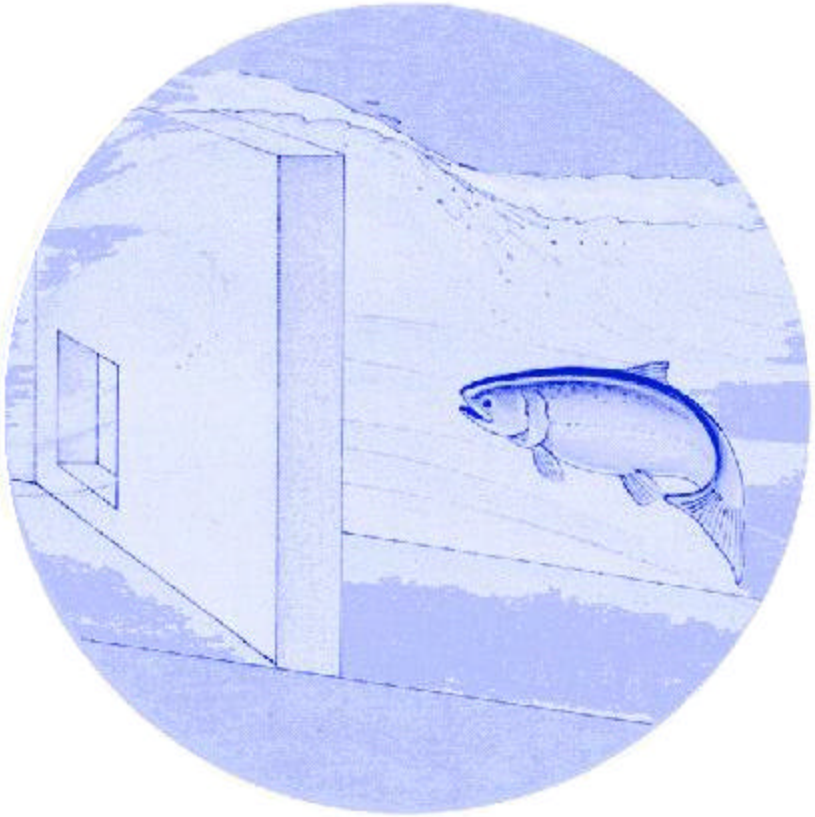
Genetic Studies in the Yakima River Basin

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report
2001 - 2002



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Yakima/Klickitat Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2002

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Executive Summary

Genetic work for 2002 was quite diverse.

- In chapter 1 we report on studies of the population genetic structure, using DNA microsatellites, of steelhead collected from different locations in the Yakima River basin (Roza Dam, Ahtanum Creek, Toppenish Creek, and Satus Creek) in 2000 and 2001. Of 28 pairwise tests of genotypic differentiation, only the 2000 and 2001 Roza Dam collections and the 2000 and 2001 Satus Creek collections did not exhibit significant differences. Similarly, pairwise tests of genetic differentiation (FST) were significant for all comparisons except the between-years comparisons of Roza Dam, Toppenish Creek, and Satus Creek collections. All tests between populations sampled from different localities were significant, indicating that these collections represent genetically differentiated stocks.
- In chapter 2 we report on genetic comparisons, again using microsatellites, of the three spring chinook populations in the Yakima basin (Upper Yakima, Naches, and American) with respect to our ability to be able to estimate the proportions of the three populations in mixed smolt samples collected at Chandler. We evaluated this both in terms of mixed fishery analysis, where proportions are estimated, but the likely provenance of any particular fish is unknown, and classification, where an attempt is made to assign individual fish to their population of origin. Simulations were done over the entire ranged of stock proportions observed in the Yakima basin in the last 20+ years. Stock proportions can be estimated very accurately by either method.
- Chapter 3 reports on our ongoing effort at cryopreserving semen from wild Upper Yakima spring chinook. In 2002, semen from 91 males, more than 50% of those spawned, was cryopreserved. Representation over the spawning season was excellent.
- Chapters 4,5, and 6 all relate to the continuing development of the domestication study design. Chapter 4 details the ISRP consultations and evolution of the design from last year's preferred alternative to the current plan of using the Naches population as a wild control, and maintaining a hatchery-only control line alongside the supplemented line. During discussions this year a major issue was the possible impact to the research and to the supplementation effort, of gene flow from precocious males from the hatchery control line into the supplemented line. At the end of the contracting period, this issue still had not been resolved. Along with the discussion of development of the domestication research design, chapter 4 presents the current monitoring plan document, with discussion of the approach to the various traits to be analyzed.
- Chapters 5 and 6 deal with experimental power of the domestication monitoring design. There is still much work to be done on power, but in chapter 5 we explore our power to detect differences among the three lines for traits measured on individual adults. Power was found to be quite good for effects of 5% per generation over three generations for traits having a coefficient of variation (CV) of 10-20%, but low if the CV was 50%. Power is higher for comparisons between the hatchery

control line and supplemented line than between the supplemented line and the wild control, a consequence of trying to avoid heavy impacts to the Naches population. Power could be improved considerably improved by sampling more Naches fish in years of high abundance.

- Chapter 6 presents the same power analysis, but attempts to explore the effect of precocious males from the hatchery control line spawning in the wild. It is clear that if gene flow from precocious males is more than one or two percent that the between-line comparisons will be biased, making the supplemented line appear to be more similar to the hatchery control line than it should and more different from the wild control line than it should. However, it was also clear that more analysis is desirable, as the heightened or diminished power is really just an enhancement or reduction of a real difference. A more straightforward analysis of the proportion of observed differences that can be attributed to precocious gene flow needs to be done.

It should be noted that a key piece of genetic analysis done this year is not reported on here at all- pedigree analysis of about 2700 juvenile spring chinook from the spawning channel. This work is reported on by Schroder et al in the 2002 Annual Report on reproductive success.

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Chapter 1

Geographic Population Genetic Structure of Steelhead in the Yakima River Basin

Janet Loxterman, WDFW
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Summary

We examined the population genetic structure of steelhead (*Oncorhynchus mykiss*) collected from different locations in the Yakima River basin (Roza Dam, Ahtanum Creek, Toppenish Creek, and Satus Creek). The primary objectives of this study were to assess spatial and temporal patterns of genetic diversity within and among these populations. To address our objectives, we used microsatellite DNA markers to analyze steelhead populations from four localities that were sampled in 2000 and in 2001. We generated multilocus genotypes for 753 individuals (371 from 2000 and 382 from 2001) using ten highly polymorphic microsatellite loci. Our results revealed significant population genetic structure among the collections with reduced genetic differentiation between years. Of 28 pairwise tests of genotypic differentiation, only the 2000 and 2001 Roza Dam collections and the 2000 and 2001 Satus Creek collections did not exhibit significant differences. Similarly, pairwise tests of genetic differentiation (F_{ST}) were significant for all comparisons except the between-years comparisons of Roza Dam, Toppenish Creek, and Satus Creek collections. All tests between populations sampled from different localities were significant, regardless of the collection year, indicating that these collections represent genetically differentiated stocks with little or no gene flow among them. While our results revealed strong geographic population structure with limited temporal structure among populations of steelhead, samples from additional localities (for example, the Naches River and its tributaries) are needed for a more comprehensive assessment of steelhead population structure in the Yakima River basin.

Introduction

Sound management and conservation of fish and wildlife species require, among other things, information about the genetic population structure and levels of genetic variation in the populations and species of concern. Such genetic information is of particular relevance when supplementation of depressed populations using artificial propagation is being contemplated.

Historically, the Yakima River basin (Figure 1), a large watershed (approximately 6,155 square miles), supported abundant populations of many salmonid fishes, including steelhead – the anadromous form of *Oncorhynchus mykiss*. However, due to a multitude of factors, summer-run steelhead and many other fish species are now much less abundant in the watershed. In fact, current steelhead stock abundance in the Yakima River basin is believed to be roughly 1% of its historical level of approximately 87,000 prior to 1890 (Howell et al., 1985). The historical spawning range of steelhead in the basin is thought to have included the mainstem Yakima River (above the confluence with Satus Creek) and portions of most major tributaries to the Yakima River from Satus Creek upstream (Howell et al. 1985; map on p.979) although it is now considerably more restricted.

The reduced abundance of steelhead has led to hatchery propagation within the basin and the introduction of non-local stocks of steelhead and rainbow trout from hatcheries outside the basin. Phelps et al. (2000) have summarized hatchery steelhead releases into the Yakima Basin as follows: 1) an average of over 65,000 smolts were released from 1961-1986; 2) smolts of Priest Rapids, Klickitat, and other unspecified Columbia River hatchery strains were released from 1961-1971; 3) only Skamania smolts were released from 1972-1986; 4) only 'Yakima' smolts derived from adults arriving at Prosser Dam have been released since 1987. Despite these introductions, there are no records of hatchery plants into the Satus Creek drainage. Over three million hatchery rainbow trout (South Tacoma and Goldendale hatchery strains) have also been released into the Yakima River basin since 1950 (Campton and Johnston 1985) and interbreeding between rainbow and steelhead is possible. Another confounding factor is potential interspecific hybridization of steelhead with introduced hatchery westslope cutthroat trout (Twin Lakes strain of *Oncorhynchus clarki lewisi*).

Our study was designed to determine whether or not steelhead collected from different locations and in multiple years in the Yakima River basin exhibited significant genetic differences that were indicative of population subdivision. For our analysis, we used microsatellite DNA loci as the genetic markers of choice for three reasons. First, data regarding microsatellite DNA loci provide an independent data set with which to compare the previously obtained allozyme data for steelhead from the same localities (cf. Phelps et al. 2000). Second, microsatellite DNA data can be readily collected using non-lethal fin-clip samples, so that it is not necessary to sacrifice the fish being sampled. Third, microsatellite DNA loci exhibit high levels of variation, high rates of mutation, and are thought to be selectively neutral so they provide a sensitive and powerful test of population subdivision. Many investigations have demonstrated the power of

microsatellite DNA markers to elucidate population structure in salmonid fishes (Estoup et al. 1998; Scribner et al. 1998; Small et al. 1998; Banks et al. 1999, 2000; Beacham et al. 1999a, b; Olsen et al. 2000a; Shaklee and Young 2000; Young and Shaklee 2000, 2001).

