality) were excluded before analysis because they did not contribute to the spawning group.

Results for the analysis of Hardy-Weinberg expectation and linkage disequilibrium for each of the analysis is shown in Table 4 while values for allelic richness and heterozygosity are shown in Table 5. Values for the allelic richness and heterozygosity do not vary and are not discussed for the results of each section.

<u>Analyses by collection years #1</u>

Tests for significant locus deviation to Hardy-Weinberg expectations for the analysis of all samples from each of the yearly collections identified significant differences at 1 – 6 loci in the collections from 1997, 2001 – 2006. The analysis of linkage disequilibrium for all samples from each of the yearly collections identified that the collections from 2001 – 2006 had significant differences at over 37% of the locus comparisons. The linkage disequilibrium at the other collection years was below 10% with the exception of the collection in 2000 (13.2% of the locus comparisons had significant linkage disequilibrium). The pairwise FST results identifies all comparisons are below 1.0%, but some significant differences from zero occur for some of the comparisons (Table 6).

<u>Analysis of the three captive brood groups #2</u>

The Hardy-Weinberg analysis found significant locus differences with the captive brood collections (from 2002 – 2006) and not in the adults that produced the captive brood or captive brood returns. The analysis of linkage disequilibrium for the adults that produced the captive brood, captive brood, and the captive brood returns revealed the most significant locus comparisons with the captive brood collections from 2002 - 2005. The number of significant locus comparisons for the adults that produced the captive brood and the collection of captive brood returns were below 10%; while the percentage of significant locus comparisons for the captive brood samples were between 13.2 and 80.2%.

Pairwise F_{ST} analysis of the three captive brood collections revealed no significant differences for the 1997 and 1998 adults that produced the captive brood to any of the captive brood collections (Table 7). The pairwise F_{ST} comparison of the 2000 and 2001 adults that produced the captive brood were significantly different from each other and the 2000 collection was significantly different from the 2005 – 2006 captive brood. The 2001 adults that produced the captive brood were significantly different from the 2002 captive brood. The 2000 captive brood did not have any pairwise F_{ST} comparisons that were significantly different. Most of the pairwise F_{ST} comparisons of the captive brood from 2001 – 2006 were significantly different from zero with the exception of the following comparisons: 2001 and 2002; 2001 and 2003; 2001 and 2006. The 2008 captive brood returns were significantly different from all captive brood collections with exception of the 2000 and 2004 captive brood collections.

The analysis of relatedness among the captive brood collections revealed between 0.20% and 2.63% of the comparisons to be 0.45 or greater indicating what could be a full-sibling relationship. The lowest relatedness value occurred in the 2008 captive brood returns and the highest relatedness value occurred in the 2000 captive brood collection. The average of the four collections of the adults that produced the captive brood was 0.67% and the average of the seven captive brood collections was 1.10%.

<u>Analysis of spawner groups (in-river and supplementation) by collection year #3</u>

The temporal in-river and supplementation samples were all in Hardy-Weinberg equilibrium with exception of the 2001 supplementation collection. The in-river collection from 2004 had the highest percentage of significant linkage disequilibrium (31.9%) while significant locus comparisons for all other in-river collections were below 3.3%. The supplementation collection from 2002 had the highest percentage of significant linkage disequilibrium at 13.2%.

Pairwise F_{ST} tests were evaluated for the temporal collections from in-river and supplementation by collection year (Table 8). Analysis of the in-river collections revealed significant pairwise differences of the 1986 natural-origin collection to all in-river collections except 1998 and 2000. Eight of the 12 significant comparisons of the in-river collections were from the 2003 and 2004 collections. The remaining four significant differences were between the 2000 - 2001, 2001 - 2007, 2001 - 2008, and 2007 – 2008. Comparison of the in-river and supplementation collections revealed that collections from the same year (1997 in-river to the 1997 supplementation) were not significantly different from zero with exception of the 2000 and 2006 collections. Other significant differences occurred; however most of the differences were below 1.0%. The majority of comparisons for the supplementation collections were significantly different from zero. The comparisons that were not significantly different were collections that were four or five years apart.

<u>Analysis of spawner groups (in-river and supplementation) by brood year #4</u>

There were no significant Hardy-Weinberg differences of the in-river and supplementation samples when they were grouped by brood year. The number of significant locus comparisons for the test of linkage disequilibrium was highest in the brood year 2000 in-river collection. The brood year 1996 – 1998 supplementation collections had more significant locus comparisons than the other collections.

The comparison of the pairwise F_{ST} values for the temporal collections of in-river and supplementation collections by brood year revealed the comparisons that were not significantly different to zero were likely a result of small samples sizes

(Table 9). Other comparisons that were not significantly different from zero were separated by four years. The brood year 2004 in-river collection had the highest pairwise F_{ST} values, but the sample size of the collection was 14 individuals. The comparison of the in-river and supplementation collections from each year (1997 in-river to the 1997 supplementation collection) again revealed that collections were not significantly different from zero.

The factorial correspondence analysis was conducted on each of the spawner groups including the collection from 1986 (Figures 1 and 2). The mean values for the individual in-river collections were plotted. The spatial distribution of the means for each of the temporal collections for the three spawners groups were independently identified into four groups: group 1 (collections from 1993, 1997, 2001, and 2005); group 2 (collections from 1998 and 2002); group 3 (collections from 1996, 2000, and 2004); and group 4 (collections from 1999 and 2003). The mean value for the four groups is around the collection from 1986. The same patterns were observed for the supplementation collections. There were more collections, but the additional collections grouped with other collections that were separated by four years.

<u>Analysis of ancestral groups (hatchery and natural-origin) by collection year #5</u>

Only the hatchery-origin collection from 2001 had loci that were not in Hardy-Weinberg equilibrium. All other hatchery and natural-origin collections were in Hardy-Weinberg equilibrium. The analysis of linkage disequilibrium for the collections of hatchery and natural-origin identified that less than 19% of the locus comparisons were significant.

The pairwise F_{ST} values for the analysis of temporal collections of hatchery-origin and natural-origin using collection year revealed many significant differences (Table 10). The pairwise F_{ST} values that were not significantly different from zero were from collections that were four or five years apart or from collections with small sample sizes. Unlike the analysis of the in-river and supplementation collections the comparisons of the hatchery-origin with the natural-origin from the same year revealed differences that were significantly different from zero between the collections from the same year.

<u>Analysis of ancestral groups (hatchery and natural-origin) by brood year #6</u>
The hatchery-origin collection from the 2002 brood year and the natural-origin collection from the 1997 brood year each had one locus that was not in Hardy-Weinberg equilibrium. All other hatchery and natural-origin collections separated into the respective brood years were in Hardy-Weinberg equilibrium. The analysis of linkage disequilibrium for the collections of hatchery and natural-origin identified that less than 24.2% of the locus comparisons were significant with the exception of the hatchery-origin collection from the 1998 brood year (42.9%).

Overall the majority of the pairwise F_{ST} values for the comparison of the hatchery and natural-origin collections by brood year were significantly different from zero (Table 11). The samples sizes for some of these comparisons were small (below 20 individuals); therefore the significance of the pairwise F_{ST} values is misleading. The collections with larger sample sizes that were not significantly different from zero were primarily from collections that were four years apart.

The factorial correspondence analysis was conducted on temporal collections of hatchery and natural-origin independently including the collection from 1986 (Figures 3 and 4). The mean values for the individual hatchery and natural-origin collections were plotted. The spatial distributions of the mean values for the temporal natural-origin collections could be grouped into polygons based the brood years that were separated by four years. The collection from 1999 and 2003 were distant to each other, but separated from the other collections. The distance between the collections from 1996, 2000, and 2004 was larger than any other group of collections, but was due to the small samples size of the collection from 2004 (N = 4). All of the other natural-origin collections were grouped closely together and near the collection from 1986. The hatchery-origin collections were more evenly distributed and were grouped into four polygons: group 1 (brood years 1993, 1997, 2001, and 2005); group 2 (collections from 1994, 1998, and 2002); group 3 (collections from 1996, 2000, and 2004); and group 4 (collections from 1995, 1999, and 2003). The polygons for three of the groups (1, 2, and 4) were around the collection from 1986. The primary age of spawners in each collection were four years old and the pattern of collections that are grouped together indicates the spawner groups are most closely related based on association with brood year. This suggests that the supplementation and captive brood program have not homogenized any of the spawner groups because they are still grouping with the collections based on their ancestry.

Analysis by spawner groups (in-river and supplementation) and ancestral groups (hatchery and natural-origin) by collection year #7

A total of forty-three collections of in-river/hatchery, in-river/natural, supplementation/hatchery and supplementation/natural were analyzed to determine if they were in Hardy-Weinberg equilibrium. Only two of the 43 collections (2001 supplementation/hatchery and 2007 supplementation/natural) had loci that were not in Hardy Weinberg equilibrium. Overall, the supplementation/hatchery collections had more significant linkage disequilibrium than the other collections.

The largest number pairwise F_{ST} comparisons that were significantly different from zero occurred in the supplementation hatchery-origin collections (approximately 70% of the temporal comparisons). The percentage of significant comparisons for the in-river natural-origin collections was 32% and the percentage of significant comparisons for the supplementation natural-origin collections was 29%. The in-river hatchery-origin collections only had one significant comparison; however the sample size for all but three of the 11 collections was below 10 individuals. Overall, the F_{ST} values for collections with samples sizes over 10 ranged between 0.00 – 0.01 which is consistent with the other analysis where the in-river and supplementation or hatchery and natural-origin collections were combined.

