Yakima/Klickitat Fisheries Project Genetic Studies
Yakima/Klickitat Fisheries Project Monitoring and Evaluation

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This report covers one of many topics under the Yakima/Klickitat Fisheries Project’s Monitoring and Evaluation Program (YKFPME). The YKFPME is funded under two BPA contracts, one for the Yakama Nation and the other for the Washington Department of Fish and Wildlife (contract number 00037649, Project Number 1995-063-25). A comprehensive summary report for all of the monitoring and evaluation topics will be submitted after all of the topical reports are completed. This approach to reporting enhances the ability of people to get the information they want, enhances timely reporting of results, and provides a condensed synthesis of the whole YKFPME. The current report was completed by the Washington Department of Fish and Wildlife.
Executive Summary

Chapter 1: A population-of-origin assignment procedure was used to estimate the percentages of unknown-origin smolts from each of five stock groups outmigrating past Chandler Trap (Yakima River) from January – July 2008. Mixture analysis was conducted on a proportional subsample of 1,200 smolts collected during the outmigration at Chandler Trap. Assignment to a population-of-origin was determined if the posterior probability of the assignment was greater than 90.0%. The largest percentage of outmigrating smolts in the first four time strata was from the upper Yakima River stock while the June – July time stratum was dominated by the lower Yakima River fall stock with 91.9% of the total assignments. Comparison of morphological assessment and genetic assignment as a spring or fall Chinook smolt conducted for all time strata indicated agreement for 1,172/1,188 (98.7%) of the smolts.

Chapter 2: The objective of this task was to identify the gender of Chinook salmon using two sets of sex-linked molecular markers. Samples of unknown gender collected at Roza Dam in 2008 were analyzed and the gender of each fish was determined. A consensus gender identification of unknown Chinook was determined for 143 out of 144 fish using the two techniques. The sex ratio of adult hatchery-origin Chinook passing Roza Dam was 69 males and 74 females. The comparison of gender identifications based on morphology and genetics resulted in 32 of 143 (22.4%) individuals that had different gender determinations. Thirty of the 32 (93.8%) differences were identified as females based on morphological characteristics and males by genetic analysis while the remaining samples were identified as males based on morphological characteristics and females by genetic analysis.

Chapter 3: We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of hatchery-origin Chinook salmon spawners collected at Roza Dam on the Yakima River. The assignments were based on genotypes from 14 microsatellite loci. A total of 1,409 hatchery-origin males (including adults, jacks, and precocial) and 793 adult hatchery-origin females collected in 2003 were genotyped and defined as the “parental” generation of this study. In 2007, 583 age-4 Chinook adults returning to Roza Dam on the Yakima River were genotyped and classified as F1 progeny. One hundred sixty-one offspring were assigned to a parental pair with 95% confidence. Two hundred thirty offspring were assigned to a mother only and 62 offspring assigned to a father only. The remaining 130 offspring with no identified parent from the 2003 brood year were assumed to be the product of two natural-origin parents. Assuming random mating and no fitness depression in the parental generation (i.e., brood year 2003), 45% of the F1 progeny should be the product of two hatchery-origin parents, 44% should have one hatchery-origin parent, and 11% should have zero hatchery-origin parents. Based on our preliminary parentage analysis of the 583 F1 offspring collected in 2007, 28% were assigned to hatchery-origin, 51% mixed hatchery and natural-origin, and 20% natural-origin. Overall, the natural-origin Chinook produced more returning F1 offspring than expected given a null hypothesis of equal production between hatchery- and natural-origin Chinook. A fundamental aspect of this project was the assumption that any unobserved parent was of natural origin. We have initiated an analysis of the 1999 hatchery broodstock from the upper Yakima supplementation program, whereby using the genotype data from the 2003 hatchery-origin
returns to Roza Dam as progeny, we can evaluate any bias in the parentage analysis that would inflate the apparent productivity of natural-origin fish.

Chapter 4: This study investigated the distribution of genetic diversity within *Oncorhynchus mykiss* collections from tributaries of the upper Yakima River. Previous genetic studies in the Yakima Basin have documented genetic differences among the anadromous (i.e., steelhead) *O. mykiss* populations; however, limited information is available regarding the genetic affinities between anadromous and resident forms of *O. mykiss* in the Yakima River. The present study analyzed known adult steelhead spawners, juvenile migrants, and resident *O. mykiss* from tributaries to the upper Yakima River. When compared to each other, Teanaway River and Taneum Creek anadromous adults had a statistically significant genic test at $\alpha = 0.05$ (i.e., different allele frequency distributions) and a pairwise estimate of $F_{ST} = 0.012$, which was statistically different from zero. Similar results were observed for juvenile migrants, suggesting the Teanaway and Taneum *O. mykiss* were genetically differentiated. Regarding resident collections, replicated collections from the same location tended to be similar, while comparisons between tributaries showed statistically significant genetic differentiation. All genic tests comparing residents from different collection locations (i.e., tributaries) were statistically different and all pairwise estimates of $F_{ST}$ were statistically different from zero. Factorial correspondence analysis was used to investigate overall genetic affinities among the various life history stages (i.e., residents, migrants, anadromous adults). Where comparisons were possible in Taneum Creek and Teanaway River, all three life history stages from the same location were more genetically similar than the same life history stage was between tributaries. In contrast to *O. mykiss* within a tributary tending to be similar, residents from the five tributaries were genetically differentiated, based on mahalanobis distances. This observation was further supported by calculation of $F_{ST}$, where the $F_{ST}$ estimated among resident collections was twice the magnitude of the $F_{ST}$ estimated among migrant and adult collections, 0.016 and 0.008, respectively. Overall, we concluded upper Yakima River *O. mykiss* were genetically differentiated, with micro population structure partitioned by river tributary and not life history form.

Chapter 5: The Yakima spring Chinook supplementation program began in 1997 but an intensive monitoring effort in natural production, genetics, and ecological interactions was begun even before the hatchery operations started, and has continued. ISRP Review coincident with the first return of adult (4-year old) fish in 2001 raised concerns that the project was not sufficiently aggressive and rigorous in evaluating domestication. The result was an expanded natural production domestication monitoring plan that began in the fall of 2002. The expanded domestication monitoring plan was first described in Busack et al.(2002), revised in 2003 (Busack et al. 2003) and in 2004 (Busack et al. 2004b). The current document is a September 1, 2009 revision. The document contains not only a description of the current effort, but also a history of modifications to the plan.

The basic design of the domestication monitoring effort is to the best of our knowledge unmatched anywhere. The performance of the supplemented Upper Yakima spring Chinook population, an integrated population with 100% natural-origin broodstock, is compared to the
performance of an Upper Yakima control line maintained under a regime of continuous hatchery culture, and to an unsupplemented wild control line in the neighboring Naches River. Performance is measured at 14 adult and 15 juvenile traits that encompass virtually the entire range of natural production domestication impacts noted in the literature. Details on the traits are presented in the Trait, Protocol and Analysis Overview section below. The only major monitoring area of the project that is not included in this document is the ecological monitoring effort.

The 2009 modifications are primarily the inclusion of detailed results on almost all traits. Results of investigations on many traits have by now appeared in the primary literature.
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Chapter 1

DNA-Based Population-of-Origin Assignments of Chinook Salmon Smolts Outmigrating Past Chandler Trap at Prosser Dam (Yakima River) in 2008

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Abstract

A population-of-origin assignment procedure was used to estimate the percentages of unknown-origin smolts from each of five stock groups outmigrating past Chandler Trap (Yakima River) from January – July 2008. Mixture analysis was conducted on a proportional subsample of 1,200 smolts collected during the outmigration at Chandler Trap. Assignment to a population-of-origin was determined if the posterior probability of the assignment was greater than 90.0%. The largest percentage of outmigrating smolts in the first four time strata was from the upper Yakima River stock while the June – July time stratum was dominated by the lower Yakima River fall stock with 91.9% of the total assignments. Comparison of morphological assessment and genetic assignment as a spring or fall Chinook smolt conducted for all time strata indicated agreement for 1,172/1,188 (98.7%) of the smolts.
Introduction

Production and survival of the Yakima River basin spring Chinook stocks (American River, Naches River, and upper Yakima River) are monitored, as part of the Yakima/Klickitat Fishery Project supplementation evaluation program. However, in the lower Yakima River, where the best facilities to collect samples exist, the three spring Chinook stocks are mixed with one another and with the Marion Drain and Yakima River fall Chinook stocks, during downstream juvenile migration. Thus, methodologies for discriminating stocks in an admixture are vital for development of stock-specific estimates. Domestication monitoring plans require discrimination of the three spring Chinook salmon stocks in the basin, and a complete analysis of migration timing and stock abundance for all Chinook requires discrimination of the two fall stocks as well. Accurate assignments of Chinook smolts captured at the Chandler fish passage facility (Prosser Dam) to population-of-origin will allow researchers and managers to estimate production by the three spring Chinook stocks, assess smolt-to-smolt survival of the three spring Chinook stocks, and could be utilized to evaluate stock-specific environmental condition factors.

The methodology used in this study to estimate the population-of-origin for individual fish in a mixture followed a partial-Bayesian approach by Rannala and Mountain (1997). This approach assumes linkage equilibrium among loci and uses the multilocus genotype of an individual to compute the probability of that genotype belonging to a population in the baseline. Others have used the methodology developed by Rannala and Mountain (1997) to provide robust population-of-origin assignments of unknown individuals (Hauser et al. 2006, Taylor and Costello 2006, and Waples and Gaggiotti 2006).

Calculation of population-of-origin for Chinook smolts trapped at Chandler trap throughout the entire outmigration (January through July) was hindered in the first few years of analysis for several reasons: non-representative temporal sampling of the downstream migration, past omission of the Marion Drain fall and lower Yakima River mainstem fall Chinook stocks from the DNA baseline, and by maintenance and other shutdowns of trap operations in December and January in many years. In the analyses of samples from 2004 - 2007, attempts were made to eliminate the problems present in previous analyses. A new sampling design was initiated to provide a proportional sample of smolts outmigrating past Chandler trap and a larger number of smolts were analyzed. Repeated multi-year samples of all five baseline stocks were used to characterize the potential sources of smolts in the Yakima River basin.

This report presents the population-of-origin assignments for outmigrating smolts collected at the Chandler trap during 2008.

Materials and Methods

Collections
There were no collections added to the Yakima River baseline this year. To establish baseline samples, since 1989, sampling crews from the Yakama Nation and WDFW collected
DNA tissue (dry-mounted scales or fin tissue preserved in 100% ethanol) from adult fish on the spawning grounds. As a result, we established multi-year baseline collections for five populations (American River spring, Naches River spring, upper Yakima River spring, Marion Drain fall, and lower Yakima River fall; Table 1 and Figure 1).

The estimated total number of smolts passing Chandler trap is based on an expansion factor from the total number of smolts actually counted at the trap. In 2008 a total of 32,482 smolts were counted from January 6 – July 18, 2008, and this number was expanded to 165,764 outmigrating smolts. The stock of origin of the natural-origin smolts was unknown. To assess relative productivity of the five baseline population, DNA samples were collected from a subset of smolts captured at Chandler. The following sampling protocol was used by the Yakima Nation fish counting team (Mark Johnston, pers. comm.): Each day, the total number of smolts at the trap was visually estimated before any processing occurred. If that number was below a predetermined threshold then a “standard” sample was taken for that day (e.g. 10 fish). If the number of smolts was above the threshold then a “peak” sample was taken for that day (e.g. 30 fish). The threshold for “standard” and “peak” days and the numbers of samples to be taken on each day varied for each of the time strata (January – February, March, April, May, and June – July). These values were determined by analyzing the number of “peak” and “standard” days counted during smolt outmigration monitoring from 2000 - 2003. Based on this sampling design, 3,245 Chinook smolt samples were collected for genetic analysis.

The total estimated number of smolts passing the Chandler Trap each day was plotted with the total number of genetic samples that had been collected. From the 3,245 smolt samples, we selected 1,200 samples for genetic analysis. The temporal distribution of the samples chosen was proportional to the temporal distribution of the outmigration. The total number of samples used for genetic analysis was based on budgetary constraints only.

**DNA Extraction Methods**
Genomic DNA was extracted by digesting a small piece of fin tissue (all smolt and some adult baseline collections) or scales (most adult baseline collections) using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

**PCR Methods**
The polymerase chain reaction mixture contained the following for a 10 µL reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units GoTaq Flexi DNA polymerase (Promega). Amplification was performed using MJ Research PTC-200 and Applied Biosystems 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94°C; 40 cycles of 15 seconds at 94°C, 30 seconds at 50-60°C, and 1 minute at 72°C; plus a final extension step at 72°C for 10 minutes, followed by a final indefinite holding step at 10°C.

Eleven microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated
DNA Technologies). Loci were combined in multiplexes to increase efficiency and decrease costs. Samples that failed to amplify a minimum of ten loci were dropped from the analysis.

Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al., 2007).

Population-of-origin Analysis
Population-of-origin assignments for the analysis of unknown-origin smolts were accomplished with GMA (Kalinowski 2003) using a partial-Bayesian Method described by Rannala and Mountain (1997). GMA estimates the probability of an individual coming from a baseline population based on the population’s estimated contribution to the mixture as a prior (Kalinowski 2003). All assignments with a posterior probability greater than or equal to 90% were accepted. Because GMA uses the estimated contribution as a prior the analysis of the known-origin samples was conducted using approximately equal numbers of samples.

Comparison of Morphological ID and Genetic Assignment
Based on smolt length, size of the eye, and snout shape, individual fish were categorized as spring or fall Chinook when they were intercepted at the Chandler Trap (Mark Johnston, Yakama Nation; pers. comm.). To determine the efficacy of assigning fish to either spring or fall stocks based on these morphological characters, we compared these assignments with those obtained from the genetic analysis.

Results

Population-of-origin Analysis
The first four time strata were dominated by the spring stocks, with the largest percentage of spring smolts assigned to the upper Yakima River (47.9 – 85.7% for the four time strata) followed by the Naches River (8.3 – 25.2%), and American River (0.0 – 8.3 (Table 3). The lower Yakima River had the highest percentage in the June - July time stratum (91.9%) (Table 3).

Comparison of Morphological ID and Genetic Assignment
A comparison of the morphological assessment to genetic assignment was conducted for all five-time strata. A total of 13 of 13 smolts in January, 8 of 8 smolts in March, 310 of 311 smolts in April, 326 of 329 smolts in May, and 531 of 539 in the June/July time strata were scored for ten or more loci, and therefore included in the analysis. Results for the time strata were as follows: January time stratum – all 13 smolts were assigned identically using morphological and genetic methods (13 spring). March time stratum – all eight smolts were
assigned identically using morphological and genetic methods (eight spring). April time stratum – all 310 smolts were assigned identically using morphological and genetic methods (310 spring). May time stratum – all 326 smolts were assigned identically using morphological and genetic methods (248 spring and 78 fall). June/July time stratum – 515 smolts were assigned identically using morphological and genetic methods (32 spring and 483 fall), seven of the remaining smolts were identified as fall by morphological methods and spring by genetic analysis and the remaining nine smolts were identified as spring by morphological analysis and fall by genetic analysis.

**Discussion**

Monitoring the relative abundances of Chinook smolts in the Yakima River from the three populations of spring Chinook (upper Yakima River, American River, and Naches River) and the two populations of fall Chinook (Marion Drain and lower Yakima River) requires the ability to estimate population composition of smolts outmigrating past Chandler trap. Because all five Chinook populations are intermingled when they pass Chandler trap, and the majority are unmarked and untagged, the only way to determine population-of-origin is by genetic analysis. This method requires that sufficient genetic differences exist among these populations in the Yakima River basin.

A baseline of 19 individual collections from the five populations in the Yakima River basin was used for the population-of-origin assignments of the outmigrating smolts. The baseline collections as a whole had higher genotyping failure compared to the Chandler smolt samples. Scales were taken from carcasses on spawning grounds for most baseline collections; therefore, DNA quality was presumably poorer than the Chandler smolt collection where tissue was collected from live fish. The upper Yakima River tissue collections were also taken from live fish at the hatchery and, therefore, genotyping success was higher for this collection than the other baseline collections.

We assigned 100% of the smolts between January and April to the three spring stocks. The upper Yakima River spring stock accounted for the highest average percentage (70.7%) of smolts present in that period. Rank in abundance of the three spring stocks was the same in the first four time strata (January-February, March, April, and May) with upper Yakima River spring stock having the most, Naches River second, and lastly the American River stock. As expected, the June-July time stratum was predominately composed of the lower Yakima River fall Chinook stock, accounting for over 91.9% of the total number of smolts.

Assessment of spring or fall smolts by morphological and genetic analysis revealed good agreement between the two methods in all time periods except for June-July. Identification as a spring or fall smolt was the same for 657 out of 657 smolts collected during the January - May time strata. The comparison in the June – July strata revealed more differences (16 differences out of 531 total smolts analyzed).
Acknowledgements

Funding for this study was provided by the Bonneville Power Administration (BPA) and by the WA State General Funds to WDFW. We would like to thank Mark Johnston and the Yakama Nation Chandler trap crew for collecting all the unknown smolts at Chandler Trap and the Yakama Nation and WDFW field sampling crews for the baseline stock collections. Doug Neeley, Dave Lind, Bill Bosch (Yakama Nation) provided Chandler Trap Chinook count and passage data.


Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA.


dinucleotide and tertanucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53:2292-2298.


Table 1. Nineteen Chinook salmon collections assembled into a baseline and used for the analysis of the known-origin and unknown-origin smolts. "*" the 05LU collection from Marion Drain was not used in the baseline, but is listed here as a collection.

<table>
<thead>
<tr>
<th>Baseline Collections</th>
<th>Collection Code</th>
<th># Processed</th>
<th># Analyzed</th>
<th>% Single Locus Genotypes Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>American River - spring</td>
<td>89AG</td>
<td>80</td>
<td>77</td>
<td>10.4%</td>
</tr>
<tr>
<td></td>
<td>91DQ</td>
<td>102</td>
<td>87</td>
<td>9.8%</td>
</tr>
<tr>
<td></td>
<td>93DO</td>
<td>18</td>
<td>17</td>
<td>3.2%</td>
</tr>
<tr>
<td></td>
<td>03EH</td>
<td>100</td>
<td>70</td>
<td>6.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>251</td>
<td>8.6%</td>
</tr>
<tr>
<td>Naches River - spring</td>
<td>89AC</td>
<td>76</td>
<td>74</td>
<td>11.4%</td>
</tr>
<tr>
<td></td>
<td>89AI</td>
<td>26</td>
<td>22</td>
<td>7.0%</td>
</tr>
<tr>
<td></td>
<td>93DQ</td>
<td>50</td>
<td>45</td>
<td>6.3%</td>
</tr>
<tr>
<td></td>
<td>93DR</td>
<td>32</td>
<td>25</td>
<td>7.3%</td>
</tr>
<tr>
<td>little Naches River - spring</td>
<td>04BI</td>
<td>42</td>
<td>41</td>
<td>2.2%</td>
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<tr>
<td></td>
<td>04EM</td>
<td>56</td>
<td>45</td>
<td>9.9%</td>
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<td></td>
<td></td>
<td>282</td>
<td>252</td>
<td>7.9%</td>
</tr>
<tr>
<td>upper Yakima River - spring</td>
<td>92DN</td>
<td>24</td>
<td>23</td>
<td>5.9%</td>
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<tr>
<td></td>
<td>97DA</td>
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<td></td>
<td>03GO</td>
<td>99</td>
<td>99</td>
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<td></td>
<td></td>
<td>246</td>
<td>237</td>
<td>3.0%</td>
</tr>
<tr>
<td>Marion Drain - fall</td>
<td>89BX</td>
<td>100</td>
<td>92</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td>92FQ</td>
<td>92</td>
<td>92</td>
<td>5.4%</td>
</tr>
<tr>
<td></td>
<td>93DY</td>
<td>8</td>
<td>8</td>
<td>8.0%</td>
</tr>
<tr>
<td></td>
<td>05LU*</td>
<td>65</td>
<td>47</td>
<td>15.3%</td>
</tr>
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<td></td>
<td></td>
<td>265</td>
<td>239</td>
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</tr>
<tr>
<td>lower Yakima River - fall</td>
<td>90DF</td>
<td>109</td>
<td>104</td>
<td>12.6%</td>
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<tr>
<td></td>
<td>93DW</td>
<td>82</td>
<td>80</td>
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<tr>
<td></td>
<td>98FB</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>252</td>
<td>234</td>
<td>10.8%</td>
</tr>
<tr>
<td>Chandler Trap Smolts - 2008</td>
<td>08AE</td>
<td>1,200</td>
<td>1,188</td>
<td>0.5%</td>
</tr>
</tbody>
</table>
Table 2. Microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci used in the analysis of Chinook from five stocks in the Yakima River Basin. Also included are the percent missing genotypes for both baseline and

<table>
<thead>
<tr>
<th>Multiplex</th>
<th>Locus</th>
<th>Annealing temp °C</th>
<th># Alleles/Locus</th>
<th>Allele Size Range (bp)</th>
<th>% missing genotypes baseline N = 1,213</th>
<th>% missing genotypes smolts N = 1,188</th>
<th>H_{0}</th>
<th>H_{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ots-M</td>
<td>Oki-100”a</td>
<td>50</td>
<td>41</td>
<td>164 - 365</td>
<td>11.4</td>
<td>0.7</td>
<td>0.913</td>
<td>0.940</td>
</tr>
<tr>
<td></td>
<td>Ots-201b”a</td>
<td>50</td>
<td>42</td>
<td>137 - 310</td>
<td>7.3</td>
<td>0.0</td>
<td>0.916</td>
<td>0.936</td>
</tr>
<tr>
<td></td>
<td>Ots-208b”b</td>
<td>50</td>
<td>52</td>
<td>158 - 342</td>
<td>9.9</td>
<td>0.1</td>
<td>0.943</td>
<td>0.954</td>
</tr>
<tr>
<td></td>
<td>Ssa-408c</td>
<td>50</td>
<td>32</td>
<td>184 - 308</td>
<td>4.0</td>
<td>0.4</td>
<td>0.827</td>
<td>0.934</td>
</tr>
<tr>
<td>Ots-N</td>
<td>Ogo-2”d</td>
<td>60</td>
<td>19</td>
<td>202 - 256</td>
<td>4.5</td>
<td>0.8</td>
<td>0.756</td>
<td>0.854</td>
</tr>
<tr>
<td></td>
<td>Ssa-197”d</td>
<td>60</td>
<td>38</td>
<td>181 - 318</td>
<td>11.9</td>
<td>0.7</td>
<td>0.915</td>
<td>0.940</td>
</tr>
<tr>
<td>Ots-O</td>
<td>Ogo-4”d</td>
<td>56</td>
<td>17</td>
<td>132 - 164</td>
<td>15.6</td>
<td>0.2</td>
<td>0.776</td>
<td>0.884</td>
</tr>
<tr>
<td></td>
<td>Ots-213”b</td>
<td>56</td>
<td>40</td>
<td>182 - 362</td>
<td>9.4</td>
<td>0.8</td>
<td>0.908</td>
<td>0.940</td>
</tr>
<tr>
<td></td>
<td>Ots-G474”f</td>
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*a = Unpublished  
b = Greig et al. 2003  
c = Cairney et al. 2000  
d = Olsen et al. 1998  
e = Oreilly et al. 1996  
f = Williamson et al. 2002  
g = Banks et al. 1999
Table 3. Stock-of-origin assignments at greater than or equal to 90% posterior probability for five stocks of Chinook in the Yakima River Basin using GMA.

