Document ID #P106829
Report covers work performed under BPA contract #34450
Report was completed under BPA contract #37649

Yakima/Klickitat Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2007
Performance Period: May, 2007-April, 2008

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Project Number 1995-063-25
Contract 00034450

May 2008

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 00034450, 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: 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 May 1, 2008 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 2008 modifications are some refinement of design of reproductive success studies, but 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. As in previous years, the entire plan is included, so the chapter can be excerpted as a stand-alone document.

Chapter 2: Samples of unknown gender Chinook collected at Roza Dam in 2007 were analyzed to determine the sex of each fish. Analysis of 140 samples of unknown gender in 2007 resulted in assignments for 137 individuals. Complete consensus agreement was determined for all individuals that were assigned. The comparison of gender identifications based on morphology and genetics resulted in 37 of 137 (27.0%) individuals in both collections that had different gender determinations. Our investigations using these two different DNA markers for gender identification in Chinook suggested high, but not 100% accuracy.

Chapter 3: We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners from the hatchery-control line (two generations of hatchery influence) and the supplementation hatchery line (SH – one generation of hatchery influence) in the Cle Elum experimental spawning channel for the 2007 brood year. The assignments were based on offspring genotypes at 12 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999992. Two thousand seven hundred and ninety-one of 2,850 fry from the 2007 brood that were genotyped at seven or more loci were assigned to a parental pair with 95% confidence.

We found no compelling evidence to suggest that un-genotyped parents spawned successfully in this year. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 453 for all males and from 0 to 225 for females. The sum of progeny attributed to the hatchery-control line males and females was 1,463, while the sum of progeny attributed to supplementation hatchery line males and females was 1,328.

Chapter 4: 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 2007. The trap was under construction from the end of January through the third week of March, therefore the January and March strata only represent a few sampling days and reduced number of outmigrating smolts. Mixture analysis was conducted on a proportional subsample of 1,500 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 87.9% 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 1,466/1,482 (98.9%) of the smolts. In addition to conducting stock-of-origin assignments for the outmigrating smolts, a collection of the Marion Drain fall stock from 2007 was assessed for genetic similarity to previous Marion Drain collections and lower Yakima River fall collections. The ability of the baseline to accurately assign individuals to each of the five stocks (3 spring and 2 fall) was also assessed using known-origin samples from the Naches River and upper Yakima River.

Chapter 5: 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. We compared the genetic similarities among collections of the same life history type. Genetic data from four collections of adult steelhead (2002, 2003, 2005, and 2006) and one collection of juvenile migrants (2006) were consistent with all collections representing a single population sample. Residents collected from Manastash Creek, Taneum Creek, and Teanaway River were distinct from each other. Insufficient sampling, cutthroat trout admixture, and kinship limited conclusions regarding the relationship among resident collections. We also compared the genetic similarities between collections differing in life history type. Anadromous *O. mykiss* collections were genetically differentiated from resident collections, and the degree of genetic differentiation appeared consistent with geographic distances, with the Teanaway steelhead being most genetically similar to the Teanaway resident collection, followed by residents from Taneum Creek then Manastash Creek.

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Chapter 1

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 May 1, 2008 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 2008 modifications are some refinement of design of reproductive success studies, but 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 May 1, 2008

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 $PHI = \frac{pHOS}{(pHOS + pNOB)}$. 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 (pNOB=1.00) and the proportion of hatchery-origin fish on the spawning grounds is approximately 50% (pHOS=0.5), PHI=.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 (pHOS=0.5), and 0.44 (pHOS=0.8). Any other program having a fixed pNOB will have a similarly limited PHI range, but fixed-PHI programs are rare. Thus even without explicit controls on pHOS, 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 pNOB-pHOS 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 pHOS range of 0.5 to 0.8 will result in an ancestry variance range of 0.058-0.087 for a program with pNOB=1.0; for a program with pNOB=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 phenotypic, due to the effect 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. Here the system of codes is defined:

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).

SH_P – 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 a WC fish.

WC_P – 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.

Trait	Tasks required	Trait	Tasks required
A1	1c, 1h, 1i, 1j, 2b	J1	1c, 1i
A2	1c, 1i	J2	1c, 1i
A3	1c, 1i, 1j, 1m, 2a, 2b	J3	1c, 1i
A4	1c, 1i, 1j, 1m, 2a, 2b	J4	1c, 1i
A5	1c, 1i, 1j, 1m, 2a, 2b	J5	1c, 1i
A6	1c, 1i	J6	1c, 1i
A7	1c, 1i, 1j	J7	1c, 1d, 1e, 1i
A8	1c, 1i	J8	1c, 1e, 1i
A9	1c, 1i, 1j	J9	1c, 1i, 1m
A10	1c, 1i	J10	1c, 1d, 1e, 1i
A11	1c, 1i	J11	1c, 1i
A12	1c, 1i, 1j	J12	1c, 1i
A13	1c, 1i	J13	1c, 1i
A14	1c, 1i	J14	1c, 1i
		J15	1c, 1i

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

2001 – A10, A11, J3, J4.
2002 – A1, A3-A9, J5, J6, J9, J11, J12.
2003 – A12, J13 (HC, SH), J14 (HC, SH).
2004 – J1, J2.
2005 – J13 (HC, SH, WC), J14 (HC, SH, WC).
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 microsatellite 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.

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

None specifically on this trait, however we have been doing pedigree analysis on fish in the spawning channel for three years (Kassler 2005), so procedures are well established except for above-noted power concerns.

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 $\pm$10% or less at α=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 (return year 2005) and are likely in 2006, although those data have not been analyzed on a brood year basis, yet. Analyses of 2005 and 2006 will be integrated with 2007 and 2008 to assess the impacts in F2 (Knudsen et al. 2007).

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 unknown. 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 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 and 2006, although the data have not been analyzed on a brood year basis, yet. Age 4 mean SH and HC 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 (Knudsen et al. 2007a).

For the first time in 9 years we observed 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 general 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)).

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.

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 and 2006, 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).

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 and 6 days for RY 2005 and 2006, respectively. SH jack median passage was 2 and 12 days later than SN jacks in RY 2005 and 2006, respectively. These were all significantly later each year in Kruskal-Wallis tests (Knudsen et al 2007a).

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. 2004). 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

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 and 2006 (Knudsen et al. 2007a).

F1: Initiation of in-river female spawning activity did not differ between SH and SN fish (RY2002-2005) (Knudsen et al. 2005a)

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. submitted).
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).

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.

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

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, in prep.). Male RE exhibited no significant difference between SH and SN fish in 2003 (p=0.54; Knudsen et al. 2004c). The trend over time (2001 to 2005) in age-4 female RE was positive and significant (p=0.01; Knudsen et al. in prep.), but explained less than 1% of the total variation in RE over time.

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 *(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

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) 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).

A13. Adult spawning behavior *(revised 12/21/05)*

Background and Justification

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.

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_p, SH, SH_p, 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_D 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_D 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_p, SH, SH_p, and HC

Protocol

Compare developmental condition at emergence (KD, 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. KD 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 KD. If domestication does occur, and egg size increases as a result, we would expect KD to increase. Thus, KD 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 KD values and egg weight for both SH_p and SN fry ($R2>0.42$, $p<0.001$; Knudsen et al. 2003c, 2004c, 2005d). The ANCOVA of KD and Egg weight for 2002, 2003 and 2004 all showed that SH_p and SN relationships had equal slopes ($p>0.26$), but significantly different means adjusted for egg weight ($p<0.02$; Knudsen et al. 2003c, 2004c, 2005d). The differences in KD means are very small and may not be biologically meaningful. However, SH origin samples (KD means ranged from 1.911 to 1.916) were consistently greater than SN samples (KD means ranged from 1.892 to 1.895).
F1: KD values of SH_p (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 KD vs. Egg weight relationships of HC_p ($n=27$) and SN ($n=26$) fry were significantly different ($p=0.050$). A t-test comparing KD HC_p (mean =1.968) and SH (mean=1.975) fry was not significant ($p=0.598$). Analysis of temporal trends has not been completed.

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, SH_p, 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

F1: In 2001, SN_p fry survived at a significantly higher rate than SH_p fry (P=0.047), while in 2004 SH_p fry had significantly higher survival (P=0.023). The other two broodyears were not significantly different (2002 and 2003, P>0.41). Thus, the effects of female origin on fry survival varied significantly across broodyears and showed no consistent trend .

F2: Egg-to-fry survival of SN_p fry (mean survival= 0.677) was approximately the same as HC_p 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.

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, SH_p, 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.

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) 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 two years and digitized, but no analysis has been done yet.

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

Background and Justification

Survival during the smolt-to-smolt stage can be used as a 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 whether 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 a) HC and SH from Clark Flats
b) SN, SH, HC from RAMF to Chandler, McNary, and John Day Dams
c) 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.

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

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. The first 3-year-old HC adults returned to the upper Yakima in 2005. Consequently it won't be until 2007 before the first broodyear to produce HC fish will have completed its return back to the Yakima.

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

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 = 300 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).

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_p, 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_P. 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_P vs. HC, WC_P 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_P, 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_P >SH>HC; scramble competition dominance HC>SH>WC; and aggressiveness WC_P >SH>HC or HC>SH> WC_P. In addition, we would expect that these differences would be accentuated with time. How aggressive and dominant WC_P 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 ($P \leq 0.05$). 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 ($P \leq 0.05$). Detailed descriptions can be found in Pearsons et al. (2004 and 2005). Trials were also conducted during 2005, 2006, and 2007 and results are presented in Pearsons et al. 2006, 2007, and 2008.

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

Groups Compared WC_p, 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, and 2007 and results are presented in Pearsons et al. 2006, 2007, and 2008. .

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.

Analytical/Statistical Methods and Issues

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

Findings to Date

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).

Literature Cited

Araki, H. and M. S. Blouin. 2005. Unbiased estimation of relative reproductive success of different groups: evaluation and correction of bias caused by parentage assignment errors. Molecular Ecology.

Bams, R.A. 1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. J. Fish. Res. Bd. Canada 27:1429-1452.

Beacham, T.D. and C.B. Murray. 1993. Fecundity and egg size variation in North American Pacific salmon (Oncorhynchus). Journal of Fish Biology 42:485-508.

Berejikian, B. A., E. P. Tezak, S. L. Schroder, C. M. Knudsen and J. J. Hard (1997). Reproductive behavioral interactions between wild and captively reared coho salmon (Oncorhynchus kisutch). ICES Journal of Marine Science 54: 1040-1050.

Blouin, M. S. (2003). Relative reproductive success of hatchery and wild steelhead in the Hood River. Final Report, Contract 9245, Project 1988-053-12. Portland, OR, Bonneville Power Administration: 1-25.

Brannon, E. 1987. Mechanisms stabilizing salmonid fry emergence timing. Canadian Special Publication Aquatic Science 96:120-124.

Brannon, E.L., M.S. Powell, T.P. Quinn, and A. Talbot. 2004. Population Structure of Columbia River Basin Chinook Salmon and Steelhead Trout. Reviews in Fisheries Science. 12:99–232.

Busack, C., G. Hart and C. M. Knudsen (2004). Initial morphometric analysis of Upper Yakima and Naches spring Chinook: progress on trait A11 of domestication monitoring plan. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2003. Portland, OR, Bonneville Power Administration: 61-77.

Busack, C., C. M. Knudsen, G. Hart, and P. Huffman (2007). Morphological differences between adult wild and first-generation hatchery Upper Yakima River spring Chinook salmon. Transactions of the American Fisheries Society 136: 1076-1087.Busack, C., G. Hart and C. M. Knudsen (2005). Morphometric differences between natural- and hatchery-origin adult spring Chinook after one generation of hatchery culture. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2004. Portland, OR, Bonneville Power Administration: 104-123.

Busack, C., S. L. Schroder and C. M. Knudsen (2002). Domestication research/monitoring design. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2001. Portland, OR, Bonneville Power Administration: 10-28.

Busack, C., S. L. Schroder and C. M. Knudsen (2003). Domestication research/monitoring design. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2002. Portland, OR, Bonneville Power Administration: 43-90.

Busack, C., S. L. Schroder, T. Pearsons, N. and C. M. Knudsen (2004). YKFP spring Chinook domestication monitoring plan development. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2004. Portland, OR, Bonneville Power Administration: 78-121.

Busack, C., B. Watson, T. Pearsons, N., C. M. Knudsen, S. R. Phelps and M. Johnston (1997). Yakima Fisheries Project spring Chinook monitoring plan. Report to Bonneville Administration DOE/BP-64878-1.

Chilcote, M. W., S. A. Leider and J. J. Loch (1986). Differential reproductive success of hatchery and wild summer steelhead under natural conditions. Transactions of the American Fisheries Society 115: 726-735.

Einum, S. and I.A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Fleming, I. A. and M. R. Gross (1989). Evolution of adult female life history and morphology in a Pacific salmon (coho: Oncorhynchus kisutch). Evolution 43(1): 141-157.
Fleming, I.A. and M.R. Gross. 1990. Latitudinal clines – a trade-off between egg number and size in Pacific salmon. Ecology 71: 1-11.
Fleming, I. A. and M. R. Gross (1992). Reproductive behavior of hatchery and wild coho salmon (Oncorhynchus kisutch): does it differ? Aquaculture 103: 101-121.
Fleming, I.A., B. Jonsson, M.R. Gross, and A. Lamberg. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (Salmo salar). Journal of Applied Ecology 33:893-905.
Fleming, I.A., A. Lamberg, and B. Jonsson. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. Behavioral Ecology 8:470-480.
Fleming, I.A., K. Hindar, I.B. Mjolnerod, B. Jonsson, T. Balstad, and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society of London B 267:1517-1523.
Fleming, I.A. and E. Petersson. 2001. The ability of released, hatchery salmonids to breed and contribute to the natural productivity of wild populations. Nordic Journal of Freshwater Research. 75: 71-98.
Folmar, L.C. and W.W. Dickhoff. 1981. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-37.
Ford, M. J. (2002). Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16(3): 815-825.
Fresh, K.L. and S.L. Schroder. 1987. Influence of the abundance, size, and yolk reserves of juvenile chum salmon (Oncorhynchus keta) on predation by freshwater fish in a small coastal stream. Canadian Journal of Fisheries and Aquatic Sciences 44:236-243.
Fresh, K.L., S.L. Schroder, E.C. Volk, J.J. Grimm, and M. Mizell. 2003. Evaluation of the Cedar River sockeye salmon hatchery: Analyses of adult otolith recoveries. Wash. Dept. of Fish and Wildlife FPL 03-01. 42pp.
Gallinat, M. J., J. Bumgarner, L. Ross and M. Varney (2001). Tucannon River spring Chinook salmon hatchery evalaution program 2000 annual report. Boise, ID, USFWS Lower Snake RiverCompensation Plan: 1-44.
Goodman, D. (2004). Salmon supplementation: demography, evolution, and risk assessment. American Fisheries Society Symposium 44: 217-232.
Grau, E.G., W.W. Dickhoff, R.S. Nishioka, H.A. Bern, and L.C. Folmar. 1981. Lunar phasing of the thyroxine surge preparatory to seaward migration of salmonid fish. Science 211: 607-609.
Groot, C. 1982. Modifications on a theme - a perspective on migratory behavior of Pacific salmon. Salmon and Trout Migratory Behavior Symposium (E.L. Brannon, and E.O. Salo, eds.). Contribution 793, School of Fisheries, University of Washington, Seattle.
Hard, J. J. 1995. Genetic monitoring of life-history characters in salmon supplementation: problems and opportunities. American Fisheries Society Symposium 15:212-225.
Hard, J. J., B. A. Berejikian, E. P. Tezak, S. L. Schroder, C. M. Knudsen and L. T. Parker (2000). Evidence for morphometric differentiation of wild and captively reared adult coho salmon: a geometric analysis. Environmental Biology of Fishes 58: 61-73.
Hard, J. J., G. A. Winans and J. C. Richardson (1999). Phenotypic and genetic architecture of juvenile morphometry in Chinook salmon. Journal of Heredity 90: 597-606.
Healy, M.C., and Heard, W.R. 1984. Inter- and intrapopulation variation in the fecundity of Chinook salmon and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.
Heath, D.D., Fox, C.W., and Heath, J.W. 1999. Maternal effects on offspring size: variation