<u>Analyses of captive brood with parents and hatchery-origin with parents using brood year #8</u>

Analyses of Hardy-Weinberg and linkage disequilibrium were conducted in the earlier analysis for these collections so they are not mentioned here. We conducted genotypic differentiation analysis for these samples to determine if there were significant differences between the parent collections, parent to offspring collections, and lastly the offspring collections. Comparison of the adults that produced the captive brood to the supplementation spawners in each of the same collection years identified no significant differences. The comparison of the adults that produced the captive brood to their offspring revealed no significant differences. The comparison of the supplementation spawners and the offspring from each brood year (these samples were released and returned) revealed a significant difference between the 1997 parents and their offspring. The last comparison of the captive brood to the supplementation offspring (identified as hatchery-origin when they returned) revealed significant differences for all comparisons.

Discussion

The values of the genetic diversity presented in this report are a consensus of results for all years (1986, 1997 – 1998, and 2000 – 2008) while each of the reports by Hawkins and Frye (2005); Kassler and Hawkins (2006, 2007, and 2008) represent results for each year of samples. The analysis of the samples collected in 1986 represent natural-origin samples that were collected prior to the return of Chinook produced by the supplementation and captive brood programs and were compared to all of the other collections.

The initial analysis of all samples from each of the collection years identified that there were significant differences in the samples from the different collections. A more in-depth analysis was therefore necessary to address the question of changes in genetic diversity among the temporal collections of in-river and supplementation spawners and hatchery and natural-origin individuals.

The second analysis was focused on the three groups of samples that were identified as part of the captive brood program. We analyzed the adults that produced the captive brood and determined that they were not differentiated from each other. The adults that produced the captive brood and the captive brood were also not differentiated suggesting that the captive brood is comprised of a random sample from the adults.

The captive brood collections however were not in Hardy-Weinberg equilibrium and had a large number of locus comparisons with significant linkage disequilibrium. Significant deviation from Hardy-Weinberg expectations suggests that there has been non-random mating or a mixture of genetically differentiated groups in a collection. Significant linkage disequilibrium can be the result of genetic drift, sampling a relatively small number of families of related individuals, assortative mating and/or analysis of an admixed collection. In the captive brood collections, the linkage disequilibria are possibly the result of pooling together genetically differentiated groups from different brood years.

Each of the captive brood collections had some loci that were not in Hardy-Weinberg equilibrium and significant locus comparisons in the analysis of linkage disequilibrium. The 2003 and 2004 captive brood collections had the most loci not in Hardy-Weinberg equilibrium and significant locus comparisons. These two groups were each produced with approximately equal number of individuals from the two brood years (Table 2). This equal mixture of individuals from the two brood years could produce a mixture of samples that appears genetically distinct and result in the large number of loci that were not in Hardy-Weinberg equilibrium and large number of locus pairs that were significantly linked. These differences result in collections that were significantly differentiated from each other.

The analysis of identity was calculated for the captive brood groups to check for relatedness of individuals that would contribute to significant differences that were detected. There is a possibility that the survival of offspring was associated with family groups in the captive brood. A relatedness value of 0.45 was used to determine full sibling relationship between two individuals. The range of full sibling relationship in the captive brood collections was between 0.20% - 2.63% suggesting that the number of sibling relationships was low. The 2000 captive brood were produced solely from adults in 1997 and therefore would have a higher likelihood of being related than offspring that were produced from multiple brood years. The captive brood were produced from a limited number of parents that were part of the captive brood program and therefore it is not surprising that the overall average relatedness was higher than detected in the captive brood parental collections.

The last group included returns of the captive brood samples in 2008. This collection was significantly different to all of the captive brood collections with exception of the 2004 collection. The 2008 captive brood return collection was comprised of mostly age-4 individuals so it was not surprising that the 2004 collection was not significantly different.

Analyses of the in-river and supplementation spawners were conducted to determine if there have been any changes to the genetic profile of these spawner groups. The collections of supplementation spawners were from broodstock of individuals used for the supplementation program and included samples of hatchery and natural-origin Chinook. The collections of in-river were taken from the river on the spawning grounds and also included individuals of hatchery and natural-origin. Analysis was conducted on the supplementation and in-river collections by collection year and by brood year to determine if genetic changes were a result of individuals from different brood years spawning together. Overall, there were no differences between the analysis of the collection years and brood years (one locus for one collection not in Hardy-Weinberg equilibrium and low number of loci comparisons with significant linkage disequilibrium). We did find pairwise F_{ST} values that were significantly different from zero; however the values were generally below 1.0% providing evidence that the collections were not highly differentiated. The factorial correspondence plots of mean values for the temporal in-river and supplementation collections with the natural-origin collection from 1986 show that the collections are differentiated, but the collection from 1986 is found in between the groups of the other temporal collections. The temporal collections grouped into clusters based on a difference of a four year cycle. The primary age of spawners in each collection were four years old and the pattern of collections that are grouped together indicates the spawner groups are most similar to collections or individuals that are four years apart. The genetic diversity of these collections has therefore not been altered from the 1986 natural-origin collection, but is maintaining a genetic difference that exists between years. This suggests that the supplementation and captive brood program have not homogenized any of the spawner groups because they are still grouping with the collections based on their spawner group or origin.

Hatchery and natural-origin samples were also analyzed to determine if there were any genetic differences that resulted between individuals that had different origins. These collections were analyzed by collection year and by brood years like the analyses that were collected for the supplementation and in-river spawners. Individuals were defined as hatchery or natural-origin if they were marked (adipose clip, CWT, visible implant elastomer) or unmarked. Even though the samples were collected as hatchery or natural-origin their parents could have had either hatchery or natural-origin ancestry. If the natural-origin collections were differentiated to hatchery-origin collections then you could suggest that there are different selection pressures on individuals based on their offspring; therefore the ancestry types have not been homogenized. The results of the analysis by collection year and brood year did not reveal any patterns in the genetic differences among collections that could be attributed to differential survival of the genetic ancestries. The differences that were detected were from differences that occurred among the collection years.

Collections were separated into smaller groupings to determine if genetic differences occurred between a specific spawner group (supplementation or in-river) and from the origin where they were produced (hatchery or natural-origin). The samples sizes for the collections of in-river natural-origin were small and therefore the results for these collections could be misleading. All of the other analyses do not indicate any pattern of genetic differentiation than what was observed for all of the earlier analyses.

The analysis of parents and offspring was conducted to determine if there was differential survival of the captive brood that were raised in captivity in comparison to a cohort of their siblings and other smolts who were released to the wild. The results of this analysis showed that there had been random mating and that the parent collections from the captive brood and supplementation spawners were not significantly different to each other or to their offspring. The significant difference between the captive brood and hatchery-origin collections from the same year occurred by a difference in the selection pressures of individuals that were held captive to those that were released. We don't know what the impacts of the selection would be, but we know that individuals that have undergone selection due to environmental influences have different selection pressures that result in differential survival.

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Table 1. Number of Tucannon spring Chinook individuals from each collection type and group used for analysis 1 - 7.

Analysis #1 - All samples by collection year

		1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	**Total**
		69	92	73	nd	120	151	297	445	403	323	200	109	263	**2545**

Analysis #2 - Analysis of the three captive brood groups

Spawner group	Spawner-origin	1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	**Total**
Adults that produced the Captive brood	Hat & Nat	na	46	42	nd	55	40	0	na	na	na	na	na	na	**183**
Captive Brood		na	na	na	nd	20	63	179	332	273	200	85	na	na	**1152**
Captive Brood returns		na	na	na	na	na	na	na	na	na	na	na	na	55	**55**

Analysis #3 - Analysis of spawner groups (in-river and supplementation) by collection year

Spawner group	Spawner-origin	1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	**Total**
In-river	Hat & Nat	69	16	11	0	27	20	36	38	52	25	32	43	117	**486**
Supplementation	Hat & Nat	0	76	62	0	73	68	82	75	78	98	83	66	91	**852**
Captive Brood		na	na	na	nd	20	63	179	332	273	200	85	na	na	**1152**

Analysis #4 - Analysis of spawner groups (in-river and supplementation) by brood year

Spawner group	Spawner-origin	1986	BY 92	BY 93	BY 94	BY 95	BY 96	BY 97	BY 98	BY 99	BY 00	BY 01	BY 02	BY 03	BY 04	BY 05	**Total**
In-river	Hat & Nat	69	nd	23	nd	nd	10	26	48	18	55	24	40	30	14	21	**378**
Supplementation	Hat & Nat	na	5	102	20	13	66	76	119	33	86	92	102	43	41	14	**812**
Captive Brood		na	na	na	na	na	na	37	126	218	295	311	145	na	na	na	**1132**

Table 1 continued.