<table>
<thead>
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<th>American R.</th>
<th>Naches R.</th>
<th>upper Yakima R.</th>
<th>Marion Drain</th>
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Figure 1. Geographic location of the Chandler trap on the Yakima River, Washington and the primary streams in the basin.
Appendix 1. Mis-assignment as a spring or fall Chinook between morphological assignment and genetic analysis in 2008.

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Chapter 2

DNA-Based Gender Determination of Hatchery-Origin spring Chinook Salmon Passing Roza Dam (Yakima River) in 2008

Todd W. Kassler
Jennifer Von Bargen

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Molecular Genetics Laboratory
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Olympia, WA 98501-1091
Abstract

The objective of this task was to identify the gender of Chinook salmon using two sets of sex-linked molecular markers. Samples of unknown gender collected at Roza Dam in 2008 were analyzed and the gender of each fish was determined. A consensus gender identification of unknown Chinook was determined for 143 out of 144 fish using the two techniques. The sex ratio of adult hatchery-origin Chinook passing Roza Dam was 69 males and 74 females. The comparison of gender identifications based on morphology and genetics resulted in 32 of 143 (22.4%) individuals that had different gender determinations. Thirty of the 32 (93.8%) differences were identified as females based on morphological characteristics and males by genetic analysis while the remaining samples were identified as males based on morphological characteristics and females by genetic analysis.
Introduction

The objective of this project is to identify the gender of Chinook salmon passing Roza Dam, using sex-linked molecular markers on the Y chromosome (normally found in males; Devlin et al. 1991, Du et al. 1993, Devlin et al. 1994, Forbes et al. 1994, Clifton and Rodriguez 1997, Devlin et al. 2002, and Brunelli and Thorgaard 2004).

We screened 140 hatchery-origin adult Chinook samples collected at Roza Dam for two DNA gender identification markers to estimate sex composition for first generation hatchery returns (hatchery produced progeny of natural-origin recruits). The DNA samples are collected as part of the YKFP hatchery-monitoring program from spring Chinook passing Roza Dam because they are abundant and provide a representative estimate of hatchery fish returns instead of sampling on spawning grounds. We also compared our genetic-based gender identification results to results of the gender identification assessment made when Chinook adults were passed over Roza Dam using morphological characteristics. The secondary sexual characteristics of the spring Chinook passing Roza Dam are not yet fully developed, therefore morphological identification of gender can lead to different gender ratio estimates.

Material and Methods

Collections of Unknown Gender
Fin-clip tissue samples were collected from Chinook salmon as they were passed at Roza Dam on the Yakima River in 2008 (N = 144 were analyzed; collection 08DJ - adults). The tissue samples were preserved in 100% ethanol and stored in pre-labeled vials.

DNA Extraction Methods
Genomic DNA was extracted from a small piece of fin tissue (approximately 2 mm²) using Macherey-Nagel nucleospin tissue kits following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

PCR and Gel Methods
Polymerase chain reaction (PCR) was used to amplify two sets of molecular markers: OT-24 (Clifton and Rodriguez 1997) and OTY2-WSU (Brunelli and Thorgaard 2004); that exist in distinct locations on the Y chromosome or are linked to the Y chromosome to identify gender of Chinook salmon. Analysis of the markers described by Clifton and Rodriguez (1997) utilized primers p551 and p559, derived from a sex specific marker (OT-24), amplifying a 950 base pair fragment in males while yielding only a fraction of the same 950 base pair fragment and potentially unobservable amount in females. A second pair of primers (p709 and p710) derived from non-sex linked HSP30 (425 base pairs) is monomorphic in Chinook. The p709 and p710 primers were multiplexed with the OT-24 primers as a PCR control to determine that there had not been a false identification as a female due to PCR failure.

Brunelli and Thorgaard (2004) identified a primer sequence OTY2-WSU (primers OTY2-f2 and OTY2-r2) that allowed gender identification in Chinook and other Pacific
salmon species. A fragment of approximately 287 base pairs amplifies in males while females do not amplify any sex-specific products. A second set of primers [GAPDH forward (AF027130, nucleotide 487 – 506) and GAPDH reverse (AF027130 nucleotide 724 – 742)] amplifying the glyceraldehyde-3-phosphate dehydrogenase gene (approximately 750 base pairs present in both males and females) was multiplexed with the OTY2-WSU primers to be a control to test for successful PCR amplification.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.1 µM of each oligonucleotide primer, and 0.05 units Taq polymerase (Promega). Amplification was performed using either a MJ Research PTC-200 or AB 9700 thermocycler. The thermal profile for both gender markers was as follows: an initial denaturation step of 3 minutes at 94ºC; 35 cycles of 15 seconds at 94ºC, 30 seconds at 48ºC (Clifton-Rodriguez) or 63 ºC (Brunelli-Thorgaard), and 1 minute at 72ºC; plus a final extension step at 72ºC for 30 minutes, followed by a final indefinite holding step at 10ºC.

Amplified products were separated electrophoretically using a 2.0% agarose gel (Agarose I (0710-100g) from AMRESCO), in 1X TBE buffer from AMRESCO with 0.4X SYBR® Gold (Molecular Probes) to visualize banding patterns using a Dark Reader™ transilluminator by Clare Chemical Research. A loading cocktail of 5µL loading dye, 1µl PCR amplified product, and 4µl sterilized dH₂O was mixed, and 8µl of this mixture was loaded into the gel. Photographs of each gel were taken with a digital camera and used for scoring.

**Scoring Methods**
For each sample, gender was identified [by two different researchers] using both the OT-24/HSP30 and OTY2-WSU systems. A questionable gender identification (M? or F?) was given for a sample if there was some ambiguity to the banding pattern. Consensus identification was reached by evaluating the six scores (three researchers using two methods). If all six scores were in agreement there was no question to the consensus identification. If there was a questionable score given by one researcher, but the other two scores were in agreement with a good score then a consensus score was given. This methodology of two good scores was applied independently for both of the methods. If there was not agreement for either of the methods for two of the researches the sample was reanalyzed. Upon reanalysis, the sample had to have two good scores for each of the techniques before a consensus score was given. In cases where there were good scores by all three researchers for each method, but scores differed by method then the individual was also reanalyzed.

Comparison of the consensus gender identification with the morphological gender identification occurred after ambiguous samples were eliminated. Elimination of these samples does potentially bias the overall percentages that were used in determining how well the techniques can correctly identify gender by decreasing the overall number of incorrect gender assignments.
Results

Analysis of Unknown Samples
Consensus gender identification was determined for the 2008 unknown samples (total of 144 fish) following the guidelines described in the Methods section, with the exception of one individual (08DJ – 1462). A list of the original scores for each technique and scorer along with the final consensus score is shown in Table 1.

Comparison of Unknown Samples Sexed by Morphology and Genetics
Sex identifications of unknown samples (08DJ) based on morphology (sexed at the time the live fish were handled and passed at the dam) and genetics were different for 32 of 143 (22.4%) individuals (Appendix 1). Thirty of the 32 (93.8%) differences were identified as females based on morphological characteristics and males by genetic analysis. The remaining individual was identified as a male using morphological characteristics and a female by genetic analysis.

Discussion
Genetic techniques can be used to identify the gender of live pre-spawned salmon when morphology-based identification is difficult. The ability of molecular techniques to correctly identify gender in Chinook was tested by analyzing samples of known gender (Kassler et al. 2004; Kassler 2006). The results of these studies identified a high rate of correct gender determination that provided confidence in using genetic analysis for this project. This analysis assessed Chinook salmon samples that were identified as males or females using morphological characteristics and then by genetic markers to determine if the morphological and genetic assessment was in agreement.

Two independent genetic methods were tested and compared to determine if the same gender identification would be revealed. We were unable to determine gender for three samples. Of the remaining samples, there was consistent agreement between the consensus scores by both methods and the scorers even when the identification was different than expected based on external morphology.

Our comparison of the morphological and genetic methods for identifying gender resulted in a total of 22.4% (32/143) of the samples identified differently. Most of these differences (93.8%) occurred when an individual was identified as a female by morphological characteristics and male by genetic analysis. This is possibly a result of mistaking the morphology of a sexually immature female and smaller male (individual that has not developed sexually dimorphic characteristics).

Conclusions
Investigations by Kassler et al. (2004) and Kassler (2006) identifying gender in Chinook salmon using two different DNA markers suggested high, but not 100% accuracy of the techniques used for this analysis. Using two DNA markers to determine the sex ratio of
143 hatchery-origin Chinook passing Roza Dam we estimated 69 adult males and 74
adult females in 2008.

Acknowledgements

Funding for this study was provided by the Bonneville Power Administration (BPA) and
by the WA State General Funds to WDFW. Mark Johnston (YN) and his crew at Roza
Dam collected samples and biological data including gender identification based on
morphological characteristics. Curt Knudsen provided background information on the
project. Ken Warheit provided editorial comments and review.
Literature Cited


Table 1. Gender determination for Chinook salmon passed at Roza Dam in 2008. Three independent gender identifications and a final consensus gender determination are shown for each method.

[methods: ‘C - R’ = Clifton & Rodriguez; ‘B - T’ = Brunelli & Thorgaard] Consensus was reached by examining scores for each technique and scorer. Question marks (?) indicate unclear or ambiguous gender id for an individual score. Individuals with two gender ID's were reanalyzed. The original gender ID is shown first and the rerun ID second. The consensus ID is based on the reanalyzed gender identification.

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Yakima River Chinook Pedigree

Todd W. Kassler
Scott M. Blankenship
Kenneth I. Warheit
Craig A. Busack

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Abstract

We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of hatchery-origin Chinook salmon spawners collected at Roza Dam on the Yakima River. The assignments were based on genotypes from 14 microsatellite loci. A total of 1,409 hatchery-origin males (including adults, jacks, and precocial) and 793 adult hatchery-origin females collected in 2003 were genotyped and defined as the “parental” generation of this study. In 2007, 583 age-4 Chinook adults returning to Roza Dam on the Yakima River were genotyped and classified as F1 progeny. One hundred sixty-one offspring were assigned to a parental pair with 95% confidence. Two hundred thirty offspring were assigned to a mother only and 62 offspring assigned to a father only. The remaining 130 offspring with no identified parent from the 2003 brood year were assumed to be the product of two natural-origin parents. Assuming random mating and no fitness depression in the parental generation (i.e., brood year 2003), 45% of the F1 progeny should be the product of two hatchery-origin parents, 44% should have one hatchery-origin parent, and 11% should have zero hatchery-origin parents. Based on our preliminary parentage analysis of the 583 F1 offspring collected in 2007, 28% were assigned to hatchery-origin, 51% mixed hatchery and natural-origin, and 20% natural-origin. Overall, the natural-origin Chinook produced more returning F1 offspring than expected given a null hypothesis of equal production between hatchery- and natural-origin Chinook. A fundamental aspect of this project was the assumption that any unobserved parent was of natural origin. We have initiated an analysis of the 1999 hatchery broodstock from the upper Yakima supplementation program, whereby using the genotype data from the 2003 hatchery-origin returns to Roza Dam as progeny, we can evaluate any bias in the parentage analysis that would inflate the apparent productivity of natural-origin fish.
Washington Department of Fish and Wildlife (WDFW) and the Yakama Nation (YN) are collaborating on a study of Chinook salmon reproductive success. Several studies have shown the relative reproductive success (RRS) of hatchery-origin fish in the natural environment is lower than that of natural-origin fish (reviewed by Araki et al. 2008). Yet, these studies do not evaluate the productivity of the returning adult progeny from hatchery-origin fish that succeeded in reproducing in the river, which is an important aspect of RRS. The present study uses genetic-based parentage analysis to examine the differences in reproductive output of natural-origin fish that differ in levels of hatchery ancestry (i.e., 0, 1, and 2 hatchery-origin parents). The key concept to understand regarding this study is that by genotyping hatchery-origin parents that have the opportunity to reproduce in the wild, parentage analysis will enable the contributions of hatchery and natural parents to subsequent generations of returning adults to be evaluated. In other words, how many adult progeny did the naturally reproducing hatchery-origin fish produce and did they contribute the expected number of progeny to subsequent generations given their abundance?

All Chinook returning to the upper Yakima River are handled when passed by Roza Dam; therefore, there is an opportunity to sample all potential parents for a brood year. Beginning in 2003, all hatchery-origin Chinook entering the upper Yakima River were tissue sampled prior to their release into the river to spawn naturally. The 2003 brood year was designated the “parental” generation for this study. The majority of the progeny from the 2003 brood year would return in 2007, as adult Chinook predominantly return to the upper Yakima River at age 4. In 2007, all Chinook adults returning to Roza Dam (i.e., hatchery- and natural-origin) were tissue sampled and had their age determined. The age 4 natural-origin adults returning in 2007 were designated the “F₁” generation for this study. To be clear, only natural-origin fish were analyzed from the 2007 brood year, as these would be the products of hatchery- and natural-origin fish reproducing naturally in the river. Obviously, there would also be age-2, age-3, and age-5 adult returns from the 2003 brood year. Yet, for this study we focused on the largest portion of the returning offspring, the age-4 adults.

The potential parents from brood year 2003 and F₁ progeny from 2007 were genotyped using microsatellite loci standardized as part of the GAPS consortium (Seeb et al. 2007). Genetics-based parentage analysis was conducted following the same protocols that have been used from 2002 - 2008 (Young and Kassler 2005, Kassler 2005, Kassler 2006, Kassler and Von Bargen 2007, 2008) analysis of Chinook at the Cle Elum spawning channel. A maximum likelihood procedure was used to determine parentage of both parents for an offspring, a male parent only, and a female parent. If an individual did not assign to any parent it was assumed to be from natural-origin parents.
Materials and Methods

Collection of Chinook – 2003 and 2007
Fin tissue was collected at Roza Dam from a total of 2,202 Chinook (793 adult females and 1,409 males (adults, jacks, and precocial parr)) in 2003, of which, all adults were hatchery-origin returns left in river to spawn naturally. In 2007, 3,081 natural-origin Chinook (1,521 adult females and 1,560 males (adults, jacks, and precocial parr)) were tissue sampled at Roza Dam on the Yakima River. Genetic analysis was conducted on all hatchery-origin sampled from the 2003 brood year (including precocial males) and 583 age 4 natural-origin adult Chinook collected in 2007.

Comparison of the multilocus genotypes among all the samples that were analyzed was conducted to determine if there were any duplicated samples using the genetic analysis program CERVUS (version 3.0; Marshall et al. 1998) and MYKISS (http://www.montana.edu/kalinowski/Software/Mykiss.htm).

DNA Extraction Methods
Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

PCR Methods
Potential parents and returning progeny were genotyped using a standardized suite of 14 microsatellite loci (Seeb et al. 2007). Parents were screened twice and scored independently at all 14 loci by two biologists to minimize potential genotyping error. The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM MgCl2, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units GoTaq Flexi DNA polymerase (Promega). Amplification was performed using MJ Research PTC-200 and AB 9700 thermocyclers. The thermal profile was as follows: an initial denaturation step of 2 minutes at 94°C; 40 cycles of 15 seconds at 94°C, 30 seconds at 49-58°C, and 1 minute at 72°C; plus a final extension step at 72°C for 10 minutes, followed by a final indefinite holding step at 4°C. Microsatellite DNA loci were amplified via the polymerase chain reaction (PCR) using fluorescently labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Loci were combined into multiplexes to increase efficiency and decrease costs. Data were collected using an AB-3730 Genetic Analyzer. Applied Biosystems GENEMAPPER v.3.7 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). Alleles were binned in GENEMAPPER using the standardized protocols established for the Chinook (Seeb et. al. 2007).

Parentage Assignments
The dataset included 38,436 single-locus genotypes. An assumed 1.0% genotyping error rate in that dataset would result in 384 incorrect single-locus genotypes. Our genotyping
error rate is unknown for this project. Since parentage analyses involve comparing genotypes of candidate parental pairs with offspring genotypes, genotyping errors can produce parent-offspring genotype mismatches and suggest exclusion of true parent-offspring pairings from consideration. Alternatively, genotyping errors can lead to failure to exclude parent-offspring pairings that are incorrect. We used a maximum likelihood procedure, implemented in CERVUS (version 3.0; Marshall et al. 1998) and MYKISS (http://www.montana.edu/kalinowski/Software/Mykiss.htm) to infer parent-offspring relationships. The procedure uses allele frequency data to assign likelihoods to parent-offspring combinations, and allows mismatching genotypic data to be evaluated concurrently with matching genotype data.

Results/Discussion

Parents
Genetic analysis was conducted on a total of 2,201 individuals and revealed that seven of the samples had a duplicate genotype to another sample (03BY 428 and 03BY 482; 03BY 432 and 03BY 484; 03BY 585 and 03BY 586; 03BY 665 and 03BY 679; 03CA 56 and 03CA 57; 03CA 56 and 03CA 58; 03CA 57 and 03CA 58; 03CA 80 and 03CA 86), therefore one of the duplicated pair (shown in bold type) was excluded from further analysis. Parents with genotype data at a minimum of 10 loci were included in the analysis (2,189 parents). A total of 14 loci were screened and used in the analysis. The number of alleles present at a microsatellite locus ranged from 6 - 44 (Ots-9 and Omm-1080 respectively) and the observed heterozygosity ranged from 0.362 – 0.937 (Ots-G474 and Omm-1080 respectively). Cumulative exclusionary power was 1.000000 for analysis using all loci when one parent was known.

Parentage Assignments
All 583 F1 progeny from the 2007 brood year were successfully genotyped at 11 or more loci and were included in the analysis. Parentage analysis was conducted using CERVUS to determine the two most likely parental pairs for the 583 progeny. Single parent-offspring pairs with that had two or more mismatches during assignment were removed. Of the 583 offspring included in the analysis, a total of 139 progeny assigned to two parents (i.e., a single male and female parent pair). The remaining 444 offspring were then evaluated for mother-only or father-only assignment. Two hundred and twenty two of the 444 assigned to a mother only and 58 assigned to a father only. There were 26 offspring that assigned to both a mother and father independently during the assignment step considering sexes separately. Yet, parent-offspring trios were not possible given the available genotypic combinations. The assignments for these 26 individuals were resolved by evaluating both the observed assignment mis-matches and consistency of assignment. For example, if a single offspring was assigned to a single sire and multiple dams, the sire-offspring pair was chosen as an assignment, which generally rendered all dam genotypes invalid as possible parents given the available alleles. For the 26 individuals, eight were determined to be from a mother only and four were determined to be father only. The parentage assignments for 14 F1 progeny were ambiguous and were excluded from the analysis.
We confirmed the parental assignments described above using the program MYKISS. The parentage analysis using MYKISS revealed an additional 22 $F_1$ progeny that assigned to a parent pair not previously identified using CERVUS. These 22 progeny were added to the assigned totals, increasing the parent-offspring trios assigned from 139 to 161. Total parentage assignments of $F_1$ progeny were 161 to trios, 230 to a mother only, 62 to a father only, and 130 not assigned to any parent.