Methods

Samples and Microsatellites

We extracted DNA from 753 tissue samples representing four subpopulations sampled in both 2000 and 2001. In both years, Yakama Indian Nation personnel collected fin clips from live fish in four subpopulations including Satus Creek, Toppenish Creek, Ahtanum Creek, and Roza Dam (Figure 1). Ten microsatellite loci were assayed using fluorescently labeled primers following multiplex protocols developed in the WDFW Genetics Lab (Table 2). Genotypes were generated from the resulting PCR products using an Applied Biosystems (ABI) 3100 automated sequencer. Microsatellite alleles were sized using an internal GS 500 ROX (ABI) size standard. We used GENESCAN (Version 3.1) and GENOTYPER (Version 2.1) software to collect and analyze the microsatellite data.

Statistical Analyses

General measures of within-population genetic diversity including average heterozygosity and allelic richness were computed for each subpopulation using MICROSAT (Version 1.5, Minch et al. 1997) and FSTAT (Version 2.9.3.2, Goudet 1995), respectively. Tests for Hardy-Weinberg proportions for each locus and genotypic linkage disequilibrium between all pairs of loci within each subpopulation were performed using GENEPOP (Version 3.3, Raymond and Rousset 1995) and statistical significance was evaluated using a Bonferroni correction of P-values (Rice 1989).

To assess population structure among steelhead subpopulations, we computed several pairwise estimates of genetic differentiation between subpopulations. We generated estimates of genotypic population differentiation using GENEPOP 3.3. In addition, we used ARLEQUIN (Version 2.000, Schneider et al. 2000) to compute measures of population subdivision between all pairs of subpopulations. These estimates use allelic and genotypic frequency data to assess differences between subpopulation pairs. Statistical significance of F_{ST} estimates was tested using 10,000 permutations and was evaluated using a Bonferroni correction of P-values (Rice 1989).

Genetic distance between pairs of subpopulations was estimated using the Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza and Edwards 1967) as performed in MICROSAT. The distance matrices (1000 bootstrap matrices) were used to construct neighbor-joining trees using the NEIGHBOR function as implemented in PHYLIP (Version 3.572, Felsenstein 1993). A bootstrap consensus tree was constructed using the CONSENSE option in PHYLIP. Trees were imported and drawn with their associated bootstrap values using the TREEVIEW program (Version 1.6.5, Page 1996).

Results and Discussion

A total of 753 individuals (371 from 2000 and 382 from 2001) were genotyped using ten microsatellite loci. All ten loci were polymorphic with the number of alleles ranging from seven at microsatellite locus Ots-103 to 59 at microsatellite locus Omm-1128. Genetic diversity as estimated by average heterozygosity was similar among all populations ranging from 0.684 in the 01Toppenish subpopulation to 0.785 in the 00Roza Dam subpopulation (Table 2). These heterozygosity estimates are in the upper portion or slightly higher than the range of average heterozygosity estimates reported for other subpopulations of steelhead (0.41 – 0.72, Wenburg et al. 1996; 0.66 – 0.72, Nielsen 1999).

A similar pattern occurred with estimates of allelic richness within each subpopulation, with richness ranging from 11.544 alleles in the 01Toppenish Creek subpopulation to 16.311 alleles in the 01Roza Dam subpopulation. While the within subpopulation estimates were not highly variable among subpopulations, the highest estimates were exhibited by the two Roza Dam subpopulations and the lowest estimates exhibited in the 01Toppenish Creek subpopulation. The higher estimates in the Roza Dam subpopulation may reflect a larger effective population relative to the other subpopulations.

Tests for Hardy-Weinberg proportions were examined to assess the validity of the underlying assumptions of the models used to interpret patterns of genetic variation. These tests revealed a few deviations from equilibrium; however, the deviations primarily involved Omy-1001, which deviated from Hardy-Weinberg expectations in three of the eight subpopulations. Since five subpopulations were in equilibrium at microsatellite locus Omy-1001, this locus was not removed from the data set. Thus, all ten microsatellite loci were used for the analysis of population genetic structure among these subpopulations of steelhead.

In addition to tests for Hardy-Weinberg proportions, we tested for linkage disequilibrium between pairs of loci in each subpopulation. Several pairs of loci exhibited significant linkage disequilibrium (Table 3). Most subpopulations exhibited little or no significant linkage disequilibrium; however, 12 of 45 possible pairs of loci deviated significantly from equilibrium in the 00Ahtanum Creek subpopulation.

While significant linkage disequilibrium likely does not indicate physical linkage, this pattern suggests that the 00Ahtanum Creek collection could have a smaller effective population size relative to the other subpopulations or that the sample could be a mixture of two or more populations of steelhead and/or a mixture of steelhead and rainbow trout. In general, small effective population size is not likely since the 01Ahtanum Creek subpopulation does not exhibit the same pattern of linkage disequilibrium, indicating that the pattern is limited to the 00Ahtanum sample. Thus, the more likely explanation for this result is that the sample is a mixture of steelhead and rainbow trout. In their review of steelhead stock structure, Phelps et al. (2000) reported that both Satus and Toppenish creeks exhibited no gene flow between hatchery-origin rainbow trout and steelhead, and the native steelhead subpopulations, likely due to limited numbers of rainbow trout. In fact, Ahtanum Creek has more resident rainbow trout relative to Satus and Toppenish

creeks (J. Hubble, pers. comm.), clearly making a mixed sample of steelhead and rainbow trout the most likely explanation. Unfortunately, the study conducted by Phelps et al. (2000) did not include samples from the Ahtanum Creek steelhead subpopulation

Pairwise tests of genotypic differentiation indicated heterogeneity in genotype distributions among most subpopulations (Table 4). Significant genotypic differentiation occurred between all subpopulations from different localities, but not between samples from the same locality in different years. The Roza Dam and Satus Creek subpopulations were not different between years and the Toppenish Creek subpopulation was only marginally significant. The two Ahtanum Creek collections exhibited significant differentiation between years. Since the 00Ahtanum Creek sample appears to be a mixture, this result was not surprising. This pattern suggests strong spatial genetic structure among the subpopulations. This contention is strengthened by the lack of differentiation between years for the subpopulations.

Significant genetic structure was further examined by testing for population subdivision using F-statistics (Table 4). While the aforementioned pairwise test uses allele frequency differences between subpopulations to determine structure, F-statistics assess population genetic structure using the differences in genotypes between subpopulations, specifically differences in heterozygosity. Similar to the pairwise tests of genotypic differentiation, the F_{ST} estimates indicate significant population structure among the different localities. Significant population structure was not present between years in the Roza Dam, Satus Creek, or Toppenish Creek subpopulations, while the two Ahtanum Creek samples were significantly different from one another and all other subpopulations (Table 4).

These results indicate that these subpopulations of steelhead represent different genetic stocks with different gene pools and potentially different evolutionary trajectories. To further illustrate these differences, we constructed a neighbor-joining tree based on genetic distance among the different subpopulations and the different collection years (Figure 2). The different subpopulations grouped together with strong bootstrap support for the subpopulation pairs, except for the two Ahtanum samples. Again, this pattern indicates little or no temporal genetic structure in these subpopulations.

Conclusion

Overall, our results strongly support the contention that these steelhead subpopulations are genetically differentiated stocks. Steelhead subpopulations from the Yakima River basin exhibit spatial genetic structure with little or no temporal genetic structure. While it is possible that steelhead sampled from consecutive years could be from the same cohort, results from our analyses suggest that genetic relatedness is not the sole factor influencing the pattern of genetic variation. Rather, the significant levels of both genotypic and genetic differentiation indicate that there is little or no gene flow among these steelhead subpopulations. Since these subpopulations are genetically distinct, any management or conservation plans involving these subpopulations should be consistent with these genetic differences. Finally, future analyses of the geographic population structure of steelhead would be enhanced by including samples from additional localities (for example, the Naches River and its tributaries).

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Table 1. PCR amplification conditions and primer references for 10 microsatellite loci used to genotype steelhead.

Multiplex	Locus	Reference	Dye Label	Annealing T (C)	Primer Conc (uM)
Omy B	One-102	Olsen et al. 2000	6fam	55	0.08
	One-114	Olsen et al. 2000	hex	55	0.20
	Ots-100	Olsen et al. 2000	ned	55	0.07
Omy C	One-108	Olsen et al. 2000	6fam	55	0.03
	Ots-103	Small et al. 1998	hex	55	0.03
	One-101	Olsen et al. 2000	ned	55	0.04
Omy F	Omy-1001	Paul Bentzen, pers. comm.	6fam	52	0.06
	Omm-1128	Rexroad III et al. 2001	hex	52	0.08
	Oki-10	Smith et al. 1998	hex	52	0.08
	One-18	Scribner et al. 1996	ned	52	0.07

Table 2. Sample size (N) and estimates of genetic diversity (Avg Het = average heterozygosity, A_o = allelic richness) in 8 collections of steelhead sampled from the Yakima River basin.

Population	N	Collection Code	Avg Het	Ao
00Toppenish Creek	100	00AB	0.723	12.239
00Roza Dam	100	00AC	0.785	15.581
00Ahtanum Creek	71	00AI	0.763	13.276
00Satus Creek	100	00CS	0.742	13.660
00Mean			0.753	13.689
01Toppenish Creek	100	01AU	0.684	11.544
01Roza Dam	100	01AV	0.784	16.311
01Ahtanum Creek	82	01AX	0.739	14.733
01Satus Creek	100	01AW	0.750	12.566
01Mean			0.739	13.788

Table 3. Tests for Hardy-Weinberg expectations at ten microsatellite loci for 8 collections of steelhead. Significant deviations (P < 0.000625 after correction) are indicated by bold type.

Locus	00Toppenish Creek	00Roza Dam	00Ahtanum Creek	00Satus Creek	01Toppenish Creek	01Roza Dam	01Ahtanum Creek	01Satus Creek
One-102	0.9868	0.3848	0.0183	0.1428	0.7256	0.3245	0.0274	0.2719
One-114	0.0875	0.2092	0.1698	0.4167	0.1742	0.2630	0.0084	0.7916
Ots-100	0.0074	0.0511	0.4515	0.1857	0.1957	0.2771	0.8161	0.0467
One-101	0.2782	0.0008	0.6096	0.0378	0.0038	0.0896	0.9362	0.5286
One-108	0.2496	**0.0000**	0.3366	0.5243	0.0046	0.0235	0.9725	0.3010
Ots-103	0.0325	0.6131	1.0000	0.5185	1.0000	0.0764	1.0000	0.2403
Oki-10	0.0568	0.5879	0.5976	0.1709	0.0253	0.4757	0.7558	0.9906
Omy-1001	0.1150	**0.0000**	1.0000	**0.0000**	0.0013	**0.0000**	0.0455	0.0377
Omm-1128	0.0010	0.6188	0.6134	0.1190	0.2520	0.9111	0.4194	0.4003
One-18	0.0088	0.0568	0.2589	0.0772	0.5368	0.7812	0.9459	0.3523

Table 4. Tests for linkage disequilibrium among ten microsatellite loci in 8 collections of steelhead from the Yakima River basin.