Analysis #5 - Analysis of ancestral groups (hatchery and natural-origin) by collection year

Spawner group	Spawner-origin	1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	**Total**
In-river & Supp	Natural	69	41	35	nd	36	49	52	73	82	68	64	75	124	**768**
In-river & Supp	Hatchery	0	51	38	nd	64	39	66	40	48	55	51	34	84	**570**
Captive Brood		na	na	na	nd	20	63	179	332	273	200	85	na	na	**1152**

Analysis #6 - Analysis of ancestral groups (hatchery and natural-origin) by brood year

Spawner group	Spawner-origin	1986	BY 92	BY 93	BY 94	BY 95	BY 96	BY 97	BY 98	BY 99	BY 00	BY 01	BY 02	BY 03	BY 04	BY 05	**Total**
In-river & Supp	Natural	69	6	66	4	nd	19	65	65	45	94	57	86	45	3	nd	**624**
In-river & Supp	Hatchery	na	1	59	18	13	57	37	102	6	47	59	56	28	52	35	**570**
Captive Brood	na	na	na	na	na	na	na	37	126	218	295	311	145	na	na	na	**1132**

Analysis #7 - Analysis by spawner groups (In-river and Supp) and ancestral groups (Hat and Nat) by collection year

Spawner group	Spawner-origin	1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	**Total**
In-river	Natural	69	10	7	nd	27	20	19	32	42	22	30	35	84	**397**
Supplemenation	Natural	na	31	28	nd	9	29	33	41	40	46	34	40	40	**371**
In-river	Hatchery	na	6	4	nd	0	0	17	6	10	3	2	8	33	**89**
Supplemenation	Hatchery	na	45	34	nd	64	39	49	34	38	52	49	26	51	**481**
Captive Brood	na	na	na	na	nd	20	63	179	332	273	200	85	na	na	**1152**
Captive Returns	na	na	na	na	na	na	na	na	na	na	na	na	na	55	**55**

Table 2. Number of individuals for each collection with the collection year and brood year.

Spawner group	Spawner-origin	Age	1997	BY	1998	BY	1999	2000	BY	2001	BY	2002	BY	2003	BY	2004	BY	2005	BY	2006	BY	2007	BY	2008	BY
In-River	Natural	3	0	94	0	95	nd	0	97	0	98	2	99	1	00	0	01	0	02	3	03	2	04	nd	
In-River	Natural	4	8	93	1	94	nd	10	96	20	97	11	98	15	99	41	00	18	01	25	02	19	03	nd	
In-River	Natural	5	2	92	6	93	nd	0	95	0	96	6	97	16	98	1	99	4	00	2	01	11	02	nd	
Supplemenation	Natural	3	0	94	0	95	nd	0	97	0	98	1	99	0	00	0	01	1	02	1	03	1	04	nd	
Supplemenation	Natural	4	27	93	3	94	nd	9	96	29	97	22	98	25	99	39	00	36	01	32	02	22	03	nd	
Supplemenation	Natural	5	4	92	25	93	nd	0	95	0	96	10	97	16	98	1	99	9	00	1	01	17	02	nd	
In-River	Hatchery	3	0	94	0	95	nd	0	97	0	98	0	99	2	00	3	01	2	02	0	03	1	04	21	05
In-River	Hatchery	4	6	93	1	94	nd	0	96	0	97	17	98	0	99	7	00	1	01	2	02	7	03	11	04
In-River	Hatchery	5	0	92	3	93	nd	0	95	0	96	0	97	4	98	0	99	0	00	0	01	0	02	1	03
Supplemenation	Hatchery	3	2	94	11	95	nd	5	97	7	98	2	99	4	00	3	01	1	02	2	03	4	04	14	05
Supplemenation	Hatchery	4	42	93	15	94	nd	57	96	32	97	47	98	3	99	34	00	51	01	46	02	17	03	36	04
Supplemenation	Hatchery	5	1	92	8	93	nd	2	95	0	96	0	97	27	98	1	99	0	00	1	01	5	02	1	03
Captive Brood		2	na		na		na	nd		12	99	35	00	32	01	0	02	0	03	0	04	nd		nd	
Captive Brood		3	na		na		na	nd		25	98	38	99	133	00	139	01	68	02	0	03	nd		nd	
Captive Brood		4	na		na		na	nd		26	97	95	98	161	99	127	00	132	01	78	02	nd		nd	
Captive Brood		5	na		na		na	nd		0	96	11	97	6	98	7	99	0	00	7	01	nd		nd	
Captive Brood R		3	na		na		na	na		na		na		na		na		na		na		na		11	05
Captive Brood R		4	na		na		na	na		na		na		na		na		na		na		na		44	04
Captive Brood R		5	na		na		na	na		na		na		na		na		na		na		na		0	03

Table 3. PCR conditions and microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci. (Also included are the observed and expected heterozygosity (H_o and H_e) for each locus.)

PCR Conditions				Locus statistics		
Poolplex	Locus	Dye Label	Annealing temp (^{O}C)	# Alleles/ Locus	Allele Size Range (bp)	References
Ots-M	*Oki-100**	vic	50	24	212 - 313	Unpublished
	*Ots-201b**	6fam	50	32	141 - 302	Unpublished
	*Ots-208b**	ned	50	35	158 - 322	Greig et al. 2003
	*Ssa-408**	pet	50	28	184 - 304	Cairney et al. 2000
Ots-N	*Ogo-2**	pet	63	11	202 - 232	Olsen et al. 1998
	*Ssa-197**	ned	63	27	189 - 305	O'Reilly et al. 1996
Ots-O	*Ogo-4**	6fam	56	14	132 - 166	Olsen et al. 1998
	*Ots-213**	ned	56	28	214 - 334	Greig et al. 2003
	*Ots-G474**	pet	56	9	156 - 204	Williamson et al. 2002
Ots-R	*Omm-1080**	vic	56	41	190 - 354	Rexroad et al. 2001
	*Ots-3M**	6fam	63	10	128 - 152	Banks et al. 1999
Ots-S	*Ots-9**	pet	63	5	103 - 111	Banks et al. 1999
	*Ots-211**	ned	63	28	208 - 327	Greig et al. 2003
	*Ots-212**	6fam	63	21	131 - 231	Greig et al. 2003

Table 4. Number of loci not in Hardy-Weinberg equilibrium out of 13 possible and number of loci with significant linkage disequilibrium (91 pairings) after Bonferroni correction of P-values (adjusted alpha p-values are shown in bold for each analysis; Rice 1989). Data is shown for seven different groupings of individuals for the analysis of the Tucannon spring Chinook data. Adjusted alpha p-value is shown in bold type at the bottom of the page.

	All samples by **Analysis #1**			Captive Brood **Analysis #2**			IN - SUPP by **Analysis #3**			IN - SUPP by **Analysis #4**	
	# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis
1986	0	0	1997 CAdult	0	5	1986 NAT	0	0	1986 NAT	0	0
1997	1	8	1998 CAdult	0	0	1997 IN	0	0	BY 1992 IN	nd	0
1998	0	1	2000 CAdult	0	3	1997 SUP	0	1	BY 1992 SUPP	0	0
2000	0	12	2001 CAdult	0	7	1998 IN	0	0	BY 1993 IN	0	2
2001	1	34	2000 CB	0	1	1998 SUP	0	0	BY 1993 SUPP	0	6
2002	2	38	2001 CB	0	14	2000 IN	0	3	BY 1994 IN	nd	0
2003	6	72	2002 CB	1	30	2000 SUP	0	0	BY 1994 SUPP	0	0
2004	6	62	2003 CB	8	73	2001 IN	0	0	BY 1995 IN	nd	0
2005	3	47	2004 CB	6	71	2001 SUP	2	8	BY 1995 SUPP	0	0
2006	2	19	2005 CB	3	52	2002 IN	0	0	BY 1996 IN	0	0
2007	0	4	2006 CB	1	12	2002 SUP	0	12	BY 1996 SUPP	0	13
2008	0	5	2008 CBR	0	0	2003 IN	0	2	BY 1997 IN	0	0
						2003 SUP	0	7	BY 1997 SUPP	0	28
						2004 IN	0	29	BY 1998 IN	0	2
						2004 SUP	0	3	BY 1998 SUPP	0	19
						2005 IN	0	1	BY 1999 IN	0	1
						2005 SUP	0	6	BY 1999 SUPP	0	1
						2006 IN	0	0	BY 2000 IN	0	29
						2006 SUP	0	5	BY 2000 SUPP	0	5
						2007 IN	0	0	BY 2001 IN	0	0
						2007 SUP	0	4	BY 2001 SUPP	0	8
						2008 IN	0	3	BY 2002 IN	0	0
						2008 SUP	0	4	BY 2002 SUPP	0	7
									BY 2003 IN	0	0
									BY 2003 SUPP	0	0
									BY 2004 IN	0	0
									BY 2004 SUPP	0	9
									BY 2005 IN	0	0
									BY 2005 SUPP	0	0
	0.0003	**0.0005**		**0.0003**	**0.0005**		**0.0002**	**0.0005**		**0.0001**	**0.0005**

Table 4 continued.

