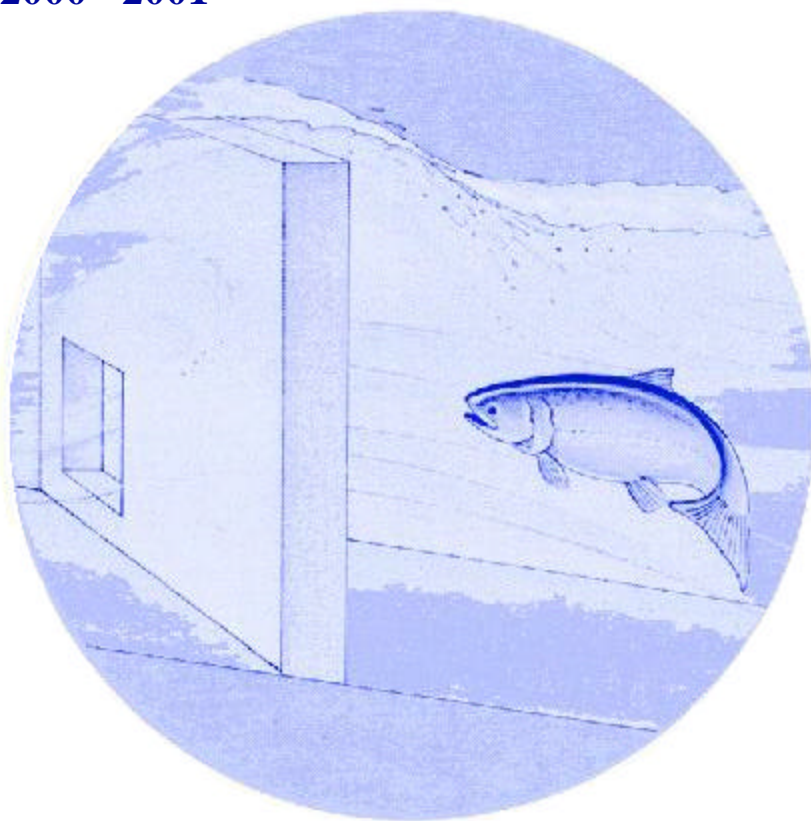


# Genetic Studies in the Yakima River Basin

## Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report  
2000 - 2001



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This report covers one of many topic areas included in 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 00004666, Project 1995-064-24). A comprehensive summary report for all the monitoring and evaluation topics will be submitted after all 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 entire YKFPME. The current report was completed by the Washington Department of Fish and Wildlife.

# **Yakima/Klickitat Fisheries Project Genetic Studies**

## **Annual Report 2001**

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## Executive Summary

Genetic work for 2001 consisted of two major phases, both reported on here. The first is a DNA microsatellite analysis of several hundred juveniles from the experimental spawning channel at the Cle Elum Supplementation Research Facility, using the genetic markers to assign the juveniles to parents, and thus judge reproductive success of individual fish. The second is a reevaluation and revision of plans for studying domestication in the spring chinook supplementation effort.

The pedigree analysis was significant in three respects. First, it showed that this approach can be successfully applied to the spawning channel research. Secondly it showed that this approach does indeed yield very useful information about the relative reproductive success of fish in the channel. Finally, it showed that this information can yield additional information about the experimental design.

- Of the 961 juveniles on which analysis was attempted, 774 yielded enough genetic information to be used in the pedigree analysis. Of these, 754 were assigned to males and females known to have been placed into the channel. Of the other 20, all were assignable to females, but sires were unknown. The genotypes of 17 of these were consistent with a single theoretical male genotype, suggesting a single precocial male sired them.
- The inferred parentage of the fish demonstrated that there had been substantial leakage of juveniles from one section of the channel into another.
- Reproductive success of females was fairly even, but success of males varied considerably. In a group of seven males (including the hypothetical one), one contributed 79% of the progeny analyzed, and three contributed none.

The domestication experimental design evaluation was prompted by a critical review of the project by the Independent Scientific Review Panel (ISRP). The ISRP review set into motion a design revision process which extended beyond the contract period; the report presented here is intended to be an account of our work through the end of the contract period, so does not include developments beyond that point. As such, combined with the upcoming 2002 report, it will provide a complete record of our process through the experimental design revision process. The current report contains the following:

- An explanation of the general concept of domestication, and why domestication is a concern in the YKFP spring chinook program.
- A discussion of the basics of experimental design for domestication.
- A history of domestication experimental design for domestication in the YKFP.
- A review of potential designs that would answer the ISRP's criticisms.
- A revised design containing the following elements:
  - A control line under continuous hatchery culture (i.e.; no spawning in the wild)
  - Use of the Naches population, where appropriate, as a wild control line.
  - Cryopreservation of sperm for later evaluation of long-term genetic trend,
  - Continuous monitoring of phenotypic trend in the supplemented line.

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# **The Analysis of Spring Chinook Reproductive Success Using DNA-Based Pedigree Reconstruction**

Sewall F. Young and James B. Shaklee

## **Introduction**

The primary focus of the DNA-based genetic analyses during the past year was on a pedigree reconstruction analysis of spring-run chinook in the Cle Elum experimental spawning channel. The overall goal of this multi-year investigation is to obtain quantitative estimates of the reproductive success of “wild” (= natural) origin adults and hatchery origin adults and compare the relative fitness of the two groups. In the past year, we conducted our initial DNA investigations in conjunction with observations of spawning behavior and analyses of behavioral and morphological attributes that were done by Steve Schroder and collaborators. Analysis of spawning behavior will be reported by Schroder and others in an accompanying report. All adult chinook in the experimental channel in this first year were “wild” (natural origin) fish. Beginning with next year’s study, both natural origin and first-generation hatchery origin fish will be used in the experimental spawning channel.

Genetic investigations in the YKFP in past years have for the most part used allozyme data (obtained by the electrophoretic analysis of enzyme variation using starch gels). However, enzyme-coding genes do not exhibit adequate levels of variation (heterozygosities are typically between 0.04 and 0.09 and the number of alleles per locus typically ranges from 2 to 6 at most polymorphic allozyme loci) to support informative pedigree reconstructions with even modest numbers of randomly chosen parents. Therefore, we chose to use microsatellite DNA markers for this study because they typically exhibit heterozygosities of over 0.50 and regularly have 6 – 40+ alleles per locus. We had previously screened approximately 15 microsatellite DNA loci in the Cle Elum Hatchery spring chinook broodstock and verified that many of these loci were highly variable in this population.

## **Materials and Methods**

**Project Design:** The experimental spawning channel was divided into two equal sections (identified as cell #1 and cell #2) by stretching fine-meshed nets across the stream channel to prevent movement of adults or fry and juveniles into or out of each section. Twenty-seven potential parents of natural origin (11 females and 16 males) were introduced into the channel in the fall of 2000. Prior to introduction into the experimental channel, a fin clip sample from each adult was obtained and preserved in absolute ethanol for subsequent DNA analysis. Six females and 10 males were placed in cell #1 and four females and six males were placed into cell #2). Extensive, detailed behavioral observations and videotapes were made throughout courtship and spawning and numerous morphological characters of each adult were documented both prior to

spawning and after death to provide other information for use in describing and evaluating the potential parents.

After spawning was completed, the fertilized eggs were allowed to develop undisturbed in the gravel of the experimental channel through hatching and eventual emergence of the fry from the gravel. At this time, emergent fry were subsampled from the two cells on a periodic basis (one or more times per week). At or slightly prior to this time, it became apparent that the constant flow of water through the experimental channel had worn a number of holes in the netting so that it was clearly possible that fry could move into or out of cells #1 and #2. The fry sampling generated more individuals than were deemed necessary for DNA analysis, so that the fry collections were subsampled to obtain sets of offspring for DNA analysis that were believed to be representative of the total production in the channel.

Microsatellite DNA analysis of the 27 known potential parents was done by screening approximately 15 loci. This initial screening revealed considerable variation at six loci and preliminary simulations using the computer program CERVUS (Marshall et al. 1998) suggested that genotyping offspring at these six loci should provide enough statistical power to assign each offspring back to a unique male and female parent (Table 1).

Table 1. Attributes of the 6 microsatellite DNA loci used in the pedigree reconstruction.

Locus	alleles <sup>1</sup>	n <sup>2</sup>	H <sub>O</sub> <sup>3</sup>	null alleles <sup>4</sup>	expected proportion excluded <sup>5</sup>	
					p = 0.95	p = 0.80
<i>Ots-101</i>	20	947	0.868	-0.0128	0.538	0.702
<i>Ots-107</i>	19	933	0.921	-0.0275	0.594	0.746
<i>Ots-108</i>	17	950	0.933	-0.0267	0.629	0.773
<i>Ogo-4</i>	10	960	0.940	-0.0832	0.453	0.629
<i>Ots-100</i>	26	957	0.953	-0.0278	0.684	0.811
<i>Ssa-197</i>	16	961	0.925	-0.0466	0.568	0.726
combined probability of non-exclusion:					<b>0.005</b>	<b>0.000</b>

<sup>1</sup> = number of alleles observed in the 27 known potential parents

<sup>2</sup> = number of fish successfully scored for this locus

<sup>3</sup> = observed heterozygosity

<sup>4</sup> = estimated frequency of null alleles

<sup>5</sup> = proportion of potential parents excluded at indicated p-value

(based on simulated individuals from the total data set; parents and offspring)

We then screened 961 offspring collected in 2001 from cell #2 (where the analysis of spawning behavior and morphological traits of adults had been completed) at these six loci and used CERVUS to conduct maximum likelihood based parentage assignments. The basic approach is to match the multilocus genotype (at up to six loci in this study) of each offspring with the female and male adults that provide the maximum likelihood of parentage. The approach (and the program CERVUS) allows genotypic mismatches to occur between offspring and assigned parents (either due to an assumed low level of



genotyping error and/or mutations) so that mismatches do not, in and of themselves, preclude a particular parentage assignment.

## Results and Discussion

The initial parentage assignments for 774 of the 961 offspring analyzed from cell #2 are summarized in Table 2. Note that we limited this analysis to offspring that had four of more loci successfully scored and 187 fry did not meet this criterion. In this table, the rows represent different females and the columns represent different males. The numbers in each cell indicate the number of individual fry assigned to each particular spawning of one female with one male. The upper left and lower right sections of the table represent spawnings occurring between adults in cell #1 and between adults in cell #2, whereas two gray areas of the table indicate theoretical spawnings involving one parent in cell #1 and one parent in cell #2. None of the 774 assigned fry were attributed to such between-cell spawnings (which were not possible because the adults in the two different cells were precluded from mixing by the presence of the nets dividing the spawning channel). However, many fry resulting from spawnings of adults restricted to cell #1 were captured in cell #2, indicating that the holes that developed in the nets over the winter definitely allowed fry to move between cells.

Table 2. Initial parentage assignments for 774 fry from cell #2.