Population	Locus 1	Locus 2	P-value
01Toppenish Creek	Ots-100	Omy-100	0.000
01Toppenish Creek	Ots-100	One-18	0.000
01Toppenish Creek	Omm-112	One-18	0.000
01Ahtanum Creek	One-102	Ots-100	0.000
01Ahtanum Creek	Ots-100	One-108	0.000
01Ahtanum Creek	Omm-112	One-18	0.000
00Toppenish Creek	One-101	Omy-100	0.000
00Ahtanum Creek	One-102	Ots-100	0.000
00Ahtanum Creek	One-114	One-101	0.000
00Ahtanum Creek	Ots-100	One-101	0.000
00Ahtanum Creek	One-102	One-108	0.000
00Ahtanum Creek	Ots-100	One-108	0.000
00Ahtanum Creek	One-102	Oki-10	0.000
00Ahtanum Creek	One-108	Oki-10	0.000
00Ahtanum Creek	One-108	Omm-112	0.000
00Ahtanum Creek	Oki-10	Omm-112	0.000
00Ahtanum Creek	One-108	Omy-100	0.000
00Ahtanum Creek	One-102	One-18	0.000
00Ahtanum Creek	Oki-10	One-18	0.000

Table 5. P-values from pairwise comparisons between pairs of subpopulations of steelhead from the Yakima River basin. Genetic estimates include genotypic differentiation above the diagonal and genetic differentiation (F_{ST}) are below the diagonal.

Population	00Toppenish Creek	00Roza Dam	00Ahtanum Creek	00Satus Creek	01Toppenish Creek	01Roza Dam	01Ahtanum Creek	01Satus Creek
00Toppenish Creek	--	< 0.00000	< 0.00000	< 0.00000	0.00015	< 0.00000	< 0.00000	< 0.00000
00Roza Dam	< 0.00000	--	< 0.00000	< 0.00000	< 0.00000	0.07945	< 0.00000	< 0.00000
00Ahtanum Creek	< 0.00000	< 0.00000	--	< 0.00000	< 0.00000	< 0.00000	< 0.00000	< 0.00000
00Satus Creek	< 0.00000	< 0.00000	< 0.00000	--	< 0.00000	< 0.00000	< 0.00000	0.10908
01Toppenish Creek	0.00684	< 0.00000	< 0.00000	< 0.00000	--	< 0.00000	< 0.00000	< 0.00000
01Roza Dam	< 0.00000	0.35059	< 0.00000	< 0.00000	< 0.00000	--	< 0.00000	< 0.00000
01Ahtanum Creek	< 0.00000	< 0.00000	< 0.00000	< 0.00000	< 0.00000	< 0.00000	--	< 0.00000
01Satus Creek	< 0.00000	< 0.00000	< 0.00000	0.82617	< 0.00000	< 0.00000	< 0.00000	--

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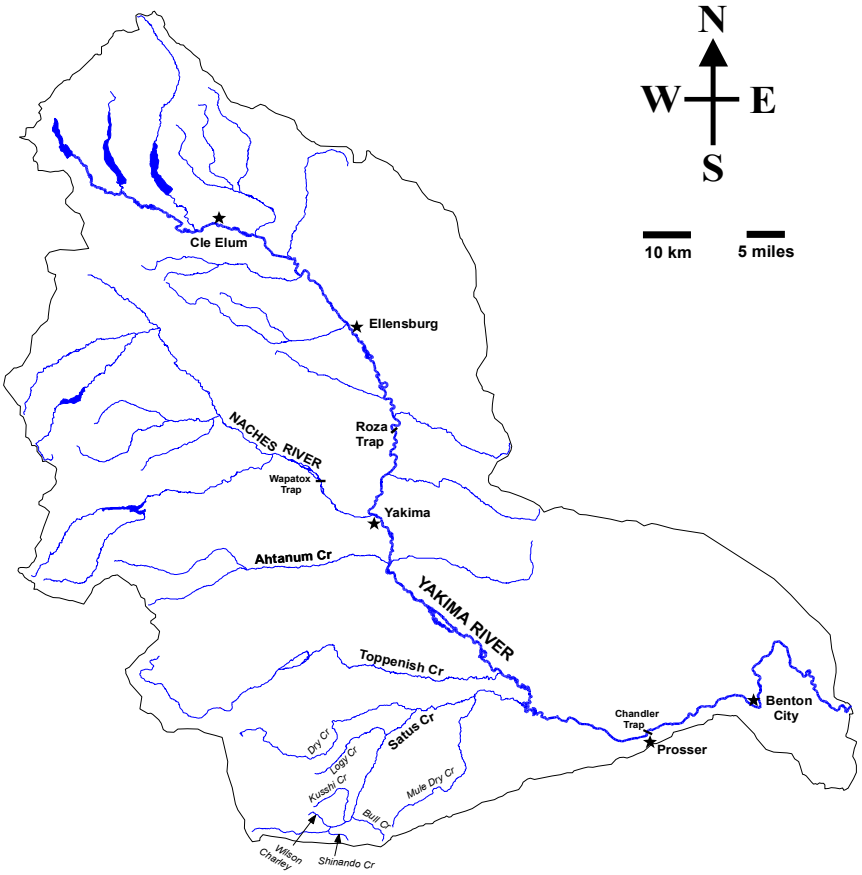

[map made from huc maps 17030003, 17030002, and 17030001 from StreamNet]

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Figure 1. Map of the Yakima River basin illustrating four steelhead collection localities

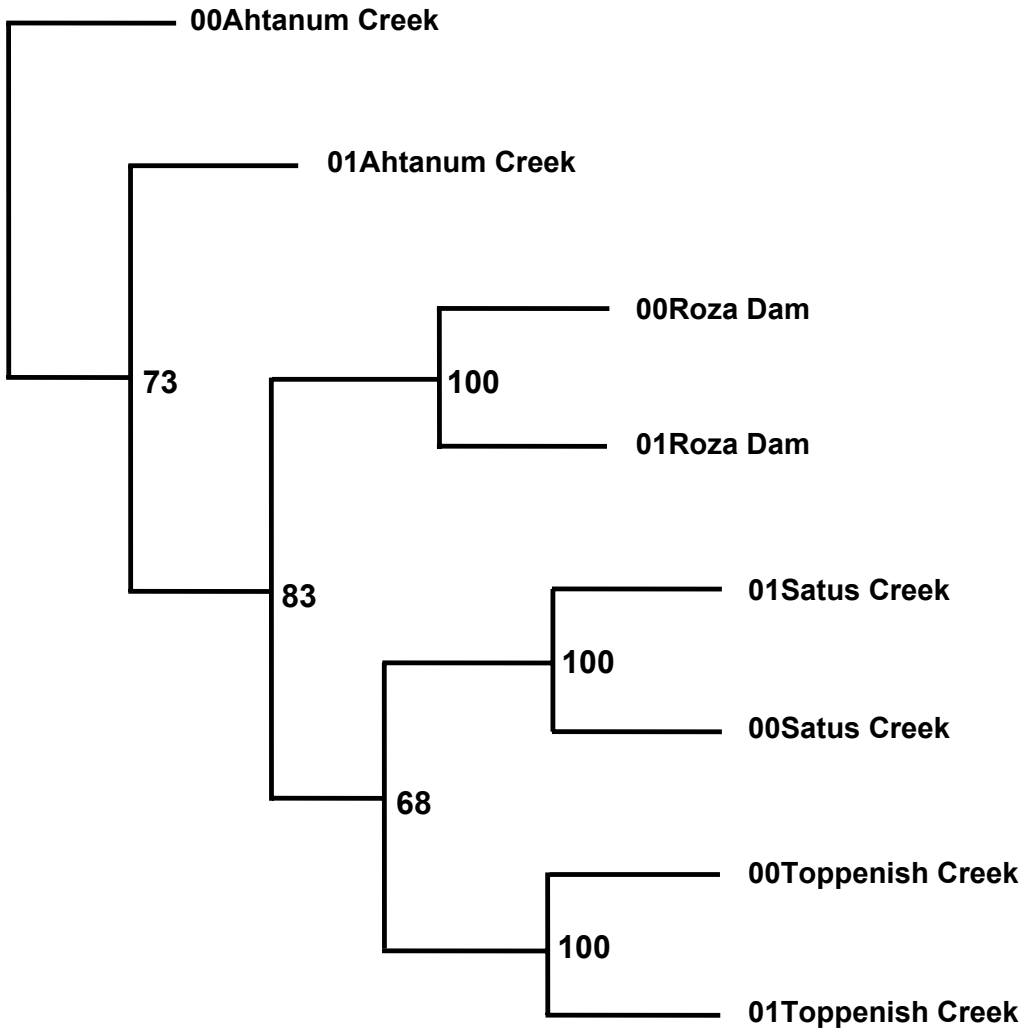

Figure 2. Consensus tree of Yakima River basin steelhead populations using Cavalli-Sforza and Edwards chord distance. Numbers represent percent bootstrap support based on 1000 replicates.

Appendix I. Allele frequencies at ten microsatellite loci sampled from four subpopulations of steelhead sampled in two years from the Yakima River basin.