	Hat - Nat by collection year			Hat - Nat by brood year			IN-HAT, IN-NAT, SUP-HAT, SUP-NAT			IN-HAT, IN-NAT, SUP-HAT, SUP-NAT	
	Analysis #5			**Analysis #6**			**Analysis #7**			**Analysis #7 cont.**	
	# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis		# Loci not in HW	Linkage Dis
1986 NAT	0	0	BY 1992 NAT	0	nd	1986 NAT	0	0	2005 In - HAT	0	0
1997 NAT	0	4	BY 1992 HAT	0	nd	1997 In - NAT	0	0	2005 Sup - NAT	0	1
1997 HAT	0	3	BY 1993 NAT	0	4	1997 In - HAT	0	0	2005 Sup - HAT	0	5
1998 NAT	0	0	BY 1993 HAT	0	6	1997 Sup - NAT	0	3	2006 In - NAT	0	0
1998 HAT	0	0	BY 1994 NAT	0	nd	1997 Sup - HAT	0	4	2006 In - HAT	0	0
2000 NAT	0	3	BY 1994 HAT	0	0	1998 In - NAT	0	0	2006 Sup - NAT	0	2
2000 HAT	0	10	BY 1995 NAT	nd	nd	1998 In - HAT	0	0	2006 Sup - HAT	0	7
2001 NAT	0	8	BY 1995 HAT	0	0	1998 Sup - NAT	0	0	2007 In - NAT	0	0
2001 HAT	2	17	BY 1996 NAT	0	1	1998 Sup - HAT	0	0	2007 In - HAT	0	0
2002 NAT	0	2	BY 1996 HAT	0	11	2000 In - NAT	0	3	2007 Sup - NAT	1	4
2002 HAT	0	7	BY 1997 NAT	1	15	2000 In - HAT	0	nd	2007 Sup - HAT	0	1
2003 NAT	0	6	BY 1997 HAT	0	12	2000 Sup - NAT	0	0	2008 In - NAT	0	1
2003 HAT	0	5	BY 1998 NAT	0	3	2000 Sup - HAT	0	14	2008 In - HAT	0	0
2004 NAT	0	16	BY 1998 HAT	0	39	2001 In - NAT	0	0	2008 Sup - NAT	0	1
2004 HAT	0	4	BY 1999 NAT	0	6	2001 In - HAT	0	nd	2008 Sup - HAT	0	2
2005 NAT	0	2	BY 1999 HAT	0	nd	2001 Sup - NAT	0	4			
2005 HAT	0	4	BY 2000 NAT	0	22	2001 Sup - HAT	2	19			
2006 NAT	0	1	BY 2000 HAT	0	4	2002 In - NAT	0	0			
2006 HAT	0	6	BY 2001 NAT	0	2	2002 In - HAT	0	0			
2007 NAT	0	1	BY 2001 HAT	0	3	2002 Sup - NAT	0	2			
2007 HAT	0	0	BY 2002 NAT	0	0	2002 Sup - HAT	0	5			
2008 NAT	0	2	BY 2002 HAT	1	10	2003 In - NAT	0	1			
2008 HAT	0	9	BY 2003 NAT	0	0	2003 In - HAT	0	0			
			BY 2003 HAT	0	0	2003 Sup - NAT	0	2			
			BY 2004 NAT	0	nd	2003 Sup - HAT	0	3			
			BY 2004 HAT	0	14	2004 In - NAT	0	0			
			BY 2005 NAT	nd	nd	2004 In - HAT	0	30			
			BY 2005 HAT	0	0	2004 Sup - NAT	0	0			
						2004 Sup - HAT	0	1			
	0.0002	**0.0005**		**0.0001**	**0.0005**	2005 In - NAT	0	0		**0.0001**	**0.0005**

Table 5. Allelic richness (A_O) and heterozygosity (H_E and H_O) for natural/hatchery-origin collections and in-river/supplementation collections by brood year and collection year. Minimum sample size for calculation of allelic richness using brood year was 17 while minimum sample size for the analysis of collection years was only 7 individuals.

	Natural/Hatchery-origin by brood year				Natural/Hatchery-origin by collection year		
	A_O	H_E	H_O		A_O	H_E	H_O
NAT 86	7.0	0.7716	0.7771	NAT 86	6.5	0.7716	0.7771
HAT 93	6.8	0.7878	0.8098	HAT 97	6.4	0.7743	0.7981
NAT 93	7.3	0.7955	0.7799	NAT 97	6.9	0.7920	0.7696
HAT 94	7.2	0.7856	0.7686	HAT 98	6.8	0.8048	0.8007
NAT 94	nd	nd	nd	NAT 98	6.8	0.7970	0.8002
HAT 95	6.6	0.7719	0.7858	HAT 00	6.7	0.7906	0.7777
NAT 95	nd	nd	nd	NAT 00	6.9	0.8040	0.7801
HAT 96	7.0	0.7868	0.7767	HAT 01	6.3	0.7794	0.7854
NAT 96	6.9	0.7920	0.7925	NAT 01	6.5	0.7978	0.7905
HAT 97	6.7	0.7773	0.7790	HAT 02	6.6	0.7977	0.8180
NAT 97	7.2	0.8049	0.7961	NAT 02	7.1	0.8177	0.7939
HAT 98	7.0	0.7961	0.8192	HAT 03	6.4	0.7892	0.7938
NAT 98	7.1	0.7927	0.8045	NAT 03	6.7	0.7951	0.8135
HAT 99	nd	nd	nd	HAT 04	6.6	0.7937	0.7994
NAT 99	7.4	0.8092	0.8008	NAT 04	6.7	0.7938	0.8371
HAT 00	6.9	0.7872	0.7933	HAT 05	6.7	0.7942	0.7970
NAT 00	7.2	0.7932	0.8284	NAT 05	6.8	0.8009	0.7816
HAT 01	7.0	0.7916	0.7990	HAT 06	6.7	0.8032	0.8110
NAT 01	7.3	0.8024	0.7862	NAT 06	7.0	0.8080	0.7978
HAT 02	7.2	0.8025	0.8037	HAT 07	6.6	0.7897	0.7952
NAT 02	7.4	0.8030	0.8004	NAT 07	6.8	0.7953	0.7969
HAT 03	6.9	0.7796	0.7900	HAT 08	6.8	0.7905	0.7654
NAT 03	7.3	0.8006	0.7917	NAT 08	6.9	0.8024	0.7958
HAT 04	6.8	0.7736	0.7597				
NAT 04	nd	nd	nd				
HAT 05	7.3	0.7955	0.7868				
NAT 05	nd	nd	nd				
	average				average		
HAT	7.0			HAT	6.6		
NAT	7.2			NAT	6.8		

Table 5 continued.

	In-river/Supplementation by brood year		
	A_o	H_E	H_O
NAT 86	7.0	0.7716	0.7771
In-river 93	7.5	0.8123	0.8311
Sup 93	7.1	0.7920	0.7855
In-river 94	nd	nd	nd
Sup 94	7.1	0.7812	0.7635
In-river 95	nd	nd	nd
Sup 95	6.6	0.7719	0.7858
In-river 96	7.1	0.7964	0.7950
Sup 96	7.1	0.7887	0.7781
In-river 97	7.1	0.8033	0.8051
Sup 97	7.2	0.7987	0.7851
In-river 98	7.2	0.8019	0.8141
Sup 98	7.1	0.7965	0.8138
In-river 99	7.8	0.8204	0.8231
Sup 99	7.2	0.7977	0.7760
In-river 00	7.2	0.7964	0.8163
Sup 00	7.1	0.7899	0.8164
In-river 01	7.3	0.8179	0.8351
Sup 01	7.1	0.7935	0.7842
In-river 02	7.3	0.8090	0.8057
Sup 02	7.3	0.8015	0.8001
In-river 03	7.3	0.8006	0.7685
Sup 03	7.2	0.7928	0.8071
In-river 04	6.5	0.7337	0.7484
Sup 04	7.0	0.7847	0.7692
In-river 05	7.3	0.8052	0.7817
Sup 05	7.5	0.7867	0.7947
	average		
In-river	7.2		
Supplementation	7.1		

	In-river/Supplementation by collection year		
	A_o	H_E	H_O
NAT 86	6.5	0.7716	0.7771
In-river 97	7.2	0.8152	0.8092
Sup 97	6.6	0.7807	0.7945
In-river 98	6.7	0.8110	0.8578
Sup 98	7.0	0.8074	0.7895
In-river 00	7.0	0.8095	0.7770
Sup 00	6.7	0.7969	0.7731
In-river 01	6.6	0.8041	0.8150
Sup 01	6.4	0.7884	0.8121
In-river 02	7.1	0.8217	0.7989
Sup 02	6.9	0.8032	0.8118
In-river 03	6.5	0.7904	0.8282
Sup 03	6.7	0.7978	0.7953
In-river 04	6.7	0.7984	0.8212
Sup 04	6.7	0.7929	0.8241
In-river 05	6.9	0.8167	0.8154
Sup 05	6.7	0.7955	0.7847
In-river 06	7.0	0.8137	0.8178
Sup 06	6.9	0.8039	0.7982
In-river 07	6.8	0.7964	0.7799
Sup 07	6.7	0.7936	0.8078
In-river 08	6.9	0.8021	0.7767
Sup 08	6.9	0.7962	0.7924
	average		
In-river	6.9		
Supplementation	6.7		

Table 6. Pairwise F_{ST} analysis (#1) of all samples from each collection year. Comparisons that are significantly different from zero are highlighted in gray.