Parentage analysis provides an opportunity to investigate reproductive success by determining the number of progeny produced by individual fish. Based on the number of hatchery- and natural-origin fish returning to Roza Dam in 2003 (minus the natural-origin fish spawning in the hatchery), the expected proportion of hatchery-origin, mixed hatchery and natural-origin, and natural-origin $F_1$ progeny returning in 2007 can be estimated. Assuming random mating and no fitness depression in the parental generation (i.e., brood year 2003), 45% of the $F_1$ progeny should be the product of two hatchery-origin parents, 44% should have one hatchery-origin parent, and 11% should have zero hatchery-origin parents. Based on our preliminary parentage analysis of the 583 $F_1$ offspring collected in 2007, 28% were assigned to hatchery-origin, 51% mixed hatchery and natural-origin, and 20% natural-origin. Overall, the natural-origin Chinook produced more returning $F_1$ offspring than expected given a null hypothesis of equal production between hatchery- and natural-origin Chinook. Furthermore, we also identified a higher proportion of hatchery-origin mothers were successful than hatchery-origin fathers. Additional age-4 adults that returned in 2007 are available for analysis, and we will analyze those individuals in a subsequent study to increase the $F_1$ generation sample size.

A fundamental aspect of this project was the assumption that any unobserved parent was of natural origin. For example, an $F_1$ individual with no parents identified from the 2003 brood year collection was by definition the product of two natural-origin parents. Yet, if for some reason the parentage assignment was in error, the contribution of natural-origin parents from the 2003 brood would be inflated. In order to quantify this potential source of bias, we have initiated an analysis of the 1999 hatchery broodstock from the upper Yakima supplementation program. The 1999 hatchery brood should contain every parent for the hatchery-origin adults returning to Roza Dam in 2003. Using the 2003 hatchery-origin returns as progeny and the 1999 broodstock collection as parents, we can evaluate any bias in the parentage analysis that would inflate the apparent productivity of natural-origin fish.

Acknowledgements

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Literature Cited


Chapter 4

Genetic Comparisons Between *Oncorhynchus mykiss* Juvenile Migrants and Mature Residents From the Upper Yakima River

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Abstract

This study investigated the distribution of genetic diversity within *Oncorhynchus mykiss* collections from tributaries of the upper Yakima River. Previous genetic studies in the Yakima Basin have documented genetic differences among the anadromous (i.e., steelhead) *O. mykiss* populations; however, limited information is available regarding the genetic affinities between anadromous and resident forms of *O. mykiss* in the Yakima River. The present study analyzed known adult steelhead spawners, juvenile migrants, and resident *O. mykiss* from tributaries to the upper Yakima River. When compared to each other, Teanaway River and Taneum Creek anadromous adults had a statistically significant genic test at $\alpha = 0.05$ (i.e., different allele frequency distributions) and a pairwise estimate of $F_{ST} = 0.012$, which was statistically different from zero. Similar results were observed for juvenile migrants, suggesting the Teanaway and Taneum *O. mykiss* were genetically differentiated. Regarding resident collections, replicated collections from the same location tended to be similar, while comparisons between tributaries showed statistically significant genetic differentiation. All genic tests comparing residents from different collection locations (i.e., tributaries) were statistically different and all pairwise estimates of $F_{ST}$ were statistically different from zero. Factorial correspondence analysis was used to investigate overall genetic affinities among the various life history stages (i.e., residents, migrants, anadromous adults). Where comparisons were possible in Taneum Creek and Teanaway River, all three life history stages from the same location were more genetically similar than the same life history stage was between tributaries. In contrast to *O. mykiss* within a tributary tending to be similar, residents from the five tributaries were genetically differentiated, based on mahalanobis distances. This observation was further supported by calculation of $F_{ST}$, where the $F_{ST}$ estimated among resident collections was twice the magnitude of the $F_{ST}$ estimated among migrant and adult collections, 0.016 and 0.008, respectively. Overall, we concluded upper Yakima River *O. mykiss* were genetically differentiated, with micro population structure partitioned by river tributary and not life history form.
Introduction

Steelhead, the anadromous form of *Oncorhynchus mykiss*, occupying the Columbia River Basin between the Wind (Washington) and Hood (Oregon) Rivers upstream to include the Yakima River are a part of the Inland Steelhead – Middle Columbia River Evolutionary Significant Unit (ESU) (Busby et al. 1997). Yakima River steelhead were included in the Middle Columbia River ESU based on life history, habitat characteristics, and genetic evidence (Busby et al. 1997; Phelps et al. 1994). The ESU contains predominantly summer steelhead, but does include the only populations of inland winter steelhead in the United States (Klickitat River and Fifteenmile Creek). Washington Department of Fish and Wildlife (WDFW) categorizes steelhead by Genetic Diversity Units (GDU), which are analogous to ESU (Leider 1995); however WDFW splits the geographic area occupied by the Middle Columbia River ESU into two GDUs. Additionally, under the Salmonid Stock Inventory (SaSI) WDFW also recognizes aggregations of populations or stocks that contribute to their respective GDU. The Yakima River has four defined steelhead SaSI stocks, Satus Creek, Toppenish Creek, Naches River, and upper Yakima (i.e., steelhead above Roza Dam), although recent genetic work conducted by WDFW suggests Ahtanum Creek may also represent a distinct population (Small et al. 2006).

The Yakima River watershed encompasses approximately 6,155 square miles in central Washington’s Columbia Basin Physiographic Province (Franklin and Dyrness 1973). The province includes Deschutes, John Day, Walla Walla and lower Snake River basins, and excludes rivers upstream of the Yakima River. Columbia River basalt formations (Miocene epoch) dominate the geology of the province, and the region includes some of the driest areas of the Pacific Northwest, where normal precipitation is generally below 40 cm annually (Jackson 1993). The Yakima River has historically supported abundant populations of many salmonid fishes, including summer-run steelhead, despite the harsh climate within the region.

The focus of this study was to characterize the distribution of genetic diversity within *O. mykiss* from tributaries of the upper Yakima River. Previous genetic studies in the Yakima Basin have documented genetic differences among steelhead populations, identifying as distinct Satus Creek, Toppenish Creek, Ahtanum Creek, Naches River, and the upper Yakima River (at Roza Dam) (Loxterman and Young 2003; Phelps et al. 2000; Small et al. 2006). Additionally, genetic studies have generally found little evidence of genetic interactions between steelhead and non-native hatchery *O. mykiss* (i.e., rainbow trout) stocked in the Yakima Basin (Campton and Johnston 1985; Phelps et al. 1994; Small et al. 2006), although Phelps et al. (2000) and Small et al. (2006) suggested non-native hatchery steelhead (i.e., Skamania) may have interacted genetically with upper Yakima River steelhead. Yet, limited information is available regarding the genetic affinities between steelhead and native resident *O. mykiss* in the Yakima River. This report presents preliminary data for known adult steelhead spawners, juvenile migrants, and resident *O. mykiss* from tributaries to the upper Yakima River. Blankenship et al. (2007) reported that genetic data for steelhead collected at Roza Dam and passed upstream were consistent with a single population sample. Therefore, the expectation is
Figure 1. Map of the upper Yakima River basin showing tributary locations for collections. Please note that only resident individuals are shown (red dots).
adult spawners and juvenile migrants should represent collections from the same underlying population, which can be compared genetically to resident *O. mykiss* collected at the same location.

**Methods and Materials**

**Samples**

Collections of known adult spawners (summer steelhead), juvenile migrants, and resident *O. mykiss* from the upper Yakima River (Figure 1) were analyzed genetically using the SPAN microsatellite suite. Designation of life history category and sampling design were as follows. WDFW’s Ecological Interactions Team conducted surveys of *O. mykiss* in the Middle Fork and North Fork Teanaway River in the spring, PIT tagging sampled fish. These same river reaches were surveyed during the summer and fall by electrofishing techniques (no PIT tagging conducted) to assess PIT tag recapture rates. Similar surveys were conducted in Taneum, Manastash, and Umtanum Creeks in the fall.

We collected fin-clips from all fish that were PIT-tagged or were known spawners. Upon collection, all tissue were immediately stored in ethanol. If a “small” body size *O. mykiss* captured during spring surveys showed signs of spawning activity or was exuding gametes upon capture, it was classified as a resident. These mature residents were generally between 150 – 200 mm in length, and were predominantly male. If a PIT tag was detected at a mainstem Yakima River or Columbia River dam, the fish was classified as a steelhead smolt. The identification of spawning location for the mature anadromous fish was determined using radio telemetry by the Yakama Nation. Only sampled fish with a verified life history (anadromous or resident) were analyzed. We analyzed a total of N=642 samples, 146 adult summer steelhead known spawners, 85 juvenile migrants, and 411 resident *O. mykiss* (Table 1). Known summer steelhead adult spawners were collected 2002, 2003, 2005, and 2006. Tissue samples were obtained from juvenile *O. mykiss* 2006 – 2008, but classification of juveniles as residents or migrants occurred variably over time. For example, an individual sampled in 2006 could have been detected out-migrating in 2007 or 2008. Please note that the date associated with juvenile collections pertains to the sampling date not the date classified. The collection locations of each resident individual analyzed are shown in Figure 1 (red dots). The collection locations of juvenile migrants are not shown, because migrants and residents were collected during the same sampling survey, and migrants were distributed identically in the river. 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**

PCR reaction volumes were 10 μL, with the reaction variables being 2 μL 5x PCR buffer (Promega), 0.6 μL MgCl₂ (1.5 mM) (Promega), 1.0 μL 10 mM dNTP mix (0.02 mM) (Promega), and 0.1 μL Go 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 47°C, and used 0.26 Molar (M) One-102, 0.14 M Oke-4, and 0.14 M Ots-100. Multiplex two had an annealing temperature of 55°C, and used 0.20 M Oki-23, 0.20 M Omy-7, and 0.28 M Ssa-408. Multiplex three had an annealing temperature of 59°C, and used 0.12 M Ots-4, 0.14 M Omm-1070, and 0.20 M Omy-1011. Multiplex four had an annealing temperature of 49°C, and used 0.14 M Omy-1001 and 0.07 M Ots-3M. Multiplex five had an annealing temperature of 59°C, and used 0.14 M Ssa-407, 0.16 M Ogo-4, and 0.24 M One-14. Multiplex six had an annealing temperature of 50°C, and used 0.20 M Ssa-289 and 0.22 M Oki-10. Thermal cycling was conducted on either PTC200 (MJ Research) or GeneAmp 9700 thermal cyclers as follows: 94°C (2 min); 30 cycles of 94°C for 15 sec., 30 sec. annealing, and 72°C for 1 min.; a final 72°C extension and then a 10°C hold. PCR products were visualized by gel electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.7 (Applied Biosystems).

**Genetic data analysis**

**Assessing within collection genetic diversity** - Heterozygosity measurements were reported using Nei’s (1987) unbiased gene diversity formula (i.e., expected heterozygosity) and Hedrick’s (1983) formula for observed heterozygosity. For each locus and collection GENEPOP 4.3 (Raymond and Rousset 1995) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles (FIS) were calculated using an exact test. A Markov chain method was employed, with the rejection zone generated for the null hypothesis (Raymond and Rousset 1995). If the exact test null hypothesis (i.e., random association) was rejected, a second test was performed to explicitly test the alternative hypothesis (i.e., heterozygote excess) (Raymond and Rousset 1995). Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.05 (Belkhir et al. 1996). Statistical significance of linkage disequilibrium (LD) was assessed using a permutation procedure implemented in GENETIX for each locus by locus combination within each collection, where results are reported as the proportion of pairwise combinations significant at α = 0.05 (p-values were adjusted for multiple comparisons).

**Assessing among collection genetic differentiation** - Differentiation of allele frequencies was assessed by the randomization chi-square test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Multi-locus genotypes were randomized between collections (2100 permutations). The G-statistic for observed data was compared to G-statistic distributions from randomized datasets (i.e., the null distribution of no allelic differentiation between collections). P-values were adjusted for multiple comparisons at α = 0.05. Population differentiation was also investigated using pairwise estimates of FST. Multi-locus estimates of pairwise FST, estimated by a “weighted” analysis of variance (Weir and Cockerham, 1984), were calculated using GENETIX version 4.05 (Belkhir et al.1996). FST was used to quantify population structure, the deviation from
statistical expectations (i.e., excess homozygosity) due to non-random mating between populations. To determine if the observed $F_{ST}$ estimate was consistent with statistical expectations of no population structure, a permutation test was implemented in GENETIX (1000 permutations). Population differentiation was assessed further using factorial correspondence analysis (FC) on allele frequencies. In brief, genetic data are transformed into a contingency table, where each individual is described by its multi-locus genotype (i.e., contingency table is individual’s X alleles). The relationship between any two individuals in n-dimensional space ($n =$ number of alleles) is represented by their $\chi^2$ distance. Specifically, the plot represents the ordination of individuals along three orthogonal vectors that represent the three largest eigenvalues derived from the weighted contingency table. Additionally, each individual was not shown, but rather the collection centroids, which are the “centers of mass” for each collection. Mahalanobis distances were calculated (over all individuals) to determine if the relative genetic distances between collection centroids were statistical significant.

Results and Discussion

**Objective 1:** Comparisons of genetic similarity among collections of the same life history type.

**Known Anadromous Spawners** – Known steelhead adult spawners were collected in 2002, 2003, 2005, and 2006 from tributaries of the Yakima River, WDFW collection codes 02NA, 03LA, 05AD, and 06AC, respectively. A total of $N=130$ adults were collected from Teanaway River and $N=16$ from Taneum Creek (Table 1). These collections were previously analyzed by Blankenship et al. (2008), but were included here for comparative purposes to new juvenile *O. mykiss* collections. Blankenship et al. (2008) reported that the genetic characteristics of adult steelhead spawners were consistent with the adults representing a single population sample, as the collection did not show evidence of being a mixture when all adults from both tributaries were combined for genetic analysis (i.e., in Hardy-Weinberg equilibrium, minimal linkage disequilibrium). Subsequent genetic analysis has suggested the existence of subtle population structure within upper Yakima River (see below). Therefore, the adults from the Teanaway River and Taneum Creek have been analyzed as separate collections in this report. Both adult collections were in Hardy-Weinberg equilibrium and showed minimal linkage (Table 1), so the adults showed random associations of alleles within and between genetic loci when they were combined into a single collection (Blankenship et al. 2008) or were analyzed separately by tributary. These results indicate that the adult steelhead from upper Yakima River are genetically similar; however, additional genetic analyses suggest that there exists some genetic differences among these tributaries. The comparison between Teanaway River and Taneum Creek adults had a statistically significant genic test at $\alpha = 0.05$ (i.e., different allele frequency distributions) and a pairwise estimate of $F_{ST} = 0.012$, which was statistically different from zero (Table 2). It is possible that the slight genetic differences now observed between the Teanaway
Table 1 Within collection genetic diversity. Collections listed are known steelhead adult spawners, known migrants, and residents from three upper Yakima River tributaries. GD is expected heterozygosity, Hz is observed heterozygosity, HWE is the probability of the null hypothesis of random associations between alleles within a locus, and LD is proportion of pairwise locus tests exhibiting statistically significant linkage disequilibrium.

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and Taneum spawners are the result of a sampling effect, where the small size of the Taneum adults collection (N= 16) was not representative of the population. Yet, the Taneum Creek adults had allele frequency distributions statistically equivalent to the other Taneum collections and two of four pairwise F_\text{ST} estimates comparing Taneum adults to Taneum juvenile collections were not statistically significant from zero (Table 2). Additionally, factorial correspondence analysis showed all Taneum Creek collections did not have statistically significant genetic differences, based on Mahalanobis distances (Figure 2). Overall, we interpret these results as there was subtle, but real genetic differentiation between the adult steelhead from the Teanaway River and Taneum Creeks.

**Juvenile Migrants** – Blankenship et al. (2008) previously analyzed N=23 *O. mykiss* juvenile migrants from the Teanaway River. These same individuals were included in this study (excluding three with cutthroat genotypes), along with an additional N=56
Teanaway River migrants (WDFW codes 06DE, 07BS, 07BT, and 08AO) (Table 1). A small collection of juvenile migrants from the Taneum Creek was available for comparison (WDFW code 07KU). All migrant collections were in Hardy-Weinberg equilibrium and showed minimal linkage disequilibrium (i.e., random associations of alleles within and between genetic loci) (Table 1). The three Teanaway River migrant collections had statistically equivalent allele frequency distributions, when compared to each other, and had pairwise $F_{ST}$ estimates not statistically different than zero (Table 2).
Table 2 Above diagonal are shown the levels of significance for pairwise tests of genic differentiation among all *O. mykiss* collections used in this analysis. * = p-value < 0.05, the nominal critical p-value for statistical significance (p-value adjusted for multiple tests); NS = non-significant. A significant result between pairs of populations indicates that the allele frequencies between the pair are significantly different. Below diagonal are shown estimates of F<sub>ST</sub>, where underlined values are statistical different from zero.

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This indicates that these collections were representative of a single population. In contrast, the Teanaway River migrants differed from Taneum Creek migrants given several genetic-based metrics. Firstly, the genic test results were mixed, where the Taneum collection allele frequency distribution was not statistically different from either the 2006 or 2007 Teanaway River collections, but was different from the 2008 Teanaway migrant collection (Table 2). Secondly, all pairwise estimates of \( F_{ST} \) comparing Taneum migrants to Teanaway migrant collections were statistically significant from zero, ranging from 0.017 to 0.028 (Table 2). Lastly, factorial correspondence analysis showed collections of migrants from Taneum Creek and Teanaway River differed genetically, based on Mahalanobis distances (Figure 2). These results were similar to those regarding adult steelhead (i.e., known anadromous spawners), suggesting the Taneum and Teanaway \( O. mykiss \) were genetically differentiated.

**Mature Resident** \( O. mykiss \) – \( O. mykiss \) individuals between 150 – 200 mm in length, showing signs of spawning activity or exuding gametes upon capture, were classified as residents. In total, \( N=411 \) mature residents were analyzed, \( N=261 \) from Teanaway River, \( N=31 \) from Swauk Creek, \( N=8 \) from Manastash Creek, \( N=92 \) from Taneum Creek, and \( N=19 \) from Umtanum Creek (WDFW codes 06DE, 07BS, 07BT, 07KU, 08AN, and 08AO). Of these \( N=411 \) \( O. mykiss \), \( N=168 \) individuals were analyzed previously by Blankenship et al. (2008), \( N=120 \) from Teanaway River, \( N=1 \) from Swauk Creek, \( N=39 \) from Taneum Creek, and \( N=8 \) from Manastash Creek (WDFW codes 06DE, 07BS, 07BT, and 07KU). Blankenship et al. (2008) reported the resident collections were genetically differentiated, so the present study analyzed collection locations separately. Additionally, temporally replicated collections from the same location were analyzed separately (Table 1). Blankenship et al. (2008) also reported resident individuals that possessed alleles classified as being indicative of \( O. clarki \) (cutthroat trout) distorted their factorial correspondence analysis plot. \( O. clarki \) alleles were identified based on the SPAN standardization process (data unpublished). The present study removed, prior to analysis, any individual possessing a purported cutthroat allele.

All resident collections were in Hardy-Weinberg equilibrium and showed minimal linkage disequilibrium (Table 1). When the replicated Teanaway River resident collections were compared to each other they had statistically equivalent allele frequency distributions and \( F_{ST} \) estimates not different from zero (Table 2). For replicated Taneum Creek resident collections, allele frequency distributions were not statistically different when compared to each other (Table 2). Pairwise estimates of \( F_{ST} \) between Taneum Creek collections were small; however the comparisons regarding the 2008 collection were statistically significant from zero (Table 2). Nevertheless, in the factorial correspondence analysis the Taneum Creek resident collections formed a cohesive cluster with themselves and the other Taneum Creek \( O. mykiss \) collections (Figure 2). The Teanaway River resident collections also formed a cohesive cluster with themselves in the factorial correspondence analysis plot (Figure 2). While replicated collections from the same location tended to similar, at the tributary level residents were genetically differentiated. All genic tests comparing different collection locations to each
Figure 2 Factorial correspondence plot of collection centroids for upper Yakima River *O. mykiss* collections. Adult, migrant, and resident collections are shown in gray, black, and red, respectively. Teanaway, Swauk, Taneum, Manastash, and Umtanum collections are shown in circles, X, triangles, square, and diamond, respectively. Collections that are not statistically different, as measured by Mahalanobis distances, are circled or joined by horizontal lines.
Objective 2: Comparisons of genetic similarity between collections differing in life history type.