		Males																female totals		
		cell #1										cell #2								
		05	09	18	19	20	23	24	25	26	27	01	04	10	17	21	22		?	
Females	cell #1	02	3	3			6												1	13
		03							6											6
		11	12	10			1	4			5									32
		12	74				14													88
		13	1	6	4		16													27
		14	15	2	2															19
		15	28	5			1											1		35
	cell #2	06															125	11		136
		07														23	89	1		113
		08															137	1		138
	16											62			9	91	5		167	
male totals		133	26	6			38	4	6		5	62			32	442	20		774	
218 fry produced in cell #1										556 fry produced in cell #2										

Reproductive success among females in cell #2 was approximately equal (range 113 – 167 offspring per female) whereas that among the six males in cell #2 was extremely variable (range: 0 – 442 offspring per male). Although fewer fry produced in cell #1 have been analyzed so far, a similar pattern appears to hold with the variance in male reproductive success being much greater than that among females.

Of the 774 offspring assigned in this initial analysis, 754 were assigned parents with exact genetic matches to identified parents. However, 20 offspring were assigned to maternal parents but had one or more mismatches to paternal parents. That is, these offspring carried one or more alleles not seen in any of the known potential male parents. There are at least three possible explanations: 1) genotyping (scoring) errors, 2) mutation, and 3) contributions from un-genotyped parents. Eleven of these ambiguous offspring were assigned to a single maternal parent (fish 00GX006). As shown in Table 3, when the genotype of the presumed female parent was compared with the offspring genotypes at the two loci with unexplained alleles, a single genotype in a hypothesized un-genotyped male parent provided an exact match for all 11 observed offspring. In fact, comparison of the genotypes of the remaining 9 juveniles that carried alleles not observed in the typed parents with the inferred genotype of the sire of the 11 fry discussed above revealed that six of the remaining fry had genotypes that were consistent with the inferred male parent's genotype. This explanation gains some support from the behavioral observations as well, because at least one small, precocial male was observed in association with adult spawning pairs in cell #2. It is our conclusion that these data provide compelling evidence that a precocial male produced at least 17 offspring in cell #2. The remaining 3 ambiguous offspring are not explained by this male and, at present, we don't have enough information to decide among alternative explanations for their presence.

Table 3. Genotypes of 11 unexpected fry, their assigned female parent, and their presumed male parent at the two informative loci.

	<b>Ots-101</b>		<b>Ots-107</b>	
genotype of assigned female parent :	197	205	208	228
unexpected fry				
01NS0040	173	197	208	<b>224</b>
01NS0256	173	205	<b>200</b>	208
01NS0314	<b>161</b>	205	<b>200</b>	208
01NS0495	173	197	<b>200</b>	228
01NS0550	<b>161</b>	197	<b>224</b>	228
01NS0559	173	205	<b>200</b>	228
01NS0634	173	205	<b>200</b>	228
01NS0869	173	197	208	<b>224</b>
01NS1172	173	197	208	<b>224</b>
01NS1196	<b>161</b>	205	<b>200</b>	228
01NS1224	<b>161</b>	197	<b>224</b>	228
presumed genotype of precocial male parent :	<b>161</b>	173	<b>200</b>	<b>224</b>

*bold type = allele not present in known potential parents*

With a single additional precocial male identified as a potential parent, the pedigree analysis was redone. This analysis classified all but 3 individual offspring to "known" parents (11 females, 16 adult males, and 1 hypothesized precocial male).

We analyzed all offspring based on the cells in which the parental matings occurred. Approximately 1/3 of the offspring sampled from cell #2 were actually produced by adult

parents spawning in cell #1. This provides a quantitative estimate of “leakage” of offspring from cell #1 into cell #2 (due to holes in the netting intended to separate the cells. Data indicate that the inferred precocial male was restricted to cell #2.

The relative male fitness (realized paternity) in cell #2 can thus be summarized as follows:

	# of offspring	% of offspring
male #22	442	79%
male #04	62	11%
male #21	32	6%
precocial	20	4%
male #01	0	0%
male #10	0	0%
male #17	0	0%

### **Future Activities**

We plan to reanalyze approximately 200 fry to obtain more complete genotypes in order to accomplish unambiguous parentage assignments for these fish.

Within the next few months, we intend to analyze an additional 1,000 offspring from 2001 (sampled from cells #1 & #3) in order to complete the picture of reproductive success in the experimental channel in 2000-2001.

Next year we will be analyzing YR 2001 adults and their YR 2002 offspring in a manner similar to that used in the current year. This group of potential parents will represent the first generation of upper Yakima hatchery origin spring chinook adults and will allow for direct comparisons of reproductive success between natural origin adults (including jacks and precocials) and single-generation hatchery fish. This next phase of the study included ninety-seven potential parents placed in the experimental spawning (21 hatchery females, 17 hatchery males, 4 hatchery jacks, 5 hatchery precocial males, 20 wild females, 23 wild males, 2 wild jacks, and 5 wild precocial males).

### **Literature Cited**

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

## Domestication Research/Monitoring Design

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**Prefatory Note:** Domestication selection monitoring is currently the subject of a considerable amount of discussion internally and with the Independent Scientific Review Panel (ISRP). Discussions with the ISRP were still going on at the end of the contract period, and as a consequence, domestication selection monitoring designs were still being revised. The material presented in this report is a primarily a summary of this process up until the end of the contract period, but also includes additional information the clarify points raised in discussion.

### Introduction

Natural selection constantly operates to change the genetic composition of populations, making them more adapted to their environments. An organism that has long existed in one environmental regime and then is placed into another can be expected to change genetically in response to the new environmental conditions. To some extent this is probably always going on as a result of environmental variability; natural selection causes the organism to be constantly at some level of flux genetically. Large genetic changes can be expected when an organism is moved from the natural environment into an artificial environment, as is the case with fish culture. Genetic change in response to the differences between natural and anthropogenic environments is called domestication<sup>1</sup>.

This general view of domestication is somewhat different from the popular view of the process, which involves using intentional artificial selection to make animals and plants better fulfill a variety of human needs. Artificial selection has been and continues to be a part of many salmon and steelhead hatchery programs. For example, winter steelhead have been intentionally selected for early spawning at Chambers Creek hatchery (Crawford 1979). Sometimes artificial selection is unintentional. The spawning timing of numerous stocks has been moved forward (Tipping and Blankenship, submitted), probably as a result of pressure to meet eggtake goals. Artificial selection of this sort is inconsistent with the concept of using hatcheries in a conservation mode, in which the goal is to increase the size of the population without causing genetic change. In modern artificial propagation projects like the YKFP spring chinook program intentional artificial selection is avoided altogether, and attempts are made to avoid unintentional artificial selection as well. This is problematic, because these practices become apparent only in

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<sup>1</sup> In all previous YKFP documents the term “domestication selection” has been used rather than “domestication”. The latter term is preferable because the process actually consists of two phases: selection and response to selection. Selection, strictly speaking, is just the relative reproductive advantage conferred on individuals based on their phenotype at one or more traits. Response to selection is the genetic consequence of this selection. Because selection can be measured irrespective of response to selection (e.g., Fleming and Gross 1994, Fleming et al. 1996), it is important in designing a monitoring plan to be precise. In the YKFP we are interested in the entire process.

hindsight or as monitoring proceeds. For example, knowing that unintentional selection for run timing has been a common problem in other programs, we meticulously avoid it by taking great pains to collect broodstock randomly over the course of the run. However, there may be other practices we currently employ that will later be shown to be selective.

Artificial selection, both intentional and unintentional, may account for a considerable amount of the domestication caused by hatcheries, and to the extent we know about it, we can avoid it. But a considerable amount of the domesticating potential of hatchery operations may be due to the differences between the hatchery and natural environments. Rearing environments are so different that it is not too great an exaggeration to say that the only obvious characteristic common to them is water. Compared to their wild counterparts, hatchery juveniles experience much higher fish densities and a much less complex environment. Differences in spawning environment may also be quite important. Natural spawners must select redd sites, dig redds, attract and compete for mates, engage in courtship, deposit gametes, and guard them. Broodstock need do none of these things. Sexual selection, the genetic result of competition for mates, is a widespread phenomenon in animals and is considered to be an important evolutionary force. It can be inferred both from the obvious sexual dimorphisms observed in salmon and steelhead and from the research done on reproductive success (e.g., Fleming and Gross 1989, 1994) that this is also an important phenomenon in salmon, but obviously one which cannot take place in the hatchery environment. Domestication caused by the hatchery environment can be divided into two components (Lynch and O'Hely 2001): 1) traits that are selected against in the natural environment are selected against less intensely in the hatchery environment (relaxation of selection); and 2) traits that are selected for in the natural environment are selected against in the hatchery environment or vice versa (antagonistic selection). Not allowing mate selection is an obvious case of relaxation of selection.

The literature on domestication in anadromous salmonids is not large. Historically this is probably because the problem was not appreciated until geneticists got involved in a big way with salmon and steelhead management in the 1970's. The reason the literature is still small is probably because the studies are difficult and expensive. There are many studies comparing wild- and hatchery-origin salmonids, but most of these lack the sophistication to make the case for the observed differences being only or largely genetic. Ford (1999) recently reviewed the literature on domestication in salmonids and evaluated 14 studies in detail. The studies dealt with a wide variety of traits: reproductive success of males and females, body morphology, egg morphology, fecundity, juvenile survival, age at maturity, predator avoidance, and agonistic behavior.

The possible domesticating effects of the hatchery environment can be countered in two fundamental ways: 1) by making the hatchery environment more like the wild environment ; and 2) limiting exposure to the hatchery environment. The YKFP spring chinook program contains examples of both approaches. In fact, these risk-containment measures are so deeply ingrained a part of YKFP culture that there may be some perception that the YKFP spring chinook program is immune to domestication. Because perception of risk in the YKFP plays a large philosophical role in how we approach the

measurement of domestication- including what proportion of our resources we dedicate to it- the striking risk containment features of the project need to be discussed in some detail.

The semi-natural treatment (SNT) currently applied to half the production raceways makes the hatchery environment more natural by including underwater feeding, underwater structure, simulated substrate, and cover. This environment should produce a fish that at least behaviorally is more similar to a naturally produced fish than will a traditional bare raceway. Our hope is that the behaviors encouraged by the SNT environment will cause SNT fish to have higher survival rates than OCT fish. How much the SNT environment will reduce domestication is another issue, however. The SNT addresses only four out of an unknown number of characteristics in which hatchery and natural environments differ. If one or more of these four factors is an important cause of domestication then perhaps SNT can significantly retard domestication. If an unaddressed factor is of major importance, SNT may still have some benefit in producing fish that are phenotypically more similar to natural-origin juveniles, but may be ineffective at retarding domestication. For example, if density is a key domesticating force, SNT may have very little impact on domestication. In fact, if density is a key factor in domestication, our ability to reduce domestication by any sort of hatchery reform may be extremely limited. A similar line of argument holds for mate selection, another factor that is not part of the SNT.