through early development of Chinook salmon. Evolution, 53: 1605–1611.
Heath, D. D., J. W. Heath, C. A. Bryden, R. M. Johnson and C. W. Fox (2003). Rapid evolution of egg size in captive salmon. Science 299: 1738-1740.
Hendry, A.P., J.E. Hensleigh, and R.R. Reisenbichler. 1998. Incubation temperature, developmental biology, and the divergence of sockeye salmon (Oncorhynchus nerka) within Lake Washington. Canadian Journal of Fisheries and Aquatic Sciences 55:1387-1394.
Hendry, A.P., T. Day, and A.B. Cooper. 2001. Optimal size and number of propagules: allowance for discrete stages, and effects of maternal size on total reproductive output and offspring fitness. The American Naturalist 157(4):387-407.
Hoar, W.S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish.Res. Board Can. 33: 1234-1252.
HSRG (2004). Hatchery reform: principles and recommendations of the HSRG. Long Live the Kings, Seattle, WA. Available at www.hatcheryreform.org.
ISRP and ISAB (2005). Monitoring and evaluation of supplementation projects: 1-11.
Jonsson, N., B. Jonsson, and I. Fleming. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? Functional Ecology 10:89-96.
Kassler, T. W. (2005). DNA based parentage assignments of Chinook salmon from the Cle Elum spawning channel in 2004. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2004. Portland, OR, Bonneville Power Administration: 20-40.
Kincaid, H.L. 1976. Inbreeding in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada 33: 2420-2426.
Kinnison, M. T., M. J. Unwin, W. K. Hershberger, and T. P. Quinn. 1998. Egg size, fecundity and development rate of two New Zealand Chinook salmon (Oncorhychus tshawytscha) populations: Can. J. Fish. Aquat. Sci. 55 1946–1953.
Kinnison, M.T., M.J. Unwin, A.P. Hendry, and T.P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution 55:1656-1667
Knudsen, C., S. Schroder, J. Rau, M. Johnston, C. Stromm, and M. Hammlin. 2002. Monitoring phenotypic and demographic traits of Yakima River hatchery and wild spring Chinook: Spawner traits. YKFP 2001 Annual Report. DOE/BP-00004666-3.
Knudsen, C. M., B.L Ben-James, S.L. Schroder, T. N. Pearsons. 2003a. Spawner and redd characteristics of wild- and hatchery-origin upper Yakima River spring Chinook in Reproductive ecology of Yakima River hatchery and wild spring Chinook. Annual Report FY 2001-2002 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-16.
Knudsen, C. M., S.L. Schroder, M. V. Johnston, T. N. Pearsons, J. A. Rau, C. R. Strom, and M. L. Hamlin. 2003b. Monitoring phenotypic and demographic traits of Yakima River hatchery and wild Spring Chinook: Spawner traits in Reproductive ecology of Yakima River hatchery and wild spring Chinook. Annual Report FY 2001-2002 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-16.
Knudsen, C. M., S.L. Schroder, T. N. Pearsons, J. A. Rau, A. L. Fritts, and C. R. Strom. 2003c. Monitoring phenotypic and demographic traits of Yakima River hatchery and wild Spring Chinook: Gametic and juvenile traits in Reproductive ecology of Yakima River hatchery and wild spring Chinook. Annual Report FY 2001-2002 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-16.
Knudsen, C. M., B. L. Ben–James, T. N. Pearsons, and S. L. Schroder. 2004a. Spawner and Redd Characteristics of Wild- and Hatchery-Origin Upper Yakima River Spring Chinook in Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook. Annual

Report to Washington Department of Fish and Wildlife. Bonneville Power Administration: Portland, Oregon. DOE/BP-00013756-4. p 69-84.

Knudsen, C. M., S. L. Schroder, M. V. Johnston, T. N. Pearsons, J. A.Rau, C. R. Strom, and M. L.Hamlin. 2004b. Monitoring Phenotypic and Demographic Traits of Yakima River Hatchery and Wild Spring Chinook: Spawner Traits in Reproductive Ecology of Yakima River hatchery and wild spring Chinook. Bonneville Power Administration: DOE/BP-00013756-4. p 3-36.

Knudsen, C. M., S. L. Schroder, T. N. Pearsons, J. A. Rau, A. L. Fritts, J. L. Scott, and C.R. Strom. 2004c. Monitoring phenotypic and demographic traits of Yakima River hatchery and wild Spring Chinook: Gametic and juvenile traits in Reproductive Ecology of Yakima River hatchery and wild spring Chinook. Annual Report to Washington Department of Fish and Wildlife. Bonneville Power Administration: DOE/BP-00013756-4. p 37-68.

Knudsen, C. M., B. B. James, T. N. Pearsons, S. L. Schroder. 2005a. Spawner and Redd Characteristics of Wild and Hatchery Origin Upper Yakima River Spring Chinook in Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook, Annual Report 2004. Bonneville Power Administration, Portland, Oregon. DOE/BP-00017478-4; p. 75-90.

Knudsen, C. M., S. L. Schroder, M. V. Johnston, C. S. Busack, T. N. Pearsons, and D. E. Fast. 2005b. A Comparison of Life-History Traits in First-Generation Hatchery and Wild Origin Upper Yakima River Spring Chinook Salmon in Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook, Annual Report 2004 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00017478-4; p. 3-28.

Knudsen, C. M., S. L. Schroder, M. V. Johnston, T. N. Pearsons, C. S. Busack, A. R. Marshall, C. R. Strom, and D. E. Fast. 2005c. Life-history and Genetic Traits of Wild Origin Yakima River Spring Chinook Salmon Populations in Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook, Annual Report 2004 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00017478-4; p. 29-61.

Knudsen, C. M., S. L. Schroder, M.V. Johnston, T. N. Pearsons, and C. R. Strom. 2005d. Monitoring Phenotypic and Demographic Traits of upper Yakima River Hatchery and Wild Spring Chinook: Gametic and Juvenile Traits. Annual Report. Bonneville Power Administration, Portland, Oregon. DOE/BP-00017478-4: p. 62-74.

Knudsen, C. M., S. L. Schroder, M. V. Johnston, C. S. Busack, T. N. Pearsons, and D. E. Fast. 2006. A comparison of life-history traits in first-generation hatchery and wild origin Upper Yakima River spring Chinook salmon. Transactions of the American Fishery Society 135: 1130–1144.

Knudsen, C. M., A. R. Marshall, M. V. Johnston, T. N. Pearsons, Craig S. Busack, S. L. Schroder, C. R. Strom, and D. E. Fast. In preparation. Life-history and genetic traits of wild origin Yakima River spring Chinook salmon populations.

Knudsen, C. M., S. L. Schroder, C. Busack, T. N. Pearsons, M. V. Johnston, and D. E. Fast. 2007a. A Comparison of Life-History Traits of Natural origin and First- and Second-Generation Hatchery Origin Upper Yakima River Spring Chinook Salmon Returning in 2006. Pages 3-19 in *ed*. C. M. Knudsen, Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook, Yakima/Klickitat Fisheries Project Monitoring and Evaluation Annual Report 2006, Project Number 1995-063-25, Document ID #P102834.

Knudsen, C. M., S. L. Schroder, M. V. Johnston, T. N. Pearsons, and C. R. Strom. 2007b. Comparison of Gametic Traits of Second Generation Hatchery- and Natural-Origin Upper Yakima River Spring Chinook Salmon. Pages 21-34 in *ed*. C. M. Knudsen, Reproductive Ecology of Yakima River Hatchery and Wild Spring Chinook, Yakima/Klickitat Fisheries

Project Monitoring and Evaluation Annual Report 2006, Project Number 1995-063-25, Document ID #P102834.

Larsen, D.A.,B.R. Beckman, K. A. Cooper, D. Barrett, M. Johnston, P. Swanson, and W. W. Dickhoff. 2004. Assessment of high rates of precocious male maturation in a spring Chinook salmon supplementation hatchery program. Transactions of the American Fisheries Society 133:98-120.

Lynch, M. and M. O'Hely (2001). Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2: 363-378.

Marshall, T. C., J. Slate, L. E. B. Kruuk and J. M. Pemberton (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7: 639-655.

Pearsons, T. N., A. L. Fritts, and J. L. Scott. 2004. The effects of domestication on predation mortality and competitive dominance. Annual Report FY 2003 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00013756-2.

Pearsons, T. N., A. L. Fritts, and J. L. Scott. 2005. The effects of domestication on predation mortality and competitive dominance. Annual Report FY 2004-2005 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00017478-2.

Pearsons, T. N., A. L. Fritts, and J. L. Scott. 2006. The effects of domestication on predation mortality and competitive dominance: Yakima/Klickitat Fisheries Project Monitoring and Evaluation. Annual Report 2005-2006. Report to Bonneville Power Administration, publication DOE/BP-00022370-2, Portland, Oregon.

Pearsons, T. N., A. L. Fritts, J. L. Scott and J. M. Vandal. 2007. The effects of domestication on predation mortality and competitive dominance. Annual Report 2006-2007. Report to Bonneville Power Administration, publication DOE/BP-102833, Portland, Oregon.

Pearsons, T. N., A. L. Fritts, and J. M. Vandal. 2008. The effects of domestication on predation mortality and competitive dominance. Annual Report 2007-2008. Report to Bonneville Power Administration, publication, Portland, Oregon.

Pearsons, T. N., A. L. Fritts, G. M. Temple, C. L. Johnson, and M. R. Schmuck. 2003. Yakima River Species Interactions Studies. Annual Report FY 2002 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-10.

Pearsons, T. N., A. L. Fritts, G. M. Temple, M. R. Schmuck, and C. L. Johnson. 2002. Yakima River Species Interactions Studies. Annual Report FY 2001 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-9.

Pearsons, T. N., A. L. Fritts, G. M. Temple, N. S. Burres, M. R. Schmuck, and C. W. Hopley. 2001b. Yakima River Species Interactions Studies. Annual Report FY 2000 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-1.

Pearsons, T. N., A. L. Fritts, K. D. Ham, G. A. McMichael, E. L. Bartrand, G. M. Temple, and C. W. Hopley. 2001a. Yakima River Species Interactions Studies. Annual Report FY 1999 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-64878-7.

Petersson, E. and T. Jarvi (1993). Differences in reproductive traits between sea-ranched and wild sea-trout (Salmo trutta) originating from a common stock. Nordic Journal of Freshwater Research 68: 91-97.

Petersson, E., T. Jarvi, N. G. Steffner and B. Ragnarsson (1996). The effect of domestication on some life history traits of sea trout and Atlantic salmon. Journal of Fish Biology 48: 776-791.

Quinn, T.P., A.P. Hendry, and L.A. Wetzel. 1995. The influence of life history tradeoffs and the size of incubation gravels on egg size variation in sockeye salmon (Oncorhynchus nerka). Oikos 74:425-438.

Quinn, T.P., M.T. Kinnison, and M.J. Unwin. 2001. Evolution of Chinook salmon (Oncorhynchus tshawytscha) populations in New Zealand: pattern, rate, and process. Genetica 112-113:493-513.

Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of hatchery and wild steelhead trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada 34: 123-128.

Reisenbichler, R. R. and S. P. Rubin (1999). Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56: 459-466.

Roff, D. A. (1997). Evolutionary quantitative genetics. New York.

Schroder, S. L., C. M. Knudsen, B. D. Watson, T. N. Pearsons, S. F. Young, and J. A. Rau. 2003a. Comparing the reproductive success of Yakima River hatchery and wild-origin spring Chinook. Annual Report FY 2001 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00004666-7.

Schroder, S. L., C. M. Knudsen, B. D. Watson, T. N. Pearsons, S. F. Young, and J. A. Rau. 2003b. Comparing the reproductive success of Yakima River hatchery and wild-origin spring Chinook. Annual Report FY 2001-2002 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-0000466-15.

Schroder, S. L., C. M. Knudsen, B. D. Watson, T. N. Pearsons, D. Fast, S. F. Young, and J. A. Rau. 2004. Comparing the reproductive success of Yakima River hatchery and wild-origin spring Chinook. Annual Report FY 2003 submitted to Bonneville Power Administration, Portland, Oregon. DOE/BP-00013756-4.

Schroder, S.L., C.M. Knudsen, T.N. Pearsons, D.E. Fast, and B.D. Watson. 2005. Comparing the reproductive success of Yakima River hatchery- and wild-origin spring Chinook. Annual Report FY 2004 submitted to Bonneville Power Administration, Portland Oregon.

Smoker, W.W., A.J. Gharrett, and M.S. Stekoll. 1998. Genetic Variation of Return Date in a Population of Pink Salmon: A Consequence of Fluctuating Environment and Dispersive Selection? Alaska Fishery Research Bulletin 5(1):46–54.

Taylor, E. B. (1986). Differences in morphology between wild and hatchery populations of juvenile coho salmon. Progressive Fish-Culturist 48: 171-176.

Taylor, E. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98:185-207.

Thompson, S. K. (1987). Sample size for estimating multinomial proportions. American Statistician 41: 32-46.

Thorpe, J.E., M.S. Miles, and D.S. Keay. 1984. Developmental rate, fecundity and egg size in Atlantic salmon, Salmo salar L. Aquaculture 43:289-305.

van den Berghe, E. P. and M. R. Gross (1989). Natural selection resulting from female breeding competition in a Pacific salmon (coho: Oncorhynchus kisutch). Evolution 43(1): 125-140.

Webb, J. H., D. W. Hay, P. D. Cunningham and A. F. Youngson (1991). The spawning behaviour of escaped farmed and wild adult Atlantic salmon (Salmo salar L.) in a northern Scottish river. Aquaculture 98: 97-110.

Wessel, M. L., W. W. Smoker and J. E. Joyce (2005). Variation of morphology among juvenile Chinook salmon of hatchery, hybrid, and wild origin. Transactions of the American Fisheries Society (in press).

Chapter 2

DNA-Based Gender Determination of Hatchery-Origin spring Chinook Salmon Passing Roza Dam (Yakima River) in 2007

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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 2007 were analyzed and the gender of each fish was determined. A consensus gender identification of unknown Chinook was determined for 137 out of 140 fish using the two techniques. The sex ratio of hatchery-origin Chinook passing Roza Dam was 64 adult males and 73 adult females. The comparison of gender identifications based on morphology and genetics resulted in 37 of 137 (27.0%) individuals that had different gender determinations. Thirty-six of the 37 (97.3%) 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 Rodriquez 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 2007 (N = 140 were analyzed; collection 07CH - 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 mm^2) 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 Rodriquez 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 Rodriquez (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 $MgCl_2$, 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 an MJ Research PTC-200 thermocycler. The thermal profile for both gender markers was as follows: an initial denaturation step of 3 minutes at 94^{o}C; 35 cycles of 15 seconds at 94^{o}C, 30 seconds at 48^{o}C (Clifton-Rodriguez) or 63 ^{o}C (Brunelli-Thorgaard), and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 30 minutes, followed by a final indefinite holding step at 10^{o}C.

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 dH_20 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 three 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. A 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 2007 unknown samples (total of 140 fish) following the guidelines described in the Methods section, with the exception of three individuals (07CH – 539, 07CH – 2423, and 07CH - 2959). 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 (07CH) based on morphology (sexed at the time the live fish were handled and passed at the dam) and genetics were different for 37 of 137 (27.0%) individuals (Appendix 1). Thirty-six of the 37 (97.3%) 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 were 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 27.0% (37/137) of the samples identified differently. Most of these differences (97.3%) 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 137 hatchery-origin Chinook passing Roza Dam we estimated 64 adult males and 73 adult females in 2007.

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. Denise Hawkins provided editorial comments and review.

Literature Cited

Brunelli, J.P. and G.H. Thorgaard. 2004. A new Y-chromosome-specific marker for Pacific salmon. Trans. Amer. Fish. Soc. 133:1247-1253.

Clifton, D.R. and R.J. Rodriquez. 1997. Characterization and application of a quantitative DNA marker that discriminates sex in Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci 54:2647-2652.

Devlin, R.H., B.K. McNeil, T.D.D. Groves, and E.M. Donaldson. 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48:1606-1612.

Devlin, R.H., B.K. McNeil, I.I. Solar, and E.M. Donaldson. 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of Chinook salmon. Aquaculture 128:211-220.

Devlin, R.H., C.A. Biagi, and D.E. Smailus. 2002. Genetic mapping of Y-chromosomal DNA markers in Pacific salmon. Genetica 111:43-58.

Du, S.J., R.H. Devlin, and C.L. Hew. 1993. Genomic structure of growth hormone genes in Chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specified pseudogene, GH-Psi. DNA and Cell Biology 12:739-751.

Forbes, S.H., K.L. Knudsen, T.W. North, and F.W. Allendorf. 1994. One of two growth hormone genes in coho salmon is sex-linked. Proceedings of the National Academy of Sciences, USA 91:1628-1631.