In general, the collection location (i.e., Teanaway, Swauk, Taneum, Manastash, and Umtanum) appeared more informative regarding genetic affinity than life history category (i.e., resident or anadromous). Where comparisons were possible in Taneum Creek and Teanaway River, all three life history stages (i.e., residents, migrants, and anadromous adults) from the same location were more genetically similar than the same life history stage was between tributaries (Figure 2). In contrast to *O. mykiss* within a tributary tending to be similar, residents from the five tributaries were genetically differentiated, based on Mahalanobis distances (Figure 2). This observation was further supported by calculation of FST, where the FST estimated among resident collections was twice the magnitude of the FST estimated among migrant and adult collections, 0.016 and 0.008, respectively. The greater divergence of resident *O. mykiss* than anadromous forms likely reflects a reduced opportunity for gene flow and not a reduced capacity for gene flow (reviewed by Utter 2001). Overall, we concluded upper Yakima River *O. mykiss* were genetically differentiated, with micro population structure partitioned by river tributary and not life history form. Based on these results, we suggest that some mechanism(s) other than genetic history is determining life histories for *O. mykiss* within the upper Yakima River system.

Acknowledgments

We would like to thank the Yakama Nation for information regarding *O. mykiss* collections in the upper Yakima River and identification of spawning location for the mature steelhead. We would also like to thank Norm Switzler for sample processing and Cheryl Dean and Maureen Small for assisting with genetic data production. We would also like to thank Ken Warheit for Mahalanobis distance statistical analysis. Funding for this study was provided by the Bonneville Power Administration (BPA) and by Washington State General Funds.
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Chapter 5

Natural Production and Domestication Monitoring of the
Yakima Spring Chinook Supplementation Program

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Abstract

The Yakima spring Chinook supplementation program began in 1997 but an intensive monitoring effort in natural production, genetics, and ecological interactions was begun even before the hatchery operations started, and has continued. ISRP Review coincident with the first return of adult (4-year old) fish in 2001 raised concerns that the project was not sufficiently aggressive and rigorous in evaluating domestication. The result was an expanded natural production domestication monitoring plan that began in the fall of 2002. The expanded domestication monitoring plan was first described in Busack et al. (2002), revised in 2003 (Busack et al. 2003) and in 2004 (Busack et al. 2004b). The current document is a September 1, 2009 revision. The document contains not only a description of the current effort, but also a history of modifications to the plan.

The basic design of the domestication monitoring effort is to the best of our knowledge unmatched anywhere. The performance of the supplemented Upper Yakima spring Chinook population, an integrated population with 100% natural-origin broodstock, is compared to the performance of an Upper Yakima control line maintained under a regime of continuous hatchery culture, and to an unsupplemented wild control line in the neighboring Naches River. Performance is measured at 14 adult and 15 juvenile traits that encompass virtually the entire range of natural production domestication impacts noted in the literature. Details on the traits are presented in the Trait, Protocol and Analysis Overview section below. The only major monitoring area of the project that is not included in this document is the ecological monitoring effort.

The 2009 modifications are primarily the inclusion of detailed results on almost all traits. Results of investigations on many traits have by now appeared in the primary literature.
Natural Production and Domestication Monitoring of the Yakima Spring Chinook Supplementation Program

Yakima/Klickitat Fisheries Project Monitoring Implementation Planning Team

Revised September 1, 2009

Introduction

The Yakima spring chinook supplementation program began in 1997 with broodstock collection at Roza Dam and spawning, incubation, and rearing at the Cle Elum Supplementation and Research Facility (CESRF). An intensive monitoring effort in natural production, genetics, and ecological interactions (Busack et al. 1997) was begun even before the hatchery operations started, and has continued. ISRP Review coincident with the first return of adult (4-year old) fish in 2001 raised concerns that the project was not sufficiently aggressive and rigorous in evaluating domestication. The result was an expanded domestication monitoring plan that began in the fall of 2002. The expanded domestication monitoring plan was first described in Busack et al.(2002), and revised in 2003 (Busack et al. 2003) and in 2004 (Busack et al. 2004).

The basic design of the domestication monitoring effort is to the best of our knowledge unmatched anywhere. The performance of the supplemented Upper Yakima spring chinook population, an integrated population sensu Hatchery Scientific Review Group (HSRG) (2004) with 100% natural-origin broodstock, is compared to the performance of an Upper Yakima control line maintained under a regime of continuous hatchery culture, and to an unsupplemented wild control line in the neighboring Naches River. Performance is measured at 14 adult and 15 juvenile traits that encompass virtually the entire range of domestication impacts noted in the literature. Details on the traits are presented in the Trait, Protocol and Analysis Overview section below.

The domestication monitoring plan last modified in 2004 was far reaching, actually covering many aspects of supplementation performance beyond domestication, but in this document we revise it even further in the direction of supplementation evaluation in response to a recent issue paper on supplementation monitoring by the ISRP/ISAB (2005) and a comprehensive overview of supplementation by Goodman (2004). This document stressed the need for supplementation projects to be evaluated in three areas: demographic benefits, long-term fitness, and ecological interactions. Ecological interaction monitoring is described elsewhere in this proposal, but two new major efforts are proposed for natural production and fitness monitoring, as recommended in the ISRP/ISAB report. The first is a pedigree study called Target Population Natural Replacement Rate (trait A2, below), in which the reproductive success in the wild of natural-origin and hatchery-origin fish can be compared. Additionally, by continuing this program over multiple generations, the possibility exists for detection of a clear signal for a genetic trend in reproductive success. The second critical change is an expansion of trait A1, now called Productivity: Female Recruits Produced per Naturally Spawning...
Female. The new revised program we propose here then consists of 14 adult and 15 juvenile traits.

Experimental Lines and General Hypotheses

A. Supplementation line (S): the Upper Yakima spring chinook population, supplemented annually by production from 16 raceways at CESRF and associated acclimation sites at Jack Creek, Easton, and Clark Flat. Broodstock collection is at the Roza Adult Monitoring Facility (RAMF) at Roza Dam. In contrast to most hatchery programs, broodstock are collected randomly throughout run, and consist of 100% natural origin fish. Other aspects of the program are as already described in numerous project documents.

B. Wild control line (WC): Naches River spring chinook. The Naches River spring chinook occur in the Naches arm of the Yakima basin. Because they will not be supplemented during the study, they are available as a wild control line. We have determined that Naches fish can be used for 7 of 14 adult traits and 6 of 15 juvenile traits in our design, provided we can adequately sample fish on the spawning grounds, and collect gametes from a minimum of 10 pairs per year for research. These gametes are used for production of juveniles for research and for evaluation of some adult traits. Spawning ground surveys are already routinely done. To minimize impacts to the control population, collection of gametes from the Naches population is minimal, semen and partial egg lots from 10-30 pairs per year, depending on run size. We anticipate that in the future we may also be able to sample and collect fish at a trap at the Cowiche Dam on the lower Naches River. This trap is designed to collect coho salmon, so some modifications to the trap or the dam itself may have to be made to facilitate the efficient capture of chinook.

C. Hatchery control line (HC): a subline of the Upper Yakima population founded from returning hatchery fish collected from throughout the 2002 adult run at the RAMF. Two of the 18 CESRF raceways (randomly chosen each year) will be dedicated to rearing of this line. These fish will be the offspring of a minimum of 36 pairs of fish, which should provide the HC line an effective size of at least 100 per generation. A larger line of HC fish was deemed to be politically untenable because of the large number of fish that would potentially have to be removed at Roza Dam. Larger effective size would be preferable, but this is far larger than the minimum of 50 for quantitative genetic studies deemed to be adequate by Roff (1997). Because the number of fish used to found the HC line is relatively small, the decision was made to have a single line to avoid the possibility of smaller replicate lines going extinct. HC fish will be reared and released exactly as will their supplementation line (S) counterparts. No HC fish will be allowed to spawn in the wild; any returnees in excess of broodstock needs will be removed at the Roza adult monitoring facility (RAMF).

By comparing the supplemented line to both controls, we will address two key questions: 1) how much domestication is incurred by a population undergoing YKFP-style supplementation?; 2) how much less domestication is incurred under YKFP-style
supplementation than would be incurred under continuous hatchery culture?. As already mentioned, because the wild control line is not an internal control we know at the outset that there will be differences in mean performance at several traits. As supplementation proceeds, if there is no discernible effect of domestication, the differences in mean trait values between the two lines should not change except for random fluctuations. If domestication does occur, however, the S line means will change and should continue to change over generations as domestication changes proceed directionally. The net effect will be a trend of increasing or decreasing differences between the supplemented and wild control line over generations. Comparisons between the hatchery control and supplemented lines will be somewhat different. Performance in the two lines should be equivalent initially because the hatchery control is an internal control. If domestication does not occur, performance of the two lines should remain the same except for random fluctuations and a small amount of drift due to the relatively low effective size of the hatchery control line. If domestication does occur, both lines will be affected, and the hatchery control line should be more affected. Thus performance at any trait should change in the same direction in both lines, but change should be greater in the hatchery control line. The rate at which the two lines diverge will be a reflection of the extent to which domestication can be retarded by the regular cycling of hatchery fish into the wild environment facilitated by the exclusive use of natural-origin broodstock. Details on expectations for individual traits are found below.

We also have cryopreserved the sperm of approximately 200 presupplementation Upper Yakima males and stored these gametes at the large cryopreservation facility at Washington State University. This will give us the potential to evaluate divergence of the supplementation line from its presupplementation state. This design concept has a number of issues associated with it, but it may be desirable to do this type of work at some level at some time in the future.

**Experimental Power Concerns**

*Hatchery Ancestry and Power*

The fact that the Yakima spring chinook program has complete control over broodstock composition and has a policy of 100% natural-origin broodstock makes this a well controlled, low variability system for monitoring cumulative effects of hatchery operations. We will deal first with the issue of control of hatchery effects. Simple modeling based on Ford (2002) and Lynch and O’Hely (2001) shows that the genetic dynamics of an integrated hatchery program is controlled by two gene flow rates: the proportion of natural-origin fish in the hatchery broodstock (pNOB), and the proportion of natural spawners comprised by hatchery-origin fish (pHOS). The proportion of time the population spends in the hatchery, called proportionate hatchery influence (PHI) is given by $\text{PHI} = \frac{p\text{HOS}}{p\text{HOS} + p\text{NOB}}$. Simulations of integrated systems show that after the initial generation or two, the rate of increase of hatchery ancestry (generations of exposure to the hatchery environment) in the natural-origin fish in the population is
equivalent to the program’s PHI. For a program like the Yakima spring chinook program, in which all broodstock are natural-origin fish \( (p_{NOB}=1.00) \) and the proportion of hatchery-origin fish on the spawning grounds is approximately 50\% \( (p_{HOS}=0.5) \), \( \text{PHI}=0.33 \). ISRP/ISAB stress the need for control of the proportion of hatchery fish on the spawning grounds, something that is typically unacceptable to project managers. It is important to point out in this regard that although the Yakima spring chinook has no control of hatchery fish on the spawning grounds except for a small selective sport fishery, because of the natural-origin only broodstock rule, the PHI of the population is likely to fluctuate only between 0.33 \( (p_{HOS}=0.5) \), and 0.44 \( (p_{HOS}=0.8) \). Any other program having a fixed \( p_{NOB} \) will have a similarly limited PHI range, but fixed-PHI programs are rare. Thus even without explicit controls on \( p_{HOS} \), the Yakima spring chinook program is fairly well “controlled”.

Now we will consider the issue of variability of response. Our simulations of the buildup of hatchery ancestry in integrated programs have highlighted one other issue related to experimental power: variation in hatchery ancestry within a generation. Assuming the performance of fish in trials of domestication is related to the amount of hatchery ancestry, the variance in response of fish to experimental situations will depend on the variance of hatchery ancestry. Interestingly, our simulations show that in an integrated program the variance builds rapidly and then reaches a constant value that does not decline. There is no obvious pattern at this point, but different \( p_{NOB} \)-\( p_{HOS} \) combinations result in different characteristic variances. Important for this study is the fact that programs with 100\% natural-origin broodstock will have considerably smaller variances than those with less than 100\%. For example, a \( p_{HOS} \) range of 0.5 to 0.8 will result in an ancestry variance range of 0.058-0.087 for a program with \( p_{NOB}=1.0 \); for a program with \( p_{NOB}=0.5 \), the range will be 0.16-0.25. For almost all types of monitoring, the project’s low variance in ancestry is an asset, but for multiple-generation pedigree analysis (see trait A2), where contemporaneous comparison of the reproductive success with a wide variety of hatchery ancestries is desired, the low variability may be problematic. We have yet to evaluate the potential impact on power in this case.

**Precocious Males**

One issue regarding this design that has been the subject of considerable discussion is “leakage” from the H line into the S line through precocious males from the H line spawning in the wild with S-line females. If this occurs at an appreciable rate, it will bias the H-S and S-W comparisons, making the supplementation treatment appear more domesticating than it is, and also, the S line will undergo more domestication than it should for the lifespan of the H line, a conservation concern. Power analysis (Busack et al. 2004) indicates that under current levels of precocity, the bias should be negligible, but work is currently underway to evaluate this risk from a variety of angles, including measures for reducing production of precocious fish (Larsen et al. 2004). The precocious males will be a source of ungenotyped fish in the pedigree study (trait A2), which can bias comparisons of relative reproductive success (Araki and Blouin 2005).
Selective Fishery Impacts

Hatchery-selective fisheries in the lower Columbia River are a relatively recent phenomenon and have the potential to bias a number of trait comparisons. This would occur when a fishery selectively removes hatchery fish (identified by their clipped adipose fin) possessing a particular phenotypic or life-history trait(s) (i.e. size-selective removal of larger fish would result in smaller size at age for those fish escaping the fishery, as well as, lower mean age at return). The magnitude of the bias is a function of both the fishery’s exploitation rate (greater rate, greater effect) and selection differential (larger selection differential, larger effect). We will use data from CWT tag recoveries of CESRF fish in the selective fisheries, e.g. lengths, ages and sex, and compare them to the SH and HC recoveries at RAMF to determine if selection is occurring and adjust our RAMF recovery data accordingly.

The impacted traits are only those involving comparisons between tagged SH or HC fish and untagged SN or WC fish. This includes size-at-age, age-at-return, sex ratio, and juvenile-to-adult survival or productivity rates. The comparisons of SH and HC fish are not affected since both groups are equally impacted by the fishery.

Trait, Protocol, and Analysis Overview

The following pages provide details in a standard format, one trait at a time, on the 14 adult and 15 juvenile traits we intend to evaluate with this design. Most traits will be evaluated annually in order to maximize power, but some may be done less frequently due to logistical limitations. Protocols may vary from year to year to allow collection of key baseline information some years, and experimental data in others. For many traits it is important to distinguish between S line fish of hatchery-origin and those of natural origin: we call these two “sublines” SH and SN in the write-ups. This distinction is made to allow a cleaner measure of genetic differences. Consider nearly any comparison of HC and S fish. Part of the difference in performance between SN and HC fish will be genetic, but part may also be due to phenotypic plasticity; the result of being reared in a hatchery. If HC fish are compared to SH fish, because they share the phenotypic effect of hatchery rearing, the performance difference will be exclusively genetic. It is important to keep in mind when reading the write-ups, however, that although we call SN and SH lines in describing experimental designs, they differ only in their rearing history. Any given pair of SN and SH fish can have the same grandparents.

Although we will make most comparisons annually, annual comparisons within a supplementation generation (slightly more than 4 years) are merely replicates. Although significant domestication effects may be detected in a single generation, we expect the big results to be trends in performance over generations, so the write-ups stress the importance of trends. Our analyses are focused on measures of central tendency (means and medians). We have not focused on variability, primarily because we have virtually no expectations based on the literature on how variability should change under domestication at individual traits. We do have a working hypothesis that variability should decline during domestication because the considerably more homogeneous
environment allows directional selection to be more effective. On the other hand, relaxation of selection caused by the hatchery environment could cause an increase in phenotypic variability. Variability at traits is therefore of interest to us. We doubt we will have enough power at any trait to detect a change in variability statistically, but we may see qualitative changes that will inspire further research.

The number of traits to be evaluated can be misleading. Many of the traits are measured on the same fish with no difference in protocol except for the measurement. Thus, the “effective” number of traits in terms of logistics and cost is considerably lower. The best example of this is the set of traits A7-A9, which are all measurements of reproductive traits on the same specimens. We list the measurements as separate traits because we consider them all important, and because we want to insure they are all done. Some traits require considerable effort and cost, whereas others will be measured in the course of ordinary fish culture operations. Our guiding philosophy was to take advantage of the opportunities offered by the CESRF and other facilities in the basin to measure as many traits relevant to domestication as feasible while minimizing impacts to the supplementation effort and the wild control population.

Nomenclature for Experimental Groups

The key to making sense of the write-ups is understanding which groups of fish are being compared. In previous versions of the domestication monitoring plan the nomenclature system for the fish to be used in the various comparisons has caused considerable confusion. In this revision we introduce a new system that should clear the confusion. Here is the new system of codes:

**SN** - naturally produced fish from the supplemented line. This designation is used for both juveniles and adults. Any natural-origin fish in the Upper Yakima qualifies as an SN fish.

**SH** – hatchery-origin fish from the supplemented line. This designation is used for both juveniles and adults produced by the CESRF as part of its normal supplementation effort (i.e., not part of HC or any experimental production group).

**SHp** – hatchery-origin progeny of SH adults. This designation is used only for juveniles. With the exception of the spawnings needed to start the HC line, no SH adults are ordinarily spawned at the CESRF. For some comparisons, however, it will be necessary to spawn small numbers of SH adults at CESRF. The juveniles produced from these spawnings will not be reared past early stages and will not be released.

**HC**- fish from the hatchery control line. This designation is used for both juveniles and adults. All HC fish are of hatchery origin. The hatchery control line was founded from first-generation hatchery returnees, so in that generation there is no distinction between SH adults and HC adults, but thereafter the distinction is clear.
**WC-natural-origin fish from the wild control line.** This designation is used for both juveniles and adults. Any natural-origin fish in the Naches qualifies as an WC fish.

**WCP – hatchery-origin progeny of WC adults.** This designation is used for juvenile fish. Small numbers of WC adults will be captured and spawned. Some of the resulting hatchery-origin progeny will be used in comparisons.

Table A.5. Tasks required for use in the adult and juvenile domestication traits.

<table>
<thead>
<tr>
<th>Trait</th>
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<tbody>
<tr>
<td>A1</td>
<td>1c, 1h, 1i, 1j, 2b</td>
<td>J1</td>
<td>1e, 1i</td>
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<tr>
<td>A2</td>
<td>1c, 1i</td>
<td>J2</td>
<td>1e, 1i</td>
</tr>
<tr>
<td>A3</td>
<td>1c, 1i, 1j, 1m, 2a, 2b</td>
<td>J3</td>
<td>1e, 1i</td>
</tr>
<tr>
<td>A4</td>
<td>1c, 1i, 1j, 1m, 2a, 2b</td>
<td>J4</td>
<td>1e, 1i</td>
</tr>
<tr>
<td>A5</td>
<td>1c, 1i, 1j, 1m, 2a, 2b</td>
<td>J5</td>
<td>1e, 1i</td>
</tr>
<tr>
<td>A6</td>
<td>1c, 1i</td>
<td>J6</td>
<td>1e, 1i</td>
</tr>
<tr>
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<td>1c, 1i, 1j</td>
<td>J7</td>
<td>1e, 1d, 1e, 1i</td>
</tr>
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<td>1c, 1i</td>
<td>J8</td>
<td>1e, 1e, 1i</td>
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<td>1c, 1i, 1j</td>
<td>J9</td>
<td>1e, 1i, 1m</td>
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<td>1c, 1i</td>
<td>J10</td>
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<tr>
<td></td>
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<td>J15</td>
<td>1e, 1i</td>
</tr>
</tbody>
</table>

Start dates for the adult and juvenile traits are as follows:

- 2002 – A1, A3-A9, J5, J6, J9, J11, J12.
- 2004 – J1, J2.
- 2006 – A2 (proposed), A13, A14, J7, J8, J10, J15.

Frequency of data collection for all traits are on an annual basis with the following exceptions:

A2 – Data collection over two or more generations with the possibility of some flexibility as to how many years within a generation need to be sampled. Analysis can occur later as funds become available.

A13 – Annually for four years.

A14 – Annually for four years.
A1. Productivity: Female recruits produced per naturally spawning female  (revised 12/21/05)

Background and Justification

The success of any supplementation effort should be based on tracking population productivity through time. One of the best measures of population-wide productivity is the number of female offspring produced per female spawner. If supplementation is succeeding, this metric will either increase or remain stable until density factors on the spawning grounds or rearing areas impose biological limits on the population. On the other hand, if the ability of hatchery-origin females to produce offspring under natural conditions has been reduced because of inadvertent domestication, then the overall productivity of a population will decrease even when density-limiting factors are not in action. To obtain estimates of productivity for Yakima River spring Chinook the number of female offspring produced from females spawning naturally in the river will be determined on a brood year basis. Productivity can vary from one brood year to the next because of environmental differences. That is why we will also track the productivity of spring Chinook females spawning in the Naches. None of these fish will have experienced any hatchery exposure and they will be spawning and rearing in areas similar to those experienced by the upper Yakima population. Thus shifts in their brood year productivity values will be a good representation of how various environmental effects influenced overall productivity.