One-102

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	186	0.0000	0.0320	0.0150	0.0690	0.0110	0.0360	0.0550	0.0220
2	190	0.0780	0.1330	0.0510	0.1130	0.0380	0.1150	0.0940	0.0500
3	194	0.0520	0.0960	0.2270	0.1940	0.0870	0.0940	0.1560	0.2890
4	198	0.0000	0.0430	0.0200	0.0690	0.0000	0.0420	0.0230	0.0220
5	202	0.0680	0.0800	0.1360	0.0560	0.1360	0.1410	0.0080	0.1110
6	206	0.2600	0.1650	0.0910	0.1810	0.2450	0.1460	0.1330	0.0610
7	210	0.0260	0.0690	0.0250	0.0440	0.0160	0.0470	0.3830	0.0170
8	214	0.4110	0.0850	0.2680	0.0560	0.3260	0.0830	0.0700	0.1890
9	218	0.0210	0.0160	0.0300	0.0750	0.0160	0.0570	0.0390	0.0280
10	222	0.0050	0.0850	0.0250	0.0440	0.0380	0.0570	0.0080	0.0780
11	226	0.0000	0.0530	0.0400	0.0130	0.0000	0.0360	0.0000	0.0170
12	230	0.0000	0.0160	0.0050	0.0190	0.0000	0.0160	0.0160	0.0220
13	234	0.0360	0.0210	0.0050	0.0000	0.0380	0.0210	0.0000	0.0110
14	238	0.0000	0.0000	0.0000	0.0130	0.0000	0.0000	0.0000	0.0000
15	242	0.0310	0.0210	0.0050	0.0060	0.0220	0.0100	0.0160	0.0060
16	246	0.0050	0.0050	0.0050	0.0000	0.0050	0.0100	0.0000	0.0110
17	250	0.0050	0.0430	0.0200	0.0380	0.0050	0.0570	0.0000	0.0000
18	254	0.0000	0.0000	0.0250	0.0000	0.0160	0.0000	0.0000	0.0440
19	258	0.0000	0.0050	0.0000	0.0060	0.0000	0.0000	0.0000	0.0060
20	262	0.0000	0.0000	0.0000	0.0060	0.0000	0.0160	0.0000	0.0060
21	266	0.0000	0.0110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
22	270	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0110
23	286	0.0000	0.0210	0.0000	0.0000	0.0000	0.0160	0.0000	0.0000

One-114

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	179	0.0000	0.0000	0.0000	0.0190	0.0000	0.0050	0.0470	0.0060
2	183	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
3	187	0.0260	0.0520	0.0730	0.1130	0.0380	0.0460	0.0390	0.0510
4	191	0.0050	0.0940	0.0260	0.0310	0.0160	0.0770	0.0630	0.0450
5	195	0.0110	0.0890	0.1090	0.0560	0.0110	0.0520	0.0000	0.1420
6	199	0.1210	0.0630	0.1040	0.1250	0.1520	0.0820	0.0780	0.1420
7	203	0.2950	0.1880	0.1770	0.0750	0.2450	0.1240	0.1250	0.1700
8	207	0.0160	0.0680	0.0420	0.1000	0.0110	0.0360	0.0390	0.0230
9	211	0.0050	0.0310	0.0470	0.0560	0.0110	0.0410	0.0390	0.0630
10	215	0.0420	0.0780	0.0730	0.0130	0.0270	0.0720	0.0080	0.0970
11	219	0.0110	0.0940	0.1150	0.1060	0.0380	0.1030	0.2030	0.0680
12	223	0.2370	0.0520	0.0680	0.0690	0.2390	0.0670	0.0860	0.0340
13	227	0.1370	0.0420	0.0260	0.0630	0.1030	0.0820	0.1480	0.0170
14	231	0.0370	0.0730	0.0050	0.0060	0.0430	0.0620	0.0230	0.0000
15	235	0.0320	0.0260	0.0570	0.0060	0.0330	0.0570	0.0000	0.0510
16	239	0.0110	0.0360	0.0210	0.0310	0.0110	0.0520	0.0310	0.0400
17	243	0.0110	0.0050	0.0100	0.0250	0.0160	0.0100	0.0080	0.0060
18	247	0.0050	0.0050	0.0000	0.0130	0.0000	0.0000	0.0000	0.0170
19	251	0.0000	0.0000	0.0360	0.0000	0.0050	0.0050	0.0080	0.0110
20	255	0.0000	0.0000	0.0050	0.0440	0.0000	0.0000	0.0310	0.0060
21	259	0.0000	0.0050	0.0000	0.0130	0.0000	0.0100	0.0000	0.0110
22	263	0.0000	0.0000	0.0000	0.0380	0.0000	0.0000	0.0230	0.0000
23	275	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000

Ots-100

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	166	0.0000	0.0530	0.0310	0.0700	0.0000	0.0560	0.0920	0.0380
2	168	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0110
3	170	0.0920	0.1530	0.1700	0.1710	0.0650	0.1070	0.1690	0.1740
4	172	0.0000	0.0050	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
5	174	0.0560	0.0470	0.1550	0.1140	0.0920	0.0610	0.0620	0.1030
6	176	0.0000	0.0000	0.0000	0.0000	0.0000	0.0100	0.0000	0.0110
7	178	0.2300	0.2050	0.2470	0.1330	0.2610	0.2190	0.1080	0.2930
8	180	0.0000	0.0000	0.0150	0.0000	0.0000	0.0000	0.0000	0.0000
9	182	0.2760	0.1580	0.1550	0.0890	0.3040	0.1020	0.1150	0.1250
10	184	0.0460	0.0530	0.0100	0.0510	0.0330	0.0200	0.0620	0.0160
11	186	0.1680	0.0840	0.1080	0.0250	0.1360	0.1480	0.1000	0.1140
12	188	0.0000	0.0160	0.0000	0.0380	0.0050	0.0050	0.0230	0.0000
13	190	0.0000	0.0210	0.0360	0.0060	0.0000	0.0460	0.0080	0.0380
14	192	0.0200	0.0160	0.0000	0.0060	0.0270	0.0260	0.0000	0.0000
15	194	0.0100	0.0420	0.0360	0.0950	0.0050	0.0870	0.0000	0.0330
16	196	0.0200	0.0260	0.0000	0.0190	0.0050	0.0100	0.0080	0.0000
17	198	0.0260	0.0470	0.0210	0.0320	0.0110	0.0460	0.0620	0.0380
18	200	0.0050	0.0370	0.0050	0.0510	0.0110	0.0200	0.0000	0.0000
19	202	0.0000	0.0050	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000
20	204	0.0260	0.0050	0.0000	0.0250	0.0380	0.0000	0.0460	0.0000
21	206	0.0000	0.0000	0.0050	0.0060	0.0000	0.0000	0.1080	0.0050
22	208	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
23	212	0.0000	0.0210	0.0000	0.0570	0.0000	0.0260	0.0310	0.0000
24	214	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	216	0.0000	0.0000	0.0000	0.0130	0.0000	0.0000	0.0080	0.0000
26	220	0.0150	0.0050	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000

One-101

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	117	0.4180	0.4950	0.6110	0.6090	0.3190	0.4430	0.5540	0.7160
2	125	0.5310	0.4170	0.3590	0.3140	0.5900	0.4590	0.2620	0.2630
3	137	0.0000	0.0000	0.0000	0.0130	0.0000	0.0000	0.0000	0.0000
4	153	0.0000	0.0210	0.0000	0.0060	0.0000	0.0050	0.0000	0.0000
5	161	0.0000	0.0050	0.0000	0.0190	0.0000	0.0000	0.0770	0.0000
6	165	0.0000	0.0160	0.0000	0.0000	0.0110	0.0210	0.0000	0.0000
7	169	0.0460	0.0160	0.0000	0.0320	0.0530	0.0050	0.1000	0.0050
8	173	0.0000	0.0160	0.0000	0.0000	0.0000	0.0360	0.0000	0.0000
9	177	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
10	181	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
11	209	0.0000	0.0050	0.0000	0.0000	0.0000	0.0150	0.0000	0.0000
12	217	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0050
13	221	0.0000	0.0100	0.0000	0.0060	0.0000	0.0050	0.0000	0.0000
14	229	0.0000	0.0000	0.0000	0.0000	0.0160	0.0000	0.0000	0.0000
15	233	0.0050	0.0000	0.0050	0.0000	0.0110	0.0000	0.0080	0.0050
16	241	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0050
17	253	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000
18	257	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000
19	261	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000

One-108

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	166	0.0160	0.0150	0.0320	0.0270	0.0000	0.0260	0.0380	0.0050
2	170	0.0050	0.0200	0.0000	0.0550	0.0210	0.0530	0.0450	0.0050
3	178	0.1420	0.0920	0.1740	0.1030	0.1320	0.0370	0.1440	0.1380
4	182	0.0370	0.1380	0.0890	0.0270	0.0320	0.1370	0.1060	0.0740
5	186	0.3050	0.1680	0.1470	0.1780	0.2580	0.1160	0.0760	0.2130
6	190	0.1420	0.2500	0.2740	0.0820	0.2320	0.2630	0.1740	0.2130
7	194	0.0320	0.0710	0.0370	0.0960	0.0420	0.1160	0.0610	0.0800
8	198	0.0050	0.0660	0.0050	0.0410	0.0320	0.0320	0.0300	0.0210
9	202	0.0470	0.0200	0.0470	0.0070	0.0050	0.0320	0.0000	0.0370
10	206	0.0110	0.0410	0.0420	0.1030	0.0050	0.0580	0.1360	0.0430
11	210	0.0160	0.0410	0.0260	0.0820	0.0260	0.0210	0.0530	0.0370
12	214	0.0260	0.0050	0.0320	0.0480	0.0160	0.0320	0.0450	0.0430
13	218	0.1580	0.0100	0.0160	0.0410	0.1370	0.0260	0.0230	0.0270
14	222	0.0050	0.0050	0.0160	0.0000	0.0050	0.0110	0.0000	0.0000
15	226	0.0000	0.0150	0.0050	0.0210	0.0000	0.0000	0.0000	0.0000
16	230	0.0000	0.0260	0.0000	0.0340	0.0000	0.0160	0.0000	0.0050
17	234	0.0000	0.0000	0.0000	0.0000	0.0000	0.0160	0.0000	0.0000
18	238	0.0210	0.0000	0.0000	0.0000	0.0110	0.0000	0.0080	0.0050
19	242	0.0000	0.0100	0.0000	0.0000	0.0050	0.0050	0.0000	0.0000
20	246	0.0260	0.0000	0.0420	0.0000	0.0320	0.0000	0.0450	0.0320
21	254	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000
22	258	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
23	262	0.0000	0.0000	0.0160	0.0000	0.0000	0.0000	0.0000	0.0210
24	314	0.0050	0.0000	0.0000	0.0410	0.0050	0.0000	0.0000	0.0000
25	322	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0150	0.0000
26	326	0.0000	0.0000	0.0000	0.0070	0.0000	0.0000	0.0000	0.0000
27	334	0.0000	0.0050	0.0000	0.0070	0.0000	0.0000	0.0000	0.0000