Kassler, T.W., A. Frye, and J.F. Von Bargen. 2004. DNA-Based Gender Determination of Hatchery-Origin Chinook Salmon Passing Roza Dam (Yakima River) in 2002 and 2003. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2004. Portland, OR, Bonneville Power Administration 6-52**.**

Kassler, T.W. 2006. DNA-Based Gender Determination of Hatchery-Origin Chinook Salmon Passing Roza Dam (Yakima River) in 2005. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2006. Portland, OR, Bonneville Power Administration 1-19**.**

Table 1. Gender determination for Chinook salmon passed at Roza Dam in 2007. Three independent gender identifications and a final consensus gender determination are shown for each method. [methods: 'C - R' = Clifton & Rodriquez; '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.

unknown gender

			C - R method			B - T method		
		Consensus	Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
1	07CH0023	**M**	F? - M	F - M	M - M	M - M	M - M	M - M
2	07CH0075	**F**	F	F	F	F	F	F
3	07CH0082	**F**	F	F	F	F	F	F
4	07CH0105	**M**	M	M	M	M	M	M
5	07CH0114	**M**	F - M	F - M	F - M	M - M	M - M	M - M
6	07CH0160	**F**	F	F	F	F	F	F
7	07CH0172	**F**	F	F	F	F	F	F
8	07CH0197	**F**	F	F	F	F	F	F
9	07CH0199	**M**	M?	M	M	M	M	M
10	07CH0233	**M**	F? - M	M - M	M? - M	M - M	M - M	M - M
11	07CH0244	**F**	F	F	F	F	F	F
12	07CH0259	**M**	F? - M	M - M	M? - M	M - M	M - M	M - M
13	07CH0282	**F**	F	F	F	F	F	F
14	07CH0312	**F**	F	F	F	F	F	F
15	07CH0321	**F**	F	F	F	F	F	F
16	07CH0346	**F**	F	F	F	F	F	F
17	07CH0359	**F**	F	F	F	F	F	F
18	07CH0420	**M**	M	M	M	M	M	M
19	07CH0468	**M**	M	M	M	M	M	M
20	07CH0486	**M**	M	M	M	M	M	M
21	07CH0506	**F**	F	F	F	F	F	F
22	07CH0514	**M**	M	M	M	M	M	M
23	07CH0530	**F**	F	F	F	F	F	F
24	07CH0539	**NO ID**	F - F	F - F	F - F	M - F?	M - M	M - F?
25	07CH0560	**M**	M	M	M	M	M	M
26	07CH0575	**M**	M	M	M	M	M	M
27	07CH0586	**F**	F	F	F	F	F	F
28	07CH0590	**F**	F	F	F	F	F	F
29	07CH0599	**M**	M	M	M	M	M	M
30	07CH0603	**F**	F	F	F	F	F	F
31	07CH0626	**F**	F	F	F	F	F	F
32	07CH0681	**F**	F	F	F	F	F	F
33	07CH0691	**F**	F	F	F	F	F	F

Table 1 continued.								
			C - R method			B - T method		
		Consensus	Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
34	07CH0698	**M**	M	M	M	M	M	M
35	07CH0716	**M**	F?	M	M	M	M	M
36	07CH0718	**F**	F	F	F	F	F	F
37	07CH0727	**F**	F	F	F	F	F	F
38	07CH0747	**F**	F	F	F	F	F?	F
39	07CH0762	**M**	M	M	M	M	M	M
40	07CH0767	**F**	F	F	F	F	M?	F
41	07CH0796	**M**	M	M	M	M	M	M
42	07CH0799	**M**	M	M	M	M	M	M
43	07CH0818	**M**	M	M	M	M	M	M
44	07CH0859	**M**	M	M	M	M	M	M
45	07CH0897	**F**	F	F	F	F	F	F
46	07CH0902	**M**	M	M	M	M	M	M
47	07CH0913	**F**	F	F	F	F	F	F
48	07CH0925	**M**	M	M	M	M	M	M
49	07CH0930	**M**	M	M	M	M	M	M
50	07CH1003	**M**	M	M	M	M	M	M
51	07CH1004	**M**	M	M	M	M	M	M
52	07CH1031	**F**	F	F	F	F	F	F
53	07CH1037	**F**	F	F	F	F	F	F
54	07CH1054	**M**	M	M	M	M	M	M
55	07CH1134	**M**	M	M	M	M	M	M
56	07CH1135	**F**	F	F	F	F	M?	F
57	07CH1152	**F**	F	F	F	F	F	F
58	07CH1192	**F**	F	F	F	F	F	F
59	07CH1198	**F**	F	F	F	F	F	F
60	07CH1224	**F**	F	F	F	F	F	F
61	07CH1268	**F**	F	F	F	F	F	F
62	07CH1273	**F**	F	F	F	F	F	F
63	07CH1284	**F**	F	F	F	F	F	F
64	07CH1291	**F**	F	F	F	F	F	F
65	07CH1298	**M**	M	M	M	M	M	M
66	07CH1302	**F**	F	F	F	F	F	F
67	07CH1316	**M**	M	M	M	M	M	M
68	07CH1357	**M**	M	M	M	M	M	M
69	07CH1387	**M**	M	M	M	M	M	M
70	07CH1419	**M**	M	M	M	M	M	M
71	07CH1432	**F**	F	F	F	F	F	F
72	07CH1446	**F**	F	F	F	F	F	F
73	07CH1451	**F**	F	F	F	F	F	F
74	07CH1462	**M**	M	M	M	M	M	M

Table 1 continued.								
			C - R method			B - T method		
		Consensus	Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
75	07CH1471	**M**	M	M	M	M	M	M
76	07CH1499	**M**	M	M	M	M	M	M
77	07CH1529	**F**	F	F	F	F	F	F
78	07CH1556	**M**	M	M	M	M	M	M
79	07CH1566	**F**	F	F	F	F	F	F
80	07CH1567	**F**	F	F	F	F	F	F
81	07CH1635	**M**	M	M	M	M	M	M
82	07CH1645	**F**	F	F	F	F	F	F
83	07CH1681	**M**	F - M	F - M	F - M	M - M	M - M	M - M
84	07CH1748	**M**	M	M	M	M	M	M
85	07CH1782	**F**	F	F	F	F	F	F
86	07CH1800	**M**	M	M	M	M	M	M
87	07CH1813	**M**	M	M	M	M	M	M
88	07CH1815	**M**	M	M	M	M	M	M
89	07CH1823	**M**	M	M	M	M	M	M
90	07CH1849	**M**	M	M	M	M	M	M
91	07CH1858	**M**	M	M	M	M	M	M
92	07CH1872	**F**	F	F	F	F	F	F
93	07CH1890	**M**	M	M	M	M	M	M
94	07CH1910	**M**	M	M	M	M	M	M
95	07CH1921	**M**	M	M	M	M	M	M
96	07CH1952	**M**	M	M	M	M	M	M
97	07CH1970	**F**	F	F	F	F	F	F
98	07CH1988	**F**	F	F	F	F	F	F
99	07CH2035	**F**	F	F	F	F	F	F
100	07CH2037	**M**	M	M	M	M	M	M
101	07CH2063	**M**	M	M	M	M	M	M
102	07CH2085	**F**	F	F	F	F	F	F
103	07CH2121	**M**	M	M	M	M	M	M
104	07CH2142	**F**	F	F	F	F	F	F
105	07CH2185	**F**	F	F	F	F	F	F
106	07CH2240	**M**	M	M	M	M	M	M
107	07CH2241	**F**	F	F	F	F	M?	F
108	07CH2246	**F**	F - F	F - F	F - F	M - F	M - F	M - F
109	07CH2251	**M**	M	M	M	M	M	M
110	07CH2264	**F**	F	F	F	F	F	F
111	07CH2323	**F**	F	F	F	F	F	F
112	07CH2325	**M**	M	M	M	M	M	M
113	07CH2353	**F**	F	F	F	F	F	F
114	07CH2364	**F**	F	F	F	F	F?	F
115	07CH2383	**F**	F - F	F - F	F - F	F - F	M - F	F? - F
116	07CH2407	**F**	F - F	F - F	F - F	M - F	M - F?	F? - F

Table 1 continued.								
			C - R method			B - T method		
		Consensus	Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
117	07CH2423	**NO ID**	F - F	F - F	F - F	M - F	M - M	M - F?
118	07CH2428	**M**	M	M	M	M	M	M
119	07CH2441	**M**	M	M	M	M	M	M
120	07CH2449	**M**	M	M	M	M	M	M
121	07CH2461	**M**	M	M	M	M	M	M
122	07CH2504	**M**	M	M	M	M	M	M
123	07CH2506	**F**	F	F	F	F	F	F
124	07CH2527	**F**	F	F	F	F	F	F
125	07CH2529	**F**	F	F	F	F	F	F
126	07CH2532	**F**	F	F	F	F	F	F
127	07CH2554	**F**	F	F	F	F	F	F
128	07CH2593	**M**	M	M	M	M	M	M
129	07CH2673	**M**	M	M	M	M	M	M
130	07CH2674	**F**	F	F	F	F	F	F
131	07CH2757	**F**	F	F	F	F	F	F
132	07CH2828	**M**	M	M	M	M	M	M
133	07CH2831	**F**	F	F	F	F	F	F
134	07CH2896	**F**	F	F	F	F	M?	F
135	07CH2938	**F**	F	F	F	F	F	F
136	07CH2948	**M**	M	M	M	M	M	M
137	07CH2959	**NO ID**	F - F	F - F	F - F	F? - F?	M - M	F - F?
138	07CH2981	**F**	F	F	F	F	M?	F
139	07CH3035	**F**	F	F	F	F	M?	F
140	07CH3066	**F**	F	F	F	F	F	F

Appendix 1. Biological data and gender determination of Chinook salmon collected at Roza Dam in 2007 using morphological characteristics and genetic analysis. Shading indicates discrepancies in gender determination between the two methods.

Date	DNA Sample #	**morph - ID**	**genetic - ID**	fork length	poh length	weight
05/11/2007	07CH0023	**F**	**M**	70	60	4.1
05/17/2007	07CH0075	**F**	**F**	69	59	3.6
05/17/2007	07CH0082	**F**	**F**	70	60	4.2
05/17/2007	07CH0105	**F**	**M**	64	54	3.2
05/18/2007	07CH0114	**M**	**M**	89	76	7.8
05/21/2007	07CH0160	**M**	**F**	72	61	4.2
05/21/2007	07CH0172	**F**	**F**	68	58	3.7
05/23/2007	07CH0197	**F**	**F**	74	64	4.7
05/23/2007	07CH0199	**M**	**M**	69	58	4.2
05/24/2007	07CH0233	**M**	**M**	73	61	4.7
05/24/2007	07CH0244	**F**	**F**	69	59	4.1
05/24/2007	07CH0259	**F**	**M**	87	76	7.9
05/24/2007	07CH0282	**F**	**F**	69	59	3.8
05/24/2007	07CH0312	**F**	**F**	68	58	3.8
05/24/2007	07CH0321	**F**	**F**	71	61	4.5
05/25/2007	07CH0346	**F**	**F**	66	56	3.9
05/25/2007	07CH0359	**F**	**F**	74	64	5.0
05/28/2007	07CH0420	**F**	**M**	68	58	3.8
05/28/2007	07CH0468	**F**	**M**	61	51	2.6
05/28/2007	07CH0486	**F**	**M**	68	58	3.5
05/28/2007	07CH0506	**F**	**F**	62	52	2.7
05/28/2007	07CH0514	**F**	**M**	70	60	4.0
05/28/2007	07CH0530	**F**	**F**	71	61	4.1
05/28/2007	07CH0539	**F**	**NO ID**	66	56	3.5
05/28/2007	07CH0560	**F**	**M**	66	56	3.4
05/29/2007	07CH0575	**M**	**M**	67	57	3.6
05/29/2007	07CH0586	**F**	**F**	75	65	5.0
05/30/2007	07CH0590	**F**	**F**	69	59	3.9
05/30/2007	07CH0599	**F**	**M**	64	54	3.3
05/30/2007	07CH0603	**F**	**F**	66	56	3.2
05/30/2007	07CH0626	**F**	**F**	70	60	4.1
05/31/2007	07CH0681	**F**	**F**	69	59	3.9
05/31/2007	07CH0691	**F**	**F**	71	61	4.2
05/31/2007	07CH0698	**F**	**M**	64	54	2.9
05/31/2007	07CH0716	**F**	**M**	70	60	4.3
05/31/2007	07CH0718	**F**	**F**	73	63	4.6
05/31/2007	07CH0727	**F**	**F**	70	60	4.0
05/31/2007	07CH0747	**F**	**F**	71	61	3.9
05/31/2007	07CH0762	**F**	**M**	70	60	3.8
05/31/2007	07CH0767	**F**	**F**	77	67	5.4
05/31/2007	07CH0796	**F**	**M**	70	60	3.8
05/31/2007	07CH0799	**F**	**M**	71	61	4.0
05/31/2007	07CH0818	**F**	**M**	61	51	2.4
06/01/2007	07CH0859	**M**	**M**	72	61	4.4
06/01/2007	07CH0897	**F**	**F**	66	56	3.4
06/01/2007	07CH0902	**F**	**M**	68	58	3.6
06/01/2007	07CH0913	**F**	**F**	68	58	3.3

Appendix 1 continued.

Date	DNA Sample #	**morph - ID**	**genetic - ID**	fork length	poh length	weight
06/02/2007	07CH0925	**F**	**M**	62	52	2.7
06/02/2007	07CH0930	**F**	**M**	61	51	2.4
06/04/2007	07CH1003	**F**	**M**	66	56	3.0
06/04/2007	07CH1004	**F**	**M**	61	51	2.8
06/04/2007	07CH1031	**F**	**F**	69	59	3.9
06/04/2007	07CH1037	**F**	**F**	74	64	4.5
06/04/2007	07CH1054	**M**	**M**	70	59	4.4
06/05/2007	07CH1134	**F**	**M**	66	56	3.5
06/05/2007	07CH1135	**F**	**F**	71	61	3.5
06/05/2007	07CH1152	**F**	**F**	68	58	3.5
06/07/2007	07CH1192	**F**	**F**	65	55	3.3
06/08/2007	07CH1198	**F**	**F**	67	57	3.4
06/08/2007	07CH1224	**F**	**F**	70	60	4.2
06/09/2007	07CH1268	**F**	**F**	60	50	2.6
06/09/2007	07CH1273	**F**	**F**	77	64	5.0
06/09/2007	07CH1284	**F**	**F**	68	58	3.7
06/09/2007	07CH1291	**F**	**F**	66	56	3.6
06/09/2007	07CH1298	**M**	**M**	73	60	4.2
06/09/2007	07CH1302	**F**	**F**	65	54	3.6
06/09/2007	07CH1316	**F**	**M**	66	56	3.0
06/09/2007	07CH1357	**M**	**M**	69	57	4.0
06/10/2007	07CH1387	**F**	**M**	66	56	3.3
06/10/2007	07CH1419	**M**	**M**	78	64	5.7
06/10/2007	07CH1432	**F**	**F**	68	58	3.8
06/10/2007	07CH1446	**F**	**F**	69	59	4.0
06/10/2007	07CH1451	**F**	**F**	68	58	3.8
06/10/2007	07CH1462	**F**	**M**	65	54	3.0
06/10/2007	07CH1471	**F**	**M**	63	52	2.9
06/11/2007	07CH1499	**F**	**M**	58	48	2.3
06/11/2007	07CH1529	**F**	**F**	73	63	4.2
06/12/2007	07CH1556	**F**	**M**	68	58	3.9
06/12/2007	07CH1566	**F**	**F**	73	63	4.8
06/12/2007	07CH1567	**F**	**F**	72	62	3.9
06/12/2007	07CH1635	**F**	**M**	66	56	3.1
06/12/2007	07CH1645	**F**	**F**	81	71	5.8
06/13/2007	07CH1681	**F**	**M**	72	62	4.2
06/14/2007	07CH1748	**M**	**M**	85	72	6.9
06/14/2007	07CH1782	**F**	**F**	70	60	4.2
06/14/2007	07CH1800	**F**	**M**	66	56	3.1
06/14/2007	07CH1813	**M**	**M**	70	58	3.7
06/14/2007	07CH1815	**F**	**M**	59	49	2.4
06/15/2007	07CH1823	**M**	**M**	77	65	5.0
06/15/2007	07CH1849	**M**	**M**	69	58	3.5
06/15/2007	07CH1858	**F**	**M**	61	51	2.6
06/15/2007	07CH1872	**F**	**F**	72	62	3.8
06/15/2007	07CH1890	**F**	**M**	64	54	2.8
06/16/2007	07CH1910	**M**	**M**	72	61	4.2
06/16/2007	07CH1921	**M**	**M**	62	52	2.7
06/16/2007	07CH1952	**M**	**M**	73	61	4.5
06/17/2007	07CH1970	**F**	**F**	72	62	4.1

Appendix 1 continued.