Location  RAMF, Prosser Dam, Upper Yakima, Naches, American spawning ground

Groups Compared  WC, SN, and SH

Protocol

At Prosser adults from all populations in the basin are counted and classified as hatchery or natural, resulting in counts for hatchery origin (HC+SH) and natural origin (SN + American + Naches (WC)) fish. At RAMF, SH, SN, and HC fish are counted, sampled for sex, age and POH length. Sex data for the HC and SN groups will come from fish captured and taken to CESRF for brood stock. Sex determinations for the SH group will be obtained from DNA samples collected at RAMF. DNA sexing is necessary because error rates of approximately 30% in males and 10% in females occur at RAMF each year based on morphological sexing of live fish (Knudsen et al. 2002, 2003). An estimate of the abundance of spring Chinook returning to the Naches and American rivers will be made by comparing Prosser and Roza counts after adjustment for harvest and incidental in-river mortality. Redd counts will be obtained from spawning ground surveys on the Naches and the American rivers. Final Naches adult counts will be calculated as the product of the Naches and American escapement and the Naches proportion of the Naches and American redd counts. Additional adjustments may be made to correct for sex ratio bias on the spawning grounds. Adult females produced per adult female spawner by brood year can be estimated for WC, HC, SH, and S natural spawners (mix of SN and SH spawning in wild). It will also be necessary to include in the analysis at least two additional factors: female spawner density and the proportion of hatchery fish spawning each year. Spawner density adjustments will require calculating a density-dependent function for each population. The proportion of hatchery fish naturally spawning each year may have a significant impact on natural productivity and should be included in the analyses.

In addition to the general productivity measures described above, critically important insights into the relative productivity of hatchery- and natural-origin females could be gained if micro-satellite DNA samples were collected on each adult processed through RAMF. In this case, each female returning to spawn could be classified as coming from an SHxSH, SNxSH, SHxSN, or SNxSN mating. The proportions of the females originating from these matings could be compared with the proportions expected to return based on the number of SH and SN adults present on the spawning grounds during their brood year.

Expectations/Hypotheses

If domestication does not occur, differences in productivity of naturally spawning females among groups will remain constant over time after adjusting for inter-annual density effects. Conversely, if domestication does occur we would expect the productivity of SH females naturally spawning to
decrease over time reducing the productivity of the aggregate mixture of naturally spawning
females. The reduction will be a function of the effects of domestication and the proportion of SH
females on the spawning grounds. Thus, the proportion of females of SH origin naturally
spawning each year must be estimated. HC fish will be intercepted at RAMF and not allowed to
naturally spawn.

**Analytical/Statistical Methods and Issues**

Within brood years no statistical analysis will be done. However, over brood years, analysis of
covariance will be used to evaluate differences in trends. Trend analysis will take into account
year-to-year spawner density effects and the proportion of SH females on the spawning grounds.

**Findings To Date**

No analyses have been completed to date. This productivity metric has just been added to the
Domestication plan.

### A2. Target Population Natural Spawning Replacement Rate

*(revised 12/21/05)*

**Background and Justification**

Part A. Relative Reproductive Success of Hatchery-origin and Natural-origin fish. According to
the ISRP and ISAB (2005), to determine whether natural production lost due to removing
spawners for hatchery production is replaced by naturally reproducing hatchery-origin fish
requires evaluation of target population natural replacement rate. They further state that to do
this the progeny of four types of matings on the spawning grounds must be enumerated: HxH,
NxN, NxH, HxN. In addition to explicitly providing this information, this effort will also provide
information that can be used in reducing bias in trait A1 (see trait A1 write-up).

Part B. Genetic Decline in Fitness

If carried out for multiple generations, because of differing levels of hatchery ancestry, genetic
impact of domestication on reproductive success can be measured by comparing the relative
reproductive success of fish with differing levels of hatchery ancestry.

**Location(s)**

RAMF

**Groups Compared**

SN fish from HxH, NxH, HxN, and NxN matings

**Protocol**

The basic idea is to sample all returning S fish (both SH and SN) at RAMF for DNA, then sample
all their progeny at RAMF a generation later. Progeny will be then be assigned to parents by
CEVUS (Marshall et al. 1998) or a similar program. For a year of parents sampled, progeny will
have to be sampled over multiple years to get complete returns (fish return at 3, 4, and 5 years of
age). All fish will be aged to assign to correct brood year. Sampling will continue through
multiple generations for Part B.

**Expectations/Hypotheses**

Based on a recent study of reproductive success of a recently created native steelhead stock in
the Hood River, OR (Blouin 2003), we expect the relative reproductive success of hatchery-origin
fish to be perhaps 15% lower than that of natural-origin fish. How much of this will be due to
genetic causes is unclear. If this is only phenotypic, we would expect this fitness difference
between natural-origin and hatchery-origin fish to remain over multiple generations. Over time we
would expect the base fitness level in the population to decrease as hatchery ancestry increases,
but at what rate it unclear. Several cases have been noted of long-established hatchery stocks
having much lower fitness in the wild than natural-origin fish (Chilcote et al. 1986; Blouin 2003),
but these were with long established nonnative stocks, and they were steelhead, not chinook.

**Analytical/Statistical Methods and Issues**

At least ten loci, the same loci used in the spawning channel pedigree study (Kassler 2005), will
be used, but potentially more will be needed because of the complexity involved in creating a
pedigree for such a large population. Ungenotyped fish is a twofold issue. There will be
ungenotyped parents because we will not be able to sample precocious males, but we will also
want to limit genotyping of returning adult fish as a means of reducing cost (there may be as
many as 10,000 returnees in some years). At this point it appears that power analysis will be
done by using CERVUS (Marshall et al. 1998), but other available programs may be used as well. Assessment of bias (Araki and Blouin 2005) will be a key part of the power analysis. Power analysis of part B will be multiple stage, as fish will essentially need to be assigned to grandparents. For analysis of part A, simple assignment by CERVUS with bias adjustment will yield per fish estimates of relative reproductive success, which will be then be grouped in results by mating type. For analysis of part B, estimates of relative reproductive success will be regressed on hatchery ancestry inferred from the pedigree to yield estimates of genetic fitness loss per generation.

**Findings to Date**

Analysis has been performed on the age-4 offspring of BY2003 assuming unassigned offspring were of natural origin (Chapter 3, this report). Additional analyses will be performed using BY1999 broodstock to evaluate any bias in the estimates of natural-origin productivity.

### A3. Age composition by sex  
(revised 12/21/05)

**Background and Justification**

Age composition or age at maturity is a trait related to fitness. For example, older females generally have higher fecundities, larger eggs and larger body sizes all of which may affect their overall fitness. Older males are also generally larger than younger ones and size in males may play a significant role in the ability of fish to successfully court and spawn with females. Age determinations are also required in order to reconstruct demographics based on brood years. While significant differences exist between natural populations of spring chinook in the Yakima River (Knudsen et al. 2006), within-population age composition is relatively stable. However, in some hatchery populations, fish may mature at younger ages, perhaps reflecting the impact of more rapid growth or a genetic change (Gallinat et al. 2001). Hence, the age of maturity of hatchery- and natural-origin fish will be tracked to see if sex-specific changes in maturity occur because of exposure to hatchery conditions.

**Location**  
RAMF, CESRF, Naches spawning grounds

**Groups Compared**  
WC, HC, SN, and SH

**Protocol**

Requires sex and age determination of adequate samples of fish. For all fish used in the hatchery (SN and HC for production, few SH for research) and for those sampled on the spawning grounds as carcasses (WC), sex can be determined visually. Sex determination based on visual inspection of green fish is not reliable, e.g. 30% of the fish classified at Roza as males are females (Knudsen et al. 2003). SH fish are sampled in low numbers as carcasses, so sex determination for SH fish will be based on DNA analysis. Age will be determined on all fish by scale analysis or tags. Minimum target sample size is 140 for WC and 200 for SH (carcasses + DNA samples). This will provide estimates of age composition with multinomial confidence intervals of +10% or less at $\alpha=0.05$ (Thompson 1987). Hatchery-selective fisheries in the lower Columbia River have the potential to bias our results by selectively removing hatchery fish with a particular phenotypic trait (i.e. size-selective removal would result in reduced age at maturity for those fish escaping the fishery). The magnitude of the bias is a function of the fisheries exploitation rate and selection differential. We will adjust our RAMF data using the method described in the selective fishery impacts section above.

**Expectations/Hypotheses**

Hatchery fish tend to return at younger ages than naturally produced fish (Gallinat et al. 2001), so younger age structures would be expected for HC and SH relative to naturally produced fish, and these differences may be only phenotypic. If domestication does not occur, differences in age structure among all four groups will remain constant over time. If domestication does occur we would expect age structure to decrease (Reisenbichler and Rubin 1999). Because HC should be most domesticated, its age structure should decrease more, but age structure of SH should decrease as well.

**Analytical/Statistical Methods and Issues**

Within years multinomial contingency tests will be used to compare age structures. Comparison
of HC and SH will be especially informative for determining genetic effects. Over years analysis of covariance will be used to evaluate differences in trends. Analysis will be complicated by the fact that age structure is in part a reflection of the genetic composition of the population, but can be strongly influenced by environmental fluctuations in brood-year survival and by hatchery selective fisheries.

**Findings To Date**

F1: Most SH and SN fish of both sexes reached maturity at age 4 (>76%), followed in magnitude by ages 3 and 5. However, SH mean age at maturation declined significantly due primarily to an increase in age 3 males over time, while SN mean age at maturation demonstrated no significant trend over time (Knudsen et al. 2006).

F2: These general trends have continued into the second generation (brood years 2002-2004) (Knudsen et al. 2009a).

### A4. Size-at-age by sex  (revised 12/21/05)

**Background and Justification**

Gallinat et al. (2001) observed that hatchery-origin adults were significantly smaller than wild cohorts that matured at the same age. How universal this phenomenon may be is unknown, but similar reductions in size have been observed in other salmon populations including those produced from the CESRF. Size at maturity is plainly influenced by environmental as well as genetic factors. Currently, the relative importance of these factors on size at maturation is showing mixed results. The HC and SH lines at the CESRF provide a unique opportunity to evaluate how additional generational exposure to a hatchery environment may affect body size. These comparisons will be put into context by also evaluating trends in body size of adults returning to the Naches spawning grounds.

**Location**  RAMF, CESRF, and Naches spawning grounds

**Groups Compared**  WC, HC, SN, and SH

**Protocol**

Protocol same as for trait A3 (same fish) but with post-orbital hypural (POH) lengths measured

**Expectations/Hypotheses**

For unknown reasons, hatchery fish have been observed on several occasions to be smaller than naturally produced fish of the same age; e.g., 2001 returnees to Cle Elum were ~2 cm shorter than naturally produced fish (Knudsen et al. 2003 and 2004; also see (Gallinat et al. 2001); Fresh et al. 2003), so smaller sizes would not be surprising in HC and SH relative to naturally produced fish, but these differences may be only phenotypic. If a reduction in size at age is primarily driven by some aspect of the hatchery environment, then we would expect an initial reduction in size of SH fish in the first generation followed by a constant difference in size between SN and SH returns over subsequent generations. In addition, there would be no difference in size between SH and HC fish over generations because they experience similar rearing environments. Assuming the smaller size observed in hatchery fish is in part a result of domestication (genetic), size can be expected to decline as domestication proceeds. Thus the size of the WC fish should remain constant, and the size of SH and HC should decline, with HC fish declining most. Hatchery-selective fisheries in the lower Columbia River have the potential to bias our results by selectively removing hatchery fish with particular phenotypic traits (i.e. size-selective removal would result in smaller size at age for those fish escaping the fishery). The magnitude of the bias is a function of the fisheries exploitation rate and selection differential. We will adjust our RAMF data using the method described in the *selective fishery impacts* section above.

**Analytical/Statistical Methods and Issues**

Within years, analysis of variance will be used to compare mean POH lengths. Comparison of HC and SH will be especially informative for determining genetic effects. Over years analysis of covariance will be used to evaluate differences in trends. If a reduction in size at age is primarily environmentally driven by some aspect of the hatchery, then we would expect an initial reduction in size of SH fish in the first generation followed by a constant difference in size between SN and SH returns over subsequent generations. In addition, there would be no difference between SH
and HC fish over generations.

**Findings To Date**

F1: For broodyears 1997 to 2000 mean lengths of 3–5-year-old SH fish were shorter than those of SN fish of the same age (differences of 2.7 cm for age 3, 1.7 cm for age 4, and 1.9 cm for age 5). Likewise, body weights of SH fish were lower than those of SN fish (differences of 0.3 kg for age 3, 0.3 kg for age 4, and 0.6 kg for age 5), representing a change in body size of between 0.5 and 1.0 standard deviation (SD) (Knudsen et al. 2006).

F2: These general trends have continued into return years 2005 through 2009. Age 4 SH and HC mean body length and weight distributions at RAMF were significantly smaller than SN adults by 1.0 to 1.3 cm and 0.2 to 0.3 kg, but did not differ significantly between each other. In contrast, HC, SH, and SN age 3’s were not significantly different and HC adults were largest in 2006 and 2008 (Knudsen et al. 2007a; Knudsen et al. 2009a).

For the first time in 9 years we observed significant sexual dimorphism in body size of age 4 upper Yakima 2006 returns. Mean female POHP lengths were significantly greater than males (SN (male = 58.0, female = 59.6), HC (male = 56.8; female = 57.9), SH (male = 56.9; female = 58.0)). Body weight dimorphism followed the same trend, but was not statistically significant between the sexes (SN (male = 3.6; female = 3.7), HC (male = 3.4; female = 3.4), SH (male = 3.4; female = 3.5)) (Knudsen et al. 2007a).

### A5. Sex ratio at age (revised 12/21/05)

**Background and Justification**

Larsen et al. (2004) observed an increase in the rate of precocious development in males at the CESRF. Early maturation in males may have been caused by rapid growth interacting with a genetic proclivity to mature early. This should mean fewer males in the hatchery population will mature at later ages causing a shift in the sex ratio of SH and HC fish. How exposure to hatchery conditions may affect age of maturation in females is unknown. If there is a tendency for hatchery-origin females to mature at early ages then the value of these fish in supplementation efforts will be reduced because of their lower fecundities and decreased ability to provide protected incubation environments (van den Berghe and Gross 1984). The incorporation of a hatchery control line once again provides us with an opportunity to evaluate how multiple generational exposure to a hatchery environment may affect another adult trait that is linked to fitness.

**Location** RAMF, CESRF, and Naches spawning grounds

**Groups Compared** WC, HC, SN, and SH

**Protocol**

Protocol same as for trait A3 (same fish)

**Expectations/Hypotheses**

If domestication does not occur we would expect to see no changes in the sex ratios of fish maturing at different ages. If domestication does occur we anticipate that the HC line will produce fewer precocious males. Consequently, greater proportions of males will mature in older age classes (e.g. 3-, 4- and 5-yr olds) in the HC line. This hypothesis is based on the fact that precocious males are not used as brood stock. Hatchery-selective fisheries in the lower Columbia River have the potential to bias our results by selectively removing hatchery fish with particular phenotypic traits (i.e. higher catch limits for age-3 jacks would result in skewed sex ratios for those SH and HC fish escaping the fishery). The magnitude of the bias is a function of the fisheries exploitation rate and selection differential. We will use sex data from CWT tag recoveries of CESRF fish in the selective fisheries and compare them to the sex ratios of recoveries at RAMF to determine if sex-selection is occurring and adjust our RAMF SH and HC recovery data accordingly.

**Analytical/Statistical Methods and Issues**

Within years, binomial test of proportions will be used. Over years analysis of covariance will be used to evaluate differences in trends.

66
Findings To Date

F1: The proportion of SH males, primarily age 3, significantly increased from 38% to 49% over time for BY 1997-2000. Conversely, the sex composition of wild fish did not exhibit a similar increasing trend. The sex composition in BY1997-2000 SN and SH fish differed in three of four brood years. Although SH males began low relative to SN fish, but ended highest (Knudsen et al. 2006).

F2: These general trends have continued into return years 2005 through 2009, although the data have not yet been analyzed on a brood year basis. Analyses of 2005 and 2006 will be integrated with 2007 and 2008 to assess the impacts in F2 (Knudsen et al. 2007a; Knudsen et al. 2009a).

A6. Migration timing to trap (revised 12/21/05)

Background and Justification

Time of spawning in Chinook salmon is a fitness related trait that is significantly influenced by water temperatures during the spawning and egg incubation periods (Brannon et al. 2004). Every spring Chinook that spawns in the Upper Yakima has to first pass through the RAMF and because those fish are inspected it is possible to document when HC, SH, and SN fish have migrated to Roza. We have found that passage date at the RAMF is either uncorrelated with spawn timing or explains no more than 4% of the variation in spawn timing (Knudsen et al. 2006). However, a population that passes RAMF later, assuming all populations spawn during the same temporal window, has fewer days on the spawning grounds to find and compete for mates and construct redds possibly having some negative fitness consequence. Therefore we plan to examine the effects of treatment origin (i.e. SH, SN, and HC) on when fish migrate to the RAMF.

Location  RAMF
Groups Compared  HC, SN, and SH
Protocol
Fish moving through the Roza Adult Monitoring Facility (RAMF) will be inspected for tags and marks making it possible to record the origin and date of passage of each fish.

Expectations/Hypotheses

No expectations on how this trait will change, but data will already be available to see if continued exposure to hatchery conditions (HC) causes a noticeable difference in when fish arrive at Roza and their ultimate spawning destination.

Analytical/Statistical Methods and Issues

Within years, a non-parametric test, either a Kolmogorov-Smirnov or Kruskal-Wallis ANOVA will be used on cumulative passage distributions. Over years, analysis of covariance will be used to compare trends in median arrival date. Run timing at RAMF is related to age, with older fish passing earlier (Knudsen et al. 2004). Therefore, if hatchery selective fisheries remove larger, older individuals that would have passed RAMF earlier, then migration timing could be biased to a later date. Again, comparison of size/age of CWT’ed fish recovered in the fishery and to those passing RAMF will help us understand if this is occurring.

Findings To Date

F1: Median arrival timing of adult (>age 4) SH and SN fish at RAMF showed no consistent difference between RY 2001 and 2004 (Knudsen et al. 2006).

F2: Adult SH and SN median passage date at RAMF differed significantly (SN earlier) by 7 to 6 days for RY 2005-2008. SH jack median passage was between 2 and 12 days later than SN jacks in RY 2005 through 2009, respectively. These differences were significantly different each year in Kruskal-Wallis tests (Knudsen et al 2007a; Knudsen et al 2009).

A7. Spawning timing (revised 12/21/05)

Background and Justification

When spring Chinook reach maturation and spawn is strongly affected by the water temperatures they encounter and the water temperatures their offspring are likely to experience (Brannon et al.
Clearly, time of spawning is a fitness related trait as the offspring of fish that spawn too early or late can suffer significant incubation and post-emergence mortality (Brannon 1987; Hendry et al. 1998; Smoker et al. 1998; Einum and Fleming 2000). We have found that natural spring Chinook populations in the Yakima River Basin exhibit differences in spawn timing that have evolved to maximize fitness (Knudsen et al. in prep.). Given this situation, an obvious question to ask is whether exposure to hatchery conditions will alter traditional maturation timing in Yakima spring Chinook. As in many of the other adult traits examined, the presence of HC, SH, SN fish as well as natural controls, will allow this question to be addressed.

**Location** CESRF, Upper Yakima and Naches spawning grounds![](image)

**Groups Compared** WC, HC, SN, and SH

**Protocol**

Monitoring this trait has two components: 1) comparing S-and WC temporal trends in redd count and carcass recovery distributions from weekly spawning ground surveys; and 2) comparing SH with HC spawn timing distributions in the hatchery.

**Expectations/Hypotheses**

Our expectation is that time of maturation will not change. Changes in spawn timing have been commonplace in hatchery operations, but this is likely tightly linked to taking eggs from the first part of the run. In this project we have made a concerted effort to take eggs in a representative fashion throughout the spawning season. Thus we do not expect to see a change in the time of spawning.

**Analytical/Statistical Methods and Issues**

Within years we will compare the temporal distributions of HC with SH spawners by using either the non-parametric Kolmogorov-Smirnov test or Kruskal-Wallis ANOVA. We will investigate whether the sexes differ significantly and require separate analyses. Within-year analyses of WC and SN fish will not be done, but median spawning/recovery dates for each of these groups will be calculated. Over years, analyses of covariance will be used on median spawning dates. One analysis will examine temporal changes in the HC and SH fish while another analysis will examine similar trends in WC, SN and SH fish. Naches information will likely not be very precise.