	1986	1997	1998	2000	2001	2002	2003	2004	2005	2006	2007	2008
1986	****	0.0023	0.0038	0.0045	0.0041	0.0048	0.0065	0.0060	0.0054	0.0059	0.0052	0.0050
1997		****	0.0029	0.0016	0.0006	0.0022	0.0038	0.0033	0.0018	0.0023	0.0045	0.0031
1998			****	0.0025	0.0003	-0.0012	0.0015	0.0025	0.0032	0.0008	-0.0004	0.0018
2000				****	0.0023	0.0018	0.0016	0.0000	0.0044	0.0047	0.0024	0.0003
2001					****	0.0012	0.0021	0.0021	0.0006	0.0027	0.0022	0.0021
2002						****	0.0015	0.0025	0.0033	0.0012	0.0015	0.0025
2003							****	0.0016	0.0041	0.0051	0.0026	0.0020
2004								****	0.0028	0.0052	0.0045	0.0006
2005									****	0.0033	0.0057	0.0032
2006										****	0.0040	0.0037
2007											****	0.0032
2008												****

Table 7. Pairwise F_{ST} analysis (#2) of the three groups for the captive brood (adults that produced the captive brood, captive brood, and captive brood returns. Comparisons that are significantly different from zero are highlighted in gray.

	86NAT	97CAdult	98CAdult	00CAdult	01CAdult	00CB	01CB	02CB	03CB	04CB	05CB	06CB	08CR
86NAT	****	0.0009	0.0029	0.0064	0.0056	0.0050	0.0048	0.0053	0.0071	0.0076	0.0063	0.0093	0.0096
97CAdult		****	0.0018	0.0017	0.0011	-0.0013	0.0002	0.0029	0.0045	0.0051	0.0015	0.0025	0.0062
98CAdult			****	0.0021	0.0027	0.0008	-0.0023	-0.0031	0.0018	0.0017	0.0038	0.0027	0.0019
00CAdult				****	0.0095	0.0049	0.0024	0.0015	-0.0004	0.0022	0.0079	0.0078	0.0050
01CAdult					****	0.0054	0.0060	0.0072	0.0057	0.0012	-0.0012	0.0067	0.0010
00CB						****	0.0020	0.0027	0.0065	0.0046	0.0040	0.0099	0.0044
01CB							****	0.0015	0.0036	0.0054	0.0051	0.0058	0.0064
02CB								****	0.0021	0.0040	0.0064	0.0055	0.0043
03CB									****	0.0025	0.0071	0.0087	0.0034
04CB										****	0.0039	0.0086	-0.0005
05CB											****	0.0036	0.0050
06CB												****	0.0096
08CBR													****

CAdult - adults that produced the captive brood
CB - captive brood
CBR - captive brood returns

Table 8. Pairwise F_{ST} analysis (#3) of the temporal collections for the in-river, and supplementation spawner collections using collection year. Comparisons that are signficantly different from zero are highlighted in gray.

	86NAT	97IN	98IN	00IN	01IN	02IN	03IN	04IN	05IN	06IN	07IN	08IN
86NAT	****	0.0050	0.0030	0.0061	0.0144	0.0075	0.0126	0.0066	0.0094	0.0111	0.0067	0.0056
97IN		****	-0.0039	-0.0032	0.0011	-0.0041	0.0009	0.0065	-0.0057	-0.0026	0.0013	0.0002
98IN			****	0.0029	0.0050	-0.0028	-0.0003	0.0060	-0.0005	0.0013	-0.0030	0.0020
00IN				****	0.0112	0.0028	0.0107	0.0068	0.0025	0.0105	0.0038	0.0034
01IN					****	0.0021	0.0121	0.0133	0.0013	0.0045	0.0099	0.0111
02IN						****	0.0040	0.0062	-0.0016	0.0008	0.0033	0.0051
03IN							****	0.0119	0.0044	0.0051	0.0013	0.0062
04IN								****	0.0085	0.0136	0.0071	0.0027
05IN									****	-0.0004	0.0054	0.0014
06IN										****	0.0045	0.0074
07IN											****	0.0023
08IN												****

	97SUP	98SUP	00SUP	01SUP	02SUP	03SUP	04SUP	05SUP	06SUP	07SUP	08SUP
97IN	0.0003	-0.0018	0.0043	0.0004	-0.0007	0.0022	0.0030	0.0006	0.0026	0.0018	0.0043
98IN	0.0040	-0.0032	0.0081	0.0039	-0.0033	-0.0014	0.0117	0.0091	-0.0028	-0.0026	0.0036
00IN	0.0071	0.0028	0.0087	0.0047	0.0055	0.0086	0.0077	0.0064	0.0044	0.0048	0.0071
01IN	0.0108	0.0048	0.0126	0.0121	0.0059	0.0097	0.0134	0.0094	0.0105	0.0115	0.0143
02IN	0.0049	-0.0017	0.0084	0.0045	-0.0001	0.0000	0.0074	0.0052	0.0022	0.0033	0.0078
03IN	0.0081	0.0023	0.0093	0.0079	0.0040	0.0013	0.0109	0.0114	0.0090	0.0025	0.0099
04IN	0.0062	0.0038	0.0012	0.0110	0.0056	0.0070	0.0014	0.0096	0.0053	0.0059	0.0021
05IN	0.0045	-0.0011	0.0060	0.0028	0.0003	0.0004	0.0072	0.0027	0.0011	0.0042	0.0062
06IN	0.0071	0.0015	0.0119	0.0068	0.0041	0.0054	0.0131	0.0070	0.0059	0.0060	0.0102
07IN	0.0078	0.0012	0.0051	0.0051	0.0042	0.0029	0.0085	0.0096	0.0062	0.0000	0.0067
08IN	0.0038	0.0018	0.0009	0.0047	0.0029	0.0036	0.0023	0.0028	0.0039	0.0029	0.0015

Table 8 continued.

	86NAT	97SUP	98SUP	00SUP	01SUP	02SUP	03SUP	04SUP	05SUP	06SUP	07SUP	08SUP
86NAT	****	0.0021	0.0035	0.0076	0.0056	0.0049	0.0082	0.0072	0.0064	0.0047	0.0044	0.0056
97SUP		****	0.0033	0.0037	0.0029	0.0023	0.0060	0.0047	0.0048	0.0042	0.0035	0.0048
98SUP			****	0.0046	0.0025	-0.0019	-0.0024	0.0050	0.0027	0.0003	-0.0016	0.0036
00SUP				****	0.0093	0.0046	0.0069	-0.0024	0.0069	0.0063	0.0047	0.0009
01SUP					****	0.0055	0.0048	0.0095	0.0001	0.0075	0.0035	0.0076
02SUP						****	0.0009	0.0049	0.0045	-0.0019	-0.0002	0.0042
03SUP							****	0.0088	0.0057	0.0035	-0.0003	0.0078
04SUP								****	0.0070	0.0061	0.0070	0.0003
05SUP									****	0.0070	0.0064	0.0058
06SUP										****	0.0029	0.0041
07SUP											****	0.0056
08SUP												****

Table 9. Pairwise F_{ST} analysis (#4) of the temporal collections for the in-river and supplementation spawner collections using brood year. Comparisons that are significantly different from zero are highlighted in gray.

	86NAT	BY93IN	BY96IN	BY97IN	BY98IN	BY99IN	BY00IN	BY01IN	BY02IN	BY03IN
86NAT	****	0.0057	0.0156	0.0088	0.0118	0.0095	0.0082	0.0124	0.0099	0.0074
BY93IN		****	0.0160	-0.0008	-0.0015	0.0015	0.0084	-0.0024	0.0016	0.0021
BY96IN			****	0.0200	0.0168	0.0195	0.0119	0.0165	0.0233	0.0059
BY97IN				****	0.0041	0.0092	0.0111	0.0024	0.0038	0.0069
BY98IN					****	0.0053	0.0118	0.0020	0.0018	0.0032
BY99IN						****	0.0088	0.0022	0.0031	0.0032
BY00IN							****	0.0124	0.0129	0.0086
BY01IN								****	0.0021	0.0046
BY02IN									****	0.0057
BY03IN										****
BY04IN										
BY05IN										

	BY04IN	BY05IN
86NAT	0.0305	0.0095
BY93IN	0.0362	0.0075
BY96IN	0.0435	0.0174
BY97IN	0.0486	0.0051
BY98IN	0.0400	0.0070
BY99IN	0.0324	0.0082
BY00IN	0.0248	0.0137
BY01IN	0.0349	0.0030
BY02IN	0.0403	0.0075
BY03IN	0.0290	0.0097
BY04IN	****	0.0308
BY05IN		****

Table 9 continued.