Date	DNA Sample #	**morph - ID**	**genetic - ID**	fork length	poh length	weight
06/17/2007	07CH1988	**F**	**F**	72	62	4.2
06/17/2007	07CH2035	**F**	**F**	73	63	5.1
06/17/2007	07CH2037	**M**	**M**	69	58	3.8
06/18/2007	07CH2063	**F**	**M**	71	61	3.8
06/18/2007	07CH2085	**F**	**F**	72	62	4.7
06/19/2007	07CH2121	**M**	**M**	81	69	5.9
06/20/2007	07CH2142	**F**	**F**	73	63	4.4
06/20/2007	07CH2185	**F**	**F**	75	65	4.8
06/21/2007	07CH2240	**F**	**M**	69	59	4.0
06/21/2007	07CH2241	**F**	**F**	73	63	4.6
06/21/2007	07CH2246	**F**	**F**	84	73	6.5
06/21/2007	07CH2251	**M**	**M**	69	58	4.1
06/21/2007	07CH2264	**F**	**F**	73	63	4.3
06/22/2007	07CH2323	**F**	**F**	72	62	4.4
06/22/2007	07CH2325	**M**	**M**	83	71	6.5
06/22/2007	07CH2353	**F**	**F**	75	65	4.4
06/22/2007	07CH2364	**F**	**F**	73	63	4.2
06/22/2007	07CH2383	**F**	**F**	74	64	5.1
06/23/2007	07CH2407	**F**	**F**	75	65	5.0
06/23/2007	07CH2423	**F**	**NO ID**	74	64	5.0
06/23/2007	07CH2428	**M**	**M**	66	56	3.0
06/23/2007	07CH2441	**F**	**M**	68	58	3.6
06/23/2007	07CH2449	**M**	**M**	72	60	3.9
06/24/2007	07CH2461	**M**	**M**	76	64	4.4
06/25/2007	07CH2504	**M**	**M**	72	61	3.7
06/25/2007	07CH2506	**F**	**F**	71	61	3.6
06/26/2007	07CH2527	**F**	**F**	66	56	2.9
06/26/2007	07CH2529	**F**	**F**	65	55	2.8
06/26/2007	07CH2532	**F**	**F**	73	63	4.2
06/27/2007	07CH2554	**F**	**F**	70	60	3.7
06/29/2007	07CH2593	**M**	**M**	74	62	4.3
07/03/2007	07CH2673	**M**	**M**	62	51	2.8
07/03/2007	07CH2674	**F**	**F**	72	62	4.2
07/07/2007	07CH2757	**F**	**F**	77	67	5.4
07/12/2007	07CH2828	**M**	**M**	68	56	3.3
07/12/2007	07CH2831	**F**	**F**	61	51	2.5
07/13/2007	07CH2896	**F**	**F**	73	60	4.1
07/19/2007	07CH2938	**F**	**F**	82	71	5.5
07/20/2007	07CH2948	**M**	**M**	77	63	5.2
07/25/2007	07CH2959	**F**	**NO ID**	77	67	4.8
08/01/2007	07CH2981	**F**	**F**	75	65	4.7
08/21/2007	07CH3035	**F**	**F**	70	60	2.9
09/24/2007	07CH3066	**F**	**F**	57	47	1.7

Chapter 3

DNA-Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2007

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Abstract

We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners from the hatchery-control line (two generations of hatchery influence) and the supplementation hatchery line (SH – one generation of hatchery influence) in the Cle Elum experimental spawning channel for the 2007 brood year. The assignments were based on offspring genotypes at 12 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999992. Two thousand seven hundred and ninety-one of 2,850 fry from the 2007 brood that were genotyped at seven or more loci were assigned to a parental pair with 95% confidence. We found no compelling evidence to suggest that un-genotyped parents spawned successfully in this year. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 453 for all males and from 0 to 225 for females. The sum of progeny attributed to the hatchery-control line males and females was 1,463, while the sum of progeny attributed to supplementation hatchery line males and females was 1,328.

Introduction

Although hatcheries have been extensively utilized in Chinook salmon management for over 100 years, only recently have rigorous experiments been developed to measure the relative reproductive success of hatchery- and natural-origin spawners in a shared natural setting. Some of the difficulty in designing informative studies has stemmed from the challenges of controlling entry to natural spawning areas and collecting representative samples of recently hatched fry. Furthermore, if control could be established over the potential spawners in the spawning area, the measurement of individual reproductive output still would require a means of associating individual fish captured in one year with individuals that spawned in a previous year. The spawning behavior of Chinook salmon adds to the complexity of quantifying individual reproductive output through behavioral observations: at a redd site, a female might be courted by several males that compete for access to the female, providing opportunities for multiple paternity in a single redd. In areas with moderate to high spawning densities, males might attend females on several adjacent redds. Microsatellites, a class of highly polymorphic, codominant DNA markers, provide a means to quantify individual spawners' reproductive output. A suite of 10 to 15 highly variable microsatellites can resolve individual identity in a moderate to large population, and through a simple inheritance model, can illuminate parent-offspring relationships.

Washington Department of Fish and Wildlife (WDFW) and the Yakama Nation (YN) are cooperating on a study of Chinook salmon reproductive success in a presumably closed access spawning observation channel at the Cle Elum Hatchery. Viewing blinds line the channel, allowing researchers to observe spawning activities.

Chinook salmon carrying visible external marks were released into the spawning channel in September 2006. Hatchery-control line (two generations of hatchery influence) males and females were released into three of six shared spawning areas and supplementation hatchery line (one generation of hatchery influence) males and females were released into the other three shared spawning areas to select and compete for mates. Prior to the release of the potential spawners, researchers collected and preserved samples of fin tissue to enable genetic characterization of the potential spawners and to allow subsequent inference of parent/offspring relationships after juveniles were collected and genotyped. One group of researchers examined morphological characteristics of these potential parents and observed and recorded spawning area behaviors and interactions. The results of the morphological and behavioral work are described in a separate report.

The potential parents' fin tissue samples and the collected progeny (fry) were delivered to the WDFW Molecular Genetics Laboratory in Olympia, Washington for genetic screening and parentage analysis following the same protocols that have been used from 2002 - 2007 (Young and Kassler 2005, Kassler 2005, Kassler 2006, and Kassler and Von Bargen 2007). The genetic analyses provide direct, quantitative estimates of fry production by individual spawning Chinook salmon. This report presents the parentage results for the 2006 – 2007 Cle Elum spawning channel experiments.

Materials and Methods

Collection of potential spawners – 2006
Fin tissue was collected from a total of 35 adult females and 37 adult males (Table 1) prior to their release into each of six sections in the spawning channel during September 2006. The genetic analysis program CERVUS (version 3.0; Marshall et al. 1998) was used to check for identical multilocus genotypes among the potential parents. Data recorded for each released fish included gender, and whether it was of hatchery-control line origin or supplementation hatchery line origin (Table 1).

Collection of Fry
Fry collections occurred from November 15, 2006 to May 18, 2007. Fry samples were collected from each section daily when fry were present. During that period a total of 7,733 fry were collected. These collections were sub-sampled to select fry for genetic analysis based on the proportional temporal representation recorded during fry collections. A total of 475 fry were included in the genetic analysis from each of the six sections (2,850 total).

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 spawners and offspring from 2007 were genotyped at 12 loci (Table 2). Potential spawners were screened twice and scored independently at all 12 loci by two biologists to minimize potential genotyping error of the parents.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* 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^{o}C; 40 cycles of 15 seconds at 94^{o}C, 30 seconds at 49-58^{o}C, and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 10 minutes, followed by a final indefinite holding step at 4^{o}C.

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 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

allele sizes established for the Chinook coastwide standardization efforts (Seeb et. al. 2007).

Parentage Assignments
The dataset included 34,200 single-locus genotypes. A genotyping error rate in that dataset of 1.0% would result in 342 incorrect single-locus genotypes. Our error rate is unknown, but possibly greater than 1%. 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) 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.

Genotyping error is not the only potential source of mismatches between the genotypes of fry and their putative parents. We would expect allele misidentification to be randomly distributed throughout the genotype dataset and not to occur in clusters. Parent-offspring mismatches can result also from germ-line mutation in which a parent passes a changed allele to its offspring, or from the inadvertent exclusion of one or more contributing parents from the parental dataset. These mismatches are due to correctly assigned but unexpected genotypes, and we expect that those genotypes should cluster in families. Distinguishing between mutation-based mismatches and mismatches that result from reproductive participation by un-genotyped parents is difficult. Assuming that all dams in the experimental channel are represented in the parental data set, we might suspect reproductive participation by one or more unrepresented sires if groups of fry that are assigned to a dam-offspring relationship with no mismatching loci, have multiple locus mismatches with all candidate sires, and no more than four alleles at a locus within the group. The data set was carefully examined for evidence of reproductive contributions by such un-genotyped parents (because evidence of ungenotyped parents had been observed in previous years).

Results

Parents
Genetic analysis revealed that all 72 fish released or found in the spawning channel had unique genotypes. There were a total of 19 hatchery control line (HC) adult males, 17 HC adult females, 18 supplementation hatchery line (SH) adult males, and 18 SH adult females. Approximately six HC males and six HC females were released into three of the six sections and approximately six SH males and six SH females were released into the other three sections (Table 1).

Loci Screened
A total of 12 loci were screened and all twelve were used in the analysis (Table 2). Number of alleles ranged from 5 - 30 (*Ots-9* and *Omm-1080* respectively) and observed

heterozygosity ranged from 0.361 – 0.972 (*Ots-G474* and *Omm-1080* respectively). Individual exclusionary power was below 47.0% for four loci (*Ogo-4*, *Ots-G474*, *Ots-3M*, and *Ots-9*) and above 63.0% for the remaining loci when neither parent was known. Exclusionary power was below 48% for three loci (*Omm-1080*, *Ots-3M*, and *Ots-9*) and above 64% for the remaining loci when one parent was known. Cumulative exclusionary power was 1.000000 for analysis using all loci when one parent was known.

Parentage Assignments

Parentage assignments were made when genotype data was available for seven or more loci. All 72 parents were genotyped at 11 or more loci while 2,840 of the 2,850 offspring were successfully genotyped at seven or more loci (Table 3).

Parentage analysis was conducted independently for the 475 fry collected from each of the six sections using all 72 adults as possible parents. Each fry was first assigned to all candidate female parents (dams) with positive LOD scores (log of odds). Assignments for fry that were unresolved were removed and assignments with three or more mismatches were also removed. The fry and dam groups were then re-analyzed to assign the two most likely males (sires). Those assignments yielded possible dam-sire-fry combinations (trios). Any fry-sire assignments with a negative LOD score were removed first and then any trios with more than two mismatching loci were excluded from further consideration. The remaining assignments to a candidate dam-sire for each fry were then sorted to determine all fry with only one possible trio. The remaining multiple dam-sire assignments to fry were then ranked by LOD scores and number of mismatches. The trio with zero mismatches and highest LOD score was then selected for each fry with multiple possible parent combinations where possible.

Of the total 2,850 fry included in the analysis a total of 2,791 fry were assigned to a single male and female parent (2,791/2,850 = 97.9%; Table 4).

Discussion

Approximately 98% success was achieved at inferring parent-offspring relationships. Examination of Table 5 reveals a very uneven pattern of reproductive success among the candidate parents. Based on the subsample of 2,791 fry that were successfully assigned parents, the range of inferred reproductive output among males was 0 - 453 fry; the range for the same period in reproductive output among females was 0 - 225 fry. Some of the dam-sire matings we inferred are well supported (there were a lot of fry assigned to them) and some are weakly supported (not many fry were assigned to them). Caution should be used when interpreting dam-sire-fry combinations that were inferred rarely. Future integration of fecundity estimates for spawners will enrich the interpretation of these estimates of reproductive output.

Interpretation of the inferred parental reproductive output based on parentage assignments by genetic analysis requires the consideration and analysis of individual fish attributes, including fecundity and body size, the closed nature of the experimental environment in which sub-dominant males had a more limited number of alternative

females to court than they might have had in an open system, and relative stocking levels and synchronicity of spawning.

Acknowledgements

Funding for this study was provided by the Bonneville Power Administration (BPA) and by the WA State General Funds to WDFW. The Cle Elum experimental spawning channel study was designed by Steve Schroder (WDFW) and Curt Knudsen (Oncor Consulting). Steve, Curt, and Yakama Nation staff collected the samples that were analyzed. Denise Hawkins provided editorial review and comments.

Literature Cited

Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon. Journal of Heredity 90: 281-288.

Cairney, M., J.B. Taggart, and B. Hoyheim. 2000. Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol. Ecol. 9: 2175-2178.

Greig, C., J.P. Jacobson, and M.A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered Chinook salmon (*Oncorhynchus tshawytscha*). Mol. Ecol. Notes 3:376-379.

Kassler, T.W. 2005. DNA Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2004. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2005. Portland, OR, Bonneville Power Administration 20-40**.**

Kassler, T.W. 2006. DNA Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2005. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2006. Portland, OR, Bonneville Power Administration 20-37**.**

Kassler, T.W. and J.F. Von Bargen. 2007. DNA Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2006. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2007. Portland, OR, Bonneville Power Administration**.**

Marshall, T.C. J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. **7**: 639-655.

Olsen, J.B., P.B. Bentzen, and J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Mol. Ecol. 7:1083-1090.

Rexroad, C.E., III, R.L. Coleman, A.M. Martin, W.K. Hershberger, and J. Killefer. 2001. Thirty-five polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Anim. Genet. 32:317-319.

Seeb, L.W., A. Antonovich, M.A. Banks, *et al.* 2007. Development of a standardized DNA database for Chinook Salmon. Fisheries 32:11.

Williamson, K.S., J.F. Cordes, and B.P. May. 2002. Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Mol. Ecol. Notes 2:17-19.

Young, S.F. and T.W. Kassler. 2005. Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2002 and 2003. Unpublished WDFW Genetics Laboratory Report Washington Department of Fish and Wildlife, Olympia, WA.

Table 1. Potential Chinook salmon spawners in the six sections of the Cle Elum experimental spawning channel in 2006. Origin is identified as hatchery-control (HC) or supplementation hatchery (SH).

Origin	Section 1 - 1 *Females*	Section 1 - 2 *Females*	Section 1 - 3 *Females*	Section 2 - 1 *Females*	Section 2 - 2 *Females*	Section 2 - 3 *Females*
HC	6	--	6	--	5	--
SH	--	6	--	6	--	6
	Males	*Males*	*Males*	*Males*	*Males*	*Males*
HC	6	--	6	--	7	--
SH	--	6	--	6	--	6

Table 2. Locus summary. The cumulative exclusionary power values for first and second parents are calculated as 1 minus the products of the individual locus non-exclusionary expectations (e.g. 1 - Excl(x)) for all loci.

					Individual locus		Cumulative		
Locus	N alleles	N parents genotyped	H_o (observed)	H_e (expected)	Excl(1)	Excl(2)	Excl(1)	Excl(2)	Estimated Null allele freq
Oki-100[a]	20	72	0.917	0.910	0.683	0.811	0.683	0.811	-0.0064
Ots-201b[a]	16	72	0.833	0.895	0.635	0.778	0.884	0.958	0.0336
Ots-208b[b]	25	72	0.944	0.952	0.800	0.889	0.977	0.995	-0.0003
Ssa-408[c]	17	72	0.667	0.909	0.676	0.806	0.993	0.999	0.1528
Ogo-4[d]	10	72	0.861	0.820	0.470	0.644	0.996	1.000	-0.0279
Ots-213[b]	19	71	0.915	0.930	0.731	0.845	0.999	1.000	0.0037
Ots-G474[e]	7	72	0.361	0.349	0.062	0.190	0.999	1.000	-0.0059
Omm-1080[f]	30	72	0.972	0.953	0.807	0.893	1.000	1.000	-0.0133
Ots-3M[g]	7	72	0.542	0.703	0.298	0.475	1.000	1.000	0.1415
Ots-211[b]	22	72	0.958	0.941	0.768	0.869	1.000	1.000	-0.0122
Ots-212[b]	20	72	0.931	0.902	0.653	0.790	1.000	1.000	-0.0191
Ots-9[g]	5	72	0.639	0.698	0.274	0.444	1.000	1.000	0.0322

[a] = Unpublished
[b] = Greig et al. 2003
[c] = Cairney et al. 2000
[d] = Olsen et al. 1998
[e] = Williamson et al. 2002
[f] = Rexroad et al. 2001
[g] = Banks et al. 1999

Table 3. Summary of genotyping efficiency in potential parents and offspring.