Ots-103

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	56	0.0050	0.0310	0.0000	0.0130	0.0310	0.0210	0.0350	0.0000
2	60	0.0000	0.0100	0.0000	0.0000	0.0000	0.0050	0.0070	0.0160
3	72	0.0000	0.0510	0.0460	0.0190	0.0000	0.0160	0.0420	0.0260
4	76	0.0000	0.0310	0.0000	0.0000	0.0000	0.0270	0.0000	0.0000
5	80	0.9750	0.8370	0.8830	0.9620	0.9430	0.8620	0.9150	0.8540
6	84	0.0050	0.0410	0.0510	0.0060	0.0210	0.0640	0.0000	0.0830
7	88	0.0150	0.0000	0.0200	0.0000	0.0050	0.0050	0.0000	0.0210

Oki-10

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	90	0.0100	0.0050	0.0000	0.0000	0.0050	0.0000	0.0000	0.0070
2	94	0.0000	0.0000	0.0000	0.0000	0.0000	0.0150	0.0000	0.0000
3	96	0.3940	0.2240	0.3760	0.0640	0.2070	0.2120	0.2460	0.4590
4	98	0.2070	0.0920	0.1460	0.0710	0.2270	0.0760	0.0290	0.1510
5	100	0.1160	0.0150	0.0510	0.0830	0.0810	0.0560	0.1300	0.0140
6	102	0.0000	0.0150	0.0390	0.1150	0.0000	0.0250	0.0510	0.0070
7	106	0.0350	0.2040	0.0790	0.2820	0.1210	0.1360	0.2320	0.1300
8	110	0.1920	0.2810	0.2420	0.2050	0.2470	0.3380	0.1740	0.2050
9	112	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0070	0.0000
10	114	0.0150	0.0660	0.0510	0.0770	0.0300	0.0510	0.0940	0.0210
11	118	0.0050	0.0360	0.0060	0.0130	0.0000	0.0300	0.0000	0.0000
12	122	0.0000	0.0360	0.0060	0.0710	0.0000	0.0450	0.0070	0.0000
13	126	0.0250	0.0050	0.0000	0.0190	0.0710	0.0000	0.0000	0.0000
14	130	0.0000	0.0050	0.0060	0.0000	0.0050	0.0000	0.0290	0.0000
15	134	0.0000	0.0150	0.0000	0.0000	0.0000	0.0150	0.0000	0.0070

Omm-1128

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	207	0.0000	0.0670	0.0200	0.0790	0.0110	0.0560	0.0070	0.0210
2	209	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0360
3	219	0.0000	0.0060	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	221	0.0000	0.0000	0.0000	0.0400	0.0000	0.0000	0.0000	0.0000
5	223	0.0270	0.0280	0.0000	0.0080	0.0110	0.0510	0.0070	0.0070
6	227	0.0000	0.0330	0.0400	0.0000	0.0160	0.0560	0.0290	0.0640
7	229	0.0050	0.0110	0.0000	0.0000	0.0000	0.0150	0.0000	0.0070
8	231	0.0430	0.0560	0.0070	0.0950	0.0870	0.0610	0.0870	0.0140
9	233	0.0000	0.0170	0.0000	0.0080	0.0000	0.0350	0.0000	0.0070
10	235	0.1220	0.0330	0.0400	0.0870	0.0710	0.0450	0.0070	0.0360
11	237	0.0000	0.0220	0.0070	0.0000	0.0000	0.0000	0.0000	0.0000
12	239	0.0270	0.0280	0.0600	0.0320	0.0050	0.0510	0.0070	0.0360
13	241	0.0000	0.0000	0.0000	0.0000	0.0000	0.0100	0.0070	0.0000
14	243	0.0370	0.0330	0.0270	0.0240	0.0650	0.0150	0.0290	0.0210
15	247	0.0000	0.0330	0.0400	0.0480	0.0000	0.0660	0.0220	0.0140
16	249	0.0000	0.0390	0.0000	0.0000	0.0000	0.0150	0.0140	0.0070
17	251	0.0530	0.0390	0.1070	0.0320	0.0270	0.0560	0.0000	0.0790
18	255	0.0000	0.0170	0.0000	0.0000	0.0050	0.0050	0.0000	0.0000
19	257	0.0000	0.0560	0.0000	0.0080	0.0000	0.0050	0.1010	0.0000
20	259	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000
21	261	0.0210	0.0220	0.0470	0.0320	0.0050	0.0100	0.1010	0.0640
22	263	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
23	265	0.0430	0.0390	0.0730	0.0160	0.0430	0.0300	0.0290	0.1140
24	267	0.0210	0.0060	0.0000	0.0000	0.0220	0.0000	0.0070	0.0000
25	269	0.0000	0.0170	0.0400	0.1030	0.0050	0.0050	0.0000	0.0710
26	271	0.0000	0.0000	0.0000	0.0080	0.0000	0.0000	0.0000	0.0000
27	273	0.0110	0.0170	0.0400	0.0000	0.0050	0.0000	0.0070	0.0640
28	277	0.0050	0.0330	0.1070	0.0160	0.0490	0.0810	0.1380	0.1070
29	279	0.0000	0.0000	0.0000	0.0080	0.0000	0.0100	0.0000	0.0000
30	281	0.0210	0.0330	0.0000	0.0160	0.0540	0.0400	0.0140	0.0070
31	283	0.0000	0.0110	0.0130	0.0080	0.0000	0.0050	0.0140	0.0000
32	285	0.1810	0.0610	0.1270	0.0870	0.1470	0.0350	0.0650	0.0210
33	287	0.0050	0.0060	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000
34	289	0.0160	0.0220	0.0270	0.0080	0.0270	0.0510	0.0000	0.0360
35	291	0.0320	0.0110	0.0000	0.0870	0.0220	0.0100	0.0070	0.0000
36	293	0.0590	0.0220	0.0070	0.0400	0.0870	0.0050	0.0510	0.0000
37	295	0.0000	0.0110	0.0200	0.0080	0.0050	0.0400	0.0140	0.0290
38	297	0.0050	0.0060	0.0000	0.0080	0.0000	0.0100	0.0290	0.0140
39	299	0.0480	0.0110	0.0600	0.0000	0.0430	0.0050	0.0070	0.0640
40	301	0.0110	0.0390	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000
41	303	0.0800	0.0110	0.0000	0.0080	0.0650	0.0050	0.0580	0.0070
42	305	0.0000	0.0060	0.0070	0.0000	0.0000	0.0150	0.0140	0.0070
43	307	0.0000	0.0060	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
44	309	0.0000	0.0390	0.0200	0.0000	0.0110	0.0100	0.0000	0.0000
45	311	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000
46	313	0.0050	0.0060	0.0000	0.0400	0.0050	0.0000	0.0000	0.0000
47	317	0.0000	0.0060	0.0000	0.0000	0.0000	0.0150	0.0800	0.0000
48	321	0.0800	0.0000	0.0200	0.0080	0.0710	0.0000	0.0000	0.0140
49	325	0.0370	0.0170	0.0470	0.0000	0.0160	0.0100	0.0070	0.0070
50	329	0.0000	0.0000	0.0000	0.0240	0.0000	0.0000	0.0000	0.0140
51	335	0.0000	0.0060	0.0000	0.0080	0.0000	0.0000	0.0000	0.0000
52	339	0.0050	0.0000	0.0000	0.0080	0.0110	0.0100	0.0070	0.0000
53	343	0.0000	0.0060	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000
54	347	0.0000	0.0170	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
55	351	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000
56	371	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
57	375	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0070
58	387	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
59	399	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000

Omy-1001

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	160	0.0000	0.0110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050
2	164	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000	0.0000	0.0000
3	168	0.0000	0.0000	0.0220	0.0000	0.0000	0.0000	0.0000	0.0160
4	170	0.0050	0.0000	0.0000	0.0400	0.0150	0.0000	0.0220	0.0000
5	172	0.0310	0.0050	0.0000	0.0000	0.0000	0.0300	0.0000	0.0110
6	176	0.1680	0.0210	0.0710	0.0730	0.1430	0.0200	0.0940	0.0380
7	178	0.0100	0.0050	0.0710	0.0070	0.0150	0.0000	0.0140	0.0930
8	180	0.0150	0.0790	0.0870	0.0470	0.0100	0.0750	0.0140	0.1430
9	182	0.0000	0.0050	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000
10	184	0.0310	0.1370	0.0920	0.1130	0.0100	0.1800	0.0510	0.0880
11	186	0.0100	0.0420	0.0920	0.0870	0.0000	0.0400	0.0360	0.0600
12	188	0.0150	0.1580	0.0330	0.0330	0.0200	0.1750	0.0220	0.0380
13	190	0.0000	0.0160	0.0330	0.0470	0.0000	0.0200	0.0000	0.0440
14	192	0.0100	0.1050	0.0600	0.1130	0.0360	0.0750	0.1880	0.0600
15	194	0.1790	0.0630	0.0920	0.1200	0.2090	0.0450	0.1160	0.0770
16	196	0.1430	0.0370	0.0760	0.1470	0.1730	0.0450	0.0360	0.0820
17	198	0.0200	0.0210	0.0000	0.0000	0.0150	0.0450	0.0510	0.0050
18	200	0.2190	0.0680	0.1680	0.0800	0.2140	0.0500	0.1520	0.1370
19	204	0.0660	0.1210	0.0270	0.0200	0.0770	0.0600	0.0070	0.0330
20	206	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
21	208	0.0050	0.0210	0.0160	0.0070	0.0050	0.0350	0.0070	0.0330
22	210	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
23	212	0.0000	0.0050	0.0000	0.0000	0.0100	0.0100	0.0000	0.0000
24	216	0.0000	0.0260	0.0220	0.0130	0.0000	0.0250	0.0000	0.0050
25	218	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000
26	220	0.0410	0.0210	0.0220	0.0130	0.0410	0.0500	0.1740	0.0220
27	222	0.0310	0.0160	0.0110	0.0000	0.0000	0.0000	0.0000	0.0050
28	224	0.0000	0.0000	0.0050	0.0000	0.0050	0.0000	0.0070	0.0000
29	246	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000	0.0000	0.0000