The effectiveness of limiting exposure to the hatchery environment as a means of retarding domestication depends on the relative strength of selection in the two environments. There is a common misperception that use of only natural-origin fish for broodstock will completely counter domestication. There is no theoretical or empirical support for this general idea, although it is possible that under very specific circumstances the result could be obtained. The most recent insight into this issue comes from the work of Lynch and O'Hely (2001). Fig. 1 below

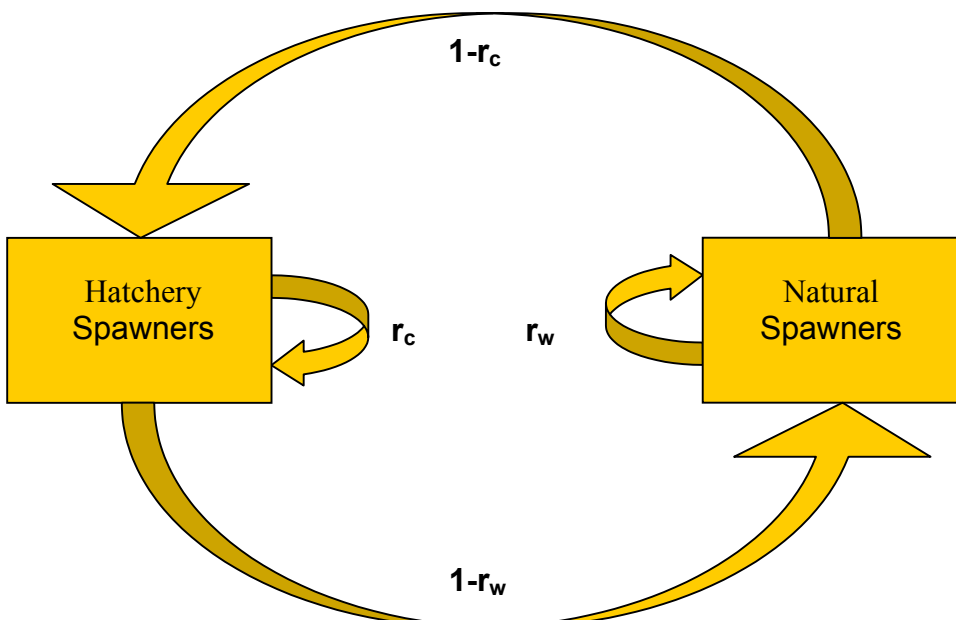


Fig. 1. Schematic of reproductive interactions between natural and hatchery subpopulations in an integrated production program (from Lynch and O'Hely, 2001)

is taken from their paper. This figure is worth reproducing here because it is a very useful schematic of hatchery programs, and as such will be of use several times in this report. Any hatchery program can be conceptualized as a population with two subpopulations, a hatchery component, and a natural component. The  $r$  coefficients on the diagram represent the proportions of spawners used in the two environments by origin. Thus  $r_c$  is the proportion of hatchery broodstock consisting of hatchery-origin fish, and  $1-r_c$  is the proportion of hatchery broodstock consisting of natural-origin fish. Thus  $r_c$  is the proportion of hatchery broodstock consisting of hatchery-origin fish, and  $1-r_c$  is the proportion of hatchery broodstock consisting of natural-origin fish. Similarly,  $r_w$  is the proportion of natural spawners consisting of natural-origin fish, and  $1-r_w$  is the proportion of natural spawners consisting of hatchery-origin fish. A typical hatchery program, in which most of the broodstock consists of returning hatchery fish, would have an  $r_c$  close to 1. In contrast, the YKFP spring chinook program has an  $r_c$  of 0. Lynch and O'Hely consider the percentage of time an allele spends in the wild as a key determinant of domestication due to relaxation selection. This is computed as

$$\frac{(1-r_c)}{(2-r_c-r_w)}.$$

Using this equation and others they present, it can be shown that

domestication under a wild-only broodstock rule is considerably less than many other regimes, but it alone will not stop domestication. Another important factor is percentage of hatchery-origin fish on the spawning grounds. This particular application of Lynch and O'Hely's work only covers domestication due to relaxation of selection and has been criticized because it is based on a genetic load argument, but the same results hold for a model of antagonistic selection recently developed by Ford (2002). In summary, there was never any support beyond an intuitive notion that domestication selection could be eliminated by using only natural-origin broodstock, and two recent papers show that using only natural-origin broodstock will lessen but not eliminate domestication.

The natural-origin only aspect of broodstock in the YKFP program makes it somewhat unique, but focusing on the aspect of the program may mask another important aspect of the program. The YKFP spring chinook program is an integrated program, in which there is considerable intentional mixing of the two subpopulations. In the YKFP we have lived with this concept for so long we are inclined to forget how novel it is. All research to date of domestication has focused on changes incurred under a more traditional regime of using largely hatchery returnees as broodstock. Summarizing the literature on domestication in salmonids, Ford (1999) concluded "At this time it appears impossible to predict the outcome of such composite systems, and no controlled empirical studies have been conducted to address this issue in salmonids."

In summary, there are good reasons to expect domestication in hatchery programs. Although the YKFP spring chinook program includes some major risk-containment measures, there are still reasons to expect domestication at some level. Finally, the issue of domestication has not been explored in "composite" systems such as ours. All this, coupled with the opportunities offered by the hatchery

## **General Considerations in Domestication Research/Monitoring Designs**

Proper development of a design for research/monitoring of domestication or any other phenomenon begins with deciding on a basic question to be asked, which is typically stated in the form of a null hypothesis. The obvious general question to be asked of the YKFP spring chinook program, with its unprecedented attention to containment of genetic risk, is: Will measurable domestication occur under the YKFP supplementation regime? We'll call this question 1. Another appropriate question is: Will less domestication occur under the YKFP supplementation regime than will occur under a more "traditional" hatchery regime? We'll call this question 2. Question 2 has received scant attention during project design, probably because project personnel were so committed to the risk-containment aspects of the project, but it is a question well-worth answering. If the time and expense involved in implementing these risk containment measures (principally a facility to collect wild broodstock ) does not appreciably reduce domestication compared to a traditional hatchery program where the origin of broodstock is not regulated, it is very important that the Region learn this.

The most straightforward way to answer any research question like this is to compare the line or population being "treated" with a control line or population, one that is as similar as possible to the treated population except for the treatment. In the case of question 1, the appropriate control is a line as identical as possible to the supplementation line, but is not subjected to hatchery influence. In other words, a wild control is needed to compare for rigorous evaluation of question 1. Following similar reasoning a "hatchery" control is necessary for rigorous evaluation of question 2.

There are often many possibilities for development of control lines. Selecting an appropriate control should be based on the following four criteria:

1. The control population must be genetically similar enough to the experimental population so that any differences observed between them can be convincingly ascribed to the experimental treatment.
2. The control population must experience an environment similar enough to the experimental population so that any differences observed between them can be convincingly ascribed to the experimental treatment.
3. The control population and experimental populations must be reproductively isolated to the extent that they do not affect each other's performance.
4. The control population has to be adequately accessible so that the differences between it and the experimental population can be measured (i.e., you have to be able to monitor it and take samples from it).

The best control line would obviously be one with the same genetic composition and subject to the same environment (except for the treatment) as the treatment line. In our case, the ideal would be a wild subline and a traditional hatchery subline founded from the Upper Yakima stock. This type of internal control may be easy to achieve in a lab



setting, but is considerably more difficult in the settings offered by anadromous salmonids, as will be seen in the discussion of design feasibility below. Only one commonly cited paper on domestication in anadromous salmonids has used an internal control, that of Reisenbichler and McIntyre (1977). These researchers had access to a new steelhead hatchery program in which an effort had been made to keep hatchery-origin and natural-origin fish reproductively isolated, and this had been going on for two generations. The next best control line situation is one using an external control, another stock which is a good genetic and environmental match to the stock of interest. Most domestication research has been of this sort. Typical of this work is the study of Berejikian et al. (1997), in which juvenile steelhead from a wild population were compared for predator-avoidance behavior with juveniles from a population subjected to several generations of hatchery culture. A weakness of this type of study is the possibility that the observed difference is not due to the effect of interest, but rather to the difference in genetic backgrounds between the two stocks. This weakness can be overcome by replicating this work in several places. Swain and Riddell (1990) used this approach in a comparison of agonistic behavior in juvenile coho. Two hatchery-wild stock pairs were used in this study. Both these studies were done in test arenas, but the approach can also be used in natural settings. A good example of this is the work of Fleming and Gross (1989), who compared morphology and fecundity in 13 wild and 5 hatchery coho populations by sampling spawners in situ. Chilcote et al. (1986) did an interesting but controversial variant of the typical external control design by using a genetically marked exogenous hatchery stock as a control in the same river as the wild stock. Weakest of all designs is not having a control at all and relying on trend, in which characteristics of the fish are simply monitored over time as hatchery operations proceed. The obvious weakness of this approach is that the researcher cannot be certain the observed changes were due to domestication. Once again, the design can be strengthened by This type of study is exemplified by Petersson et al. (1996), who monitored several traits in a salmon stock as it underwent 20 years of hatchery culture.

New technologies make at least two other approaches to evaluating domestication possible, both of which rely on internal “quasi-controls” rather than actual control lines. The first involves use of DNA microsatellites to evaluate the reproductive success of individual fish. Fish returning to spawn are identified as to origin (hatchery or natural) and then sampled for DNA. When their progeny return, they are sampled for DNA and then assigned to parents. Domestication can be measured as the difference in reproductive success between fish of similar origin but different hatchery-wild ancestry. This technique is currently being applied in several projects, most notably the joint NMFS/WDFW Minter Creek coho project. Limitations are that only the one trait can be considered, the approach is expensive, the number of genetic classes grows over time so power drops, and only question 1 can be explored. The approach can be very valuable in producing information on selection differential, however. The other approach is using cryopreserved sperm to compare fish in the supplementation line  $n$  generations apart. To do the evaluation, the performance of progeny sired by contemporaneous sperm is compared to that of progeny sired by cryopreserved sperm. This approach is very appealing, but it has a number of real and theoretical drawbacks. It assumes that whatever genetic change is observed is due to domestication, when in fact there may be

other possibilities. It also assumes that there is no selective effect of the cryopreservation process itself. A final problem is that it only approaches question 1.

### **History of Domestication Research/Monitoring Planning in the YKFP Spring Chinook Program**

The preceding section was a short travelogue of theoretical ways to approach domestication research and monitoring. In attempting to explain our current situation with respect to monitoring domestication we have to consider these designs in light of the constraints imposed by the system, but also the history of our approach to developing monitoring measures. Historically the most important factor in developing all monitoring in the project has been the project purpose, as stated in a letter from the Northwest Power Planning Council (NPPC 1990):

*“In its action [giving conditional approval to the project] the Council reiterated that the purpose of the Yakima/Klickitat Production Project is to test the assumption that new artificial production can be used to increase harvest and natural production while maintaining genetic resources. It also emphasized that careful evaluation of supplementation and employment of adaptive management methods will be needed to accomplish this purpose. Such an approach should add the benefits of learning about supplementation and hatchery systems while contributing to the Council’s goal of increasing salmon and steelhead runs in the Columbia River Basin.”*

This statement makes clear that the project has three goals: more fish, more harvest, and containment of genetic risk. An additional goal, implied by the RASP definition of supplementation (RASP 1992) is the containment of ecological risk. Exactly how monitoring should be done is not stated except that it is clear that monitoring must be comprehensive to provide maximal value to the Region. Note that there is no implication of the preeminence of one goal over the other. Certainly there is no suggestion that this is to be primarily a genetic study. Based on these marching orders and the corporate culture of the project, the implicit assumption made in the monitoring plan of 1997 (Busack et al. 1997) is that the project was to be a working supplementation project. A heavily monitored working supplementation project, but a working supplementation project nevertheless.