Loci genotyped	Parents (06EW)	Offspring (07EN)
12	71	2,544
11	1	179
10	0	66
9	0	31
8	0	16
7	0	4
6	0	4
5	0	1
4	0	0
3	0	1
2	0	1
1	0	0
0	0	3
	72	2,850

Table 4. Parentage among spawning pairs from each of the six sections inferred with 95% confidence for 2,791 Chinook salmon fry spawned in fall of 2006. The row and column headers describe the adult males, females, the origin, and section where each male and female were released. "N" for males indicates total number of progeny assigned to this male, "N" for females indicates a subtotal of the number of progeny assigned to this female (totals can be obtained by summing female "N" across pages [see Table 5 for totals]). Boxes indicate fry assigned to parents released in the section where fry were collected.

Section 1 - 1			**Male**	06EW 02	06EW 03	06EW 09	06EW 10	06EW 11	06EW 12
	Origin			HC	HC	HC	HC	HC	HC
		Section		1-1	1-1	1-1	1-1	1-1	1-1
Female			**N =**	5	386	36	17	0	0
06EW01	HC	1-1	151	-	151	-	-	-	-
06EW04	HC	1-1	107	-	104	-	3	-	-
06EW05	HC	1-1	30	5	25	-	-	-	-
06EW06	HC	1-1	8	-	-	8	-	-	-
06EW07	HC	1-1	106	-	106	-	-	-	-
06EW08	HC	1-1	42	-	-	28	14	-	-

Section 1 - 2			**Male**	06EW 02	06EW 03	06EW 09	06EW 13	06EW 14	06EW 16	06EW 17	06EW 20	06EW 21	06EW 29	06EW 32
	Origin			HC	HC	HC	SH	SH	SH	SH	SH	SH	HC	HC
		Section		1-1	1-1	1-1	1-2	1-2	1-2	1-2	1-2	1-2	1-3	1-3
Female			**N =**	1	57	2	113	47	30	181	0	24	2	7
06EW01	HC	1-1	25	-	25	-	-	-	-	-	-	-	-	-
06EW04	HC	1-1	12	-	12	-	-	-	-	-	-	-	-	-
06EW05	HC	1-1	18	1	17	-	-	-	-	-	-	-	-	-
06EW07	HC	1-1	3	-	3	-	-	-	-	-	-	-	-	-
06EW08	HC	1-1	2	-	-	2	-	-	-	-	-	-	-	-
06EW15	SH	1-2	94	-	-	-	-	8	-	86	-	-	-	-
06EW18	SH	1-2	0	-	-	-	-	-	-	-	-	-	-	-
06EW19	SH	1-2	55	-	-	-	55	-	-	-	-	-	-	-
06EW22	SH	1-2	101	-	-	-	-	-	13	70	-	18	-	-
06EW23	SH	1-2	103	-	-	-	58	39	-	-	-	6	-	-
06EW24	SH	1-2	42	-	-	-	-	-	17	25	-	-	-	-
06EW26	HC	1-3	3	-	-	-	-	-	-	-	-	-	-	3
06EW28	HC	1-3	1	-	-	-	-	-	-	-	-	-	-	1
06EW31	HC	1-3	3	-	-	-	-	-	-	-	-	-	-	3
06EW34	HC	1-3	2	-	-	-	-	-	-	-	-	-	2	-

Table 4 continued.

Section 1 - 3			**Male**	06EW 03	06EW 09	06EW 13	06EW 14	06EW 16	06EW 17	06EW 21	06EW 29	06EW 30	06EW 32	06EW 33	06EW 35	06EW 36
	Origin			HC	HC	SH	SH	SH	SH	SH	HC	HC	HC	HC	HC	HC
		Section		1-1	1-1	1-2	1-2	1-2	1-2	1-2	1-3	1-3	1-3	1-3	1-3	1-3
Female			**N =**	7	1	12	4	5	28	1	259	11	121	4	17	0
06EW01	HC	1-1	3	3	-	-	-	-	-	-	-	-	-	-	-	-
06EW05	HC	1-1	3	3	-	-	-	-	-	-	-	-	-	-	-	-
06EW07	HC	1-1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
06EW08	HC	1-1	1	-	1	-	-	-	-	-	-	-	-	-	-	-
06EW15	SH	1-2	15	-	-	-	1	-	14	-	-	-	-	-	-	-
06EW19	SH	1-2	9	-	-	9	-	-	-	-	-	-	-	-	-	-
06EW22	SH	1-2	14	-	-	-	2	-	11	1	-	-	-	-	-	-
06EW23	SH	1-2	4	-	-	3	1	-	-	-	-	-	-	-	-	-
06EW24	SH	1-2	8	-	-	-	-	5	3	-	-	-	-	-	-	-
06EW25	HC	1-3	32	-	-	-	-	-	-	-	32	-	-	-	-	-
06EW26	HC	1-3	29	-	-	-	-	-	-	-	-	11	18	-	-	-
06EW27	HC	1-3	116	-	-	-	-	-	-	-	108	-	-	4	4	-
06EW28	HC	1-3	45	-	-	-	-	-	-	-	-	-	45	-	-	-
06EW31	HC	1-3	60	-	-	-	-	-	-	-	-	-	50	-	10	-
06EW34	HC	1-3	130	-	-	-	-	-	-	-	119	-	8	-	3	-

Section 2 - 1			**Male**	06EW 03	06EW 17	06EW 29	06EW 32	06EW 37	06EW 38	06EW 40	06EW 42	06EW 44	06EW 45	06EW 50
	Origin			HC	SH	HC	HC	SH	SH	SH	SH	SH	SH	HC
		Section		1-1	1-2	1-3	1-3	2-1	2-1	2-1	2-1	2-1	2-1	2-2
Female			**N =**	2	1	12	1	14	277	22	0	50	92	2
06EW01	HC	1-1	2	2	-	-	-	-	-	-	-	-	-	-
06EW15	SH	1-2	1	-	1	-	-	-	-	-	-	-	-	-
06EW25	HC	1-3	5	-	-	5	-	-	-	-	-	-	-	-
06EW27	HC	1-3	3	-	-	3	-	-	-	-	-	-	-	-
06EW28	HC	1-3	1	-	-	-	1	-	-	-	-	-	-	-
06EW34	HC	1-3	4	-	-	4	-	-	-	-	-	-	-	-
06EW39	SH	2-1	91	-	-	-	-	-	38	-	-	-	53	-
06EW41	SH	2-1	36	-	-	-	-	13	23	-	-	-	-	-
06EW43	SH	2-1	60	-	-	-	-	-	5	19	-	36	-	-
06EW46	SH	2-1	53	-	-	-	-	-	-	-	-	14	39	-
06EW47	SH	2-1	61	-	-	-	-	-	61	-	-	-	-	-
06EW48	SH	2-1	154	-	-	-	-	1	150	3	-	-	-	-
06EW49	HC	2-2	1	-	-	-	-	-	-	-	-	-	-	1
06EW52	HC	2-2	1	-	-	-	-	-	-	-	-	-	-	1

Table 4 continued.

Section 2 - 2			Male	06EW 03	06EW 38	06EW 50	06EW 51	06EW 53	06EW 56	06EW 57	06EW 59	06EW 60	06EW 67
	Origin			HC	SH	HC	HC	HC	HC	HC	HC	HC	SH
		Section		1-1	2-1	2-2	2-2	2-2	2-2	2-2	2-2	2-2	2-3
Female			N =	1	20	374	3	5	0	36	0	28	3
06EW01	HC	1-1	1	1	-	-	-	-	-	-	-	-	-
06EW39	SH	2-1	1	-	1	-	-	-	-	-	-	-	-
06EW47	SH	2-1	3	-	3	-	-	-	-	-	-	-	-
06EW48	SH	2-1	16	-	16	-	-	-	-	-	-	-	-
06EW49	HC	2-2	100	-	-	64	3	2	-	30	-	1	-
06EW52	HC	2-2	205	-	-	205	-	-	-	-	-	-	-
06EW54	HC	2-2	51	-	-	24	-	-	-	-	-	27	-
06EW55	HC	2-2	90	-	-	81	-	3	-	6	-	-	-
06EW58	HC	2-2	0	-	-	-	-	-	-	-	-	-	-
06EW61	SH	2-3	2	-	-	-	-	-	-	-	-	-	2
06EW66	SH	2-3	1	-	-	-	-	-	-	-	-	-	1

Section 2 - 3			Male	06EW 29	06EW 38	06EW 50	06EW 51	06EW 57	06EW 60	06EW 62	06EW 63	06EW 64	06EW 67	06EW 68	06EW 69
	Origin			HC	SH	HC	HC	HC	HC	SH	SH	SH	SH	SH	SH
		Section		1-3	2-1	2-2	2-2	2-2	2-2	2-3	2-3	2-3	2-3	2-3	2-3
Female			N =	1	3	57	1	2	5	0	19	56	230	0	96
06EW34	HC	1-3	1	1	-	-	-	-	-	-	-	-	-	-	-
06EW48	SH	2-1	3	-	3	-	-	-	-	-	-	-	-	-	-
06EW49	HC	2-2	4	-	-	3	1	-	-	-	-	-	-	-	-
06EW52	HC	2-2	19	-	-	19	-	-	-	-	-	-	-	-	-
06EW54	HC	2-2	9	-	-	4	-	-	5	-	-	-	-	-	-
06EW55	HC	2-2	33	-	-	31	-	2	-	-	-	-	-	-	-
06EW61	SH	2-3	68	-	-	-	-	-	-	-	4	10	51	-	3
06EW65	SH	2-3	37	-	-	-	-	-	-	-	-	-	37	-	-
06EW66	SH	2-3	81	-	-	-	-	-	-	-	15	-	66	-	-
06EW70	SH	2-3	126	-	-	-	-	-	-	-	-	16	21	-	89
06EW71	SH	2-3	13	-	-	-	-	-	-	-	-	9	-	-	4
06EW72	SH	2-3	76	-	-	-	-	-	-	-	-	21	55	-	-

Table 5. Total number of offspring assigned to females and males from each of the six sections in the spawning channel and the life stage (HC - hatchery control line; SH - supplementation hatchery line) for each fish.

Females	Section	HC or SH	Total Offspring
06EW0001	1 - 1	HC	182
06EW0004	1 - 1	HC	119
06EW0005	1 - 1	HC	51
06EW0006	1 - 1	HC	8
06EW0007	1 - 1	HC	110
06EW0008	1 - 1	HC	45
06EW0015	1 - 2	SH	110
06EW0018	1 - 2	SH	0
06EW0019	1 - 2	SH	64
06EW0022	1 - 2	SH	115
06EW0023	1 - 2	SH	107
06EW0024	1 - 2	SH	50
06EW0025	1 - 3	HC	37
06EW0026	1 - 3	HC	32
06EW0027	1 - 3	HC	119
06EW0028	1 - 3	HC	47
06EW0031	1 - 3	HC	63
06EW0034	1 - 3	HC	137
06EW0039	2 - 1	SH	92
06EW0041	2 - 1	SH	36
06EW0043	2 - 1	SH	60
06EW0046	2 - 1	SH	53
06EW0047	2 - 1	SH	64
06EW0048	2 - 1	SH	173
06EW0049	2 - 2	HC	105
06EW0052	2 - 2	HC	225
06EW0054	2 - 2	HC	60
06EW0055	2 - 2	HC	123
06EW0058	2 - 2	HC	0
06EW0061	2 - 3	SH	70
06EW0065	2 - 3	SH	37
06EW0066	2 - 3	SH	82
06EW0070	2 - 3	SH	126
06EW0071	2 - 3	SH	13
06EW0072	2 - 3	SH	76
			2,791

Males	Section	HC or SH	Total Offspring
06EW0002	1 - 1	HC	6
06EW0003	1 - 1	HC	453
06EW0009	1 - 1	HC	39
06EW0010	1 - 1	HC	17
06EW0011	1 - 1	HC	0
06EW0012	1 - 1	HC	0
06EW0013	1 - 2	SH	125
06EW0014	1 - 2	SH	51
06EW0016	1 - 2	SH	35
06EW0017	1 - 2	SH	210
06EW0020	1 - 2	SH	0
06EW0021	1 - 2	SH	25
06EW0029	1 - 3	HC	274
06EW0030	1 - 3	HC	11
06EW0032	1 - 3	HC	129
06EW0033	1 - 3	HC	4
06EW0035	1 - 3	HC	17
06EW0036	1 - 3	HC	0
06EW0037	2 - 1	SH	14
06EW0038	2 - 1	SH	300
06EW0040	2 - 1	SH	22
06EW0042	2 - 1	SH	0
06EW0044	2 - 1	SH	50
06EW0045	2 - 1	SH	92
06EW0050	2 - 2	HC	433
06EW0051	2 - 2	HC	4
06EW0053	2 - 2	HC	5
06EW0056	2 - 2	HC	0
06EW0057	2 - 2	HC	38
06EW0059	2 - 2	HC	0
06EW0060	2 - 2	HC	33
06EW0062	2 - 3	SH	0
06EW0063	2 - 3	SH	19
06EW0064	2 - 3	SH	56
06EW0067	2 - 3	SH	233
06EW0068	2 - 3	SH	0
06EW0069	2 - 3	SH	96
			2,791

Chapter 4

DNA-Based Population-of-Origin Assignments of Chinook Salmon Smolts Outmigrating Past Chandler Trap at Prosser Dam (Yakima River) in 2007

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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 2007. The trap was under construction from the end of January through the third week of March, therefore the January and March strata only represent a few sampling days and reduced number of outmigrating smolts. Mixture analysis was conducted on a proportional subsample of 1,500 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 87.9% 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 1,466/1,482 (98.9%) of the smolts. In addition to conducting stock-of-origin assignments for the outmigrating smolts, a collection of the Marion Drain fall stock from 2007 was assessed for genetic similarity to previous Marion Drain collections and lower Yakima River fall collections. The ability of the baseline to accurately assign individuals to each of the five stocks (3 spring and 2 fall) was also assessed using known-origin samples from the Naches River and upper Yakima River.

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 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 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 - 2006, 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 2007. We also present results of a comparison between a 2007 collection of fall Chinook salmon from Marion Drain to previous collections from Marion Drain and the lower Yakima River. Fall Chinook from Marion Drain collected in 2007 were compared to samples collected at Marion Drain in 1989, 1992, 1993, and 2005. The majority of samples from the 2005 Marion Drain collection assigned to lower Yakima River with over 90% posterior probability (Kassler and Von Bargen 2007). The new collection from 2007 was analyzed to determine if the 2007 Marion Drain samples assigned to Marion Drain or to the lower Yakima River. Additionally, three collections of known-origin spring Chinook samples from the Naches River and upper Yakima River were analyzed to evaluate the ability of the baseline to accurately assign individuals to the correct population-of-origin.

Materials and Methods

Collections

There were no collections added to the Yakima River baseline this year. Since 1989, sampling crews from the Yakama Nation and WDFW have collected adult spawning ground tissue samples to be included in the baseline. The tissue samples consisted of dry-mounted scales or fin tissue preserved in 100% ethanol from five baseline stocks collected across multiple years (American River spring, Naches River spring, upper Yakima River spring, Marion Drain fall, and lower Yakima River fall; Table 1 and Figure 1).

A total of 27 fall Chinook were collected from the wheel on Marion Drain. The wheel is located approximately three miles from the confluence of Marion Drain and the lower Yakima River. It is the location where broodstock is taken for production of the Marion Drain Chinook and is within the spawning location of the naturally spawning Marion Drain Chinook population.

Known-origin Chinook samples were collected in two years from the Naches River (2006 & 2007) and one year from the upper Yakima River (2007). A total of 25 samples from the Naches River were analyzed and 35 samples from the upper Yakima River.