One-18

Code	Size (bp)	01Toppenish	01Roza	01Satus	01Ahtanum	00Toppenish	00Roza	00Ahtanum	00Satus
1	164	0.0000	0.0100	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050
2	168	0.1620	0.2140	0.3840	0.1090	0.1750	0.2270	0.1300	0.3510
3	170	0.0000	0.0050	0.0000	0.0060	0.0000	0.0050	0.0000	0.0050
4	172	0.3940	0.1630	0.2630	0.3780	0.4100	0.2070	0.3990	0.2840
5	174	0.2170	0.3010	0.1890	0.3140	0.1600	0.2420	0.0430	0.1860
6	176	0.0000	0.0310	0.0000	0.0190	0.0050	0.0050	0.0220	0.0000
7	178	0.0510	0.1730	0.1160	0.0640	0.0550	0.1770	0.2610	0.1390
8	180	0.1570	0.0770	0.0470	0.0580	0.1800	0.0910	0.0940	0.0260
9	184	0.0200	0.0260	0.0000	0.0510	0.0150	0.0450	0.0510	0.0050

Chapter 2

Determining Stock Proportions and Classifying Upper Yakima, Naches, and American River Chinook: Simulations of Mixture Analysis and Classification

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Introduction

Production and survival of the Yakima basin spring chinook stocks are important characteristics to monitor, but in the lower Yakima River where the best facilities to collect samples exist, the three stocks are commingled, both during adult upstream migration and during downstream juvenile migration. Thus, methodologies for discriminating stocks in an admixture are vital for development of stock-specific estimates. In the past we have used allozyme markers to estimate the proportions of Upper Yakima and Naches/American smolts passing Chandler to estimate smolt production in the two major arms of the basin. To avoid non-lethal sampling, several years ago we began developing DNA methodologies for mixture analysis. This effort was hindered by a difficulty getting adequate resolution of DNA microsatellite loci in the American and Naches stocks.

The new domestication monitoring design has made the need for DNA discrimination of the three stocks even greater. In addition to the ongoing U. Yakima vs. Naches/American analysis, information is now required on smolt production for the Naches stock separate from the American, requiring that Naches proportions be estimated in mixtures apart from the American production. Furthermore and proposed stock-specific monitoring of post-Chandler smolt-smolt survival would require more than mixture analysis. It would require the ability to classify individual fish to stock of origin with high accuracy.

Although resolution difficulties with the American and Naches samples have not been entirely cleared up, enough data has now been collected on these stocks to permit us to analyze, via simulations, our ability to use microsatellite data to do precise and unbiased mixture analysis and classification.

Methods

All stock-of-origin assignments in this study were accomplished with an Excel spreadsheet implementation of Expectation Maximization (the EM algorithm) to simultaneously estimate admixture proportions and assign individuals to candidate donor stocks. Our implementation of EM uses iterative approximations of admixture

proportions and individual assignments to stock-of-origin coupled with assessments of congruence of those estimates to increase assignment accuracy over previously described tests (Paetkau et al. 1995, Banks and Eichert 2000).

Those earlier assignment tests assume that the individual genotype frequencies in each baseline population (candidate source population) are in Hardy-Weinberg equilibrium (H-W), that the loci are independent, and that the likelihood that an unknown individual originated from a candidate source population is equal to the H-W frequency of its multilocus genotype in that population. Inherent in that last assumption is an expectation that the various potential source populations have an equal likelihood of contributing individuals to the unknown mixture sample. Using this basic approach, each unknown is assigned to the baseline population where its multilocus genotype has the highest probability of occurrence. However, when the multilocus genotype of an individual from the mixture has similar probabilities in the two most likely source populations, such a simple allocation is unreliable.

Unequal population sizes and divergent migration timing are among factors that probably commonly cause deviations from the expectations of equal admixture proportions that the previously described tests require. The EM-based procedure used here is intended to minimize the bias expected when the baseline stocks do not have equal probabilities of contributing individuals to the mixture.

We calculate estimates of probability of membership (Pm) for each individual in each candidate source population.

$$Pm_a = ra_a \times GT_a ,$$

where Pm_a = probability of membership in population a, ra_a = relative abundance of population a in the admixture, and GT_a = H-W expected genotype frequency in population a. We provisionally assign each individual in the mixture sample to the population with the highest Pm. We then tally the provisional individual assignments to re-estimate the relative abundance of each candidate stock in the admixture. We repeat this process until the estimates of population relative abundances do not change from one iteration to the next.

Pella and Masuda (2000) independently described a Bayesian solution to the problem of unequal mixture proportions that is similar conceptually to our approach.

We used 15 microsatellite DNA loci (*Ogo-2, Ogo-4, One-8, Ots-1, Ots-107, Ots-108, One-114, Ots-2M, Ots-101, Ots-3M, Ocl-1, Ssa-197, One-13M, Ots-100, and Ots-G474B*) to characterize spring chinook spawning populations (baseline samples) from American River, Naches River and upper Yakima River, and then to perform mixture analysis and individual assignments on smolt samples collected at the Chandler trap in the lower Yakima River. The collections used in this analysis included many degraded samples. A variety of laboratory steps failed to generate data from 35 of 96 American River individuals and 24 of 96 Naches River individuals. Work is ongoing to increase the baseline sample.

We performed simulations to evaluate the precision and accuracy of estimates of admixture stock compositions and individual assignments to stock-of-origin. The simulations are based on baseline data sets of 61 American River individuals, 72 Naches River individuals, and 86 upper Yakima individuals. We bootstrapped genotypes from those data sets one locus at a time and constructed novel multilocus genotypes that presumably reflect the genotypic characteristics of the three stocks. Each simulated admixture composition included 100 iterations of 500 individuals drawn from the simulated genotypes. Nineteen different mixtures of the three stocks were simulated, representing the actual estimated escapement proportions from 1982 to 2000 (data provided by B. Watson, Yakama Nation).

Results and Discussion

Results are presented in two groups of tables, Tables 1a-d for the mixture analyses and Tables 2a-d for the classification analyses. Although there are differences, there are a number of similarities that making giving an overview useful. In both cases the reason for four tables is different levels of critical value. This is a threshold measure determining which genotypes are actually used, based on how common they are in the three populations. With a critical value of 1, all genotypes are used. With a higher value, ten for example (Tables 1b and 2b), only genotypes that are ten times more common in one population than the others get used in the mixture analysis or get classified. Values of 10, 100, and 1000 are used in tables 1-2b, 1-2c, and 1-2d, respectively. The higher the critical value used, the more certain you can be about the origin of the genotype, but the number of unused animals increases, creating additional uncertainty. The lower the critical value that gives the desired level of accuracy and precision, the better.

On both sets of tables there are two things to look for, accuracy and precision. Accuracy is the extent to which the mean estimated proportions in the columns agree with the true simulated proportions in the left most columns. For example, consider the penultimate mixture in Table 1a. The true mixture is 25% American, 70% Naches, and 5% Upper Yakima. The mean estimated proportions are 27% American, 69%Naches, and 5% Upper Yakima, indicating a bias: American proportions are being overestimated and Naches proportions are being underestimated. The values in parentheses indicate the 90^{th} percentile band, the lowest and highest values encountered in the simulations after the bottom 5% and top 5% are cropped off. This shows the precision of the estimate. In this example it can be seen that the maximum variation from a mean estimate is 0.01, indicating high precision.

Table 1a basically tells the entire story on mixture analysis. There was high precision and low bias throughout the 19 simulated scenarios. The example just discussed represents the highest bias seen. Tables 1b-d show the same situations but for higher critical levels. As critical level increased, so did bias and imprecision. This is not a problem because there is no need to resort to the higher critical values, given the low bias and high precision seen in Table 1a.

Tables 2a-d include the same sort of information as Tables 1a-d, but also show where misclassification occurs. This can be somewhat misleading to read, so some explanation is in order. Consider again the penultimate simulation scenario, where the true proportion is 25% American, 70% Naches, and 5% Upper Yakima. In Table 2a we see that 27% were classified as American (25% correctly and 2% incorrectly), 69% as Naches (68% correctly and 1% incorrectly), and 5% Upper Yakima (all correctly classified). So again there is a bias, with too many fish being assigned to American and too few to Naches. This seems quite in line with the mixture analysis in the earlier graphs, but has a greater practical consequence. If this particular stock mixture is occurring, 1 out of 12 fish you classify as American will actually be from another stock. Consider now the same situation with a higher critical value (Table 2b). The estimated proportion of American fish is 23%, and the true proportion is 25%, but there are no fish incorrectly classified as American, or any other stock. The higher critical value resulted in 9% of the fish being classified as unknowns, however. So with this true mixture, if you tag 100 fish, all fish classified will be classified correctly at a cost of 9 tags being put on unclassifiable fish. Looking Table 2b over more carefully, we see that there is never any incorrect classification, and the maximum number of unclassifiable fish is 10%. Tables 2c and 2d are include for completeness, showing how higher critical values will work, but there is no new important information on them. It's impossible to increase classification accuracy over 100%. The basic message is that with a critical level of 10 you can correctly classify 90% or more of the fish without error.

We analyzed the samples collected from the year 2002 spring chinook smolt emigration past Chandler and estimated the relative contributions of the three candidate source populations at American River = 0.04, Naches River = 0.27, and upper Yakima River = 0.69. Our simulations suggest that at these mixture proportions, our estimates are unbiased and within 0.01 of the true values (Table 1a).

Literature cited

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Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4:347-354.

Pella, J. and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fish. Bull. 99:151-167.