	BY92SUP	BY93SUP	BY94SUP	BY95SUP	BY96SUP	BY97SUP	BY98SUP	BY99SUP	BY00SUP	BY01SUP
BY93IN	-0.0035	0.0004	0.0096	0.0033	0.0090	0.0015	0.0008	0.0017	0.0104	0.0045
BY96IN	0.0260	0.0123	0.0276	0.0272	0.0168	0.0155	0.0183	0.0242	0.0207	0.0183
BY97IN	0.0066	0.0030	0.0147	0.0170	0.0109	0.0074	0.0061	0.0084	0.0135	0.0069
BY98IN	0.0035	0.0041	0.0062	0.0092	0.0110	0.0069	0.0003	0.0088	0.0126	0.0083
BY99IN	0.0037	0.0037	0.0135	0.0035	0.0079	0.0024	0.0054	-0.0034	0.0098	0.0088
BY00IN	0.0056	0.0059	0.0141	0.0078	0.0014	0.0124	0.0081	0.0101	0.0033	0.0131
BY01IN	0.0031	0.0023	0.0095	0.0076	0.0104	0.0019	0.0023	0.0041	0.0116	0.0037
BY02IN	0.0045	0.0047	0.0085	0.0110	0.0127	0.0077	0.0040	0.0058	0.0160	0.0079
BY03IN	0.0051	0.0037	0.0137	0.0075	0.0052	0.0049	0.0039	0.0055	0.0106	0.0092
BY04IN	0.0244	0.0280	0.0404	0.0363	0.0180	0.0302	0.0344	0.0400	0.0185	0.0317
BY05IN	0.0119	0.0045	0.0062	0.0126	0.0085	0.0039	0.0048	0.0133	0.0103	-0.0037

	BY02SUP	BY03SUP	BY04SUP	BY05SUP
BY93IN	0.0016	0.0013	0.0145	0.0101
BY96IN	0.0139	0.0175	0.0228	0.0315
BY97IN	0.0069	0.0082	0.0210	0.0126
BY98IN	0.0049	0.0036	0.0208	0.0151
BY99IN	0.0067	0.0016	0.0141	0.0092
BY00IN	0.0066	0.0092	0.0061	0.0138
BY01IN	0.0039	0.0041	0.0198	0.0102
BY02IN	0.0047	0.0039	0.0233	0.0078
BY03IN	0.0064	-0.0016	0.0180	0.0155
BY04IN	0.0294	0.0332	0.0097	0.0228
BY05IN	0.0089	0.0087	0.0204	-0.0065

Table 9 continued.

	86NAT	BY92SUP	BY93SUP	BY94SUP	BY95SUP	BY96SUP	BY97SUP	BY98SUP	BY99SUP	BY00SUP
86NAT	****	-0.0024	0.0022	0.0103	0.0092	0.0079	0.0058	0.0066	0.0102	0.0097
BY92SUP		****	-0.0018	0.0018	0.0106	0.0031	-0.0056	0.0000	0.0081	0.0013
BY93SUP			****	0.0096	0.0077	0.0044	0.0015	0.0015	0.0067	0.0061
BY94SUP				****	0.0148	0.0128	0.0103	0.0061	0.0137	0.0146
BY95SUP					****	0.0077	0.0098	0.0041	-0.0032	0.0117
BY96SUP						****	0.0091	0.0070	0.0112	-0.0024
BY97SUP							****	0.0060	0.0100	0.0096
BY98SUP								****	0.0055	0.0088
BY99SUP									****	0.0162
BY00SUP										****
BY01SUP										
BY02SUP										
BY03SUP										
BY04SUP										
BY05SUP										

	BY01SUP	BY02SUP	BY03SUP	BY04SUP	BY05SUP
86NAT	0.0066	0.0046	0.0056	0.0129	0.0070
BY92SUP	0.0043	-0.0033	0.0021	-0.0057	-0.0006
BY93SUP	0.0036	0.0022	0.0016	0.0129	0.0070
BY94SUP	0.0087	0.0086	0.0102	0.0201	0.0050
BY95SUP	0.0111	0.0032	0.0061	0.0099	0.0111
BY96SUP	0.0098	0.0072	0.0049	0.0057	0.0066
BY97SUP	0.0009	0.0060	0.0034	0.0149	0.0047
BY98SUP	0.0052	0.0003	0.0004	0.0172	0.0087
BY99SUP	0.0113	0.0059	0.0015	0.0198	0.0161
BY00SUP	0.0114	0.0084	0.0089	0.0036	0.0067
BY01SUP	****	0.0079	0.0056	0.0182	-0.0008
BY02SUP		****	0.0038	0.0125	0.0081
BY03SUP			****	0.0169	0.0082
BY04SUP				****	0.0111
BY05SUP					****

Table 10. Pairwise F_{ST} analysis (#5) of the temporal collections for the hatchery-origin and natural-origin collections using collection year. Comparisons that are significantly different from zero are highlighted in gray.

	86NAT	97HAT	98HAT	00HAT	01HAT	02HAT	03HAT	04HAT	05HAT	06HAT	07HAT	08HAT
86NAT	****	0.0059	0.0053	0.0089	0.0088	0.0060	0.0145	0.0093	0.0083	0.0082	0.0084	0.0079
97HAT		****	0.0062	0.0073	0.0099	0.0074	0.0156	0.0060	0.0097	0.0124	0.0149	0.0087
98HAT			****	0.0067	0.0094	-0.0026	0.0029	0.0071	0.0066	0.0030	0.0048	0.0069
00HAT				****	0.0123	0.0064	0.0101	-0.0026	0.0118	0.0100	0.0053	0.0033
01HAT					****	0.0102	0.0145	0.0115	0.0042	0.0158	0.0075	0.0140
02HAT						****	0.0019	0.0061	0.0088	0.0020	0.0033	0.0102
03HAT							****	0.0100	0.0096	0.0107	0.0026	0.0144
04HAT								****	0.0099	0.0093	0.0097	0.0027
05HAT									****	0.0125	0.0127	0.0068
06HAT										****	0.0075	0.0089
07HAT											****	0.0120
08HAT												****

	97NAT	98NAT	00NAT	01NAT	02NAT	03NAT	04NAT	05NAT	06NAT	07NAT	08NAT
97HAT	0.0110	0.0090	0.0138	0.0116	0.0071	0.0094	0.0122	0.0069	0.0070	0.0095	0.0104
98HAT	0.0043	0.0004	0.0070	0.0027	0.0008	0.0005	0.0080	0.0014	0.0004	0.0010	0.0032
00HAT	0.0061	0.0070	0.0101	0.0130	0.0094	0.0100	0.0015	0.0042	0.0095	0.0078	0.0024
01HAT	0.0056	0.0094	0.0112	0.0164	0.0101	0.0104	0.0163	0.0099	0.0091	0.0073	0.0088
02HAT	0.0064	-0.0018	0.0069	0.0057	0.0049	0.0044	0.0084	0.0016	0.0026	0.0026	0.0044
03HAT	0.0112	0.0004	0.0099	0.0098	0.0099	0.0075	0.0129	0.0056	0.0085	0.0072	0.0089
04HAT	0.0078	0.0057	0.0085	0.0096	0.0062	0.0095	0.0049	0.0035	0.0095	0.0078	0.0046
05HAT	0.0069	0.0069	0.0082	0.0038	0.0089	0.0106	0.0148	0.0034	0.0075	0.0093	0.0085
06HAT	0.0075	0.0036	0.0075	0.0112	0.0005	0.0085	0.0080	0.0069	0.0069	0.0105	0.0075
07HAT	0.0041	-0.0016	0.0043	0.0114	0.0082	0.0043	0.0069	0.0079	0.0049	0.0033	0.0013
08HAT	0.0078	0.0077	0.0091	0.0130	0.0073	0.0116	0.0042	0.0042	0.0100	0.0097	0.0065

Table 10 continued.

	86NAT	97NAT	98NAT	00NAT	01NAT	02NAT	03NAT	04NAT	05NAT	06NAT	07NAT	08NAT
86NAT	****	0.0044	0.0027	0.0059	0.0101	0.0080	0.0093	0.0070	0.0069	0.0062	0.0049	0.0060
97NAT		****	0.0033	0.0029	0.0066	0.0031	0.0063	0.0065	0.0054	0.0041	0.0041	0.0036
98NAT			****	-0.0004	0.0028	0.0036	0.0007	0.0052	0.0019	-0.0011	-0.0012	0.0013
00NAT				****	0.0086	0.0061	0.0108	0.0068	0.0074	0.0053	0.0045	0.0033
01NAT					****	0.0077	0.0084	0.0149	0.0022	0.0063	0.0080	0.0086
02NAT						****	0.0031	0.0091	0.0049	0.0031	0.0046	0.0064
03NAT							****	0.0121	0.0078	0.0041	0.0012	0.0051
04NAT								****	0.0083	0.0100	0.0095	0.0034
05NAT									****	0.0037	0.0061	0.0044
06NAT										****	0.0018	0.0036
07NAT											****	0.0038
08NAT												****

Table 11. Pairwise F_{ST} analysis (#6) of the temporal collections for the hatchery-origin and natural-origin collections using brood year. Comparisons that are significantly different from zero are highlighted in gray. Collections with less than 20 individuals are in bold type.