**Findings To Date**

F1: Maturation timing of SH fish averaged 5.2 days earlier than SN fish at CESRF (RY2001-2004) (Knudsen et al. 2006).
F2: This trend has continued into return years 2005 through 2009 (Knudsen et al. 2007a; Knudsen et al. 2009a).
F1: Initiation of in-river female spawning activity did not differ between SH and SN fish (RY2002-2005) (Knudsen et al. 2005a)

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**A8. Fecundity (revised 12/21/05)**

**Background and Justification**

Significant changes in locally adapted traits due to hatchery influences, whether of genetic or environmental origin, will likely be maladaptive, resulting in reduced population productivity and fitness (Taylor 1991; Hard 1995). Fecundity or the total number of eggs produced by a female, significantly affects maternal reproductive success and fitness in salmonids (Healey and Heard 1984; Fleming and Gross 1990; Beacham and Murray 1993). Fecundity, egg mass and egg size also reflect local adaptations to the conditions present on spawning grounds (Taylor 1991; Hendry et al. 1998; Quinn et al. 2001). Investigations that have examined how domestication may influence fecundity in hatchery populations have shown that egg number can be reduced (Fleming and Gross 1992; Petersson et al. 1996). Whether environmental or genetic effects cause such reductions is not currently known. Comparing the fecundities of HC, SH, and SN females, however, will provide information about the existence of genetic change due to repeated exposure to hatchery conditions.
### Location
CESRF

### Groups Compared
HC, SN, and SH

### Protocol
Enumerate eggs from at least 30 females of each type (i.e. SH, HC, and SN). This means that some SH origin females (a minimum of 30) will have to be held to maturity at CESRF. Conversely, fecundity samples from SN and HC females will be taken from fish being held for broodstock in the two lines. WC fish are not included because we intend to collect only partially spawned females and thus will not be able to get total egg counts. Fecundity will be estimated using a gravimetric methodology and corrected for bias based on a correction factor derived from a comparison of estimated fecundity (gravimetric) to known fecundity (hand counts) for a sample of females. Each year, corrected fecundity estimates of 10 females will be compared to their hand counts to determine whether our gravimetric estimation methodology is changing over time.

### Expectations/Hypotheses
If domestication does not occur, fecundity will remain constant. However, Fleming and Gross (1989; 1992) predicted that under hatchery culture fecundity will decrease, at least for coho salmon. Thus, we would expect fecundity to decrease in the SH and HC lines, and the decrease should be greater in HC.

### Analytical/Statistical Methods and Issues
Within years, analysis of covariance will be used to compare body traits vs. fecundity within age classes. Analysis of variance will be used within years to compare absolute fecundities within age classes. Over years analysis of covariance will be used on mean fecundity by age to detect trend differences among groups.

### Findings To Date
- **F1:** After adjusting for broodyear and length differences, SN females averaged 234 more eggs than SH females for broodyears 1997 through 2001 (Knudsen et al. 2008).
- **F2:** After adjusting for POHP, mean fecundity of HC (3,319.7 eggs; n=38) and SN (3,328.8 eggs; n=208) origin age 4 females were not significantly different (p=0.923). Mean fecundity in BY2002 was the lowest we have observed, reflecting the fact that age 4 body size was also the smallest recorded since beginning the collection of fecundity data (Knudsen et al. 2007b). In 2008, HC female fecundity (mean = 3897 eggs) was significantly greater than S females (mean = 3643 eggs) (Knudsen et al. 2009b).

#### A9. Egg weight  (revised 12/21/05)

### Background and Justification
Heath et al. (2003) concluded that egg weight in Chinook salmon decreased by 27% after five generations of captive rearing. Furthermore, Heath et al (2003) speculate that exposure to hatchery conditions will decrease egg size in hatchery-origin females (see also Fleming et al. 2000). Alternatively, Fleming and Gross (1989; 1992) and Petersson et al. (1996) reported that egg size in hatchery salmonids increased. Egg weight is a very important biological trait as it has a significant effect on emergent fry size, yolk reserves at emergence (Thorpe et al. 1984; Hendry et al. 2001), incubation rates, and emergence timing (Beacham and Murray 1993; Quinn et al. 1995). Obviously, all of these egg-size related traits can clearly affect the survival and ultimate reproductive success of salmonids. Consequently localized natural selection pressures undoubtedly strongly influence this trait (Taylor 1991; Hendry et al. 1998; Quinn et al. 2001). As mentioned above, hatchery environments appear to affect egg size in a non-consistent manner. The goal of monitoring this trait is to determine whether egg size change is occurring because of exposure to hatchery conditions, and if so, to ascertain the rate and direction of that change.

### Location
CESRF, Naches spawning grounds

### Groups Compared
WC, HC, SN, and SH

### Protocol
Measure weight of individual eggs originating from WC, HC, SH, and SN females. Same fish used for trait A7. Requires holding some SH origin females (a minimum of 30) to maturity at hatchery in addition to the SN females that will be held for SN broodstock and the HC females that will be
used for HC broodstock. Also requires sampling eggs from a maximum of 10 Naches females on spawning grounds. The coefficient of variation associated with egg weights from individual females is typically less than 2%. Consequently, five individual egg weights will be obtained from each sampled female.

**Expectations/Hypotheses**

If domestication does not occur egg weight will not change. However, Heath et al. (2003) observed that egg weights declined in captive chinook populations while Fleming and Gross (1989, 1992) and Petersson et al. (1996) observed that under hatchery culture egg size increased. We would expect egg weight to change in SH and HC, and the change should be greater in HC. The direction of change is not known because of differing reports in the literature.

**Analytical/Statistical Methods and Issues**

Within years, analysis of covariance will be used to compare body traits vs. egg weight within age classes. Analysis of variance will be used within years to compare egg weights within age classes. Over years analysis of covariance will be used on mean egg weight by age to detect trend differences between groups. Naches females, because there will be so few of them, should represent a variety of sizes.

**Findings To Date**

No consistent difference in mean egg weights of SH and SN origin females has been observed (Knudsen et al. 2002, 2003c, 2004c, 2005d, in prep.). In both SN and SH females, eggs of age-5 fish are significantly larger than age-4’s (ANOVA; p<0.01). Trends in age-specific egg weights over time were not significant for either group (p>0.35). Eggs of WC females are significantly heavier than eggs of SH and SN females of the same size (p<0.05; Knudsen et al. 2005d). In 2006, 4-yr-old females from the HC line will be available for the first time making it possible to evaluate how two generations of exposure to hatchery conditions may affect egg size. There was a significant difference in HC and S female egg mass means after adjusting for body size, (equality of means p=0.037) with S origin adjusted mean egg mass 9 to 10% heavier than in HC eggs (Knudsen et al. 2009b).

**A10. Reproductive effort** (revised 12/21/05)

**Background and Justification**

The biomass of gametes produced per unit body size indicates how populations have optimized allocation of energy between somatic growth, gametes, migration, competition and mating (Heath et al. 1999; Kinnison et al. 1998; Kinnison et al. 2001). In a hatchery setting, significant relaxation of selection pressures on reproductive effort (gonad weight divided by total body weight) may occur. Hatchery females, for example, do not have to allocate energy toward nest construction, spawning, guarding, and post-spawn redd sculpting. Similarly hatchery males do not have to invest energy into searching for and defending females and conducting courtship activities. In theory this energy could be reallocated and placed into gonads making the reproductive effort of hatchery fish higher than that seen in wild cohorts. An increase in reproductive effort (RE) has been observed in hatchery origin fish. If it occurs in our situation it could reduce the capacity of hatchery fish to reproduce under natural conditions because the energy they need to carry out reproductive behaviors would be irretrievably allocated to gametes. The goal of this trait evaluation is to determine if reproductive effort is increasing in our hatchery origin fish or whether this trait remains stable even when selection pressures affecting its expression have been notably relaxed.

**Location** CESRF

**Groups Compared** HC, SN, and SH

**Protocol**

Reproductive effort is calculated by dividing gonad weight by body weight. To collect this information, testes and total egg mass weights (sans ovarian fluid) will be measured in HC, SH, and SN fish. Testes weights will be collected from un-spawned HC, SH, and SN males. The acquisition of RE data in SH fish requires that some (a minimum of 30 pairs) be held at CESRF to maturity. Additionally, data from SN and HC fish will be taken from individuals that are being used as broodstock. WC fish will not be included in this analysis because partially spawned WC...
fish are being used as donors for our WC line and therefore it is not possible to measure the total weight of their unspawned gametes.

**Expectations/Hypotheses**

If domestication does not occur reproductive effort will remain constant. However, Fleming and Gross (1989, 1992) and Jonsson et al. (1996) observed that under hatchery culture reproductive effort does increase. Thus, we would expect reproductive effort to increase in SH and HC, and the increase should be greater in HC over time.

**Analytical/Statistical Methods and Issues**

Within years, analysis of covariance will be used to compare body traits vs. reproductive effort within age classes. Over years analysis of covariance will be used on mean reproductive effort by age to detect trend differences between groups. We cannot collect data on total gamete mass in Naches (WC) females (they are all partially spawned prior to collection), so we will not be able to estimate their reproductive effort.

**Findings To Date**

F1: From 2001 through 2005 there were no differences between SH and SN origin age-4 females (2-way ANOVA; Origin effects p=0.64; Knudsen et al. 2002, 2003c, 2004c, 2005d, Knudsen 2008). The trend over time (2001 to 2005) in age-4 female RE was positive and significant (p=0.01; Knudsen et al. 2008), but explained less than 1% of the total variation in RE over time.

F2: Male RE exhibited no significant difference between SH and SN fish in 2003 (p=0.54; Knudsen et al. 2004c). However, in 2007 and 2008 we estimated that NO males (mean GSI=0.070) produced 9.4% more testes biomass than similarly sized HC males (mean GSI=0.064). The GSI means were significantly different (p=0.046) after adjusting for POHP length, which was negatively correlated (regression p<0.01) with GSI while POHP length explained 32.8% of the total variation in GSI (Knudsen et al. 2009b).

**A11. Male and female fertility  (revised 12/21/05)**

**Background and Justification**

How fertility is affected by exposure to hatchery conditions is unknown and plausible arguments can be raised that it may be reduced or increased in hatchery fish. Because this trait is so closely linked to fitness it is important to understand if viability is influenced by hatchery exposure

**Location** CESRF

**Groups Compared** HC, SN, and SH

**Protocol**

The fertility of HC, SH, and SN fish will be estimated by creating *inter se* (within group) factorial crosses using 2x2 or 3x3 mating designs. Gametes from the fish used for trait A9 will be used. Some (a minimum of 30 pairs) SH origin males and females will have to be held to maturity at the hatchery in order to make the SH crosses. In addition gametes from fish being held for SH and HC fish broodstock that will be used to make the crosses necessary for these populations. When 2x2 crosses are performed a total of 4 families (2 for each male and female used) are created while 3x3 crosses generate six families, three for each fish used. Two hundred eggs are used to create each family and standardized fertilization methods are employed. Therefore, 400 eggs per female are used in the 2x2 crosses and 600 in the 3x3 crosses. Each single-pair mating of approximately 200 eggs is incubated in its own isolette. If male or female gamete quality is poor, it is readily discerned by this approach, since it allows both males and females to produce zygotes with multiple mates.

**Expectations/Hypotheses**

If domestication does not occur fertility will remain constant. However, under hatchery culture selection for fertility may be relaxed considerably, especially in males. If so, fertility could decrease in both the SH and HC lines, but at a faster rate in the HC line.

**Analytical/Statistical Methods and Issues**

Within years, analysis of variance will be used to compare fertility of individual animals within groups. Over years analysis of covariance will be used on mean fertility to detect trend differences between groups.
### Findings To Date

Of the pre-hatching mortalities we collected from isolettes in 2004, the vast majority were not fertilized (98% of the SH and 97% of the SN mortalities). Thus, on average only 2-3% died after fertilization. Egg survival to the eyed-egg stage averaged 76% and 86% for hatchery and wild females, respectively. Analysis of temporal trends has not yet been completed.

### A12. Adult morphology at spawning

**Background and Justification**

Based on earlier work (see expectations/hypotheses), domestication can be expected to cause changes in body shape, especially those aspects of shape that are secondary sexual characteristics.

**Location(s)**

CESRF and possibly some effort on Naches spawning grounds

**Groups Compared**

WC, HC, SN, SH

**Protocol**

Collect digitized measurement data from lateral image landmarks on photos of adults. Develop orthogonal variables with which to compare WC, HC, SH, and SN fish. Same fish used for traits A7-A10. Requires holding some SH origin males and females (about 30 pairs) to maturity at hatchery in addition to the SN fish that will be held for S broodstock and the HC fish that will be used for HC broodstock. Data on Naches fish will be collected from carcasses on spawning grounds. Program TPSDig ([http://life.bio.sunysb.edu/morph/index.html](http://life.bio.sunysb.edu/morph/index.html)) will be used to mark the coordinates of 13 landmarks. These are the same 13 used by Hard et al. (2000): 1) tip of snout, 2) base of skull, 3) anterior dorsal insertion, 4) posterior dorsal insertion, 5) anterior adipose insertion, 6) dorsal caudal insertion, 7) posterior end of body, 8) ventral caudal insertion, 9) posterior anal fin insertion, 10) anterior anal fin insertion, 11) anterior insertion of pelvic, 12) anterior insertion of pectoral, 13) distal tip of maxillary.

**Expectations/Hypotheses**

If domestication does not occur no changes in morphology will occur. If domestication does occur, we expect secondary sexual characteristics in both sexes to become less pronounced; e.g., reduced kype length, reduced body depth, less fusiform body shape, smaller adipose fins (Webb et al. 1991; Fleming and Gross 1992; Petersson and Jarvi 1993; Petersson et al. 1996; Berejikian et al. 1997; Hard et al. 2000). We would thus expect these types of changes in the S and HC lines, with greater changes in the HC line.

**Analytical/Statistical Methods and Issues**

Analysis closely follows Hard et al. (2000) and Wessel et al. (2005). Principal and partial warps were generated by TPSRelW. Warp scores were then used in MANOVA, MANCOVA, and discriminant function analysis in Systat to evaluate differences between groups (sexes, origins, and years). TPSRegr was used to regress warp scores on centroid size, and to generate consensus shapes for visual comparison. Use of IMP program Standard6 is being explored as a means of further reducing influence of size on shape.

**Progress to Date**

Four-year old adult wild upper Yakima spring Chinook were compared morphologically to first-generation SH fish over three consecutive brood years (1998-2000) using thin-plate spline analysis on 12 digitized landmarks, and an analysis of 27 truss characters based on the landmarks. Canonical discriminant analysis (CDA) of sex-specific partial warp scores correctly classified females to origin (hatchery or wild) with 75% accuracy (up to 84% accuracy for one brood year) and males to origin with 65% accuracy (up to 89% accuracy for one brood year). Classification to brood year using sex- and brood year specific partial warps was 62% accurate for both females and males. Results of truss analysis were very close to those from the thin-plate spline analysis. Sex-specific CDA’s correctly classified 75% of females to origin, and 68% of males. Correct classification to brood year was 61% for females and 58% for males. Consensus shapes based on partial warps suggested that hatchery fish of both sexes have longer and deeper heads, and shallower mid-bodies than wild fish. They also appear to be somewhat shorter in the posterior body. ANOVA of individual truss characters led to the same general
conclusion, but also found evidence of hatchery fish having wider anal fins. There was no
evidence of sex-specific differences between hatchery and wild fish. Body proportion differences
between hatchery and wild fish at the eight most diagnostic truss characters averaged 0.31
standard deviations in females and 0.35 standard deviations in males. In terms of actual
measurement, these differences amounted to an average of 1.4% of the wild mean, providing
little discriminatory power. Results were published in Busack et al. (2007).

<table>
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<tr>
<th>A13. Adult spawning behavior  (revised 12/21/05)</th>
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<td><strong>Background and Justification</strong></td>
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| A critical assumption associated with supplementation is that hatchery-origin adults possess
behavioral traits that allow them to spawn under natural conditions at a level that is comparable to
natural-origin fish. Previous work that examined the spawning behavior of wild and first
generation hatchery spring Chinook at CESRF showed that hatchery-origin fish were not as
successful at producing offspring as wild fish. Such a comparison does not allow the relative
importance of environmental and genetic effects to be evaluated. Here the reproductive behavior
of first- and second-generation hatchery spring Chinook will be compared. In this instance, the
early-life history of the fish will be similar (both will have been reared in a hatchery) and thus any
differences observed can be attributed to genetic changes caused by inadvertent domestication.
Such differences can also be linked to a single generation of additional exposure to hatchery
conditions. Documenting the magnitude of any genetic changes observed will significantly
increase our understanding of the biological costs associated with supplementation programs that
rely on hatcheries prior to release. |
| **Location**  Observation stream located at the Cle Elum Supplementation Research Facility |
| **Groups Compared**  HC and SH |
| **Protocol**  Homogenous spawning populations consisting of pure SH or HC adults will be introduced into 4.9
m wide by 15.2 m long sections of an observation stream while still in an immature state. An
opaque, temporary wall will subdivide each of these sections into two, 2.5 m by 15.2 m
subsections. Three pairs of fish will be placed into each subsection and all subsections will be
filled on the same day. Fish will be weighed, measured, tagged, and DNA sampled prior to being
liberated into their designated locations. Traits measured in females will include total life time in
the observation stream (longevity and egg retention at death. Longevity in males will also be
assessed. |
| **Expectations/Hypotheses**  Second generation hatchery fish are expected to be less competent at spawning than first-
generation or SH individuals. Fleming et al (1996) and Fleming et al (2000), for example, found
that fifth generation hatchery Atlantic salmon were 20 to 40% less effective than wild cohorts at
reproducing under natural conditions. If significant domestication occurs, second generation
hatchery females are expected to have shorter life-times and greater egg retentions Second
generation males are also expected to have shorter life-times. Fleming et al. (1996) discovered
that hatchery Atlantic salmon males ignored key behavioral signals provided by females as they
approached ovi-deposition and consequently their ability to fertilize eggs was severely
compromised. |
| **Analytical/Statistical Methods and Issues**  Both non-parametric and parametric analyses will be utilized. For example, longevity data will be
ordinal in nature because of how it is collected and consequently Kruskal-Wallis one-way
ANOVAs or Mann-Whitney U tests will be used to examine whether differences exist in the
longevity of SH and HC adults. In those instances where the response variable is at the interval
or ratio scale, nested ANOVAs will be employed. The fixed treatment in these analyses will be
adult type (SH or HC), the first order group would be the year that the experiment was performed,
subgroups would be the sections in the observation stream that were used, while the items in the
subgroups would be values obtained from the individuals placed into a section. An example of
this type of analysis would be egg retention in SH and HC females. The random variable in this case would be the percentage of a female’s expected fecundity she retained at death. The expectation would be that HC females would not be as effective at depositing their eggs as SH fish. The Nested ANOVA design would be used to test this expectation after the arc sin square root transformation was used on the raw data.

An analysis was performed to determine the experimental design that would provide the most power to detect differences in spawning behavior between SH and HC fish. The results of this analysis indicated that power would increase if the number of homogenous populations of SH and HC were increased. To accomplish that we have subdivided sections in the observation stream making it possible for six independent spawning populations of SH and HC fish to be evaluated each year. The decision to place three pairs in each of these populations was based on two factors, the need for replication within each population and the effects of instantaneous density on spawning success. If more pairs were placed into each population it is likely that intrasexual competition among the females would become intense enough to prevent some of them from spawning.

Findings To Date

The effect behavioral traits in females on their ability to produce offspring was evaluated in NOR and SH spring Chinook in the observation stream. Of these traits, longevity and redd tenure proved to be the most important. Females that guarded a single redd location produced more offspring than those that were evicted or otherwise abandoned their redd locations. Also a positive relationship was found to exist between how long a female lived and her ability to convert her eggs to offspring. Longevity in this case served as a surrogate for energy reserves, long-lived females apparently have greater stores of energy and therefore can complete tasks like territory acquisition, nest construction, redd development, and post-spawn guarding. The reproductive success of males was primarily linked to their aggressiveness. Individuals that instigated attacks on rivals were generally more successful at producing offspring than fish expressing lower levels of agonistic behavior. For a complete description of these results see Schroder et al. 2003a, 2003b, 2004, and 2005.

A14. Adult spawning success (revised 12/21/05)

Background and Justification

A significant challenge associated with evaluating salmonid supplementation is comparing the productivity of supplemented and non-supplemented populations. The ISRP and ISAB (2005) suggest comparisons could be accomplished if such populations were placed in a common experimental setting. For the past five years we have simultaneously introduced wild upper Yakima spring Chinook along with first generation hatchery fish in an observation stream and compared their capacities to produce offspring. This was done by performing pedigree analyses on the juveniles produced by these populations via micro-satellite DNA. These analyses estimated the number of offspring each adult fish produced. Differences were observed (see below) but it is unknown what proportion was caused by environmental differences in early life history or by genetic change caused by inadvertent domestication. The only way that we can quantify the effects of potential genetic change caused by exposure to hatchery life is to compare the reproductive success of salmon that have experienced different levels of hatchery exposure. In this case, the reproductive success of SH (first generation hatchery fish) will be compared with HC (second generation hatchery fish). Both types of fish will have experienced similar early life histories. Therefore differences between their capacities to produce offspring will be a reflection of genetic change brought about by hatchery conditions. The results of such an appraisal will provide managers with a way to estimate the genetic costs to recipient populations that are being supplemented by adult fish with varying degrees of hatchery ancestry.