Because of the understanding that the project was a supplementation project and not just a study of supplementation, the monitoring measures developed were minimally intrusive. We considered using the Naches and/or American River stocks as external controls for the supplementation effort in general, but concluded they would not serve well as controls (Busack et al. 1997):

The YFP has long been popularized as a project that will “test” supplementation. Accordingly, the first consideration must be the extent to which the measures in this plan actually evaluate supplementation. What usually comes to mind when a scientific test is spoken of is a controlled experiment. The optimal situation for

testing the effects of YFP supplementation on harvest opportunities, natural production, genetic impacts, and ecological impacts would be one of replicated treatments, where one treatment is supplementation, and the other is no supplementation. Unfortunately, the experimental situation offered by the YFP supplementation project is far from this optimum. There is no spatial replication, and there is no control. Monitoring the unsupplemented Naches and American River stocks may provide useful correlative data, but these stocks are too different from the Upper Yakima stock in life history traits (e.g., age structure, spawning timing, juvenile migratory pattern) and habitat to serve as controls. The unsupplemented Yakima basin spring chinook stocks are thus considered reference, not control, populations.

The wording of this paragraph seems very strong now, five years later, but our intent was to develop monitoring measures as rigorous as possible while not overpromising results. We did not consider an internal control line at this time because it would interfere with the supplementation effort. Thus we felt we had no real opportunity for a control line. At this time we were thinking only of question 1.

We eventually arrived at the following package of monitoring measures for domestication (Busack et al. (1997). Measures fell into three categories: selection potentials, trend, and direct measurement of change:

#### Selection potentials

- 1) Distribution by sex, size, age, and date of capture of prespawning mortality
- 2) Comparison of wild and hatchery spawners at selected traits that are likely to impose or reflect significant selection pressures (e.g., size, age at maturity, fecundity, geographical spawning distribution)
- 3) Comparison of wild and hatchery spawners at selected traits that are likely to impose or reflect significant selection pressures (e.g., size, age at maturity, fecundity, geographical spawning distribution)
- 4) Comparison of wild and hatchery juveniles at selected traits that are likely to impose or reflect significant selection pressures (e.g., size, migration timing)

#### Genetic trend

Comparison of means and variances of selected quantitative traits (e.g., size, age at maturity, spawning and migration timing, percentage of winter migrants) with baseline values in this stock and with contemporaneous data in reference stocks

#### Direct measurement of genetic change

- 1) Performance of juveniles generated by test crosses in hatchery (h<sub>xh</sub>, h<sub>xw</sub>, w<sub>xw</sub>) at selected traits

## 2) Performance of adults generated by test crosses in hatchery (h<sub>xh</sub>, h<sub>xw</sub>, w<sub>xw</sub>) at selected traits

This package would not at all be considered a strong package were domestication evaluation the major thrust of the monitoring effort, but was a reasonable non-intrusive approach. The selection differential approach is for the most part a detailed comparison of hatchery-origin and natural-origin fish, with the hope that the differences could in time be linked to selection, by coupling these changes with trends. The trend approach was coupled with some measures on the reference stocks, essentially a comparison with a wild control line. Because of existing initial genetic differences between the Upper Yakima stock and the reference stocks, we would expect the means at any trait to be different, but at a trait affected by domestication, the mean would change over time. In the unsupplemented control it would not be expected to change except through random variation. The direct measurement portion of the work is the most solid measurement of genetic change in the entire package. It mimics the approach of Reisenbichler and McIntyre (1977), and is therefore called the “Reisenbichler” approach. The plan was to periodically do these test crosses at whatever scale is required for adequate power. Because the number of fish generated by these crosses is likely to be quite high, they would have to become part of the releases. Thus, in doing the work, the domestication effect was likely to be increased somewhat. One interesting omission from the suite of measures is a “Reisenbichler” style experiment using cryopreserved sperm, as described in an earlier section. This has substantial advantages over the design proposed. As long as progeny from the test crosses are released it would interfere with the “natural” progress of domestication from supplementation, but the effect would be to retard domestication rather than increase it.

### **Revision of Domestication Research/Monitoring Designs**

No specific action was taken on the domestication aspects of the monitoring plan in the early years of the project because there were not hatchery-origin fish returning (no 4-year olds would return until 2001). Some feasibility work was done on the measures proposed for direct measurement of genetic change because although intended to be minimally disruptive, they would necessitate bringing hatchery-origin broodstock into the CESRF.

In January 2001 we began planning in earnest by holding a workshop to discuss the domestication and reproductive success study designs. Given the amount of development that had been taking place in DNA technology and the importance of the project, we thought it best to solicit comments from a wide variety of experts. Geneticists and other scientists interested in hatchery/wild interactions from WDFW, CRITFC, NMFS, USFWS, NWIFC, and USGS attended. The overwhelming opinion of attendees is that we needed to have an unsupplemented control line. There was some discussion of using the Naches and/or American stocks as control lines, but the majority of the discussion focused on developing an internal control line in a sequestered part of the basin. The Keechelus reach (from Easton dam to Keechelus dam). Other topics were discussed as well. The most noteworthy of these was a concern voiced by Reisenbichler that the

Reisenbichler-type design we planned for direct measurement of genetic change would not be powerful enough to detect a signal generation of domestication. He pointed out that in his 1977 study (Reisenbichler and McIntyre 1977) his hatchery-origin and natural-origin fish were two generations apart.

A few months later, the Independent Scientific Review Panel (ISRP) echoed and expanded upon the concerns voiced by workshop participants, arguing that the design should include both an unsupplemented control and a hatchery-only control (i.e., one maintained by mating hatchery-origin fish inter se). The ISRP recommended that funding be withheld until these concerns were addressed. The ISRP felt very strongly about the promise of the project, but felt that it would not live up to the promise without the control lines. Implicit but not directly stated in their response was the assertion that studying domestication needed to be a major part of a serious supplementation monitoring effort.

In response both to the ISRP and to the workshop, we embarked on a comprehensive analysis of potential domestication study designs, examining a total of 13 possible designs and combinations of designs. The designs spanned a range of complexity and scientific rigor from the full design the ISRP recommended- supplementation tested against both wild and hatchery control lines- to simply monitoring phenotypic trend. Brief overviews of all designs considered are included as an appendix. Based on our discussions of these designs we reached the following major conclusions:

1. An internal wild control line is not feasible. We agreed with the ISRP that the best design for monitoring domestication would include both a hatchery control line and a wild control for reasons explained earlier in this report. However, we concluded that a wild control line is not feasible. We considered two approaches to maintenance of a control line, and found neither one to be workable. The first approach was spatial sequestering, in which a portion of the basin was devoted to maintenance of the control line (designs 1A and 1B in appendix). The only possible area where this could potentially be accomplished- the Keechelus reach- is not a representative area of the basin, is not highly productive, and is subject to hydrological disruptions beyond our control. We felt it was unlikely we could sustain a run here, and even if we could, we doubted that it would be a convincing control line. A second problem was control of juveniles. For the wild control to work properly, all or nearly all juveniles would have to be trapped and marked so that they could be identified and returned to the sequestered area upon their return. Any untrapped fish would become leakage into the supplementation line. Also, these untrapped fish are essentially a loss to the control line, so the line would have to be very productive to maintain itself. This is a subtle point that requires some explanation. If smolt-adult survival is 1.5% (historically a reasonable value for Upper Yakima spring chinook), then a line of 200 spawners will produce about 13,000 smolts to sustain itself. If only marked fish are allowed to become spawners in this line, and only 80% (for example) of the smolts can be captured and marked, then only 160 adults identifiable to this line can be used. The net effect of trapping inefficiency is equivalent to an increase in mortality: the line will get smaller each generation.

In order to get around these trapping problems we also considered designs involving a temporally sequestered approach, where the entire basin was devoted to a wild control line one out of four years, and the supplementation line limited to the other three years (designs 2A and 2B). This approach required the wild line to have an obligate 4-year age structure, which would involve culling 3-year olds and 5-year olds from that line, and from some brood years of the supplementation line. Not only was the need to do this questionable scientifically as it would create a “selected” wild control, but it would also likely result in the removal of large numbers of fish from the system. A second problem is the fact that spring chinook exhibit precocial maturation to some degree, so there would be basin-wide leakage- at some level- in “off” years of wild control fish into the supplementation because of precocial males on the spawning grounds. Other problems were that since the wild fish would be returning in years when the supplementation line was not, direct comparisons of the two would not be possible, and data on the wild line would accrue slowly due to their appearance only one out of four years.

2. There are two feasible approaches to getting some level of wild control without maintenance of a wild control within the Upper Yakima population. The first is using the nearby Naches population as a wild control (designs 3A and 3B). Because of some key life history differences between it and the Upper Yakima population, it will be a convincing control for only a small number of traits, but using Naches fish for these traits is feasible. The second approach is cryopreservation of sperm from truly wild (i.e. presupplementation) males (design 5). Several generations after supplementation begins this sperm can be used for test crosses, and the differences in performance between fish sired with contemporaneous sperm and those sired with cryopreserved sperm will reflect domestication over multiple generations. Both these elements have been incorporated into our preferred design (design 9).

3. A hatchery control line is feasible. All juveniles can be marked before release, and thus all returning adults will be identifiable. Those not needed for broodstock can be removed at Roza Dam, so none will end up on the spawning grounds. There will still be a leakage problem at some level due to precocials from the hatchery control line fish remaining in the basin and spawning with supplementation line fish.

4. A design that has a hatchery control line as its foundation is considerably more relevant and powerful than the design we had been considering (designs 4 and 8). This was an approach very similar to that of Reisenbichler and McIntyre (1977), in which no control line is maintained but test crosses are made periodically with natural and hatchery fish from the supplementation line. As previously mentioned, this approach was originally chosen largely because it offered the most power with minimal disruption to the supplementation effort, but it would only have measured single-generation effects of hatchery rearing. This may in itself not seem a problem, but a subtle essential opportunity is missed using the Reisenbichler approach, and that is to show the effects of the supplementation regime rather than just hatchery rearing. Under supplementation we would expect the hatchery to cause some domestication, but this effect to be diluted by the regular cycling of fish through the natural environment. The Reisenbichler approach

will not pick up this “back-selection” effect. The control line approach has thus become the key element in the preferred design.