An estimated total of 159,252 smolts passed the lower Yakima River at Chandler from January 9 – July 9, 2007. This estimate, derived by Doug Neeley and staff (Yakama Nation) was based on expansion of the total number of smolts counted at the Chandler trap (17,965) to account for trap efficiency, etc. Unknown-origin smolts were collected at Prosser Dam (Chandler Trap) following a sampling design that would identify a proportional number of smolt samples that represents the entire smolt outmigration. The trap was not operational during the end of January through the end of March therefore the proportional sampling design is based on times when samples were collected while the trap was being operated. The following five time strata (January, March, April, May, and June – July) were used for analysis. Samples were collected from January 9 – January 29 and resumed on March 22 and continued until July 9, 2007. These samples were genetically analyzed to get reliable estimates of population proportions. 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" day's sample was taken (e.g. 10 fish). If the number of smolts was above the threshold then a "peak" day's sample was taken (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. These values were determined by analyzing the number of "peak" and "standard" days counted during four years of smolt outmigration monitoring. Based on this sampling design, 2,361 Chinook smolt samples were collected for genetic analysis.

The total estimated numbers of smolts passing the Chandler Trap each day were plotted with the total number of genetic samples that had been collected. A process was then employed to proportionalize the available genetic samples with the daily counts to provide a representative number of smolts that were outmigrating from January – July. A total of 1,500 smolts were identified for analysis.

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 $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units Go*Taq* 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^{o}C; 40 cycles of 15 seconds at 94^{o}C, 30 seconds at 50-60^{o}C, and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 10 minutes, followed by a final indefinite holding step at 10^{o}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.

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, the fall Chinook collections, and the known-origin-smolts were accomplished with GMA (Kalinowski 2003) using a 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
Smolts were categorized as spring or fall Chinook when they were intercepted at the Chandler Trap based on morphological characteristics. Three morphological features (length, size of the eye, and snout shape) were used to identify smolts as spring or fall (Mark Johnston, Yakama Nation; pers. comm.).

Results

Collections

A total of 1,500 unknown Chinook smolts were selected and analyzed from those collected at Chandler Trap. A total of 26 known-origin adult samples were analyzed from Marion Drain (one sample was dropped because of missing data), 25 adult samples from Naches River (0 samples were dropped because of missing data), and 35 adult samples from the upper Yakima River (0 samples were dropped because of missing data). Smolt samples that were missing data for five or more loci (N = 18) were dropped from statistical analyses.

Population-of-origin Analysis

The mixture composition estimates for the entire 2007 smolt outmigration indicated that the largest overall percentage of spring smolts was from the upper Yakima River followed by the Naches River and American River in the first four strata for samples that assigned with the 90% posterior probability threshold. During the migration from January – May, the proportion of the Naches River and upper Yakima River stocks was between 18.2 and 72.7% while the American River spring stock and the two fall stocks were between 0.0 – 18.7% (Table 3). The lower Yakima River had the highest percentage in the June - July time stratum (87.9%).

The samples from the 2007 collection of Marion Drain fall Chinook were analyzed using a baseline that included the 1989 collection from Marion Drain and 1990 collection from the lower Yakima River. A total of 15 samples from the 2007 collection from Marion Drain assigned at the 90% threshold and six of those samples assigned to Marion Drain (Table 4).

The known-origin samples from Naches and the upper Yakima River were assigned using a baseline that included populations from the American River, Naches R, upper Yakima River, Marion Drain (without the 2005 and 2007 collections), and the lower Yakima River. These known samples were analyzed with approximately equal numbers of known-origin samples from the Naches River and upper Yakima River. A total of 16 of the 25 samples from the Naches River assigned at the 90% threshold and 14 of those assigned to the Naches River (Table 4). Thirty-two of the 35 samples from the upper Yakima River assigned at the 90% threshold and all 32 assigned to the upper Yakima River.

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 40 of 40 smolts in January, 86 of 87 smolts in March, 801 of 811 smolts in April, 355 of 359 smolts in May, and 200 of 203 in the June/July time strata were scored for six or more loci, and therefore included in the analysis (Appendix 1). Results for the time strata were as follows: January time stratum – all 40 smolts were assigned identically using morphological and genetic methods (40 spring). March time stratum – all 86 smolts were assigned identically using morphological and genetic methods (86 spring). April time stratum – all 801 smolts were assigned identically using morphological and genetic methods (800 spring and 1 fall). May time stratum - 354 smolts were assigned identically using morphological and genetic methods (287 spring and 67 fall), the remaining smolt was identified as a fall Chinook by morphological methods and a spring Chinook by genetic analysis. June/July time stratum – 185 smolts were assigned identically using morphological and genetic methods (19 spring and 166

fall), 4 of the remaining smolts were identified as fall by morphological methods and spring by genetic analysis and the remaining 11 smolts were identified as spring by morphological analysis and fall by genetic analysis.

Discussion

Collection of smolts at the Chandler Trap in 2007 utilized a sampling design intended to yield a sample that was proportional to the number of smolts passing the Chandler Trap. Sampling a proportional number of smolts was important to determine an accurate percentage of smolts from each stock that were outmigrating from the basin. Developing the sampling strategy for identifying a "standard" versus "peak" day of smolts that were in the trap and applying a sampling goal for those days allowed for a proportional sample. Subsampling the smolts collected for genetic analysis provided a best fit to the actual passage of smolts for a given day.

Monitoring the relative abundances of Chinook smolts in the Yakima River from the three different 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 vast 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 known-origin samples and 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 and the known-origin collections 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.

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 1,280 out of 1,281 smolts collected during the January - May time strata. The comparison in the June – July strata revealed more differences (15 differences out of 200 total smolts analyzed).

Over 99% of the assignments from the early time stratum (January) were from the three spring stocks with 0.2% assigning to the lower Yakima River fall stock. The upper Yakima River spring stock accounted for the highest percentage (72.7%) of smolts present. Rank in abundance of the three spring stocks was the same in the first four time strata (January, 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 87.9% of the total

number of smolts. These genetic results should allow a reliable estimation of relative stock-specific smolt production in this system.

A baseline of the 1989 collection from Marion Drain and the 1990 collection from the lower Yakima River was used when evaluating the 2005 and 2007 samples from Marion Drain to represent what fall Chinook in Marion Drain would be most like. Comparison of the 2005 and 2007 Marion Drain samples to the three earlier collections from Marion Drain and to the collections from the lower Yakima River indicates that more individuals assign to the lower Yakima River population than Marion Drain. Only three samples (3/29 = 10.3%) from the 2005 Marion Drain assigned to Marion Drain and six individuals (6/15 = 40.0%) from the 2007 Marion Drain collection assigned to Marion Drain. This identifies the presence of Marion Drain fall Chinook that are mixed in with lower Yakima River fall Chinook.

The known-origin samples from the Naches River and upper Yakima River assigned a majority of the samples to each of their respective populations-of-origin. Twelve of the 14 samples (85.7%) from the Naches River collections correctly assigned back to the Naches River and all 32 samples from the upper Yakima River correctly assigned back to the upper Yakima River. The high assignment rate back to the correct population-of-origin provides confidence in the baseline to correctly assign unknown samples to the correct population-of-origin.

Assessment of DNA Mixture Assignments from 2000 – 2007

Mixed stock analysis has been conducted on Chandler smolts since 2000 (Young 2004, Kassler et al. 2005, Kassler 2006, and Kassler and Von Bargen 2007), however the sampling design for samples collected in 2000 – 2003 was not proportionalized during the run. The yearly assignments are therefore not comparable from those years. Beginning in 2004, staff at the Chandler trap utilized a sampling protocol to provide a number of smolts that was relative to the percentage of smolts passing that day. Samples were then subsampled at WDFW to provide a proportional number of samples that would represent the overall passage to be 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. Melinda Davis (YN) and staff provided the 2007 samples from Marion Drain. Doug Neeley and Dave Lind (Yakama Nation) provided Chandler Trap Chinook count and passage data. We also thank Ken Warheit for the methodology of the proportional subsampling scheme.

Literature cited

Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon. Journal of Heredity 90: 281-288.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 2001. *Genetix, logiciel sous Windows* TM *pour la genetique des populations*. Laboratoire Genome, Populations, Interactions: CNRS UMR 5000, Universite de Montpellier II, Montpellier, France.

Cairney, M., J.B. Taggart, and B. Hoyheim. 2000. Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Molecular Ecology 9: 2175-2178.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

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

Greig, C., J.P. Jacobson, and M.A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3:376-379.

Hauser, L., T.R. Seamons, M. Dauer, K.A. Naish, and T.P. Quinn. 2006. An empircal verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. Molecular Ecology 15(11): 3157-3173.

Kalinowski, S. 2003. Genetic Mixture Analysis 1.0. Department of Ecology, Montana State University, Bozeman, MT 59717. Available for download from http://www.montana.edu/kalinowski

Kassler, T.W., M. Johnston, S.F. Young, and J.B. Shaklee. 2005. DNA based stock-of-origin assignments of Chinook salmon smolts outmigrating past Chandler Trap (Yakima River) in 2004. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2005. Portland, OR, Bonneville Power Administration 41-77**.**

Kassler, T.W. 2006. DNA based stock-of-origin assignments of Chinook salmon smolts outmigrating past Chandler Trap (Yakima River) in 2005. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2006. Portland, OR, Bonneville Power Administration 138-181**.**

Kassler, T.W. and J. Von Bargen. 2007. DNA based stock-of-origin assignments of Chinook salmon smolts outmigrating past Chandler Trap (Yakima River) in 2006. Yakima/Klickitat Fisheries Project Genetic Studies. Annual Report 2007. Portland, OR, Bonneville Power Administration**.**

Olsen, J.B., P.B. Bentzen, and J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1083-1090.

O'Reilly, P.T., L.C. Hamilton, S.K. McConnell, and J.M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotideand tertanucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53:2292-2298.

Page, R.D.M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Computer Application Biosciences 12:351-358.
Rannala, B. and J.L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA 94, 9197-9201.
Raymond, M. and F. Rousset. 1995. GENEPOP (ver. 1.2): A population genetics software for exact test and ecumenicism. Journal of Heredity 86:248-249.
Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.
Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.
Seeb, L.W., A. Antonovich, M.A. Banks, *et al.* 2007. Development of a Standardized DNA Database for Chinook Salmon. Fisheries 32:11.
Taylor, E.B. and A.B. Costello. 2006. Microsatellite DNA analysis of coastal populations of bull trout (*Salvelinus confluentus*) in British Columbia: zoogeographic implications and its application to recreational fishery management. Canadian Journal of Fisheries and Aquatic Sciences 63(5):1157-1171.
Waples, R.S. and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15(6):1419-1439(21).
Williamson, K.S., J.F. Cordes, and B.P. May. 2002. Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2:17-19.
Young, S.F. 2004. Year 2003 Chandler Chinook Smolt Stock-of-Origin Assignments. WDFW Genetics Laboratory unpublished report (Washington Department of Fish and Wildlife) Olympia, WA.

Figure 1. Geographic location of the Chandler trap on the Yakima River, Washington and the primary streams in the basin.

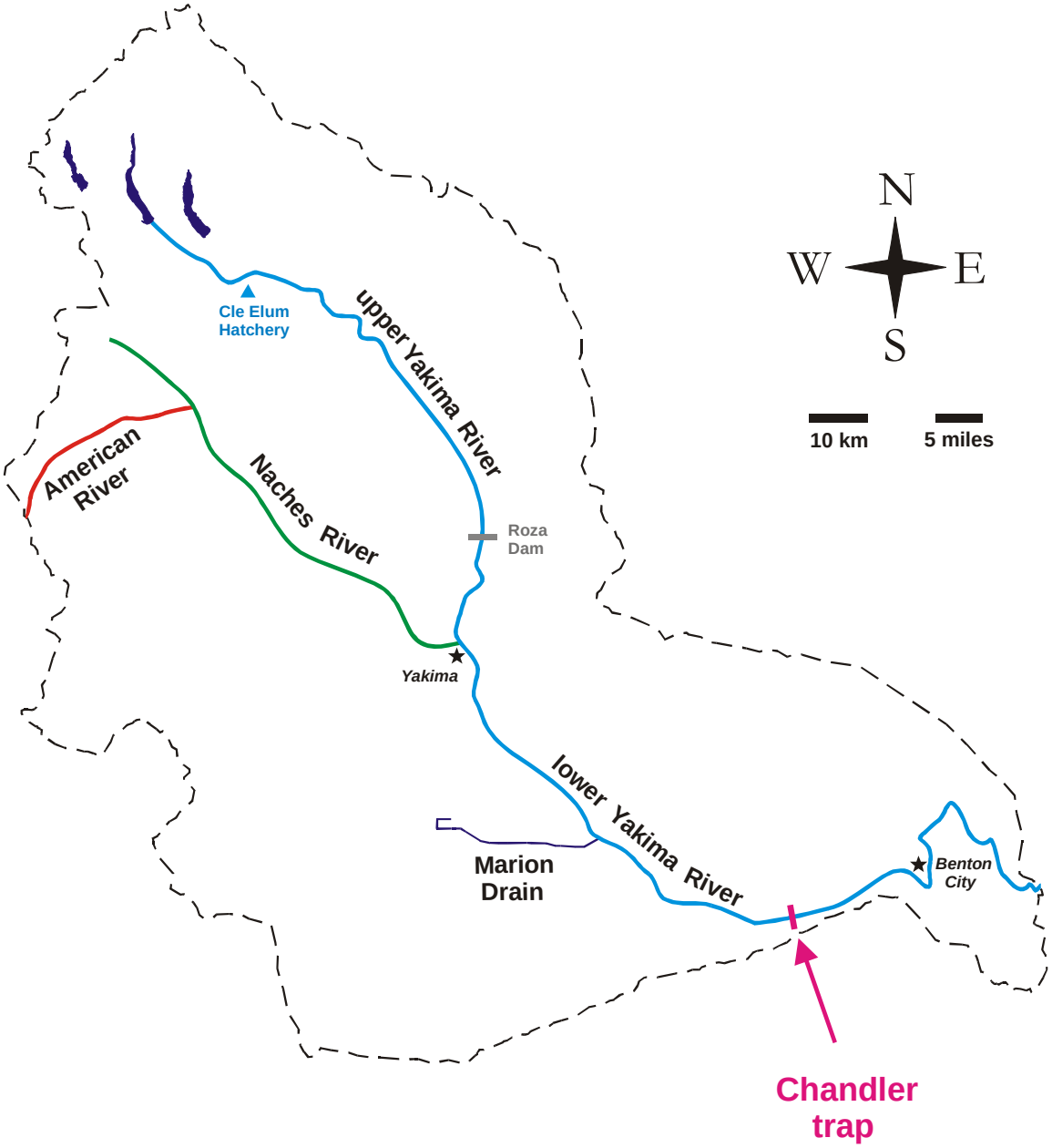

Table 1. Nineteen Chinook salmon collections assembled into a baseline and used for the analysis of the known-origin and unknown-origin smolts. "*" the 05LU collection from Marion Drain was not used in the baseline, but is listed here as a collection from Marion Drain. The percentage of single locus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

Baseline Collections	Collection Code	# Processed	# Analyzed	% Single Locus Genotypes Missing
American River - spring	89AG	80	77	10.4%
	91DQ	102	87	9.8%
	93DO	18	17	3.2%
	03EH	100	70	6.6%
		300	251	8.6%
Naches River - spring	89AC	76	74	11.4%
	89AI	26	22	7.0%
	93DQ	50	45	6.3%
	93DR	32	25	7.3%
little Naches River - spring	04BI	42	41	2.2%
	04EM	56	45	9.9%
		282	252	7.9%
upper Yakima River - spring	92DN	24	23	5.9%
	97DA	123	115	3.9%
	03GO	99	99	1.4%
		246	237	3.0%
Marion Drain - fall	89BX	100	92	8.3%
	92FQ	92	92	5.4%
	93DY	8	8	8.0%
	05LU*	65	47	15.3%
		265	239	8.6%
lower Yakima River - fall	90DF	109	104	12.6%
	93DW	82	80	9.8%
	98FB	61	50	8.7%
		252	234	10.8%
Chandler Trap Smolts - 2007	07AE	1,500	1,482	1.2%
Known-origin samples				
Marion Drain - fall	07HP	27	26	2.1%
Naches River - spring	06IM	4	4	0.0%
Naches River - spring	07KS	21	21	1.4%
upper Yakima River - spring	07CH	35	35	3.5%

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 smolts collections and heterozygosity (observed (H_o) and expected (H_e)) for each locus.