Table 1a. Mixture analysis simulation results: Proportions of mixture assigned to each candidate source population with a critical likelihood ratio of 1.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).			
American River	Naches River	Upper Yakima	American River	Naches River	Upper Yakima River	Unassigned
0.02	**0.06**	**0.92**	0.02 (0.02 - 0.02)	0.06 (0.06 - 0.06)	0.92 (0.92 - 0.93)	0.00 (0.00 - 0.00)
0.07	**0.09**	**0.84**	0.08 (0.07 - 0.08)	0.09 (0.08 - 0.09)	0.84 (0.84 - 0.84)	0.00 (0.00 - 0.00)
0.11	**0.22**	**0.68**	0.11 (0.11 - 0.11)	0.21 (0.20 - 0.21)	0.68 (0.68 - 0.68)	0.00 (0.00 - 0.00)
0.10	**0.19**	**0.71**	0.10 (0.10 - 0.10)	0.19 (0.18 - 0.19)	0.71 (0.71 - 0.71)	0.00 (0.00 - 0.00)
0.18	**0.33**	**0.49**	0.19 (0.19 - 0.19)	0.32 (0.31 - 0.32)	0.49 (0.49 - 0.49)	0.00 (0.00 - 0.00)
0.16	**0.33**	**0.51**	0.17 (0.17 - 0.17)	0.32 (0.31 - 0.32)	0.51 (0.51 - 0.52)	0.00 (0.00 - 0.00)
0.25	**0.40**	**0.36**	0.26 (0.26 - 0.27)	0.39 (0.38 - 0.39)	0.35 (0.35 - 0.36)	0.00 (0.00 - 0.00)
0.14	**0.26**	**0.61**	0.14 (0.14 - 0.15)	0.25 (0.25 - 0.25)	0.61 (0.60 - 0.61)	0.00 (0.00 - 0.00)
0.12	**0.26**	**0.62**	0.12 (0.12 - 0.13)	0.25 (0.25 - 0.25)	0.63 (0.62 - 0.63)	0.00 (0.00 - 0.00)
0.14	**0.24**	**0.62**	0.15 (0.14 - 0.15)	0.23 (0.23 - 0.24)	0.62 (0.62 - 0.63)	0.00 (0.00 - 0.00)
0.08	**0.20**	**0.72**	0.08 (0.08 - 0.09)	0.19 (0.19 - 0.20)	0.72 (0.72 - 0.72)	0.00 (0.00 - 0.00)
0.19	**0.30**	**0.51**	0.20 (0.20 - 0.20)	0.29 (0.29 - 0.30)	0.51 (0.50 - 0.51)	0.00 (0.00 - 0.00)
0.18	**0.37**	**0.45**	0.19 (0.19 - 0.20)	0.36 (0.36 - 0.37)	0.45 (0.45 - 0.45)	0.00 (0.00 - 0.00)
0.19	**0.24**	**0.57**	0.20 (0.20 - 0.20)	0.23 (0.23 - 0.24)	0.57 (0.57 - 0.57)	0.00 (0.00 - 0.00)
0.06	**0.35**	**0.59**	0.07 (0.07 - 0.07)	0.34 (0.34 - 0.35)	0.59 (0.58 - 0.59)	0.00 (0.00 - 0.00)
0.14	**0.30**	**0.56**	0.15 (0.15 - 0.16)	0.29 (0.28 - 0.29)	0.56 (0.56 - 0.56)	0.00 (0.00 - 0.00)
0.32	**0.38**	**0.30**	0.33 (0.33 - 0.34)	0.37 (0.37 - 0.38)	0.30 (0.29 - 0.30)	0.00 (0.00 - 0.00)
0.04	**0.25**	**0.71**	0.05 (0.04 - 0.05)	0.24 (0.24 - 0.25)	0.71 (0.71 - 0.71)	0.00 (0.00 - 0.00)
0.02	**0.25**	**0.73**	0.02 (0.02 - 0.02)	0.25 (0.25 - 0.25)	0.73 (0.73 - 0.74)	0.00 (0.00 - 0.00)
0.05	**0.45**	**0.00**	0.05 (0.05 - 0.06)	0.45 (0.44 - 0.45)	0.50 (0.50 - 0.50)	0.00 (0.00 - 0.00)
0.70	**0.25**	**0.05**	0.72 (0.72 - 0.73)	0.23 (0.23 - 0.24)	0.05 (0.05 - 0.05)	0.00 (0.00 - 0.00)
0.25	**0.70**	**0.05**	0.27 (0.26 - 0.27)	0.69 (0.68 - 0.69)	0.05 (0.05 - 0.05)	0.00 (0.00 - 0.00)
0.60	**0.05**	**0.35**	0.61 (0.61 - 0.61)	0.04 (0.04 - 0.04)	0.35 (0.35 - 0.35)	0.00 (0.00 - 0.00)

Table 1b. Mixture analysis simualtion results: Proportions of mixture assigned to each candidate source population with a critical likelihood ratio of 10.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).			
American River	Naches River	Upper Yakima	American River	Naches River	Upper Yakima River	Unassigned
0.02	**0.06**	**0.92**	0.02 (0.01 - 0.02)	0.05 (0.04 - 0.05)	0.94 (0.93 - 0.94)	0.03 (0.02 - 0.03)
0.07	**0.09**	**0.84**	0.07 (0.07 - 0.08)	0.07 (0.06 - 0.07)	0.86 (0.85 - 0.86)	0.04 (0.03 - 0.04)
0.11	**0.22**	**0.68**	0.11 (0.11 - 0.11)	0.18 (0.18 - 0.19)	0.71 (0.70 - 0.71)	0.06 (0.06 - 0.07)
0.10	**0.19**	**0.71**	0.10 (0.10 - 0.10)	0.16 (0.16 - 0.17)	0.74 (0.73 - 0.75)	0.06 (0.05 - 0.07)
0.18	**0.33**	**0.49**	0.19 (0.19 - 0.20)	0.29 (0.29 - 0.30)	0.51 (0.51 - 0.52)	0.09 (0.08 - 0.10)
0.16	**0.33**	**0.51**	0.17 (0.17 - 0.17)	0.29 (0.29 - 0.30)	0.54 (0.53 - 0.54)	0.08 (0.08 - 0.09)
0.25	**0.40**	**0.36**	0.26 (0.26 - 0.27)	0.37 (0.36 - 0.37)	0.37 (0.36 - 0.37)	0.10 (0.08 - 0.10)
0.14	**0.26**	**0.61**	0.14 (0.14 - 0.15)	0.22 (0.22 - 0.23)	0.63 (0.63 - 0.64)	0.07 (0.07 - 0.08)
0.12	**0.26**	**0.62**	0.12 (0.12 - 0.12)	0.23 (0.22 - 0.23)	0.65 (0.65 - 0.66)	0.08 (0.07 - 0.08)
0.14	**0.24**	**0.62**	0.15 (0.14 - 0.15)	0.20 (0.20 - 0.21)	0.65 (0.64 - 0.66)	0.07 (0.06 - 0.08)
0.08	**0.20**	**0.72**	0.08 (0.08 - 0.08)	0.17 (0.16 - 0.18)	0.75 (0.74 - 0.75)	0.06 (0.05 - 0.07)
0.19	**0.30**	**0.51**	0.20 (0.20 - 0.21)	0.27 (0.26 - 0.27)	0.53 (0.53 - 0.54)	0.08 (0.07 - 0.09)
0.18	**0.37**	**0.45**	0.19 (0.19 - 0.20)	0.34 (0.33 - 0.35)	0.47 (0.46 - 0.47)	0.09 (0.08 - 0.10)
0.19	**0.24**	**0.57**	0.20 (0.20 - 0.21)	0.20 (0.19 - 0.21)	0.60 (0.59 - 0.60)	0.07 (0.07 - 0.08)
0.06	**0.35**	**0.59**	0.06 (0.06 - 0.07)	0.33 (0.32 - 0.33)	0.61 (0.60 - 0.62)	0.08 (0.07 - 0.09)
0.14	**0.30**	**0.56**	0.15 (0.15 - 0.16)	0.26 (0.26 - 0.27)	0.59 (0.58 - 0.59)	0.08 (0.07 - 0.09)
0.32	**0.38**	**0.30**	0.34 (0.34 - 0.35)	0.35 (0.34 - 0.35)	0.31 (0.31 - 0.31)	0.10 (0.09 - 0.11)
0.04	**0.25**	**0.71**	0.04 (0.04 - 0.04)	0.23 (0.22 - 0.23)	0.73 (0.73 - 0.74)	0.06 (0.06 - 0.07)
0.02	**0.25**	**0.73**	0.01 (0.01 - 0.02)	0.23 (0.23 - 0.24)	0.75 (0.75 - 0.76)	0.06 (0.05 - 0.06)
0.05	**0.45**	**0.00**	0.05 (0.05 - 0.05)	0.44 (0.43 - 0.44)	0.51 (0.51 - 0.52)	0.08 (0.07 - 0.09)
0.70	**0.25**	**0.05**	0.75 (0.74 - 0.75)	0.20 (0.20 - 0.21)	0.05 (0.04 - 0.05)	0.07 (0.06 - 0.08)
0.25	**0.70**	**0.05**	0.26 (0.26 - 0.27)	0.69 (0.69 - 0.70)	0.05 (0.04 - 0.05)	0.09 (0.09 - 0.10)
0.60	**0.05**	**0.35**	0.62 (0.62 - 0.62)	0.03 (0.02 - 0.03)	0.35 (0.35 - 0.36)	0.03 (0.02 - 0.03)