### **Revised Preferred Design**

The preferred design (design 9 in appendix) has four key elements, each of which is discussed in detail below:

*1. The supplementation line will be tested against a continuous hatchery control line to measure the retarding effect of natural selection on domestication over multiple generations.*

The essential difference between supplementation and traditional hatchery culture is that in supplementation there is an opportunity for domestication to be reduced by natural selection in the wild. The difference in performance between fish reared under the supplementation regime and those reared under a regime of continuous hatchery culture will be a measure of this natural “back” selection. Two of the raceways (randomly chosen each year) will be dedicated to the hatchery control (H) line, which will be started from hatchery returnees in 2002. These fish will be the offspring of a minimum of 30 pairs of fish, which should provide the H line an effective size of at least 100 per generation (assuming a 3:1 Nc/Nb ratio and 30% boost from factorial mating (Busack et al, in prep). This is well above the minimum of 50 recommended by Roff (1997) for minimization of drift during evaluation of quantitative traits. H line fish will be reared and released exactly as will their supplementation line (S) counterparts. No H line fish will be allowed to spawn in the wild; any returnees in excess of broodstock needs will be removed at the adult collection facility at Roza Dam. H and S line fish will be compared at a large number of adult and juvenile traits (see table below) each year, the suite of traits chosen includes the range of traits that have thus far been the subject of domestication studies, reproductive success (e.g. Chilcote et al. 1986; Fleming and Gross 1992,1993; Fleming et al. 1996), morphology (Fleming and Gross 1989), juvenile growth and survival (Reisenbichler and McIntyre 1977), juvenile predator avoidance behavior (Berejikian 1995, Berejikian et al. 1997), and juvenile agonistic behavior (Swain and Riddell 1990). All traits to be examined are fitness-related quantitative traits. The table explains in some detail how the various traits will be measured, but some mention should be made of key elements. In adult comparisons it is essential to make sure that H-line fish are compared to hatchery fish from the supplementation line (SH). Therefore, for comparisons of reproductive traits, 30 pairs of SH fish will be brought into the hatchery to be spawned for gamete and fertility comparisons or used in the experimental spawning channel for behavioral comparisons. Comparisons of juvenile growth and morphology in the hatchery environment will be made in the raceways, but comparisons in the wild will be made in the slough adjacent to the hatchery. Juvenile behavioral comparisons will be made in test arenas at the hatchery. The H line is small compared to the S line, and in any given year this may result in experimental power problems, but this will be overcome by doing the comparisons annually.

*2. Where appropriate, fish of the Naches stock will be used as wild controls to measure the amount of domestication the supplemented Upper Yakima stock has undergone over multiple generations.*

Comparing the S and H lines will show how much less domestication is incurred under supplementation than other traditional hatchery culture, but will not measure how much domestication is taking place. This can only be done with wild controls. A wild control line not being feasible (see Designs Evaluated section above) one approach is to use nearby similar stocks. The closest such stock is the Naches population. It differs considerably in age structure (Knudsen 1991) and some other respects from the Upper Yakima population so much so that its use as a control is precluded for many traits. For some traits- notably juvenile behavior- it seems likely that the differences between the Naches and Upper Yakima populations will be negligible, and the Naches stock will serve as a credible wild control. But this needs to be evaluated. Beginning this year, behavioral comparisons of Naches and wild Upper Yakima juveniles will be carried out in test arena aquaria to make sure the Naches stock can be used as a wild control. Assuming the result is positive, comparing Naches juveniles with natural-origin Upper Yakima juveniles will be a routine part of juvenile trait monitoring.

*3. Sperm from a large number of wild males will be cryopreserved, and then used in test crosses several generations later to measure the amount of divergence the stock has undergone during the project over multiple generations.*

Our main approach to measuring how much domestication has been incurred over multiple generations of supplementation will be through use of cryopreserved sperm. Sperm from 200 males will be cryopreserved as per Wheeler and Thorgaard (1991) both for gene banking and for this effort. The evaluation will be made after several generations, but the exact timing has not yet been decided, as discussed below. To control for inter-female variation in maternal effects, which will affect early growth, egg lots from individual females will be split, with 1/3 being fertilized by a contemporaneous male and 2/3 by a cryopreserved male. Assuming that half the eggs fertilized by cryopreserved sperm will be nonviable (Thorgaard, pers. comm.), this will result in equal numbers of juveniles in the two treatments. The juveniles will then be compared for all the juvenile traits listed in the table in a manner similar to that of Reisenbichler and McIntyre (1977). There are several details of this effort yet to be decided, but because of the parallel gene banking purpose of this activity, cryopreservation efforts began in 2001. The most important detail is when to do the evaluation. Although the cryopreserved sperm will be  $n$  generations older than the females it is used to fertilize, the genetic effects of the  $n$  generations of domestication will be halved in the hybrid fish. The design has to be powerful enough to detect  $n/2$  generations of domestication rather than  $n$  generations. Thus, it is likely that this evaluation will not be done until at least the fifth generation of supplementation. Sizing is an issue not just because of experimental power, but also because this work will impact the normal supplementation operation spatially, and because 1/3 of the eggs from any female involved will be lost. A final issue is disposition of the fish sired by cryopreserved sperm.



*4. A great deal of phenotypic data, already being collected, will continue to be collected to measure performance trends over multiple generations of supplementation.*

It is important not to lose sight of the fact that because this entire hatchery effort was designed around the concept of evaluating supplementation, a huge amount of data on this population was collected before the hatchery was built, and much of these data continue to be collected annually. Unlike other attempts at addressing domestication, where researchers have gone in “mid-stream” and measured differences between the progeny of wild and hatchery fish or compared a population influenced by a hatchery with a nearby wild one, here we are developing a complete record of changes the population undergoes at many traits as supplementation proceeds. Indeed, much of the domestication evaluation is merely expanding the measurements we have already been doing to include a hatchery control line. Trends in themselves are not proof of anything, but a trend in the direction of changes that have been observed or hypothesized to occur under domestication will put observations that are not statistically significant in context. Also, this extensive and detailed time series of performance information will strengthen the inferences we make from the explicitly experimental parts of the work, and provide insights that may well direct modifications of the monitoring effort.

### **Traits to be Evaluated**

The preferred design allows the possibility of monitoring numerous traits in adults and juveniles. Table 1 provides an overview of traits proposed for monitoring. Together these traits encompass nearly all traits previously explored in domestication studies in salmonids. 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. The reason for the distinction is simple: 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.

We list 11 adult traits and 11 juvenile traits to be evaluated, but this 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. 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, but at the same time minimize the impact to the supplementation effort.

Table 1. Traits to be evaluated in YFP spring chinook domestication selection monitoring. (H= hatchery line, SH=hatchery-origin fish from supplementation line, SN=natural-origin fish from supplementation line)		
Trait	Location	Activity
<b>Adult Traits</b>		
Adult to adult survival	Roza	Enumerate adults of each type (SH, H, and SN) passing upstream and taken into the hatchery. Based on age, allocate to respective broodyear and calculate recruits/spawner.
Age composition by sex	Roza – scale and DNA collection. Hatchery – SH/H origin spawners	Collecting scales and DNA subsamples for sexing at Roza. Record hatchery and SN origin adult age and sex at hatchery. Analyzing DNA samples to determine sex of 140 fish per SH and H.
Size-at-age by sex	Roza – scale and DNA collection. Hatchery – SH/H origin spawners	Collecting biological data from fish sampled for scales and DNA at Roza. Recording hatchery and SN origin adult sex at hatchery.
Sex ratio by age	Roza – scale and DNA collection. Hatchery – SH/H origin spawners	Recording sex of fish sampled for scales and DNA at Roza. Recording hatchery origin adult sex at hatchery
Migration timing to Roza	Roza	Sampling fish passing for marks and recording origin and date of passage.

Migration/spawning timing to spawning grounds	River	Daily snorkel trips over a limited area, i.e. Easton to Game Ramp, to look at the ratio of SH and SNs. Compare to SH/SN ratio observed at Roza. Compare spawning temporal distributions via K-S test.
Fecundity	Hatchery	Enumerate eggs from hatchery origin and SN females. Requires holding SH females to maturity at hatchery. Once mature H Control line fish return these will need to be held on station, as well.
Egg size	Hatchery	Measure egg sizes of hatchery origin and SN females. Requires holding SH origin females to maturity at hatchery. Once mature H Control line fish return these will need to be held on station, as well.
Reproductive effort	Hatchery	Weigh gametes of hatchery origin and SN fish. Requires holding SH origin fish to maturity at hatchery. Once H Control line mature fish return these will need to be held on station, as well.
Male and female fertility	Hatchery	Within year comparison of survival of eggs from SH, H and SNs using isolettes. Long term trends over time.
Adult morphology at spawning	Hatchery (collection), Lab (analyses)	Collect truss measurement data from adult digital photos. Use sheared principle components analysis to compare SH, H, and SN by sex. N=100 images per group.
<b>Juvenile Traits</b>		
Emergence timing	Hatchery incubation room	Compare differences in emergence timing of SH, H and SN females from test incubation units
KD at emergence	Hatchery incubation room	Weight and length at emergence. Regression Length vs Weight. ANCOVA to test differences in slopes. ANOVA to test for Origin effects.
Egg-fry survival	Hatchery incubation room	Enumerate mortalities of SH, H and SN origin females.
Fry-smolt survival	Production raceways	Enumerate mortalities of H and SN origin fingerlings in hatchery raceways.
	Oxbow – The “Wild” side of Reisenbichler design	Enumerate % survivors of SH, H and SN origin fry as they develop and emigrate from oxbow. In addition, growth, and migration timing will be documented.
Smolt-to-smolt survival	Acclimation sites to McNary, JD, Bonn.	Estimate PIT tag survival of SH, H and SN to various dam juvenile PIT tag detectors
Smolt-to-adult	Acclimation to Roza	Based on the number of smolts released calculate the number surviving back to Roza by age and sex
Out migration timing	Acclimation sites to McNary, JD, Bonn.	Estimate PIT tag migration timing of SH, H and SN to various dam juvenile PIT tag detectors
Condition factor/Growth rate	Production raceways	Weight and length collected at marking, Jan. QC, and release. Regression Length vs Weight. ANCOVA to test differences in slopes. ANOVA to test for H vs SH line effects.
Agonistic-Competitive behavior	Test tanks	Challenge H, SH and SN fish in pairs. Note behaviors. Use Naches fish at some time as “wild” control, also.

Predator avoidance	Test tanks	Challenge H, SH and SN fish to predator exposure. Enumerate survivors. Note behaviors.
	Channel – June-July use possible	Challenge H, SH and SN fish to predator exposure. Enumerate survivors. Note emigration timing.
Precocialism	Production raceways	At release, note proportion of precocials from H and SH groups. At final “push” on May 30 sample and estimate number precocials remaining in SH and H groups.

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## Appendix: Overviews of Domestication Monitoring/Research Designs

### Design 1A. Supplementation with Continuous Wild and Hatchery Control Lines-“Spatial Sequestering”

**General Description:** Supplementation proceeds, but 1) a section of natural environment is sequestered for use by a continuous wild control line, and 2) some of the hatchery facilities are dedicated to the production of a hatchery control line (H). Wild control (W) line is maintained by allowing only wild-line (W) adults into the sequestered area, tagging as many of the resulting juveniles as possible as they leave the sequestered area. The W line will be maintained by two methods. First, the capture and DNA-typing of all wild fish passed into the sequestered area in generation one of the W line, and in all subsequent generations. DNA-typing allows progeny of wild spawners volunteering into a trap at the mouth of the sequestered area to be recognized by pedigree. An undetermined number of W line progeny may not, however volunteer into the sequestered area trap. Thus, the second measure entails marking as many as possible of the W line juveniles as they leave the sequestered area. Such fish would, as returning adults, be identified at a downstream trap and transported to the sequestered area.