Multiplex	Locus	Annealing temp ^oC	# Alleles/ Locus	Allele Size Range (bp)	% missing genotypes baseline N = 1,213	% missing genotypes smolts N = 1,482	Heterozygosity H_o	Heterozygosity H_e
Ots-M	*Oki-100* [a]	50	41	164 - 365	11.4	1.0	0.913	0.940
	Ots-201b [a]	50	42	137 - 310	7.3	0.5	0.916	0.936
	Ots-208b [b]	50	52	158 - 342	9.9	5.0	0.943	0.954
	Ssa-408 [c]	50	32	184 - 308	4.0	2.9	0.827	0.934
Ots-N	*Ogo-2* [d]	60	19	202 - 256	4.5	0.5	0.756	0.854
	Ssa-197 [e]	60	38	181 - 318	11.9	0.3	0.915	0.940
Ots-O	*Ogo-4* [d]	56	17	132 - 164	15.6	0.1	0.776	0.884
	Ots-213 [b]	56	40	182 - 362	9.4	1.4	0.908	0.940
	Ots-G474 [f]	56	15	152 - 212	3.8	0.7	0.507	0.697
Ots-R	*Ots-3M* [g]	53	15	128 - 158	2.9	0.3	0.601	0.672
Ots-S	*Ots-9* [g]	60	8	99 - 113	5.0	1.1	0.668	0.709

[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.

	American R.	Naches R.	upper Yakima R.	Marion Drain	lower Yakima R.	Total
January	3	6	24	0	0	33
March	9	20	33	0	0	62
April	42	153	424	0	1	620
May	36	64	115	2	50	267
June-July	1	1	18	1	153	174

	American R.	Naches R.	upper Yakima R.	Marion Drain	lower Yakima R.
January	9.1%	18.2%	72.7%	0.0%	0.0%
March	14.5%	32.3%	53.2%	0.0%	0.0%
April	6.8%	24.7%	68.4%	0.0%	0.2%
May	13.5%	24.0%	43.1%	0.7%	18.7%
June-July	0.6%	0.6%	10.3%	0.6%	87.9%

Table 4. Population-of-origin assignments for the known-origin individuals from Marion Drain, Naches River, and the upper Yakima River. Samples with a posterior probability of 90% or greater are shown in bold type.

Marion Drain

Individual in Mixture	Best Estimate	Posterior Probability	2nd Best Estimate	Posterior Probability	3rd Best Estimate	Posterior Probability
07HP0001	low Yakima R	0.9991				
07HP0002	low Yakima R	0.9900				
07HP0003	low Yakima R	0.7449	Marion Drain	0.2551		
07HP0004	Marion Drain	0.7768	low Yakima R	0.2232		
07HP0005	low Yakima R	0.9564	Marion Drain	0.0436		
07HP0006	low Yakima R	0.9968				
07HP0007	low Yakima R	0.5708	Marion Drain	0.4292		
07HP0008	Marion Drain	0.9985				
07HP0009	Marion Drain	0.8720	low Yakima R	0.1280		
07HP0010	low Yakima R	0.9999				
07HP0011	low Yakima R	0.8107	Marion Drain	0.1892		
07HP0012	low Yakima R	0.9137	Marion Drain	0.0863		
07HP0013	Marion Drain	0.8569	low Yakima R	0.1431		
07HP0014	low Yakima R	0.9999				
07HP0015	Marion Drain	0.9490	low Yakima R	0.0510		
07HP0016	Marion Drain	0.9792	low Yakima R	0.0208		
07HP0017	low Yakima R	1.0000				
07HP0018	low Yakima R	0.9895	Marion Drain	0.0105		
07HP0019	Naches River	0.5642	Marion Drain	0.2390	low Yakima R	0.1787
07HP0020	low Yakima R	0.9948				
07HP0021	low Yakima R	0.5363	Marion Drain	0.4637		
07HP0022	low Yakima R	0.5752	Marion Drain	0.4248		
07HP0023	Marion Drain	0.6744	low Yakima R	0.3256		
07HP0025	low Yakima R	0.7729	Marion Drain	0.2271		
07HP0026	low Yakima R	1.0000				
07HP0027	low Yakima R	0.7884	Marion Drain	0.2116		

Naches River

Individual in Mixture	Best Estimate	Posterior Probability	2nd Best Estimate	Posterior Probability	3rd Best Estimate	Posterior Probability
06IM0001	Naches River	0.9990				
06IM0002	Naches River	0.9983				
06IM0003	Naches River	0.6567	up Yakima River	0.3428		
06IM0004	Naches River	0.4521	up Yakima River	0.3785	American River	0.1694
07KS0001	Naches River	0.8164	up Yakima River	0.1831		
07KS0002	Naches River	0.9102	up Yakima River	0.0727	American River	0.0171
07KS0003	American River	0.7322	Naches River	0.2669		
07KS0004	Naches River	0.9966				
07KS0005	Naches River	0.9339	up Yakima River	0.0659		
07KS0006	American River	0.9712	Naches River	0.0287		
07KS0007	American River	0.6029	Naches River	0.3969		
07KS0008	Naches River	0.9684	up Yakima River	0.0313		
07KS0009	Naches River	0.9825	American River	0.0164		

Table 4 continued.

Naches River continued.

Individual in Mixture	Best Estimate	Posterior Probability	2nd Best Estimate	Posterior Probability	3rd Best Estimate	Posterior Probability
07KS0010	Naches River	0.9277	up Yakima River	0.0723		
07KS0011	Naches River	0.9684	up Yakima River	0.0313		
07KS0012	Naches River	0.6208	up Yakima River	0.3791		
07KS0013	Naches River	0.9372	up Yakima River	0.0628		
07KS0014	Naches River	0.9964				
07KS0015	Naches River	0.9999				
07KS0016	up Yakima River	0.6077	Naches River	0.3919		
07KS0017	up Yakima River	0.5140	Naches River	0.4859		
07KS0018	up Yakima River	0.7645	Naches River	0.2354		
07KS0019	Naches River	0.9907				
07KS0020	Naches River	0.9556	up Yakima River	0.0345		
07KS0021	up Yakima River	0.9907				

upper Yakima River

Individual in Mixture	Best Estimate	Posterior Probability	2nd Best Estimate	Posterior Probability	3rd Best Estimate	Posterior Probability
07CH0023	up Yakima River	0.9860	Naches River	0.0140		
07CH0114	up Yakima River	0.9275	Naches River	0.0725		
07CH0199	up Yakima River	0.9820	Naches River	0.0180		
07CH0282	up Yakima River	0.9881	Naches River	0.0119		
07CH0359	Naches River	0.5955	up Yakima River	0.4045		
07CH0506	up Yakima River	1.0000				
07CH0560	up Yakima River	0.9668	Naches River	0.0332		
07CH0599	up Yakima River	0.9142	Naches River	0.0858		
07CH0691	up Yakima River	1.0000				
07CH0727	up Yakima River	0.9962				
07CH0796	up Yakima River	1.0000				
07CH0897	up Yakima River	0.9434	Naches River	0.0566		
07CH0930	up Yakima River	1.0000				
07CH1037	up Yakima River	0.9800	Naches River	0.0200		
07CH1152	up Yakima River	0.5665	Naches River	0.4334		
07CH1268	up Yakima River	0.9262	Naches River	0.0738		
07CH1298	up Yakima River	0.9983				
07CH1387	up Yakima River	0.9423	Naches River	0.0577		
07CH1451	up Yakima River	0.9998				
07CH1529	up Yakima River	0.9988				
07CH1635	up Yakima River	0.9774	Naches River	0.0226		
07CH1782	up Yakima River	0.9991				
07CH1823	up Yakima River	0.9935				
07CH1890	up Yakima River	0.9865	Naches River	0.0135		
07CH1970	up Yakima River	1.0000				
07CH2063	up Yakima River	0.9896	Naches River	0.0103		
07CH2185	up Yakima River	0.9990				

Table 4 continued.

upper Yakima River continued.

Individual in Mixture	Best Estimate	Posterior Probability	2nd Best Estimate	Posterior Probability	3rd Best Estimate	Posterior Probability
07CH2251	Naches River	0.7928	up Yakima River	0.2068		
07CH2353	up Yakima River	0.9735	Naches River	0.0265		
07CH2423	up Yakima River	0.9741	Naches River	0.0259		
07CH2461	up Yakima River	1.0000				
07CH2529	up Yakima River	0.9974				
07CH2673	up Yakima River	1.0000				
07CH2831	up Yakima River	0.9967				
07CH2959	up Yakima River	0.9896	Naches River	0.0104		

Appendix 1. Mis-assignment as a spring or fall Chinook between morphological assignment and genetic analysis in 2007.

January-February Time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment	Length (mm)	Weight (g)
None					

March Time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment	Length (mm)	Weight (g)
None					

April Time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment	Length (mm)	Weight (g)
None					

May Time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment	Length (mm)	Weight (g)
05/23/2007	07AE1748	SP	F	105	12.0

June-July Time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment	Length (mm)	Weight (g)
06/16/2007	07AE2116	SP	F	102	12.0
06/17/2007	07AE2162	F	SP	122	17.9
06/17/2007	07AE2165	F	SP	119	17.6
06/18/2007	07AE2166	F	SP	130	22.5
06/18/2007	07AE2170	F	SP	121	17.5
06/19/2007	07AE2171	F	SP	115	14.9
06/19/2007	07AE2174	SP	F	109	14.0
06/20/2007	07AE2177	F	SP	121	18.2
06/23/2007	07AE2245	SP	F	93	8.7
06/25/2007	07AE2293	F	SP	116	16.2
06/25/2007	07AE2294	F	SP	120	17.7
06/25/2007	07AE2295	F	SP	120	16.7
06/27/2007	07AE2302	F	SP	112	15.0
06/27/2007	07AE2303	F	SP	130	23.6
06/28/2007	07AE2309	SP	F	103	10.1

Chapter 5

Genetic Comparisons Between Spawning Adult Steelhead, Juvenile Migrants, and Mature Resident *O. mykiss* From Teanaway River and Taneum Creek

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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. We compared the genetic similarities among collections of the same life history type. Genetic data from four collections of adult steelhead (2002, 2003, 2005, and 2006) and one collection of juvenile migrants (2006) were consistent with all collections representing a single population sample. Residents collected from Manastash Creek, Taneum Creek, and Teanaway River were distinct from each other. Insufficient sampling, cutthroat trout admixture, and kinship limited conclusions regarding the relationship among resident collections. We also compared the genetic similarities between collections differing in life history type. Anadromous *O. mykiss* collections were genetically differentiated from resident collections, and the degree of genetic differentiation appeared consistent with geographic distances, with the Teanaway steelhead being most genetically similar to the Teanaway resident collection, followed by residents from Taneum Creek then Manastash Creek.

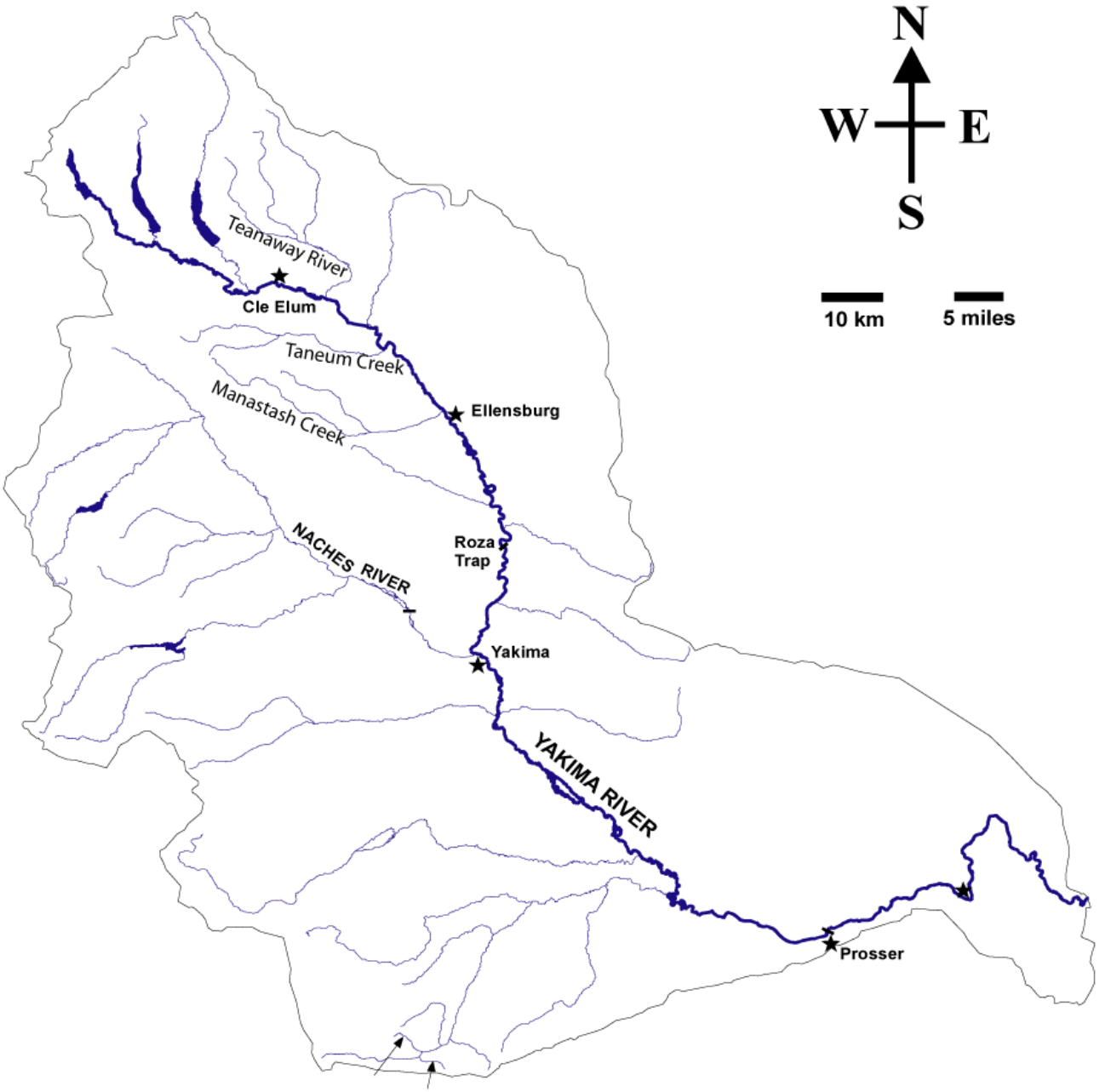
Introduction

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)(Figure 1). 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 two 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

that 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.



[map made from huc maps 17030003, 17030002, and 17030001 from StreamNet]

Figure 1. Map of the Yakima River basin showing tributary locations for collections.

Methods and Materials

Samples

Collections of known adult spawners (summer steelhead), juvenile migrants, and resident *O. mykiss* from Teanaway River and Taneum Creek (i.e., upper Yakima River) 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 and Manastash Creeks in the fall. All fish that were PIT tagged or were known spawners had a fin clip collected. Upon collection, all tissue was immediately stored in ethanol. If a "small" *O. mykiss* captured during spring surveys was exuding gametes, it was classified as a resident. These mature residents were generally between 150 – 200 mm in length, and were all 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 were analyzed. We analyzed a total of N=337 samples, 146 adult summer steelhead known spawners, 23 juvenile migrants, and 168 resident *O. mykiss* (Table 1). Known summer steelhead adult spawners were collected 2002, 2003, 2005, and 2006, collections of juvenile migrants occurred in 2006, and resident *O. mykiss* were collected in 2006 – 2007. 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

Polymerase chain reaction (PCR) amplification was performed using 16 fluorescently end-labeled microsatellite marker loci, *Ogo*-4 (Olsen et al. 1998), *Oke*-4 (Buchholz et al. 2001), *Oki*-10 and 23 (Smith et al. 1998), *Omm*-1070 (Rexroad et al. 2001), *Omy*-7 (K. Gharbi, pers. comm.), *Omy*-1001 and 1011 (Spies et al. 2005), *One*-14 (Scribner et al. 1996), *One*-102 (Olsen et al. 2000), *Ots*-3M and 4 (Banks et al. 1999), *Ots*-100 (Nelson and Beacham 1999), *Ssa*-289 (McConnell et al. 1995), *Ssa*-407 and 408 (Cairney et al. 2000). PCR reaction volumes were 10 µL, with the reaction variables being 2 µL 5x PCR buffer (Promega), 0.6 µL $MgCl_2$ (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 denaturing polyacrylamide 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. Both tests were implemented using the microsatellite toolkit (Park 2001). For each locus and collection FSTAT version 2.9.3.2 (Goudet 1995) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles were calculated using a randomization procedure. Alleles were randomized among individuals within collections (900 randomizations for this dataset) and the F_{IS} (Weir and Cockerham 1984) calculated for the randomized datasets were compared to the observed F_{IS} to obtain an unbiased estimation of the probability that the null hypothesis was true. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.05 (Belkhir et al. 1996). Statistical significance of linkage disequilibrium results was assessed using a permutation procedure implemented in GENETIX for each locus by locus combination within each collection.