Location(s) Observation stream located at the Cle Elum Supplementation Research Facility
Groups Compared HC and SH
Protocol Homogenous populations consisting of pure SH or HC spring Chinook adults will be introduced
into 2.5 m wide by 15.2 m long subsections of an observation stream just prior to becoming mature. Fish will be weighed, measured, tagged, and DNA sampled prior to being liberated into their designated subsections. Three fish of each sex will be placed into each subsection and all subsections will be filled on the same day. The fish in each subsection will be allowed to spawn naturally. An estimate will be made of the fecundity of each female to predict her potential egg deposition (PED) and her actual egg deposition (AED) will be estimated by subtracting any eggs she retained at death from her predicted fecundity. Modified fyke nets with floating live boxes will be installed at the end of every subsection to capture juveniles as they emerge and begin to migrate downstream. The fry traps will be checked daily, the number of fry caught will be counted and 10% of them will be preserved in 100% ethanol for later micro-satellite DNA analyses. At the end of the emergence period, electro-shocking gear and seines will be used to remove any remaining juveniles. A pedigree analysis will be performed using DNA samples from the adults and juveniles to estimate the number of offspring each adult produced. Results from the pedigree assessments will allow us to estimate the egg-to-fry survival rates (both PED and AED) of each female placed into a channel section. The capacity to produce offspring depends on the ability of females to choose appropriate nest sites, to construct and guard their nests, and on the ability of males to successfully match their gamete releases to when a female spawns her eggs. If either sex is unable to complete a specific series of tasks productivity will decrease. That is why we will be looking at two egg-to-fry survival measures. The first one (PED-to-fry survival) is a measure of how successfully a female was able to convert the eggs she brought into a spawning ground to fry. The second one (AED-to-fry survival) looks at how successful the eggs deposited by a female are converted into juveniles.

**Expectations/Hypotheses**

The effects of domestication are expected to increase in cultured populations that have prolonged artificial rearing periods and that are continuously recycled back into a hatchery. Given this expectation, we hypothesize that the HC populations will be less productive at producing fry than those comprised of SH individuals. The degree of difference will reflect the genetic cost associated with one additional generational exposure to hatchery conditions.

**Analytical/Statistical Methods and Issues**

Mixed model Nested ANOVAs will be used to compare the productivity of HC and SH populations. In these analyses, the fixed treatment will be the adult origin of the population, i.e. SH or HC. The first random group will be year that the experiment was performed, the random subgroup below year will be the subsection in the channel where the population spawned, and the items in that subgroup will be female specific values for either PED-to-fry survival or AED-to-fry survival. The goal is to have three females in each population for all years of the study in an effort to create a balanced design. Thus every subgroup would have three replicate values of either PED or AED survival to the fry stage. This design will reveal how much variation in productivity can be accounted for by channel subsection, year, and adult origin. Even if the channel subsections or years add a significant amount of variation to the analyses, we will still be able to evaluate whether the variation caused by adult origin is greater than expected. Four years of such comparisons are planned. Therefore over the duration of this study, a total of 96 HC and SH males and females will be used (24 males and females of each type per year).

The number of adults that will be placed into each population was based on previous studies in the observation stream from 2000-2005. This work suggests that three spring Chinook females are able to spawn simultaneously in 2.5 m wide x 15.2 m long stream sections. When higher numbers are present, significant intrasexual competition among females for space occurs. In most instances, instantaneous densities of spawning females in supplemented populations will be low. Consequently, three females represent a compromise between the need for replication and the desire to mimic natural spawning densities. Refinements to nested ANOVA designs are based on assessments of how much variation exists in each of the random groups and subgroups. As in the Trait 12A we expect that the most variable portion will be the individual values obtained from the females placed into the observation stream. If necessary, adjustments to the number of fish used in each population will be made after the first study year has been completed. For example, up to six females could be placed into each subsection. However, at these loading densities, a number of females may be prevented from spawning or might only be
able to partially spawn. Consequently the desire for replication would actually increase variance and subsequently reduce power.

**Findings To Date**

Beginning in 2001 we created heterogeneous populations of wild- and hatchery-origin spring Chinook and allowed them to spawn naturally in the observation stream. Altogether, seven independent test groups were placed into the stream from 2001 through 2005. No differences were detected in the egg deposition rates of wild and hatchery females ($P = 0.228$). Pedigree assignments based on microsatellite DNA, however, showed that the eggs deposited by wild females survived to the fry stage at a 7% higher rate ($P = 0.01$) than those spawned by hatchery females. Subtle differences between hatchery and wild females in redd abandonment, egg burial, and redd location choice may have been responsible for the difference observed. Body size did not affect the ability of females to spawn or the survival of their deposited eggs. How long a female lived was positively related to her breeding success but female origin did not affect longevity. The density of females spawning in portions of the stream affected both egg deposition and egg-to-fry survival. No difference, however, was found in the overall distribution patterns of the two types of females. We also discovered that reproductive success in males is often twice as variable as that found in females. For example, the coefficient of variation in male success ranged from 90 to 200% whereas for females it varied from 34% to 77%. An analysis examining the importance of male origin and behavior on their ability to produce offspring is ongoing (Schroder et al. 2009).

**J1. Emergence timing (revised 12/21/05)**

**Background and Justification**

When a juvenile emerges has a direct affect on its potential survival. Therefore rate of development is subject to strong natural selection pressures. Fish that emerge early will encounter little competition for territorial sites but may experience low food availability. Conversely, late emerging individuals will have to compete with prior residents and may be forced to make lengthy downstream migrations in order to find open habitat areas for rearing. In most production hatcheries fish are not allowed to emerge from their incubation devices. Moreover, when they are introduced into juvenile rearing areas the capacity to find and hold a feeding territory is not relevant. Hence, selection pressures that have finely tuned when natural-origin fish emerge are greatly relaxed in hatcheries. We are uncertain how or whether developmental rate will be affected by domestication. The goal of this evaluation is to determine if exposure to incubation and early rearing conditions in a hatchery will alter the rate that embryos develop into free-swimming fry.

**Location**  Cle Elum Supplementation and Research Facility incubation room

**Groups Compared**  WC<sub>p</sub>, SH, SH<sub>p</sub>, and HC

**Protocol**

Compare emergence timing of fish from different groups produced by *inter se* matings (same matings in trait A10). Eggs will be housed in 100-egg upwelling incubation chambers that allow fish to volitionally exit. Number of fish exiting will be noted daily. Eggs used will be those from the studies of adult reproductive traits.

**Expectations/Hypotheses**

If domestication does not occur, we would expect no changes in emergence timing or duration of emergence. If domestication does occur, we would expect duration of emergence to be compressed due to the more homogeneous environment presented by the hatchery, however, this trait has not been examined by other investigators so if or how emergence timing may be altered is unknown. If our supposition is correct, the emergence period for HC and SH would be reduced but more so in HC. Also if egg size increases as a result of domestication (see trait A8), then time to emergence will increase in SH and HC, with HC showing a greater increase. This would occur because it takes embryos originating from large eggs longer to develop into fry than those produced by smaller eggs.
Analytical/Statistical Methods and Issues

Two within-year analyses will be performed: 1) a nonparametric or parametric analysis of variance will be used to compare duration of emergence. If egg size and duration are correlated, then analysis of covariance will be used to correct for this factor; 2) analysis of covariance will be used to compare median date of emergence among groups. Over years, analysis of covariance will be used to examine differences in trends in these two variables.

Findings To Date

Results from 2002 and 2003 were reported in Knudsen et al. 2003c and 2004c. However, due to problems with uncontrolled water temperatures during those years we believe our earlier analyses were compromised. Our attempts to control water temperature across vessels using a single mixing head box delivery system have not been completed, yet.

J2. K<sub>D</sub> at emergence (revised 12/21/05)

Background and Justification

The amount of yolk reserves a juvenile possess at emergence can affect its survival in two opposing ways. First, yolk material can serve as an important food reserve as an individual transitions from an endogenously feeding fish to one that must rely on external prey. Second, yolk materials may also make an individual conspicuous, reduce its swimming speed, and therefore increase the risk that a predator will consume it (Fresh and Schroder 1987). Therefore, the amount of yolk material a fish has at emergence is likely a compromise between these two competing selection pressures. Under hatchery conditions these pressures will be relaxed and it is uncertain how K<sub>D</sub> will respond. If it changes in either direction negative survival consequences could occur when fish incubate and emerge under natural conditions.

Location Cle Elum Supplementation and Research Facility incubation room

Groups Compared WC<sub>p</sub>, SH, SH<sub>p</sub>, and HC

Protocol

Compare developmental condition at emergence (K<sub>D</sub>, Bams 1970) of fish from different groups produced by inter se matings (same fish as in J1). Eggs will be housed in 100-egg upwelling incubation chambers that allow fish to volitionally exit. K<sub>D</sub> will be measured daily on fish as they exit. Eggs used will be those from the studies of adult reproductive traits.

Expectations/Hypotheses

If domestication does not occur, we would expect no changes in K<sub>D</sub>. If domestication does occur, and egg size increases as a result, we would expect K<sub>D</sub> to increase. Thus, K<sub>D</sub> would increase in SH and HC, but more so in HC.

Analytical/Statistical Methods and Issues

Within years analysis of covariance (with egg size as covariate) will be used to compare slopes and adjusted means among groups. Over years, analysis of covariance will be used to examine differences in trends in these two variables.

Findings To Date

There was a significant positive relationship between K<sub>D</sub> values and egg weight for both SH<sub>p</sub> and SN fry (R<sup>2</sup>&gt;0.42, p&lt;0.001; Knudsen et al. 2003c, 2004c, 2005d). The ANCOVA of K<sub>D</sub> and Egg weight for 2002, 2003 and 2004 all showed that SH<sub>p</sub> and SN relationships had equal slopes (p&lt;0.26), but significantly different means adjusted for egg weight (p&lt;0.02; Knudsen et al. 2003c, 2004c, 2005d). The differences in K<sub>D</sub> means are very small and may not be biologically meaningful. However, SH origin samples (K<sub>D</sub> means ranged from 1.911 to 1.916) were consistently greater than SN samples (K<sub>D</sub> means ranged from 1.892 to 1.895).

F1: K<sub>D</sub> values of SH<sub>p</sub> (overall mean = 1.98) and SN (overall mean=1.99) fry did not differ significantly (P=0.126) in a 2-way ANOVA testing for Origin and Brood year effects.

F2: ANCOVA of BY2006 indicated that K<sub>D</sub> vs. Egg weight relationships of HC<sub>p</sub> (n=27) and SN (n=26) fry were significantly different (p=0.050). A t-test comparing K<sub>D</sub> HC<sub>p</sub> (mean =1.968) and SH (mean=1.975) fry was not significant (p=0.598). Analysis of temporal trends has not been completed.

In 2008, mean K<sub>D</sub> values were highest for HC fry (mean=1.964) followed by S (mean=1.958) and
NO (mean=1.946) fry suggesting that HC eggs produce leaner (higher KD) fry than S or NO fry. The p-value for the comparisons of HC, S, and NO fry KD means was just above significance (p=0.082) (Knudsen et al. 2009b).

**J3. Egg-fry survival (revised 12/21/05)**

**Background and Justification**

Egg-to-fry survival is the culmination of a continuous series of ontological events that depend upon gamete quality. In general, fertilization must occur along with successful hatching and conversion of yolk to body tissues. Natural selection pressures affect eggs and alevins that incubate in nests created by their maternal parent. We assume that these same selection pressures will be muted in a hatchery and that a new set will be imposed. Thus over time adaptations that increase the survival of hatchery fish to their new incubation environment are expected to evolve. As a result survival may increase in a hatchery setting but may decrease under natural conditions.

**Location** Cle Elum Supplementation and Research Facility incubation room

**Groups Compared** SH, SHp, and HC

**Protocol**

Compare egg-to-fry survival of fish from different groups produced by inter se matings (same matings in trait A10). Eggs will be housed in 200-egg isolettes (see trait A10). At the eyed-egg stage mortalities in each isolette will be counted. Then 100 live eggs from a sunset of females will be placed into the upwelling chambers described in J-1 and 2. The remaining eggs will be returned to their isolettes and mortality will be assessed at yolk absorption. In addition, mortality will be assessed in the upwelling chambers after emergence has been completed.

**Expectations/Hypotheses**

If domestication does not occur, we would expect no changes in egg-to-fry survival. If domestication does occur, we would expect survival of HC fish to increase over time as they adapt to hatchery selection pressures during incubation (Reisenbichler and McIntyre 1977). Survival of SH fish should also increase but not as rapidly as HC and SN fish will show a smaller or no increase.

**Analytical/Statistical Methods and Issues**

Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent survival in each isolette. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in survival diverge over time.

**Findings To Date**

F2: Egg-to-fry survival of SNp fry (mean survival= 0.677) was approximately the same as HCp fry (mean survival= 0.681) for BY2002. Origin effects were not significant (t-test p=0.849). This was the poorest in-hatchery egg-to-fry survival recorded since we began monitoring in 1997 (Knudsen et al. 2007b).

Analysis of temporal trends has not been completed.

Mean survival to the fry stage in 2007 and 2008 showed that HC eggs had the highest survival (8.3 to 10.8% greater than NO fry survival), S eggs were intermediate each year (1.0 to 7.8% greater than NO fry survival), and NO fry survival was lowest (Knudsen et al.2009). The 2-way ANOVA (Origin x Year) results show that the differences in mean survivals due to Origin effects, p=0.080, was just above α = 0.05 level. However, in both broodyears the HC, S and NO survival reaction norms fell in the order expected if in-hatchery domestication were occurring and increasing with each additional generation of domestication.

**J4. Occurrence of developmental abnormalities (revised 12/21/05)**

**Background and Justification**

Abnormalities in juvenile salmonids are caused by environmental perturbations as well as by genetic factors such as inbreeding. In theory, the founding populations of hatcheries should be diverse enough to limit inadvertent inbreeding. However, large variances in family size can occur
in salmonids and therefore it is possible that genetic diversity can be significantly reduced over time, increasing the likelihood of inbreeding. Here we will monitor the occurrence and type of abnormalities in populations that have experienced differing levels of hatchery exposure. Such an evaluation may allow us to indirectly measure loss of genetic diversity. Conversely, HC fish may be better adapted to the physical conditions experienced during hatchery incubation and therefore express fewer abnormalities than SH embryos.

**Location**  Cle Elum Supplementation and Research Facility incubation room

**Groups Compared**  SH, SH₀, and HC

**Protocol**  Compare the percentage of abnormally appearing alevins originating from each group using the progeny produced from the *inter se* matings (same matings in trait A10). Eggs will be housed in 200-egg isolettes (see trait A10). After yolk absorption abnormal appearing alevins in each isolette will be counted.

**Expectations/Hypotheses**  If domestication does not occur, we would expect no changes in the occurrence of abnormal fry. If it does occur we may see more or fewer abnormalities in HC fish. More abnormalities would be expected in the HC fish if genetic diversity is reduced and inbreeding heightened (Kincaid 1976). Less would occur if HC fish were adapting to the selection pressures present during the hatchery incubation period. If inbreeding occurs the proportion of abnormal offspring present in the SH and SN groups is also expected to increase but at a lower rate than that expressed by the HC line. Alternatively, fewer abnormalities may be expressed in SH and SN lines over time if the fish are adapting themselves to hatchery incubation conditions. The WC line will not be included in this trait due to the significantly different manner in which eggs are handled post-fertilization which might, through mechanical perturbations, cause developmental abnormalities.

**Analytical/Statistical Methods and Issues**  Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent abnormalities in each isolette. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in percent abnormalities diverge over time.

**Findings To Date**  Occurrences of abnormalities in emergent fry have been very low (<0.9%; Knudsen et al. in prep.). In general, no differences were observed in the incidence of abnormalities in offspring produced by SH and SN origin adults in 2002, 2003, and 2004 (Knudsen et al. 2003c, 2004c, 2005d). In 2001, SH values were significantly greater than SN by 0.5% (ANOVA; p=0.04; Knudsen et al. 2002). Analysis of temporal trends has not been completed.

### J5. Fry-smolt survival in a hatchery environment  (revised 12/21/05)

**Background and Justification**  Survival from the unfed fry stage to smolt can be used as a indicator of domestication. Presumably, individuals that originated from hatchery-origin parents should experience higher survival rates in raceways than those originating from natural-origin fish if domestication is occurring.

**Location**  Cle Elum Supplementation and Research Facility

**Groups Compared**  SH and HC

**Protocol**  The fry-to-smolt survival of supplementation and hatchery control line fish being reared in a hatchery environment will be compared. HC and SH fish will be reared in separate raceways under comparable conditions (loading densities, feeding rates, water temperatures, flows, etc.). Mortalities will be counted throughout the entire rearing period until volitional release begins. This comparison will not include WC juveniles because there is no intention to raise WC fish to the smolt stage. Raising WC fish to the smolt stage would require additional hatchery facilities and these fish would have to be sacrificed rather than released. Also, taking enough eggs to have sufficient WC fry to fill a raceway at standard rearing densities would have an unacceptably high
impact on the Naches population.

**Expectations/Hypotheses**

If domestication does not occur, we would expect mortality rates to be comparable in the HC and SH groups. If domestication does occur, we would expect HC fish to have lower mortality rates during the rearing period (Reisenbichler and McIntyre 1977).

**Analytical/Statistical Methods and Issues**

Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent mortality experienced over the entire rearing period by raceway. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in mortalities diverge over time. Since at present there are only two HC raceways within year tests will not be statistically robust. However, over time replicates will take place increasing the power of this evaluation.

**Findings To Date**

Problems associated with bias in fecundity estimates used to estimate the number of initial fry have been resolved and the data are now being analyzed.

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**J6. Juvenile morphology at release** *(revised 12/21/05)*

**Background and Justification**

Based on earlier work (see expectations/hypotheses), domestication can be expected to cause changes in body shape, especially those aspects of shape that are secondary sexual characteristics, but differences may also be seen in juveniles because shape has heritable components (Hard et al. 1999).

**Location(s)** HC Acclimation site

**Groups Compared** SH, HC

**Protocol**

Photograph 50 fish from each raceway at acclimation site, for a total of 100HC and 200 SH fish. Collect digitized measurement data from lateral image landmarks on photos. Program TPSDig ([http://life.bio.sunysb.edu/morph/index.html](http://life.bio.sunysb.edu/morph/index.html)) will be used to mark the coordinates of 13 landmarks. These are the same 13 used by Hard et al. (2000): 1) tip of snout, 2) base of skull, 3) anterior dorsal insertion, 4) posterior dorsal insertion, 5) anterior adipose insertion, 6) dorsal caudal insertion, 7) posterior end of body, 8) ventral caudal insertion, 9) posterior anal fin insertion, 10) anterior anal fin insertion, 11) anterior insertion of pelvic, 12) anterior insertion of pectoral, 13) distal tip of maxillary.

**Expectations/Hypotheses**

If domestication does not occur no changes in morphology will occur. If domestication does occur, SH and HC morphology will diverge. We would expect that HC fish would become more fusiform (Taylor 1986).

**Analytical/Statistical Methods and Issues**

Analysis will closely follow Hard et al. (2000) and Wessel et al. (2005). Principal and partial warps will be generated by TPSRelW. Warp scores will then be used in MANOVA, MANCOVA, and discriminant function analysis in Systat to evaluate differences between groups (origins and years). TPSRegr will be used to regress warp scores on centroid size, and to generate consensus shapes for visual comparison. Use of IMP program Standard6 is being explored as a means of further reducing influence of size on shape.

**Findings to Date**

Fish have been photographed for six years and digitized, but no analysis has been done yet.

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**J7. Smolt-to-smolt survival** *(revised 12/21/05)*

- a) SH and HC from Clark Flats acclimation site to Chandler
- b) SN, SH and HC from RAMF to Chandler
- c) SN, SH, HC, WC from Chandler to McNary and John Day dams

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## Background and Justification

Survival during the smolt-to-smolt stage can be used as an indicator of domestication. Individuals that originate from hatchery environments are known to experience lower survival rates during freshwater emigration than natural origin smolts. We are monitoring and comparing the survival of hatchery and wild origin smolts in the Yakima River to ascertain the biological cost of hatchery rearing on smolt survival. Quantification of this cost requires that the in-stream survival of fish exposed to varying levels of artificial culture be simultaneously evaluated. Consequently, the survival of SH and HC smolts released from Clark Flats will be measured as they migrate past Chandler, and two lower Columbia River Dams. The survival of SN smolts will also be assessed to provide a relative measure of hatchery smolt quality. If survival rates between HC and SH smolts are comparable then no genetic effect has occurred. Moreover, comparing the survival rates of HC, SH, and SN smolts can determine hatchery conditions affect smolt survival. In this case, if HC and SH survival is relatively low when compared to SN smolts then environmental factors associated with hatchery life are most likely responsible. Obviously, the proportion of naturally spawning hatchery-origin adults in the parental generation could influence the quality of SN smolts. However, WC smolts will not be affected in this manner and will thus serve as wild controls.

### Location

Clark Flat Acclimation site, RAMF, Chandler, McNary and John Day dams

### Groups Compared

1. HC and SH from Clark Flats
2. SN, SH, HC from RAMF to Chandler, McNary, and John Day Dams
3. SH, HC, SN, and WC from Chandler to McNary and John Day Dams

### Protocol

a) HC and SH pre-smolts reared at Clark Flats will receive PIT tags prior to being released. PIT tag detectors will monitor their passage through Chandler, McNary, and John Day dams. Tag recovery will be downloaded and analyzed to compare the survival rates of HC and SH smolts.

b) A sub-sample of SN, SH, and HC fish will receive PIT tags at Roza (RAMF). Survival rate comparisons of SN, SH, and HC fish will only occur among individuals that passed through the Roza juvenile trap during the same time period. WC smolts do not migrate past the RAMF and therefore will not be included in this analysis.

c) Additional fish will be tagged at Chandler, including Naches and American smolts (identified by DNA micro-satellites). Comparisons of survival rates among these fish will be based on PIT tag recoveries at monitoring sites located at McNary, John Day, and any other suitably equipped downstream sites.