**Facility Requirements:** A controlled-reach of the Upper Yakima basin must be sequestered for exclusive use of W line by the construction of an 100% efficient adult trap. Juvenile trapping facilities are also needed below the sequestered area that can catch a large proportion of outmigrating juveniles for marking. The sequestered reach must be capable of supporting at least 400 spawners, and must provide sufficient rearing habitat to retain juveniles until they reach marking size. Juvenile marking must occur year-round. A portion of the hatchery facilities (probably about 1/3) will have to be devoted to incubation and rearing of the H line. Additional activity at Roza trap, in addition to sorting by line, will be transporting W line fish to sequestered reach and surplus excess H line fish.

**Tagging Requirements:** The three lines will have to be marked unambiguously so that adults collected at Roza can be identified as to line. So altogether, there will be four groups to sort out: W, H, S-line hatchery fish, and naturally-spawned S-line fish (“natural origin recruits”, or NOR’s).

**Pros:** 1) Scientifically the most interesting of all designs we are considering. The control lines allow supplementation to be tested against no hatchery influence and against heavy hatchery influence. 2) Allows the comparisons of different levels of hatchery impact to be evaluated continuously.

**Cons:** 1) The only possible reach identified for sequestering is the Easton reach. It is unclear whether it can support enough fish and whether it is sustainable. It is also a nonrepresentative production area. 2) Unless all W juveniles can be trapped and marked, “leakage” will occur from W to S line. This will downwardly bias the differences in performance between the W and S line, upwardly bias the S-H comparison, and result in underestimates for W line productivity. 3) Returning H line adults in excess of broodstock needs will have to be removed from the system whenever they occur. There could be many surplus fish in some years.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)				
Year	Natural Environment		Hatchery Environment	
	Sequestered Natural Area	Rest of Basin	Remaining Resources	Dedicated Hatchery Resources
1	W	S	S	H
2	W	S	S	H
3	W	S	S	H
4	W	S	S	H
5	W	S	S	H
6	W	S	S	H
7	W	S	S	H
8	W	S	S	H
9	W	S	S	H
10	W	S	S	H



### Design 1B. Supplementation with Continuous Wild Line- “Spatial Sequestering”

**General Description:** Same as 1A, but without hatchery control line. Supplementation proceeds, but a section of natural environment is sequestered for use by a continuous wild control line. Wild control (W) line is maintained by allowing only wild-line (W) adults into the sequestered area, tagging as many of the resulting juveniles as possible as they leave the sequestered area. The W line will be maintained by two methods. First, the capture and DNA-typing of all wild fish passed into the sequestered area in generation one of the W line, and in all subsequent generations. DNA-typing allows progeny of wild spawners volunteering into a trap at the mouth of the sequestered area to be recognized by pedigree. An undetermined number of W line progeny may not, however volunteer into the sequestered area trap. Thus, the second measure entails marking as many as possible of the W line juveniles as they leave the sequestered area. Such fish would, as returning adults, be identified at a downstream trap and transported to the sequestered area.

**Facility Requirements:** A controlled-reach of the Upper Yakima basin must be sequestered for exclusive use of W line by the construction of an 100% efficient adult trap. Juvenile trapping facilities are also needed below the sequestered area that can catch a large proportion of outmigrating juveniles for marking. The sequestered reach must be capable of supporting at least 400 spawners, and must provide sufficient rearing habitat to retain juveniles until they reach marking size. Juvenile marking must occur year-round. Additional activity at Roza trap, in addition to sorting by line, will be transporting W line fish to sequestered reach.

**Tagging Requirements:** The two lines will have to be marked unambiguously so that adults collected at Roza can be identified as to line. So altogether, there will be three groups to sort out: W, S-line hatchery fish, and naturally-spawned S-line fish (“natural origin recruits”, or NOR’s).

**Pros:** 1) The control lines allow supplementation to be tested against no hatchery influence, probably the key question we’re interested in. and against heavy hatchery influence.  
2) Requires no routine surplus of fish.

**Cons:** 1) The only possibility for a sequestered reach is the Easton reach. The situation here is risky, and this reach is not good representative habitat.

2) Unless all W juveniles can be trapped and marked, “leakage” will occur from W to S line. This will downwardly bias the differences in performance between the W and S line, and result in underestimates for W line productivity.

Year	Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)		
	Natural Environment		Hatchery Environment
	Sequestered Natural Area	Rest of Basin	
1	W	S	S
2	W	S	S
3	W	S	S
4	W	S	S
5	W	S	S
6	W	S	S
7	W	S	S
8	W	S	S
9	W	S	S
10	W	S	S

### Design 1C. Supplementation with Continuous Hatchery Control Line

**General Description:** Supplementation proceeds, but in addition to being used for ordinary supplementation operations, the hatchery is also used for culture of a hatchery control line. Hatchery control line (H) is maintained by tagging the progeny in a way that will distinguish them from the regular supplementation line (S) production of the hatchery. When H adults return to Roza they will be taken as broodstock or surplused, but under no circumstances released upstream.

**Facility Requirements:** A portion of the hatchery facilities (probably about 1/3) will have to be devoted to incubation and rearing of the H line. Additional activity at Roza trap, in addition to sorting by line, will be transporting W line fish to sequestered reach and surplusing excess H line fish.

**Tagging Requirements:** The two lines will have to be marked unambiguously so that adults collected at Roza can be identified as to line. So altogether, there will be three groups to sort out: H, S-line hatchery fish, and naturally-spawned S-line fish ("natural origin recruits", or NOR's).

**Pros:** 1) Scientifically interesting. The control line allows supplementation to be tested against heavy hatchery influence.  
2) Definitely feasible, and the only definitely feasible control line design.

**Cons:** 1) Hypothesis to be tested (supplementation against continuous hatchery culture) is not a key hypothesis of the project, and may be of little interest to the region.

2) Returning H line adults in excess of broodstock needs will have to be removed from the system whenever they occur. There could be many surplus fish in some years.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)			
Year	Natural Environment	Hatchery Environment Remaining Resources	Dedicated Hatchery Resources
1	S	S	H
2	S	S	H
3	S	S	H
4	S	S	H
5	S	S	H
6	S	S	H
7	S	S	H
8	S	S	H
9	S	S	H
10	S	S	H

## Design 2A. Supplementation with Single Brood Year Wild and Continuous Hatchery Control Lines- “Temporal Sequestering”, “1 in 4”

**General Description:** Supplementation proceeds normally three out of four years, but 1) every fourth year the natural production area is used only for a single-brood year wild control line, and 2) in addition to being used for ordinary supplementation operations, the hatchery is also used for culture of a hatchery control line. Key to success of this approach is genetically isolating the Wild control (W) line from the Supplementation (S) line. This will be done by removal at Roza of 3 and 5 year old fish from the S line that return in the “on” years, and removal of all 3 and 5 year old fish produced by W line (these will always return in off years). Hatchery (H) control line is maintained by collecting H fish as broodstock at Roza and removing all fish in excess of broodstock needs. Surplus H fish will under no circumstances be released upstream.

**Facility Requirements:** A portion of the hatchery facilities (probably about 1/3) will have to be devoted to incubation and rearing of the H line. In “on” years, since there will be S line production, the entire hatchery is available for H-line production. Additional activity at Roza trap, in addition to sorting by line, will be removal of 3’s and 5’s from W line and surplus excess H line fish.

**Tagging Requirements:** W line will not be tagged. S line will be tagged as it is presently, but H line will have to be tagged so H fish can be separated from S-line hatchery fish.

**Pros:** 1) The control lines allow supplementation to be directly tested against heavy hatchery influence 3 out of 4 years, and wild against heavy hatchery influence 1 out of 4 years. Supplementation can be tested against no hatchery influence more or less continuously but across years.  
2) Does not require a controlled-access sequestered stream reach.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)			
Year	Natural Environment	Hatchery Environment	
		Remaining Resources	Dedicated Hatchery Resources
1	W	H	H
2	S	S	H
3	S	S	H
4	S	S	H
5	W	H	H
6	S	S	H
7	S	S	H
8	S	S	H
9	W	H	H
10	S	S	H

**Cons:** 1) Environmental variability will weaken comparisons of S vs W (but continuous comparison of S vs H will help control for this).  
2) Removal of 3’s and 5’s is selection on age structure that will make the W line differ genetically from the other lines more than would be expected due to drift. This selection conceivably could depress the fitness of this line as well.  
3) The “1 in 4” approach guarantees slow accrual of data on comparisons of W vs other lines.  
4) Returning H line adults in excess of broodstock needs will have to be removed from the system whenever they occur. There could be many surplus fish in some years.

## Design 2B. Supplementation with Single Brood Year Wild and Hatchery Control Lines- “Temporal Sequestering”, “1 in 4”

**General Description:** Same as 2A, but with H line being maintained on “1 in 4” basis. Supplementation proceeds normally three out of four years, but 1) every fourth year the natural production area is used only for a single-brood year wild control line, and 2) in addition to being used for ordinary supplementation operations, the hatchery is also used for culture of a hatchery control line. Key to success of this approach is genetically isolating the Wild control (W) line from the Supplementation (S) line. This will be done by removal at Roza of 3 and 5 year old fish from the S line that return in the “on” years, and removal of all 3 and 5 year old fish produced by W line (these will always return in off years). Hatchery (H) control line is maintained by collecting H fish as broodstock at Roza and removing all fish in excess of broodstock needs. Surplus H fish will under no circumstances be released upstream.

**Facility Requirements:** A portion of the hatchery facilities (probably about 1/3) will have to be devoted to incubation and rearing of the H line. In “on” years, since there will be S line production, the entire hatchery is available for H-line production. Additional activity at Roza trap, in addition to sorting by line, will be removal of 3's and 5's from W line and surplus excess H line fish.

**Tagging Requirements:** W line will not be tagged. S line will be tagged as it is presently, but H line will have to be tagged so H fish can be separated from S-line hatchery fish.

**Pros:** 1) Wild can be tested against heavy hatchery influence 1 out of 4 years. Supplementation can be tested against two levels of hatchery influence more or less continuously but across years.  
2) Does not require a controlled-access sequestered stream reach.  
3) Fewer H fish will have to be surplus than with design 2A.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)			
Year	Natural Environment	Hatchery Environment	
		Remaining Resources	Dedicated Hatchery Resources
1	W	H	H
2	S	S	S
3	S	S	S
4	S	S	S
5	W	H	H
6	S	S	S
7	S	S	S
8	S	S	S
9	W	H	H
10	S	S	S

**Cons:** 1) Environmental variability will weaken comparisons of S vs W, and having H line in 1 of 4 years only will not help alleviate this.  
2) Removal of 3's and 5's is selection on age structure that will make the W line differ genetically from the other lines more than would be expected due to drift. This selection conceivably could depress the fitness of this line as well.  
3) The “1 in 4” approach guarantees slow accrual of data on comparisons of W vs other lines.  
4) Returning H line adults in excess of broodstock needs will have to be removed from the system whenever they occur. There could be many surplus fish in some years.