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. 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). Population differentiation was also investigated using pairwise estimates of F_{ST}. Multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984), were calculated using GENETIX version 4.05 (Belkhir et al.1996). F_{ST} 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 (FCA) 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 individuals X alleles). The relationship between any two individuals in n-dimensional space (n = number of alleles) is represented by their χ^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.

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=128 adults were collected from Teanaway River, N=16 from Taneum Creek, and N=2 from Stafford Creek. All collections were initially combined for genetic analysis, creating a sample of N=146 adult steelhead (Table 1). Substantial genetic diversity was observed, with an observed heterozygosity of 77%, and 12.9 mean number of alleles over 15 microsatellite loci. The within-locus genetic diversity was consistent with random association of alleles, as the observed F_{IS} was not significantly different from the Hardy-Weinberg null

Table 1 Within collection genetic diversity. Collections listed are known steelhead adult spawners, known migrants, and residents from three upper Yakima River tributaries Manastash Creek, Taneum Creek, and Teanaway River. MNA is mean number of alleles per locus, FIS p-value 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 ($\alpha = 0.01$) linkage disequilibrium.

	Sample	Heterozygosity			FIS	LD
Collection	size	Unbiased	Observed	MNA	p-value	% < 0.01
Spawners	146	0.79	0.77	12.93	0.34	0.019
Migrants	23	0.77	0.78	9.20	0.85	0.019
Manastash	8	0.76	0.77	5.60	0.80	0.010
Taneum	39	0.77	0.78	9.87	0.97	0.048
Teanaway	117[a]	0.78	0.79	12.80	0.83	0.057

[a] Sample size excludes four residents pronounced genetic outliers (see text).

expectation (p-value = 0.34). The association between alleles at different loci was also minimal, with linkage disequilibrium observed for 1.9% of the pairwise locus comparisons (i.e., 2 out of 105). We interpret these data as the collections of adult steelhead adult spawners represent samples from the same underlying population, and it is appropriate to combine all collections into a single collection for analysis.

Juvenile Migrants – A total of N=23 *O. mykiss* individuals (tissue sampled and PIT tagged) were detected exiting the Yakima River system and classified as migrants (WDFW code 06DE) (Table 1). All individuals were sampled from the Teanaway River. This collection had levels of genetic diversity similar to the spawner collection, with 78%

observed heterozygosity, although the small sample size lowered the mean number of alleles per locus. The genetic diversity was consistent with null expectations of random associations of alleles within and between loci, with observed F_{IS} p-value=0.85 and minimal linkage disequilibrium (i.e., 2 out of 105 comparisons).

Mature Resident *O. mykiss* – *O. mykiss* individuals between 150 – 200 mm in length, exuding gametes upon capture, were classified as residents. In total, N=168 individuals were collected, 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). Initially, all residents collected were combined into a single sample for analysis. The combined resident collection showed evidence of being a mixture, with significant linkage disequilibrium (i.e., associations between alleles at different loci) observed for approximately 22% of the pairwise locus tests (data not shown). The resident collection was separated into three groups, Manastash, Taneum, and Teanaway. The singleton from Swauk Creek was combined with the Teanaway River collection. Analysis of genetic diversity was repeated, and we observed the linkage disequilibrium remained within the Teanaway collection (data not shown). We conducted a FCA on the allele frequency data and observed that several resident individuals from the Teanaway River collection substantially influenced the primary FCA axis (Figure 2). The individual labeled A on Figure 2 was the singleton from Swauk River, and contained 11 alleles that are characteristic of cutthroat trout (*O. clarki*)(WDFW unpublished data). Individuals labeled B – D on Figure 2 contained 4, 5, and 4 cutthroat trout alleles, respectively (WDFW unpublished data). This information suggests the primary FCA axis was affected by admixture between *O. mykiss* and *O. clarki*. Excluding individuals labeled A – D, there are three other resident individuals within the Teanaway collection that contain putative cutthroat trout alleles, however these individuals are not genetic outliers (data not shown). The three migrant individuals influencing FCA axis 2 were related individuals with rare genotypes for this dataset.

Excluding the four outlying individuals from the Teanaway collection (i.e., diamonds on Figure 2), the observed heterozygosity increased from 77% to 79% and the observed linkage disequilibrium was substantially reduced from 22% to 5.7% (Table 1). From this result, we chose to perform all further genetic analyses excluding the four outlier individuals. Please note that our exclusion decision was somewhat arbitrary, since: (i) putative cutthroat alleles remained in the Teanaway collection, (ii) the "true" background level of cutthroat admixture is unknown, and (iii) "hybrid-looking" individuals are observed in the system, but purposefully not collected. Further investigation is required to elucidate issues pertaining to the interaction between *O. mykiss* and *O. clarki*. Regarding the Manastash and Taneum collections, the observed heterozygosity measured 77% and 78% for these resident collections, respectively (Table 1). The observed F_{IS} for both collections was not significantly different from null expectations derived from the permutation test. The observed linkage disequilibrium for Manastash and Taneum collections were 1% and 4.8%, respectively.

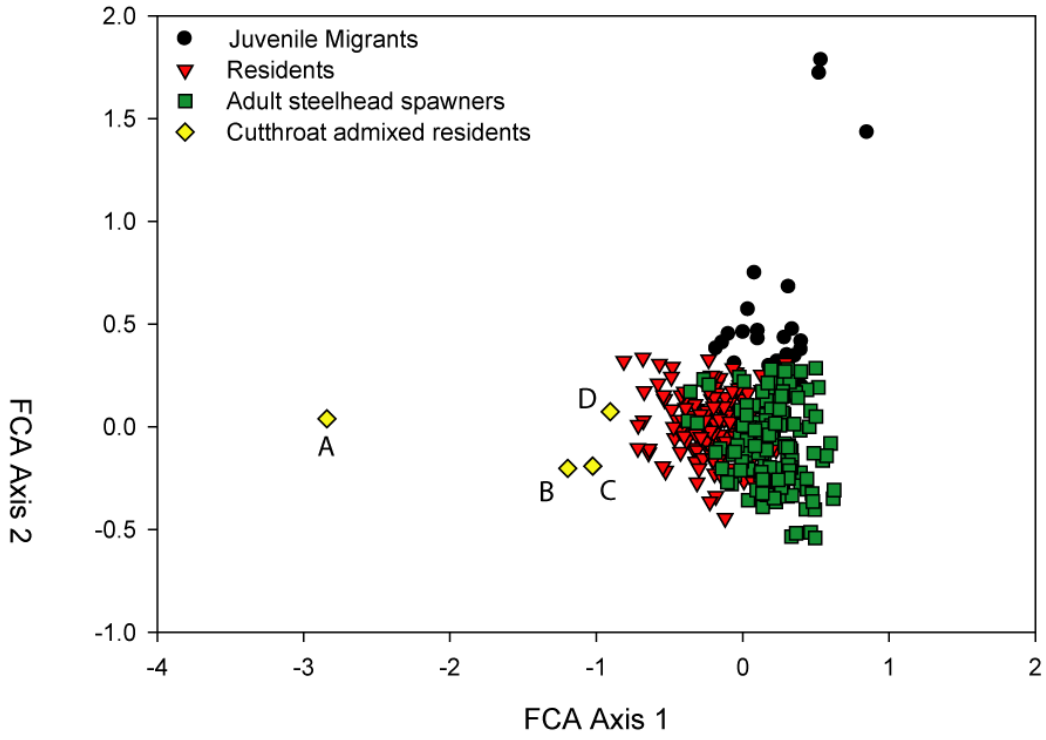


Figure 2 2D FCA plot of genetic data. *O. mykiss* residents are labeled with triangles. Individuals labeled A – D are residents with differing levels of cutthroat trout admixture (see text).

Differentiation between Mature Resident *O. mykiss* – The resident collections from Manastash Creek, Taneum Creek, and Teanaway River were genetically differentiated from each other (Table 2). Comparisons of allele frequencies made using randomization tests all had p-values that suggested differentiation among collections. Additionally, estimates of F_{ST} were all statistically different from zero, and ranged from 0.014 (Taneum vs. Teanaway) to 0.042 (Manastash vs. Taneum). A relationship between genetic diversity and geographic distance was not observed. For example, the resident collection from Taneum Creek, which is located geographically between Teanaway River and Manastash Creek, is not more genetically similar to Manastash Creek *and* Teanaway River than the Teanaway River collection is to Manastash Creek. Sample size is likely an issue, as a collection with a small sample size may not accurately estimate allele frequencies. Additionally, there is evidence of kinship within the *O. mykiss* collections (data not shown), which may also alter genotype frequencies. More comprehensive sampling of *O. mykiss* residents is required if the distribution of genetic diversity within *O. mykiss* from the upper Yakima is to be characterized.

Objective 2: Comparisons of genetic similarity between collections differing in life history type.

Known Anadromous Spawners vs. Juvenile Migrants – Genetic differentiation between known steelhead adult spawners and juvenile migrants from the Teanaway River was investigated. The p-value for the randomization chi-square test was not statistically significant, which suggested the allele frequencies for both the spawner and migrant collections represented samples from the same underlying allele-frequency distribution (Table 2). This result was consistent with the observed F_{ST}=0.003, which was not statistically different from zero. We interpret these results as the spawner and migrant collections represent a single population.

Table 2 Between collection genetic analyses. Above diagonal are p-values for randomization chi-square population differentiation tests, where p=0.005 was the chosen threshold to cease the assessment of significance. Below diagonal are pairwise estimates of F_{ST}. Bolded values are statistically significant.

	Spawners	Migrants	Manastash	Taneum	Teanaway
Spawners	-	0.06	**0.005**	**0.005**	**0.005**
Migrants	0.003	-	**0.005**	**0.005**	**0.005**
Manastash	**0.026**	**0.041**	-	**0.005**	**0.005**
Taneum	**0.013**	**0.018**	**0.042**	-	**0.005**
Teanaway	**0.005**	**0.008**	**0.026**	**0.014**	-

Steelhead vs. Mature Resident *O. mykiss* – The known adult spawner collection was differentiated from each resident collection based on microsatellite allele frequencies and estimated values of F_{ST} (Table 2). All pairwise comparisons of allele frequencies using a randomization chi-square test were statistically significant. All estimates of F_{ST} were statistically different from zero, and ranged from a slight 0.005 (Spawners vs. Teanaway) to 0.026 (Spawners vs. Manastash). The above results were similar to those for the comparisons between the migrants and residents, where all comparisons of allele frequencies were statistically different. Similarly, estimates of F_{ST} were non-zero, however they were slightly larger for migrant – resident comparisons than for spawner – resident comparisons. We attribute the increased F_{ST} to small sample sizes. In general, the Teanaway steelhead are most similar to the Teanaway resident collection, followed by Taneum Creek then Manastash Creek, which is consistent with geographic distances. This result contrasts with the observation regarding resident collections, where a relationship between genetic diversity and geographic distance was not apparent. We interpret these results as the steelhead and resident collections are genetically differentiated. Yet, these results also suggest that the residents and steelhead from the Teanaway River interact substantially.

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. Funding for this study was provided by the Bonneville Power Administration (BPA) and by Washington State General Funds.

Literature Cited

Banks MA, Blouin MS, Baldwin BA, Rashbrook VK, Fitzgerald HA, Blankenship SM, Hedgecock D (1999) Isolation and inheritance of novel microsatellites in chinook salmon (Oncorhynchus tschawytscha). *Journal of Heredity,* 90:281-288.

Belkhir K, Borsa P, Chikhi L et al (1996) 1996-2001 GENETIX 4.05, logiciel sous Windows TM pour la Génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Blankenship SM, Dean CA, and Small MP (2007) Comparison of Adult Steelhead and Resident Trout Collected at Roza Dam in 2006 to Available DNA-based Microsatellite Genetic Data. Yakima/Klickitat Fisheries Project Genetic Studies. 2007 Annual Report, Bonneville Power Adminstration, Portland, OR.

Busby PJ, Wainwright TC, Bryant GJ et al (1997) Status Review of West Coast Steelhead from Washington, Idaho, Oregon, and California. U.S. Dept. of Commerce, NMFS-NWFSC-27, Pp. 275.

Buchholz WG, Miller SJ, Spearman WJ (2001) Isolation and characterization of chum salmon microsatellite loci and use across species. Animal Genetics **32**: 162

Cairney M, Taggart JB, and Hoyheim B (2000) Characterization of microsatellite and minisatellite loci in Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. Molecular Ecology 9, 2175–2178.

Campton DE, and Johnston JM (1985) Electrophoretic evidence for a genetic admixture of native and nonnative rainbow trout in the Yakima River, Washington. Trans. Am. Fish. Soc. 114:782-793.

Franklin JF, and Dyrness CT (1973) Natural vegetation of Oregon and Washington. USDA Forest Service General Technical Report PNW-8, 417 p. (Available from U.S. Department of Agriculture, Pacific Northwest Forest and Range Experiment Station, P.O. Box 3890, Portland, OR 97208.)

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Hedrick PW (1983) Genetics of Populations, Science Books International, Boston

Jackson PL (1993) Climate. In P. L. Jackson and A. J. Kimerling (editors), Atlas of the Pacific Northwest, p. 48-57. Oregon State Univ. Press, Corvallis.

Leider SA, Phelps SR, and Hulett PL (1995) Genetic analysis of Washington steelhead: Implications for revision of genetic conservation management units. Wash. Dep. Fish Wildl. Prog. Rep., 21 p. (Available from Washington Department of Fish and Wildlife, Fish Management Program, 600 Capitol Way N, Olympia, WA 98501-1091.)

Loxterman J, and Young S (2003) Geographic population genetic structure of steelhead in the Yakima River basin. Pages 6-23 in C.Busack, A. Fritts,J. Loxterman, J. Shaklee, S. Young, C. Knudsen, and J. Rau. Yakima/Klickitat Fisheries Project Genetic Studies. 2002 Annual Report, Bonneville Power Adminstration, Portland, OR.

McConnell S, Hamilton L, Morris D, Cook D, Paquet D, Bentzen P, and Wright P (1995) Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. Aquaculture 137, 19–30.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York, NY

Nelson RJ and Beacham TD (1999) Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics 30:228-229.

Olsen JB, Bentzen P, Seeb JE (1998) Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7 (8): 1087-1089

Olsen JB, Wilson SL, Kretschmer EJ, Jones KC, Seeb JE (2000) Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. *Molecular Ecology* 9, 2185-2187.

Park SDE (2001) Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis], University of Dublin

Phelps SR, Baker BM, Hulett PL, and Leider SA (1994) Genetic analysis of Washington steelhead: Initial electrophoretic analysis of wild and hatchery steelhead and rainbow trout. Washington Dep. Fish and Wildlife, Fisheries Management Program Report 94-9. (Available from Washington Department of Fish and Wildlife, Fisheries Management Program, 600 Capitol Way N., Olympia, WA 98501-1091.)

Phelps SR, Baker BM, and Busack CA (2000) Genetic relationships and stock structure of Yakima River basin and Klickitat River basin steelhead populations. Washington Dep. Fish and Wildlife, Fisheries Science Program Report. 19pp. (Available from Washington Department of Fish and Wildlife, Fisheries Management Program, 600 Capitol Way N., Olympia, WA 98501-1091.)

Rexroad CE, Coleman RL, Martin AM, Hershberger WK, Killefer J (2001) Thirty-five polymorphic microsatellite markers for rainbow trout (Oncorhynchus mykiss). *Animal Genetics,* 32:317-319.

Scribner KT, Gust JR, Fields RL (1996) Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Candian Journal of Fisheries and Aquatic Sciences.* 53, 833-841.

Smith CT, Koop BF, Nelson RJ (1998) Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. *Molecular Ecology* **7** : 1614-1617.

Spies IB, Brasier DJ, O'Reilly PT et al (2005) Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (Oncorhynchus mykiss). Molecular Ecology 5:278-281

Small MP, Loxterman JL, and Young SF (2006) Microsatellite DNA Study of Population Genetic Structure among Yakima Basin Steelhead. Washington Dep. Fish and Wildlife, Fisheries Science Program Report. (Available from Washington Department of Fish and Wildlife, Fisheries Management Program, 600 Capitol Way N., Olympia, WA 98501-1091.)

Weir BS (1979) Inferences about linkage disequilibrium. Biometrics 35:235-254.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38:1358-1370.