### Expectations/Hypotheses

If domestication does not occur, we would expect smolt-to-smolt survivals of HC and SH groups to be comparable. SN fish are expected to survive at higher rates. This phenomenon has been observed in many other salmonid populations. If domestication does occur, we would expect SH smolts to survive at higher rates than HC individuals, but not as well as SN fish. The comparisons involving SN need to be interpreted carefully, because they include only SN fish that migrate during the spring. Winter migrants, another major life history strategy, will not be included. The survival of WC smolts is expected to be free of hatchery influence.

### Analytical/Statistical Methods and Issues

Within-year analyses will be performed by using logistic regression analysis. Analysis of covariance will be used to ascertain if trends in survival diverge over time.

### Findings To Date

None.

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### J8. Natural Smolt Production  (revised 12/21/05)

#### Background and Justification

Smolt productivity, which we define as the number of smolts produced per female spawner, is being monitored to evaluate the effect of supplementation on the Upper Yakima spring Chinook population. Smolt productivity values from Naches and the American River are expected to remain relatively constant over time after adjusting for spawner densities. It is unknown what effect supplementation will have on the smolt productivity level of females spawning in the upper...
Yakima River. Varying proportions of hatchery-origin females will be spawning in this area. If they are less capable of producing smolts productivity of the whole population will decline. On the other hand, if hatchery females can produce smolts at the same rate as wild cohorts then the productivity of this population segment will remain constant.

**Location** Chandler Smolt Facility

**Groups Compared** WC, SN, SH, and HC

**Protocol**
Out-migrating smolts made up of a mixture of WC, SN, SH and HC fish will be sub-sampled as they migrate past the Chandler facility. These samples will be used to estimate the proportion of smolts that have originated from each of these groups. Marks and tags will be used to identify hatchery-origin fish. DNA samples will be collected on unmarked individuals and used to estimate the proportion of smolts produced by the American River, Naches River and upper Yakima populations. Chinook smolts migrate past Chandler year around, however spring Chinook typically migrate by this facility from March through June. Samples proportionate to smolt abundance will be collected during this period. Total smolt passage numbers will be estimated during the trapping period and allocated to each group based on the results of the DNA analyses and mark recoveries. These estimates will be summed across the migration period to get indices of total smolt production for the WC, SN, SH and HC groups.

**Expectations/Hypotheses**
If domestication does not occur, we would expect the density adjusted productivity of the upper Yakima population to remain constant. If domestication does occur, we would expect the productivity of that population to decline over time. And the rate of decline would be positively linked to the prevalence of naturally spawning SH fish in the upper Yakima. The density-adjusted productivity of the WC population will remain constant and it will be used as a wild control benchmark against which the productivity of the upper Yakima population will be compared.

**Analytical/Statistical Methods and Issues**
Within year analysis will consist of comparing the density-adjusted productivities of each population. This will require that an estimate be made of the total number of smolts produced per population. In addition we need to know how many females produced those smolts. The annual density of female spawners and the proportion of SH females spawning in the upper Yakima will need to be accounted for to help explain variation in productivity. The adjusted smolts/female values will be analyzed with ANCOVA to determine trends in productivity over time. The relationship between the number of spawning females versus the number of smolts/spawner will be used to describe the density-dependent productivity function for each group.

**Findings To Date**
No data have yet been collected for this trait

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**J9. Smolt-to-adult survival of hatchery-origin fish** *(revised 12/21/05)*

**Background and Justification**
Previous studies (Fleming and Petersson 2001; Fleming et al. 1996; Fleming et al.1997; Fleming et al. 2000) have shown that populations that have been repeatedly recycled through a hatchery are more likely suffer from inadvertent domestication than those that have not been continuously exposed to hatchery conditions. Moreover, salmonids with prolonged hatchery rearing periods are more likely to undergo domestication than those that are reared for shorter periods. Because spring Chinook are kept in culture for over a year they may be susceptible to inadvertent domestication, particularly if they are continuously recycled back into a hatchery environment. In this trait, we examine whether the smolt-to-adult survival of HC fish differs from SH individuals. Any difference detected will reflect a genetic change caused by hatchery exposure as both populations will have been incubated and reared in comparable hatchery environments prior to release into the upper Yakima River.
Location  Clark Flat Acclimation Site to RAMF
Groups Compared  SH and HC
Protocol
Prior to release, every SH and HC fish will be tagged so that its origin can be identified. An estimate of the number of smolts leaving each raceway will be made via continuous PIT tag monitoring. The numbers of adult fish produced from each raceway returning to Roza will be recorded by inspecting fish for tags and marks. Scale samples will be taken to assign an age to each returning adult. The survival of fish by age class will be calculated for each raceway by broodyear. This will be done by dividing the number of 3, 4, or 5 year-olds originating from a raceway/broodyear combination by the total number of fish released from that raceway. WC fish will not be included for reasons outlined under J5.

Expectations/Hypotheses
If domestication does not occur, we would expect HC and SH fish to have equivalent survival rates. If domestication does occur, we would expect SH-origin fish to have higher survivals than HC individuals.

Analytical/Statistical Methods and Issues
Differences in overall survival will be examined by using a mixed model two-way ANOVA. The fixed treatment will by smolt origin, either HC or SH, and the random treatment will be brood year. The random variable in this ANOVA will be the percentage of smolts that survived to the adult stage. Additional mixed model two-way ANOVAs will be performed to see if age at maturation varied due to smolt origin. In these analyses, smolt type (HC or SH) will be fixed and brood year will once again be a random treatment. The response variable will be the percentage of smolts that matured at a given age within the same sex. For example, one of these ANOVAs would compare the percentage of 3-yr-old males produced by the HC and SH lines. These tests will not only allow us to examine whether shifts in age at maturation are occurring due to domestication they may also help explain any differences seen in overall survival. Finally, ANCOVA will be used to ascertain if trends in survival by age in HC and SH fish diverge over time.

Findings To Date
Analyses on this trait have not yet started.

J10. Smolt out-migration timing and rate  (revised 12/21/05)
Background and Justification
Both exogenous and endogenous factors regulate the onset and duration of seaward migrations in natural origin smolts (Groot 1982). Chief among the endogenous factors would be an increase in hypo-osmotic regulatory capacity, elevated levels of thyroxine, and hormones regulating growth (Folmar and Dickhoff 1981). Important exogenous factors would include water temperature, day length, and lunar phases during the spring (Grau et al. 1981). Clearly, the temporal occurrence and speed of downstream migration can have significant survival effects on juvenile salmonids (Hoar 1976). One concern associated with artificial rearing has been whether exogenous cues are obscured by hatchery conditions. For example, facilities that use spring water are likely denying their fish the opportunity to detect seasonal changes in water temperature. This could affect the timing of smoltification and their readiness to migrate. Here we compare the timing and speed of migration of smolts originating from three different sources. Two of these will be hatchery-origin fish that have different levels of hatchery exposure (HC and SH lines). The third group represents individuals that have been produced under natural conditions, the SN line.

Location  From the Clark Flat Acclimation site to downstream monitoring sites
Groups Compared  SN, SH, and HC
Protocol
Two comparisons of migration speed will be made. In the first, a sub-sample of SN, SH, and HC fish will receive PIT tags as they are collected at the Roza juvenile trap. Their subsequent migration rates past downstream sampling locations will be compared. Furthermore, to account for probable differences in migration speed due to seasonal effects, comparisons will be restricted to individuals that passed through the Roza juvenile trap during the same time period. In the
second comparison, migration speeds of HC and SH fish will be made that include all PIT tagged
fish released from the Clark Flat acclimation site. The timing and abundance of these fish as they
move downstream past Roza, Chandler, McNary, and John Day dams will be recorded and
compared. Migration timing of SN, SH, and HC smolts will be evaluated by documenting their
temporal occurrence and abundance at the Roza Adult Monitoring Facility. WC fish will not be
included for reasons outlined under J5.

**Expectations/Hypotheses**

If domestication does not occur, HC and SH fish are expected to migrate at the same time and
rate. If it does occur, we are uncertain what effect it might have. However, since both HC and
SH fish will experience comparable juvenile histories it will be possible to assign any discovered
difference to additional exposure to hatchery conditions. In the first migration rate comparison,
HC, SH, and SN smolts are expected to migrate at equivalent rates because they all are actively
migrating smolts. However, hatchery conditions may delay smoltification or create differences in
morphology and energy reserves that could cause HC and SH smolts to migrate at slower speeds
than SN fish. Currently, it is unknown whether the migration timing of SH and HC fish will be
influenced by the rearing and release protocols they experience. We are evaluating this trait
because of its close linkage to smolt-to-adult survival. Thus, if timing differences are noted they
may help explain any differences seen in the survival rates of SN, SH, and HC smolts to the adult
stage.

**Analytical/Statistical Methods and Issues**

Within year analysis of migration speed and timing will use Kolmogorov-Smirnov tests. Analysis
of covariance will be used to ascertain if genetically based trends in median out-migration timing
occur in HC and SH fish.

**Findings To Date**

None, study will begin in 2006

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**J11. Food conversion efficiency**  *(revised 12/21/05)*

**Background and Justification**

As fish become adapted to the hatchery environment, one aspect of adaptation may be the ability
to more efficiently metabolize the artificial feeds used in the hatchery

**Location(s)**

Cle Elum Supplementation and Research Facility and smolt acclimation sites

**Groups Compared**

SH and HC

**Protocol**

This trait is a surrogate for growth rate. HC and SH fish will experience normal hatchery rearing
procedures, which includes being fed at a rate based on size. The quantity of food supplied to
each raceway from ponding to release will be recorded. Two random samples of fish will be
removed from each raceway, one at the time of tagging (after 8 months of rearing) and another
just prior to release (approximately 12 months of rearing). Individual weights will be taken on 200
fish from each raceway. The weight data will be used to estimate the biomass of fish in each
raceway at the time of sampling. Food conversion efficiencies will be determined by dividing total
biomass of fish by total weight of food delivered to a raceway. WC fish will not be included for
reasons outlined under J5.

**Expectations/Hypotheses**

If domestication does not occur, we would expect HC and SH fish to have equivalent food
conversion rates at tagging and again just prior to release. If domestication does occur, we would
expect HC fish to have greater food conversion efficiencies than SH fish (Reisenbichler, pers.
comm.).

**Analytical/Statistical Methods and Issues**

Within year analyses will use one-way ANOVAs (per sample period) to examine food conversion
rates in HC and SH raceways. A single within year analysis will have low power because there
are only two HC raceways. However, by analyzing multiple years with two-way ANOVAs power
will be increased, allowing us to examine year and treatment effects. Within-year analyses of
conversion rate will be done by two-way fixed treatment ANOVAs estimating origin, raceway, and
interaction effects. In addition, analysis of covariance will be used to ascertain if trends in food conversion in these two groups diverge over time. With only one measurement per raceway, and only two HC raceways, this is not a powerful design, so it may well be dropped in the future.

**Findings to Date**
Data are available, but have not yet been analyzed.

### J12. Juvenile Length-Weight Relationships *(revised 12/21/05)*

**Background and Justification**
Multiple-generational exposure to hatchery conditions is expected to modify traits in juvenile salmonids, making them better adapted to artificial rearing conditions. One potential adaptation would be an increased capacity to convert artificial foods into biomass. Such a difference could be expressed by possessing a more robust body shape (greater weight for a given length). Since the groups being compared will by HC and SH fish any differences seen are likely to be genetically based and thus trait can be another measure of domestication.

**Location** CESRF and smolt acclimation sites

**Groups Compared** SH and HC

**Protocol**
HC and SH fish will experience normal hatchery rearing procedures. Two random samples of fish will be removed from each raceway, one at the time of tagging (after 8 months of rearing) and another just prior to release (after approximately 12 months of rearing). Individual lengths and weights will be taken on 200 fish from each raceway. WC fish will not be included for reasons outlined under J5.

**Expectations/Hypotheses**
If domestication does not occur, we would expect HC and SH fish to have equivalent length/weight relationships at tagging and again just prior to release. If domestication does occur, we would expect HC fish to either have steeper slopes (greater biomass increase per unit length) than SH fish or greater mean body weight at a standardized length.

**Analytical/Statistical Methods and Issues**
Within year analyses will compare the length/weight relationships found in SH and HC juveniles by using ANCOVA. In addition, analysis of covariance will be used to ascertain if trends in mean length and weight in these two groups diverge over time.

**Findings To Date**
F1: ANCOVA using egg mass as a covariate showed that SHp fry were significantly (P=0.002) heavier (mean body weight across all years = 317 mg) than SNp fry (mean body weight across all years = 313 mg) in each of the four years.

F2: We tested for Origin effects in BY2002 fry body weight distributions by ANCOVA using egg mass as a covariate. HCP fry (mean body weight = 304 mg) were slightly smaller than SNp fry (mean body weight = 304 mg), but there was no significant Origin effect (p=0.209) indicating that, given eggs of the same weight, hatchery fry will have equivalent body mass as wild fry in 2006 (Knudsen et al. 2007b).

In 2008 there was no significant difference in adjusted fry body size means between S, HC, and NO fry (equal adjusted means p=0.450; Knudsen et al. 2009b).

### J13. Agonistic-competitive behavior *(revised 12/21/05)*

- **a) Contest competition**
- **b) Scramble competition**
- **c) Aggression**

**Background and Justification**
Competition and aggression has been demonstrated to be influenced by domestication.

**Location(s)** Cle Elum Supplementation and Research Facility
### Groups Compared
WC<sub>p</sub>, SH, and HC

#### Protocol
Juvenile fish produced from the crosses used in J3 will be test subjects. Dominance and aggressiveness will be compared to the WC<sub>p</sub>. Two types of dominance experiments will be performed. The first will test for contest competition (14a) and the second scramble competition (14b). In this behavioral assay, three group comparisons will be made: WC<sub>p</sub> vs. HC, WC<sub>p</sub> vs. SH, and SH vs. HC. Size-matched pairs of fish (each fish represents a different group) will be simultaneously introduced into tanks. In the test of contest competition, fish will be placed into tanks that have one optimal location (possessing one piece of cover and a single tube used to introduce food and velocity in the water column). Dominance will be assigned to the fish that obtains the most food, dominates the majority of the agonistic contests, and spends the most time adjacent to the food tube and cover. In the test of scramble competition, no cover will be provided, water will be introduced through a tube as before, and food will be introduced in different locations on the surface of the water. Dominance will be assigned to the fish that eats the most food items. Replicate trials will be conducted for 7 days. Aggression (14c) will be examined by comparing the rates of agonistic interactions initiated during competition trials in 14a and 14b. In the event that the desired number of replicates cannot be achieved, then contest competition will be prioritized over scramble competition. Approximately 250 trials will be conducted every year.

#### Expectations/Hypotheses
If domestication does not occur, we would expect HC, WC<sub>p</sub>, and SH fish to have equivalent levels of aggression and dominance. If domestication does occur, we would expect the following results ordered from most to least: contest competition dominance WC<sub>p</sub> &gt; SH &gt; HC; scramble competition dominance HC &gt; SH &gt; WC; and aggressiveness WC<sub>p</sub> &gt; SH &gt; HC or HC &gt; SH &gt; WC<sub>p</sub>. In addition, we would expect that these differences would be accentuated with time. How aggressive and dominant WC<sub>p</sub> fish may be is unknown, but their behavior is not expected to change over time and therefore they will act as a valuable reference.

#### Analytical/Statistical Methods and Issues
Within a year paired comparisons between hatchery and wild fish of the percentages of food pellets eaten in the water column, fish in the best habitat, interactions initiated, agonistic interactions dominated, interaction type, and overall dominance will be made for each replicate using a two-tailed Wilcoxon matched pairs test. The test for total dominance in the contest trials will be a matched comparison of the sums of the percentages of the food acquisition, habitat used, and interactions initiated. Paired comparisons of growth and interaction rate (average interactions per minute for all tanks) will be compared using a two-tailed paired student’s t-test. A paired sign test will be used to compare whether fish in each replicate that grew the most were also classified as dominant. Analysis of covariance will be used to ascertain if trends in dominance among the comparisons diverge over time.

#### Findings to Date
Offspring of wild origin fish dominated 4% more contests than offspring of hatchery origin fish (P<0.05). Dominance was not significantly different in the scramble competition trials (P>0.05). Wild fish initiated more agonistic interactions than hatchery fish in both contest and scramble trials. There were no differences in the frequency of different types of agonistic interactions that were used by hatchery and wild fish. We also found that dominant fish grew more than subordinate fish in both contest and scramble trials (P<0.05). Detailed descriptions can be found in Pearsons et al. (2004 and 2005). Trials were also conducted during 2005, 2006, 2007, and 2008 with varying dominance between years. Detailed results are presented in Pearsons et al. 2006, 2007, 2008, and Fritts et al. 2009.

### J14. Predator avoidance
(revised 12/21/05)

#### Background and Justification
Predation has been demonstrated to be influenced by domestication

#### Location(s)
Cle Elum Supplementation and Research Facility

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Groups Compared  WC, SH, and HC

Protocol
Predator challenges will be conducted in net pens to determine if domestication affects the survival of fry. To avoid pseudo-replication, multiple arenas possessing different individual fish predators will be established. There will be 8 arenas, which will consist of 8 x 10 foot net pens. Net pens will be placed in a single hatchery raceway. Between 67 (3 line comparison) and 100 (2 line comparison) size-matched fish from each line will be simultaneously introduced into an arena containing 2 rainbow trout and 2 torrent sculpin predators. Prior to introduction, fish from each line will be differentially marked or tagged. After a designated period of time has elapsed, which corresponds to approximately 50% of the introduced fish having been eaten (e.g., 4 days), survivors will be removed from each arena and enumerated. Fish predators will be changed after each trial to avoid pseudo-replication. We will also attempt to measure differences in innate antipredator behaviors between the groups. Behaviors will be assessed in aquaria described for J15, but torrent sculpin predators will be introduced along with the chinook salmon.

Expectations/Hypotheses
If domestication does not occur, we would expect fish from all lines to survive at equal rates. In addition, the expression and use of innate anti-predator behaviors should remain constant within a line over time. If domestication does occur, we would expect WC fish to have the highest survival rates followed by SH, and HC individuals in that order.

Analytical/Statistical Methods and Issues
Wilcoxon matched pairs tests will be used for within year analyses between SH and HC. For years when WC are available, Wilcoxon matched paired tests will be used between SH and WC and between HC and WC, using WC as a baseline to measure differences in SH and HC survival within year. Analysis of covariance will be used to determine if trends in survival are manifested over time in both assays.

Findings to Date
There was no significant difference in survival between the SH and HC fry during 2003 (P=0.051) or 2004 (P=0.122). SH fry were found to have a 2.15% survival advantage over HC fry when 2003 and 2004 data were combined to increase statistical power (P=0.016). Detailed description can be found in Pearsons et al. (2004 and 2005). Trials were also conducted in 2005, 2006, 2007, and 2008 and results are presented in Pearsons et al. 2006, 2007, 2008, and Fritts et al. 2009.

J15. Incidence of precocity in production raceways  (revised 12/21/05)

Background and Justification
Larsen et al. (2004) observed that 37 to 49% of the males released from CESRF acclimation sites had matured precociously. They felt this was caused by early rapid growth interacting with a genetic proclivity to mature early. Precocious males are not used as hatchery broodstock therefore if precocious development has a genetic basis it should decrease when a population is repeatedly exposed to a hatchery environment. The occurrence of precocious males in HC and SH fish will be compared to see if this expectation is realized.

Location(s)  Clark Flat Acclimation site

Groups Compared  SH and HC

Protocol
Just prior to release, two hundred fish from the six raceways located at an acclimation site will be examined to determine the percentage of the males that are precociously maturing. One acclimation site is being used because there are only two raceways of HC fish. Additionally, by using one acclimation site the environmental conditions the fish experience will be standardized. WC fish will not be included as none will be reared in raceways, for reasons mentioned earlier.

Expectations/Hypotheses
If domestication does not occur, we would expect HC and SH fish to have equivalent rates of precocial development. If domestication does occur, we would expect HC fish to have a lower incidence of precocialism.
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<tr>
<th><strong>Analytical/Statistical Methods and Issues</strong></th>
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<tr>
<td>Within year analysis will use one-way ANOVAs. Analysis of covariance will be used to ascertain if trends in the production of precocial males in these two lines diverge over time</td>
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<th><strong>Findings to Date</strong></th>
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<tr>
<td>Mean precocity rates of male progeny from first generation hatchery parents were 14% (brood year 2002) and 11% (brood year 2003). Mean precocity rates of male progeny for natural origin parents were 40% (brood year 2002) and 21% (brood year 2003).</td>
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