**Design 3A. Supplementation with a HxH control line, and using the Naches stock as a Wild control**

**General Description:** Supplementation proceeds, but 1) the Naches system is used as a wild control line, and 2) in addition to being used for ordinary supplementation operations, the hatchery is also used for culture of a hatchery control line. Hatchery control line (H) is maintained by tagging the progeny in a way that will distinguish them from the regular supplementation line (S) production of the hatchery. When H adults return to Roza they will be taken as broodstock or surplused, but under no circumstances released upstream. Tagged returning S adults will be counted at Roza and released to spawn naturally.

**Facility Requirements:** Adult and jack recruits will be counted at Prosser and Naches system production estimated by subtracting out fish passing Roza. A portion of the hatchery facilities (probably about 1/3) will have to be devoted to incubation and rearing of the H line. Additional activity at Roza trap will be surplusing excess H line fish.

**Tagging Requirements:** The two hatchery origin lines will have to be marked unambiguously so that adults collected at Roza can be identified as to H or S line.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)			
Year	Natural Environment	Upper Yakima	Dedicated Hatchery Resources
1	W	S	H
2	W	S	H
3	W	S	H
4	W	S	H
5	W	S	H
6	W	S	H
7	W	S	H
8	W	S	H
9	W	S	H
10	W	S	H

**Pros:** 1) The wild Naches control line is reproductively isolated from the Upper Yakima system's supplemented and hatchery lines, so a comparison of W vs H vs S lines is possible each year.

**Cons:** 1) The Naches system is not a good representation of upper Yakima habitat. Thus, natural selection pressures differ resulting in significant differences in life history traits of the two stocks, e.g. age composition and size at age. The Naches has a larger proportion of older age classes and are also larger at age. We assume these life history differences are due to the differences in population-specific selection pressures each stock experiences. The older age classes mean that portions of the two populations originating from the same cohort will occupy different spatio-temporal strata and experience different marine environments, adult in-river conditions, fishing selectivity and pressure, etc. making comparisons of quantitative traits influenced by environmental variation suspect.

2) Returning H line adults in excess of broodstock needs will have to be removed from the system whenever they occur. There could be many surplus fish in some years.

3) Sampling Naches fish for many reproductive traits (i.e. fecundity, reproductive effort, total gamete weight, viability) will be impossible unless unspawned individuals are collected for lethal sampling. No facility or satisfactory method of collecting a representative sample of unspawned Naches fish exists.

**Design 3B. Supplementation with the Naches stock as a Wild Control Line**

**General Description:** Like 3A, but without the hatchery control line. Supplementation proceeds, but the Naches system is used as a wild control line.

**Facility Requirements:** Adult and jack recruits will be counted at Prosser and Naches system production estimated by subtracting out fish passing Roza.

**Tagging Requirements:** No new tagging required; S line tagged as it presently is.

Physical Location of Production Lines during Period of Research (S=supplemented, W= wild control, H=hatchery control)			
Year	Natural Environment		Hatchery Environment
	Naches System	Upper Yakima	
1	W	S	S
2	W	S	S
3	W	S	S
4	W	S	S
5	W	S	S
6	W	S	S
7	W	S	S
8	W	S	S
9	W	S	S
10	W	S	S

**Pros:** 1) The wild Naches control line is reproductively isolated from the Upper Yakima system's supplemented and hatchery lines, so a comparison of W vs S lines is possible each year.

**Cons: 1)** The Naches system is not a good representation of upper Yakima habitat. Thus, natural selection pressures differ resulting in significant differences in life history traits of the two stocks, e.g. age composition and size at age. The Naches has a larger proportion of older age classes and are also larger at age. We assume these life history differences are due to the differences in population-specific selection pressures each stock experiences. The older age classes mean that portions of the two populations originating from the same cohort will occupy different spatio-temporal strata and experience different marine environments, adult in-river conditions, fishing selectivity and pressure, etc. making comparisons of quantitative traits influenced by environmental variation suspect.

2) Given the differences between the two stocks, not using a hatchery control line could result in a significant loss of information about hatchery effects.

3) Sampling Naches fish for many reproductive traits (i.e. fecundity, reproductive effort, total gamete weight, viability) will be impossible unless unspawned individuals are collected for lethal sampling. No facility or satisfactory method of collecting a representative sample of unspawned Naches fish exists.

#### Design 4. Within-Generation Reaction Norms-“Reisenbichler”

**General Description:** Supplementation proceeds without control lines, but in generations 1,3, and 5 (tentatively) of supplementation, test crosses are made of hatchery-origin ( $S_H$ ) and wild-origin ( $S_W$ ) fish ( $S_H \times S_H$ ,  $S_H \times S_W$ , and  $S_W \times S_W$ ) are made, and the progeny of the crosses are compared in both hatchery and natural environments. All fish produced by crosses are considered hatchery production; none need be removed.

**Facility Requirements:** Some of the hatchery will have to be dedicated to rearing fish from test crosses during the years the study is done. How much of the hatchery needs to be used for this depends on the traits to be evaluated. Also, one or more sequestered reaches will be needed for fish to incubate and rear (a single reach can be used if we can replicate temporally). A trap at reach outfall must be capable of trapping a high proportion of outmigrants. The spawning channel and hatchery slough could be used for this purpose.

**Tagging Requirements:** Depends on which traits will be evaluated. Assuming evaluations will include measurements released fish, fish need to tagged with raceway-specific tags, as is now done in OCT-SNT comparisons. DNA microsat will be used to identify fish in or leaving sequestered reach(es). In other years fish can be tagged in any other way.

**Pros:** 1) Will allow testing of single-generation domestication effects at several juvenile traits, without the logistical difficulty of control lines.

2) Because a large portion of the hatchery can potentially be used for the comparisons, probably many traits can be evaluated with high power.

3) Logistically simple to carry out (at least in hatchery), and flexible. Can be done as often as desired (but see Con#4).

**Cons:** 1) Will detect only single-generation effects; and lack of controls means committing to this approach (aside from augmentation with a cryo approach [design 5]) will preclude opportunities for looking at changes over multiple generations.

2) Approach is simple for traits to be evaluated in hatchery but this only gets at half the comparisons of interest. More interesting is the evaluation of fitness changes in the wild, and for this, stable sequestered reaches are needed.

3) Unlikely that changes can be detected in adult performance because adults will have to be “grown” from test cross progeny, and to achieve sufficient power, entire hatchery would have to be used for crosses.

4) May result in use of many hatchery-origin fish in hatchery during years the study is being done, and this will cause the population to undergo more domestication than it would otherwise.

Physical Location of Production Lines during Period of Research (S=supplemented, crosses = $S_H \times S_H$ , $S_H \times S_W$ , $S_W \times S_W$ juveniles). This layout assumes (for illustration only) that the study is done once per generation.				
Year	Natural Environment Sequestered Natural Area	Rest of Basin	Hatchery Environment Remaining Resources	Dedicated Hatchery Resources
1	crosses	S	S	Crosses
2	S	S	S	S
3	S	S	S	S
4	S	S	S	S
5	crosses	S	S	crosses
6	S	S	S	S
7	S	S	S	S
8	S	S	S	S
9	crosses	S	S	Crosses

## Design 5. Multi-generation Reaction Norms-“Cryo”

**General Description:** Supplementation proceeds without control lines, but in generations 1,3, and 5 (tentatively) of supplementation, test crosses are made, using the natural-origin females taken for broodstock and sperm from contemporaneous males and cryopreserved sperm from pre-supplementation wild fish. Probably the most powerful design would be to split each female's egg lot, and fertilize half with a contemporaneous male and half with a cryo male. The progeny of the crosses are compared in both hatchery and natural environments. All fish produced by crosses are considered hatchery production; none need be removed.

**Facility Requirements:** Some of the hatchery will have to be dedicated to rearing fish from test crosses during the years the study is done. Also, one or more sequestered reaches will be needed for fish to incubate and rear (a single reach can be used if we can replicate temporally). A trap at reach outfall must be capable of trapping a high proportion of outmigrants. The spawning channel could be used for this purpose.

**Tagging Requirements:** Depends on which traits will be evaluated. Assuming evaluations will include measurements released fish, fish need to tagged with raceway-specific tags, as is now done in OCT-SNT comparisons. DNA microsat will be used to identify fish in or leaving sequestered reach(es). In other years fish can be tagged in any other way.

**Pros:** 1) Will allow testing of multiple-generation domestication effects at several juvenile traits, without the logistical difficulty of control lines.  
2) Logistically simple to carry out (at least in hatchery), and flexible. Can be done as often as desired so long as the cryopreserved sperm lasts (but see Con#1).  
3) By infusion of genes from presupplementation years, evaluation will slow domestication somewhat.

**Cons:** 1) Cryopreserved sperm tend to have low fertility (a good planning average is 50%). Thus, we can expect half the eggs fertilized with cryopreserved sperm to be lost. Assuming we want to

have equal progeny numbers of each type from each female, we will lose 33% of the eggs from each female involved.

2) Approach is simple for traits to be evaluated in hatchery but this only gets at half the comparisons of interest. More interesting is the evaluation of fitness changes in the wild, and for this, stable sequestered reaches are needed.

3) Unlikely that changes can be detected in adult performance because of low power. Testing for changes at adult traits will be operationally the same as current OCT-SNT comparisons.

4) Because the only presupplementation genes will be from males, the genetic effect that will be expressed is only half of what has taken place. In other words, the “signal” from genetic change will only be half of what it would be based on comparison of supplementation with a control line.

Physical Location of Production Lines during Period of Research (S=supplemented, crosses= HxH, HxW, WxW juveniles). This layout assumes (for illustration only) that the study is done once per generation.			
Year	Natural Environment		Hatchery Environment
	Sequestered Natural Area	Rest of Basin	
1	crosses	S	crosses
2	S	S	S
3	S	S	S
4	S	S	S
5	crosses	S	crosses
6	S	S	S
7	S	S	S
8	S	S	S
9	crosses	S	crosses
10	S	S	S



Design 6. Phenotypic Trend

**General Description:** Supplementation proceeds without control lines, but measurements of performance for several traits are made in both the hatchery and natural environments. Genetic change is inferred from differences between current and historical performance. This approach is the minimally intrusive “see what happens” approach.

**Facility Requirements:** No specific modifications of hatchery operations will have to be made.

**Tagging Requirements:** No large-scale specific tagging will have to be done explicitly for this purpose.

**Pros:** 1) Simple, minimally intrusive. Does not require special facilities or tagging, or sequestered reaches.

**Cons:** 1) This will be a totally uncontrolled experiment. Attributing change to genetic effects will be difficult since much observed change could be due to an environmental trend (stronger by also tracking Naches stock).

Physical Location of Production Lines during Period of Research (S=supplemented). This layout assumes (for illustration only) that the study is done once per generation.		
Year	Natural Environment	Hatchery Environment
1	S	S
2	S	S
3	S	S
4	S	S
5	S	S
6	S	S
7	S	S
8	S	S
9	S	S
10	S	S

### Design 7. Single-Generation Reaction Norms with Continuous Hatchery Line and Naches as wild control for selected traits

**General Description:** Supplementation proceeds, but in generations 1,3, and 5 (tentatively) of supplementation, test crosses are made of hatchery-origin ( $S_H$ ) and wild-origin ( $S_W$ ) fish ( $S_H \times S_H$ ,  $S_H \times S_W$ , and  $S_W \times S_W$ ) are made, and the progeny of the crosses are compared in both hatchery and natural environments. All fish produced by crosses are considered hatchery production; none need be removed. A hatchery-only control line will also be maintained. The Naches stock will be used as a wild control for some traits (mainly juvenile behavior) to provide a multi-generation supplementation vs wild comparison.