	86NAT	**BY92HAT**	BY93HAT	**BY94HAT**	**BY95HAT**	BY96HAT	BY97HAT	BY98HAT	**BY99HAT**	BY00HAT
86NAT	****	-0.0184	0.0072	0.0074	0.0092	0.0096	0.0128	0.0096	0.0014	0.0130
BY92HAT		****	-0.0155	-0.0165	-0.0268	-0.0161	-0.0244	-0.0261	-0.0686	-0.0190
BY93HAT			****	0.0123	0.0129	0.0099	0.0138	0.0091	0.0012	0.0104
BY94HAT				****	0.0106	0.0111	0.0211	0.0061	0.0004	0.0144
BY95HAT					****	0.0094	0.0185	0.0052	-0.0152	0.0123
BY96HAT						****	0.0180	0.0102	-0.0064	-0.0031
BY97HAT							****	0.0204	0.0101	0.0193
BY98HAT								****	-0.0021	0.0111
BY99HAT									****	-0.0054
BY00HAT										****
BY01HAT										
BY02HAT										
BY03HAT										
BY04HAT										
BY05HAT										

	BY01HAT	BY02HAT	BY03HAT	BY04HAT	BY05HAT
86NAT	0.0085	0.0094	0.0131	0.0167	0.0096
BY92HAT	-0.0226	-0.0311	-0.0247	-0.0079	-0.0067
BY93HAT	0.0083	0.0125	0.0191	0.0205	0.0098
BY94HAT	0.0113	0.0085	0.0177	0.0208	0.0052
BY95HAT	0.0127	0.0080	0.0103	0.0155	0.0131
BY96HAT	0.0141	0.0124	0.0104	0.0094	0.0098
BY97HAT	0.0096	0.0220	0.0173	0.0251	0.0178
BY98HAT	0.0094	0.0061	0.0061	0.0253	0.0106
BY99HAT	0.0047	-0.0026	-0.0079	0.0040	0.0035
BY00HAT	0.0141	0.0146	0.0130	0.0105	0.0109
BY01HAT	****	0.0147	0.0163	0.0220	0.0006
BY02HAT		****	0.0168	0.0181	0.0113
BY03HAT			****	0.0315	0.0188
BY04HAT				****	0.0202
BY05HAT					****

Table 11 continued.

	BY92NAT	BY93NAT	**BY94NAT**	BY95NAT	**BY96NAT**	BY97NAT	BY98NAT	BY99NAT	BY00NAT	BY01NAT
BY92HAT	-0.0097	-0.0300	-0.0193	nd	0.0018	-0.0307	-0.0316	-0.0240	-0.0170	-0.0264
BY93HAT	0.0058	0.0085	0.0192	nd	0.0187	0.0065	0.0055	0.0121	0.0130	0.0064
BY94HAT	0.0013	0.0064	-0.0011	nd	0.0190	0.0077	0.0081	0.0124	0.0119	0.0056
BY95HAT	0.0159	0.0058	0.0236	nd	0.0172	0.0128	0.0113	0.0004	0.0101	0.0097
BY96HAT	0.0064	0.0066	0.0195	nd	0.0155	0.0113	0.0128	0.0135	0.0013	0.0085
BY97HAT	0.0030	0.0107	0.0169	nd	0.0228	0.0188	0.0157	0.0182	0.0190	0.0170
BY98HAT	0.0131	0.0036	0.0098	nd	0.0151	0.0063	0.0083	0.0077	0.0121	0.0033
BY99HAT	-0.0023	-0.0105	-0.0160	nd	0.0016	-0.0021	-0.0037	-0.0134	-0.0030	-0.0054
BY00HAT	0.0086	0.0089	0.0172	nd	0.0135	0.0109	0.0125	0.0154	0.0060	0.0091
BY01HAT	0.0083	0.0061	0.0127	nd	0.0180	0.0035	0.0114	0.0119	0.0149	0.0040
BY02HAT	-0.0003	0.0054	0.0259	nd	0.0130	0.0083	0.0080	0.0095	0.0099	0.0101
BY03HAT	0.0174	0.0045	0.0061	nd	0.0107	0.0127	0.0125	0.0067	0.0145	0.0103
BY04HAT	0.0025	0.0164	0.0235	nd	0.0180	0.0226	0.0214	0.0238	0.0066	0.0216
BY05HAT	0.0048	0.0093	0.0122	nd	0.0198	0.0053	0.0108	0.0149	0.0130	0.0014

	BY02NAT	BY03NAT	BY04NAT	BY05NAT
BY92HAT	-0.0315	-0.0231	0.0482	nd
BY93HAT	0.0064	0.0059	0.0231	nd
BY94HAT	0.0047	0.0102	0.0212	nd
BY95HAT	0.0044	0.0081	0.0121	nd
BY96HAT	0.0101	0.0082	0.0044	nd
BY97HAT	0.0147	0.0113	0.0112	nd
BY98HAT	0.0028	0.0062	0.0300	nd
BY99HAT	-0.0097	-0.0076	0.0037	nd
BY00HAT	0.0120	0.0093	0.0143	nd
BY01HAT	0.0085	0.0078	0.0249	nd
BY02HAT	0.0063	0.0113	0.0205	nd
BY03HAT	0.0075	0.0067	0.0224	nd
BY04HAT	0.0205	0.0170	0.0158	nd
BY05HAT	0.0100	0.0102	0.0120	nd

Table 11 continued.

	86NAT	**BY92NAT**	BY93NAT	**BY94NAT**	BY95NAT	**BY96NAT**	BY97NAT	BY98NAT	BY99NAT	BY00NAT
86NAT	****	0.0028	0.0028	0.0126	nd	0.0109	0.0078	0.0107	0.0104	0.0080
BY92NAT		****	-0.0016	0.0018	nd	0.0038	0.0016	0.0024	0.0084	0.0042
BY93NAT			****	0.0092	nd	0.0074	0.0016	0.0054	0.0042	0.0055
BY94NAT				****	nd	0.0132	0.0081	0.0131	0.0128	0.0208
BY95NAT					****	nd	nd	nd	nd	nd
BY96NAT						****	0.0142	0.0168	0.0171	0.0122
BY97NAT							****	0.0074	0.0071	0.0129
BY98NAT								****	0.0104	0.0125
BY99NAT									****	0.0139
BY00NAT										****
BY01NAT										
BY02NAT										
BY03NAT										
BY04NAT										
BY05NAT										

	BY01NAT	BY02NAT	BY03NAT	BY04NAT	BY05NAT
86NAT	0.0080	0.0050	0.0057	0.0082	nd
BY92NAT	0.0059	0.0043	-0.0003	0.0016	nd
BY93NAT	0.0033	0.0002	0.0014	0.0141	nd
BY94NAT	0.0011	0.0017	0.0032	0.0158	nd
BY95NAT	nd	nd	nd	nd	nd
BY96NAT	0.0145	0.0118	0.0077	0.0189	nd
BY97NAT	0.0000	0.0039	0.0054	0.0268	nd
BY98NAT	0.0085	0.0038	0.0030	0.0175	nd
BY99NAT	0.0089	0.0057	0.0054	0.0156	nd
BY00NAT	0.0127	0.0096	0.0110	0.0131	nd
BY01NAT	****	0.0029	0.0052	0.0177	nd
BY02NAT		****	0.0011	0.0178	nd
BY03NAT			****	0.0079	nd
BY04NAT				****	nd
BY05NAT					****

Figure 1. Factorial correspondence plot of Tucannon spring Chinook in-river collections from brood years 1993, 1996 – 2005 and 1986 natural-origin.

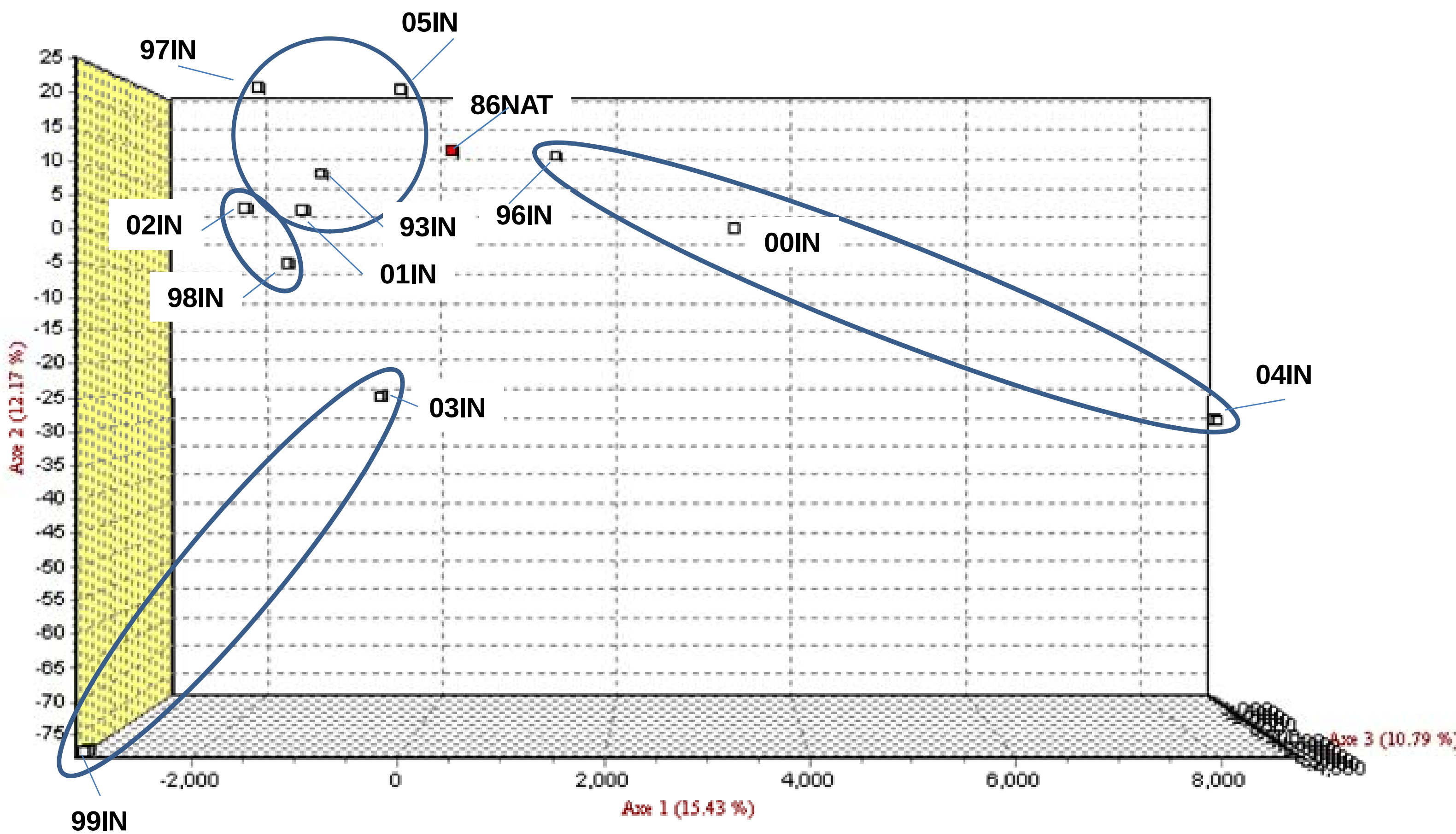

Figure 2. Factorial correspondence plot of Tucannon spring Chinook supplementation collections from brood years 1992 – 2005 and 1986 natural-origin.

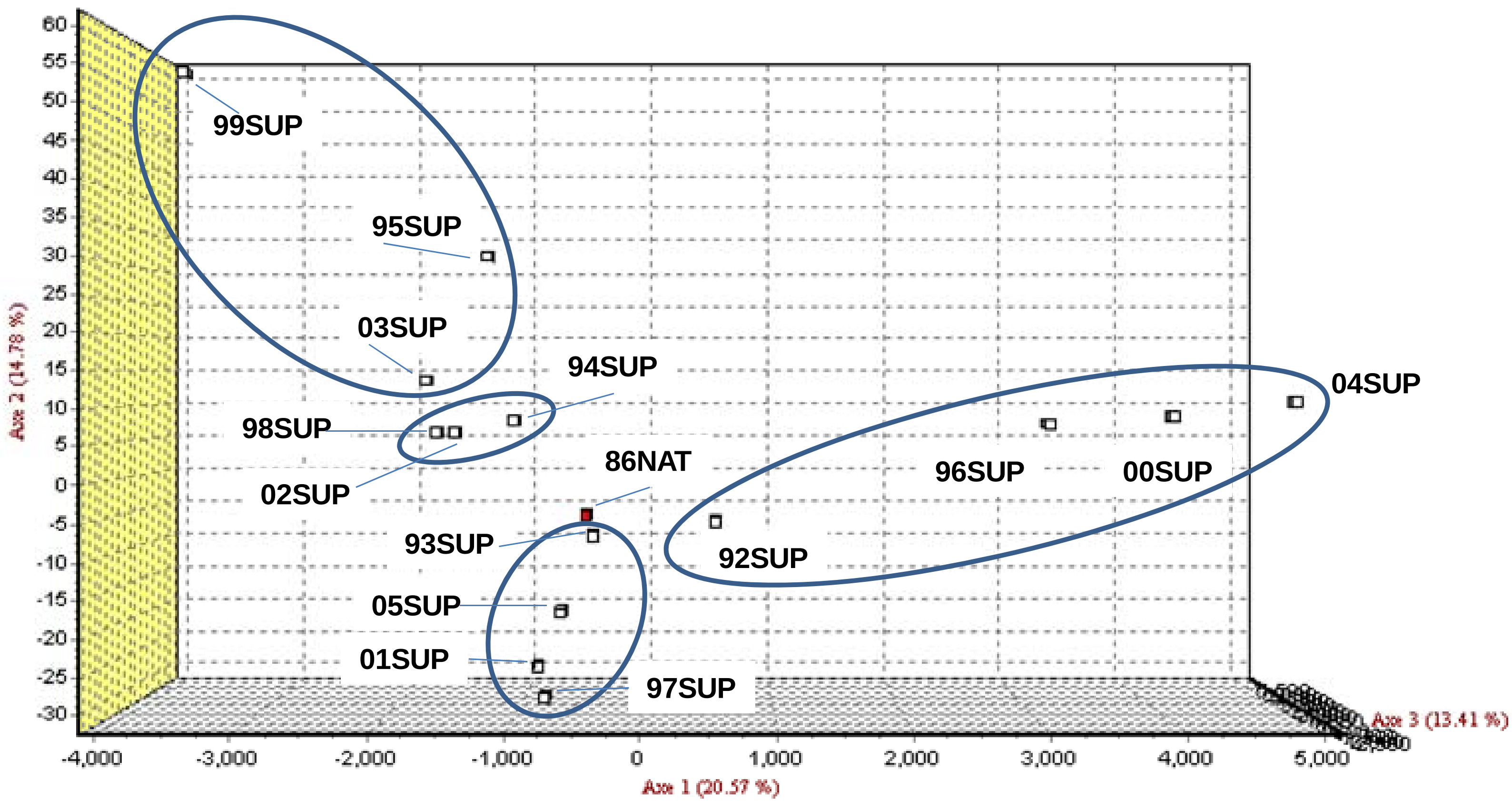

Figure 3. Factorial correspondence plot of Tucannon spring Chinook hatchery-origin collections from brood years 1993 – 2005 and 1986 natural-origin.

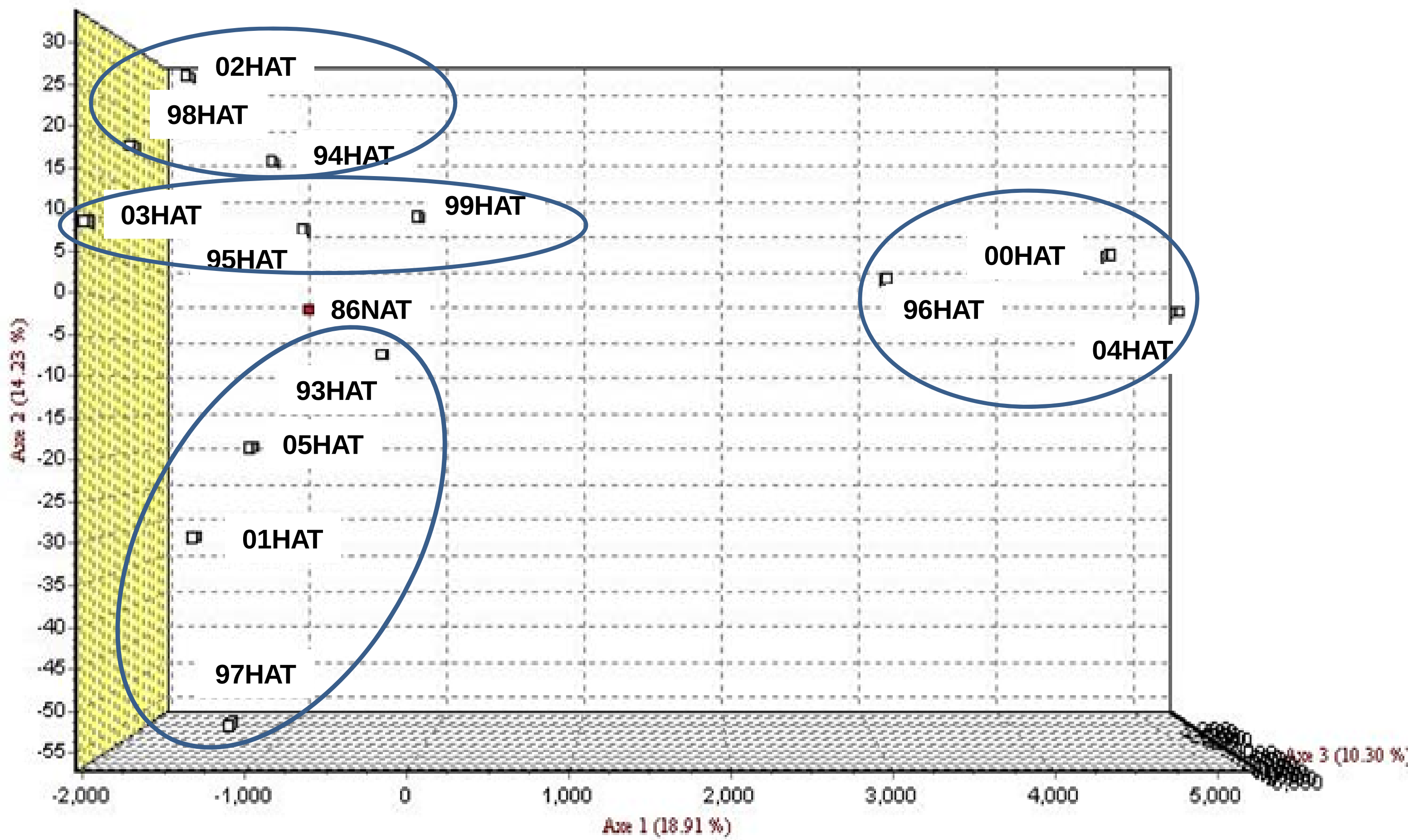

Figure 4. Factorial correspondence plot of Tucannon spring Chinook natural-origin collection from 1986 and from brood years, 1992 – 1994, 1996 - 2004.