**Facility Requirements:** Some of the hatchery will have to be dedicated to rearing fish from test crosses during the years the study is done. How much of the hatchery needs to be used for this depends on the traits to be evaluated. A third of the hatchery will have to be dedicated to production of the hatchery control line. Also, one or more sequestered reaches will be needed for fish to incubate and rear (a single reach can be used if we can replicate temporally). A trap at reach outfall must be capable of trapping a high proportion of outmigrants. The spawning channel and hatchery slough will be used for this purpose. Surplus H line fish will have to be removed at Roza.

**Tagging Requirements:** Depends on which traits will be evaluated. Assuming evaluations will include measurements released fish, fish need to be tagged with raceway-specific tags, as is now done in OCT-SNT comparisons. DNA microsats will be used to identify fish in or leaving sequestered reach(es). In other years fish can be tagged in any other way. H line fish will have to be marked distinctly.

**Pros:** 1) Will allow testing of single-generation domestication effects at several juvenile traits and multiple generation test s of domestication differences between supplementation and pure hatchery culture.

2) Because a large portion of the hatchery (up to 2/3) can potentially be used for the comparisons, probably many traits can be evaluated with high power.

3) Logistically simple to carry out (at least in hatchery), and flexible. Can be done as often as desired (but see Cont#4).

**Cons:** 1) Will detect only single-generation effects; and lack of controls means committing to this approach (aside from augmentation with a cryo approach [design 5]) will preclude opportunities for looking at changes over multiple generations, except for those traits for which Naches is a suitable control.  
2) Approach is simple for traits to be evaluated in hatchery but this only gets at half the comparisons of interest. More interesting is the evaluation of fitness changes in the wild, and for this, stable sequestered reaches are needed.  
3) Unlikely that single-generation changes can be detected in adult performance because adults will have to be “grown” from test cross progeny, and to achieve sufficient power, entire hatchery would have to be used for crosses.  
4) May result in use of many hatchery-origin fish in hatchery during years the study is being done, and this will cause the population to undergo more domestication than it would otherwise.  
5) H-line fish surplus to broodstock needs will have to be removed from system.

Physical Location of Production Lines during Period of Research (S=supplemented, crosses = $S_H \times S_H$ , $S_H \times S_W$ , $S_W \times S_W$ juveniles). This layout assumes (for illustration only) that the study is done once per generation.					
Year	Natural Environment		Hatchery Environment		
	Sequestered Natural Area (Spawning channel and slough)	Rest of Basin	Remaining Resources	Other Dedicated Hatchery Resources	Dedicated Hatchery Resources
1	crosses	S	S	H	crosses
2	S	S	S	H	S
3	S	S	S	H	S
4	S	S	S	H	S
5	crosses	S	S	H	crosses
6	S	S	S	H	S
7	S	S	S	H	S
8	S	S	S	H	S
9	crosses	S	S	H	crosses
10	S	S	S	H	H

**Design 8. Single-Generation Reaction Norms with Naches as wild control for selected traits, with use of cryopreserved sperm for multi-generation evaluation of domestication.**

**General Description:** Supplementation proceeds, but in generations 1,3, and 5 (tentatively) of supplementation, test crosses are made of hatchery-origin ( $S_H$ ) and wild-origin ( $S_W$ ) fish ( $S_H \times S_H$ ,  $S_H \times S_W$ , and  $S_W \times S_W$ ) are made, and the progeny of the crosses are compared in both hatchery and natural environments. All fish produced by crosses within the supplementation line are considered hatchery production; none need be removed. One or more small, sequestered areas will be used for juvenile testing (probably the spawning channel and slough). The Naches stock will be used as a wild control for some traits (mainly juvenile behavior) to provide a multi-generation supplementation vs wild comparison. After several generations (probably a minimum of 5), test crosses will be done with cryopreserved sperm to evaluate how much the supplementation line has diverged from the founding population. At that time, a portion of the hatchery will have to be dedicated to rearing these test cross fish (the exact needs will have to be determined by power analysis).

Physical Location of Production Lines during Period of Research (S=supplemented, crosses = $S_H \times S_H$ , $S_H \times S_W$ , $S_W \times S_W$ juveniles). This layout assumes (for illustration only) that the study is done once per generation. After several generations, cryopreserved sperm will be used for test crosses				
Year	Natural Environment		Hatchery Environment	
	Sequestered Natural Area (Spawning channel and slough)	Rest of Basin	Remaining Resources	Dedicated Hatchery Resources
1	crosses	S	S	Crosses
2	S	S	S	S
3	S	S	S	S
4	S	S	S	S
5	crosses	S	S	crosses
6	S	S	S	S
7	S	S	S	S
8	S	S	S	S
9	crosses	S	S	Crosses
10	S	S	S	H

**Facility Requirements:** Some of the hatchery will have to be dedicated to rearing fish from test crosses during the years the study is done. How much of the hatchery needs to be used for this depends on the traits to be evaluated. One or more sequestered reaches will be needed for fish to incubate and rear (a single reach can be used if we can replicate temporally). A trap at reach outfall must be capable of trapping a high proportion of outmigrants. The spawning channel and hatchery slough will be used for this purpose. If these areas are used, the trap at the slough will have to be modified to exclude other fish. Also, if the slough is to be used for this purpose, it should not be used for coho rearing.

**Tagging Requirements:** Depends on which traits will be evaluated. If evaluations will include measurements on released fish, fish need to be tagged with raceway-specific tags, as is now done in OCT-SNT comparisons. Juveniles used in sequestered areas will need to be otolith marked.

### Design 8 (continued)

**Pros:** 1) Will allow testing of single-generation domestication effects at several juvenile traits and some adult traits, and multiple generation tests of domestication for traits that can appropriately be tested with Naches fish.

2) Because a large portion of the hatchery can potentially be used for the comparisons, probably many traits can be evaluated with high power.

3) Logistically simple to carry out (at least in hatchery), and flexible. Can be done as often as desired (but see Con#4).

4) Unlike #9, no fish need be removed from system routinely.

5) Does not require portion of the hatchery to be continuously dedicated to a control line.

**Cons:** 1) The single-generation orientation of the supplementation vs wild aspect of this approach may limit our ability to find differences that we could find over multiple generations if we had a wild control line.

2) The lack of a hatchery control line makes the number of multiple-generation effects we can look at very limited. Design is considerably weaker than #9 in this regard.

3) Approach is simple for traits to be evaluated in hatchery but this only gets at half the comparisons of interest. For many traits, the “hatchery “ half is all we can expect to be able to do. More interesting is the evaluation of fitness changes in the wild, and for this, stable sequestered reaches are needed, and these are feasible only for juvenile traits.

4) Unlikely that single-generation changes can be detected in adult performance because adults will have to be “grown” from test cross progeny, and to achieve sufficient power, entire hatchery would have to be used for crosses.

5) May result in use of many hatchery-origin fish in hatchery during years the study is being done, and this will cause the population to undergo more domestication than it would otherwise.

6) EIS may have to be modified to allow use of hatchery fish in hatchery for generating test crosses.

7) Use of slough for domestication testing will make it unavailable for coho rearing.

**Design 9. Supplementation with 1) Continuous Hatchery Line, 2) Naches as wild control for selected traits, and 3) cryopreserved sperm for multiple-generation evaluation of supplementation vs wild.**

**General Description:** Supplementation proceeds, but a hatchery-only control line will also be maintained. One or more small, sequestered areas will be used for juvenile testing of H and S line fish (probably the spawning channel and slough). The Naches stock will be used as a wild control for some traits (mainly juvenile behavior) to provide a multi-generation supplementation vs wild comparison. After several generations (probably a minimum of 5), test crosses will be done with cryopreserved sperm to evaluate how much the supplementation line has diverged from the founding population. At that time, a portion of the hatchery will have to be dedicated to rearing these test cross fish (the exact needs will have to be determined by power analysis).

Physical Location of Production Lines during Period of Research This layout does not include the testcrosses using cryopreserved sperm that will be done after perhaps 5 generations.				
	Natural Environment		Hatchery Environment	
Year	Sequestered Natural Area (Spawning channel and slough)	Rest of Basin	Remaining Raceways	2 raceways
1	S,H	S	S	H
2	S,H	S	S	H
3	S,H	S	S	H
4	S,H	S	S	H
5	S,H	S	S	H
6	S,H	S	S	H
7	S,H	S	S	H
8	S,H	S	S	H
9	S,H	S	S	H
10	S,H	S	S	H

**Facility Requirements:** Two raceways will have to be continuously dedicated to production of the hatchery control line. Also, one or more sequestered reaches will be needed for fish to incubate and rear (a single reach can be used if we can replicate temporally). A trap at reach outfall must be capable of trapping a high proportion of outmigrants. The spawning channel and hatchery slough will be used for this purpose. If these areas are used, the trap at the slough will have to be modified to exclude other fish. Also, if the slough is to be used for this purpose, it should not be used for coho rearing. Surplus H line fish will have to be removed at Roza. Some of the hatchery will have to be dedicated to rearing fish from test crosses using cryopreserved sperm after several generations. How much of the hatchery needs to be used for this will be determined later as familiarity with the expected levels of change increases.

**Tagging Requirements:** Depends on which traits will be evaluated. If evaluations will include measurements on released fish, fish need to be tagged with raceway-specific tags, as is now done in OCT-SNT comparisons. H line fish will have to be marked distinctly. Juveniles in sequestered natural area will be otolith marked.

### Design 9 (continued)

**Pros:** 1) Will allow multiple generation tests of domestication differences between supplementation and pure hatchery culture at several juvenile traits and many more adult traits. The difference between supplementation and pure hatchery culture is that supplementation promotes a regular exchange of fish between the two environments, and the comparison between the S and H line will reflect this.

2) Because of the continuous multiple generation control line, probably many traits can be evaluated with high power.

3) Logistically simple to carry out.

4) Can look at S vs W using cryopreserved sperm after a few generations.

**Cons:** 1) Except for tests involving cryopreserved sperm, no comparison shows absolute amount of domestication supplementation incurs.

2) Approach is simple for traits to be evaluated in hatchery but this only gets at half the comparisons of interest. For many traits, the “hatchery “ half is all we can expect to be able to do. More interesting is the evaluation of fitness changes in the wild, and for this, stable sequestered reaches are needed, and these are feasible only for juvenile traits.

3) H-line fish surplus to broodstock needs will have to be removed from system

4) EIS will have to be modified to allow use of part of hatchery to be dedicated to H line., and perhaps to allow hatchery fish to be taken into hatchery for generation of test crosses.

5) Dedication of 2 raceways to H line means less supplementation effort

6) Use of slough for domestication testing will make it unavailable for coho rearing.