Table 1c. Mixture simulation analysis: Proportions of mixture assigned to each candidate source population with a critical likelihood ratio of 100.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).			
American River	Naches River	Upper Yakima	American River	Naches River	upper Yakima River	Unassigned
0.02	**0.06**	**0.92**	0.01 (0.01 - 0.02)	0.03 (0.02 - 0.03)	0.96 (0.95 - 0.96)	0.07 (0.06 - 0.07)
0.07	**0.09**	**0.84**	0.07 (0.07 - 0.08)	0.03 (0.03 - 0.04)	0.89 (0.89 - 0.90)	0.10 (0.09 - 0.11)
0.11	**0.22**	**0.68**	0.11 (0.11 - 0.12)	0.13 (0.12 - 0.14)	0.76 (0.75 - 0.77)	0.18 (0.17 - 0.19)
0.10	**0.19**	**0.71**	0.10 (0.10 - 0.10)	0.11 (0.10 - 0.12)	0.79 (0.78 - 0.80)	0.17 (0.16 - 0.18)
0.18	**0.33**	**0.49**	0.20 (0.19 - 0.21)	0.23 (0.22 - 0.25)	0.56 (0.55 - 0.57)	0.23 (0.22 - 0.25)
0.16	**0.33**	**0.51**	0.18 (0.17 - 0.18)	0.23 (0.22 - 0.25)	0.59 (0.58 - 0.60)	0.23 (0.22 - 0.24)
0.25	**0.40**	**0.36**	0.29 (0.28 - 0.30)	0.30 (0.29 - 0.32)	0.41 (0.40 - 0.42)	0.26 (0.25 - 0.28)
0.14	**0.26**	**0.61**	0.15 (0.14 - 0.15)	0.16 (0.15 - 0.18)	0.69 (0.68 - 0.70)	0.20 (0.19 - 0.21)
0.12	**0.26**	**0.62**	0.12 (0.12 - 0.13)	0.17 (0.16 - 0.18)	0.71 (0.70 - 0.72)	0.20 (0.19 - 0.21)
0.14	**0.24**	**0.62**	0.15 (0.15 - 0.16)	0.14 (0.13 - 0.15)	0.71 (0.70 - 0.72)	0.20 (0.19 - 0.21)
0.08	**0.20**	**0.72**	0.08 (0.08 - 0.09)	0.12 (0.11 - 0.13)	0.80 (0.79 - 0.81)	0.17 (0.16 - 0.18)
0.19	**0.30**	**0.51**	0.22 (0.21 - 0.22)	0.20 (0.19 - 0.21)	0.58 (0.57 - 0.59)	0.22 (0.21 - 0.24)
0.18	**0.37**	**0.45**	0.20 (0.20 - 0.21)	0.28 (0.27 - 0.30)	0.51 (0.50 - 0.53)	0.25 (0.24 - 0.26)
0.19	**0.24**	**0.57**	0.21 (0.21 - 0.22)	0.14 (0.13 - 0.15)	0.65 (0.64 - 0.65)	0.20 (0.19 - 0.21)
0.06	**0.35**	**0.59**	0.06 (0.05 - 0.07)	0.28 (0.27 - 0.29)	0.66 (0.65 - 0.67)	0.22 (0.21 - 0.23)
0.14	**0.30**	**0.56**	0.16 (0.15 - 0.16)	0.20 (0.19 - 0.21)	0.64 (0.63 - 0.65)	0.22 (0.21 - 0.23)
0.32	**0.38**	**0.30**	0.38 (0.37 - 0.39)	0.28 (0.27 - 0.29)	0.34 (0.33 - 0.35)	0.26 (0.24 - 0.27)
0.04	**0.25**	**0.71**	0.04 (0.03 - 0.04)	0.18 (0.17 - 0.19)	0.78 (0.77 - 0.79)	0.18 (0.17 - 0.18)
0.02	**0.25**	**0.73**	0.01 (0.01 - 0.01)	0.20 (0.19 - 0.21)	0.79 (0.78 - 0.80)	0.16 (0.15 - 0.17)
0.05	**0.45**	**0.00**	0.04 (0.04 - 0.05)	0.41 (0.40 - 0.42)	0.55 (0.54 - 0.56)	0.23 (0.21 - 0.24)
0.70	**0.25**	**0.05**	0.81 (0.80 - 0.82)	0.14 (0.13 - 0.16)	0.05 (0.04 - 0.05)	0.18 (0.17 - 0.18)
0.25	**0.70**	**0.05**	0.27 (0.26 - 0.28)	0.69 (0.68 - 0.70)	0.04 (0.03 - 0.05)	0.25 (0.24 - 0.27)
0.60	**0.05**	**0.35**	0.63 (0.63 - 0.64)	0.01 (0.00 - 0.01)	0.36 (0.35 - 0.36)	0.06 (0.05 - 0.06)

Table 1d. Mixture simulation analysis: Proportions of mixture assigned to each candidate source population with a critical likelihood ratio of 1000.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).			
American River	Naches River	Cle Elum	American River	Naches River	upper Yakima River	Unassigned
0.02	**0.06**	**0.92**	0.01 (0.01 - 0.02)	0.01 (0.00 - 0.01)	0.98 (0.98 - 0.98)	0.09 (0.07 - 0.11)
0.07	**0.09**	**0.84**	0.08 (0.07 - 0.09)	0.01 (0.00 - 0.01)	0.92 (0.91 - 0.92)	0.13 (0.09 - 0.15)
0.11	**0.22**	**0.68**	0.12 (0.11 - 0.13)	0.04 (0.02 - 0.05)	0.84 (0.83 - 0.85)	0.29 (0.28 - 0.30)
0.10	**0.19**	**0.71**	0.11 (0.10 - 0.11)	0.03 (0.01 - 0.04)	0.87 (0.86 - 0.88)	0.26 (0.25 - 0.28)
0.18	**0.33**	**0.49**	0.23 (0.22 - 0.24)	0.10 (0.07 - 0.12)	0.67 (0.66 - 0.69)	0.41 (0.40 - 0.42)
0.16	**0.33**	**0.51**	0.20 (0.18 - 0.21)	0.10 (0.09 - 0.13)	0.70 (0.68 - 0.71)	0.41 (0.40 - 0.42)
0.25	**0.40**	**0.36**	0.34 (0.33 - 0.36)	0.15 (0.12 - 0.19)	0.50 (0.48 - 0.52)	0.47 (0.46 - 0.48)
0.14	**0.26**	**0.61**	0.16 (0.15 - 0.17)	0.05 (0.04 - 0.07)	0.79 (0.78 - 0.80)	0.34 (0.33 - 0.35)
0.12	**0.26**	**0.62**	0.13 (0.12 - 0.14)	0.05 (0.04 - 0.07)	0.81 (0.80 - 0.83)	0.34 (0.33 - 0.35)
0.14	**0.24**	**0.62**	0.16 (0.15 - 0.17)	0.05 (0.03 - 0.06)	0.79 (0.78 - 0.80)	0.32 (0.31 - 0.34)
0.08	**0.20**	**0.72**	0.08 (0.07 - 0.09)	0.04 (0.02 - 0.05)	0.88 (0.87 - 0.89)	0.28 (0.26 - 0.29)
0.19	**0.30**	**0.51**	0.24 (0.23 - 0.26)	0.07 (0.05 - 0.09)	0.69 (0.67 - 0.70)	0.39 (0.38 - 0.39)
0.18	**0.37**	**0.45**	0.23 (0.22 - 0.25)	0.14 (0.11 - 0.17)	0.63 (0.60 - 0.64)	0.45 (0.43 - 0.46)
0.19	**0.24**	**0.57**	0.23 (0.22 - 0.24)	0.04 (0.02 - 0.05)	0.73 (0.72 - 0.74)	0.32 (0.30 - 0.33)
0.06	**0.35**	**0.59**	0.06 (0.05 - 0.07)	0.17 (0.14 - 0.19)	0.78 (0.76 - 0.80)	0.41 (0.40 - 0.42)
0.14	**0.30**	**0.56**	0.17 (0.16 - 0.19)	0.08 (0.06 - 0.10)	0.75 (0.73 - 0.76)	0.38 (0.37 - 0.39)
0.32	**0.38**	**0.30**	0.46 (0.45 - 0.48)	0.12 (0.10 - 0.15)	0.41 (0.40 - 0.43)	0.46 (0.44 - 0.47)
0.04	**0.25**	**0.71**	0.04 (0.03 - 0.04)	0.08 (0.06 - 0.11)	0.88 (0.86 - 0.90)	0.33 (0.32 - 0.34)
0.02	**0.25**	**0.73**	0.01 (0.01 - 0.01)	0.13 (0.10 - 0.15)	0.86 (0.85 - 0.89)	0.32 (0.30 - 0.33)
0.05	**0.45**	**0.50**	0.04 (0.03 - 0.05)	0.32 (0.29 - 0.34)	0.65 (0.62 - 0.67)	0.45 (0.43 - 0.46)
0.70	**0.25**	**0.00**	0.90 (0.89 - 0.92)	0.05 (0.04 - 0.07)	0.05 (0.04 - 0.05)	0.30 (0.29 - 0.31)
0.25	**0.70**	**0.05**	0.28 (0.26 - 0.30)	0.68 (0.66 - 0.71)	0.04 (0.03 - 0.05)	0.50 (0.48 - 0.51)
0.60	**0.05**	**0.35**	0.64 (0.64 - 0.65)	0.00 (0.00 - 0.00)	0.36 (0.35 - 0.36)	0.06 (0.05 - 0.08)

Table 2a. Classification simulation analysis: Accuracy of individual assignments to each candidate source population with a critical likelihood ratio of 1.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).											
AMER	NACH	UYAK	Correctly assigned to American River		Incorrectly assigned to American River		Correctly assigned to Naches River		Incorrectly assigned to Naches River		Correctly assigned to upper Yakima River		Incorrectly assigned to upper Yakima River		Assigned	
0.02	0.06	0.92	0.02	(0.02 - 0.02)	0.00	(0.00 - 0.00)	0.06	(0.05 - 0.06)	0.00	(0.00 - 0.00)	0.92	(0.92 - 0.92)	0.00	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.07	0.09	0.84	0.07	(0.07 - 0.07)	0.00	(0.00 - 0.01)	0.08	(0.08 - 0.09)	0.00	(0.00 - 0.00)	0.83	(0.83 - 0.84)	0.00	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.11	0.22	0.68	0.11	(0.10 - 0.11)	0.01	(0.00 - 0.01)	0.20	(0.20 - 0.21)	0.00	(0.00 - 0.01)	0.67	(0.67 - 0.68)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.10	0.19	0.71	0.10	(0.09 - 0.10)	0.01	(0.00 - 0.01)	0.18	(0.18 - 0.19)	0.01	(0.00 - 0.01)	0.71	(0.70 - 0.71)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.18	0.33	0.49	0.18	(0.18 - 0.18)	0.01	(0.01 - 0.02)	0.31	(0.31 - 0.31)	0.01	(0.00 - 0.01)	0.48	(0.48 - 0.49)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.16	0.33	0.51	0.16	(0.16 - 0.16)	0.01	(0.01 - 0.02)	0.31	(0.30 - 0.31)	0.01	(0.00 - 0.01)	0.51	(0.51 - 0.51)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.25	0.40	0.36	0.24	(0.24 - 0.25)	0.02	(0.01 - 0.02)	0.38	(0.37 - 0.38)	0.01	(0.00 - 0.01)	0.35	(0.35 - 0.35)	0.00	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.14	0.26	0.61	0.13	(0.13 - 0.14)	0.01	(0.01 - 0.01)	0.24	(0.24 - 0.25)	0.01	(0.00 - 0.01)	0.60	(0.60 - 0.60)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.12	0.26	0.62	0.11	(0.11 - 0.12)	0.01	(0.01 - 0.01)	0.24	(0.24 - 0.25)	0.01	(0.00 - 0.01)	0.62	(0.62 - 0.62)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.14	0.24	0.62	0.14	(0.14 - 0.14)	0.01	(0.01 - 0.01)	0.22	(0.22 - 0.23)	0.01	(0.00 - 0.01)	0.62	(0.61 - 0.62)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.08	0.20	0.72	0.08	(0.08 - 0.08)	0.01	(0.00 - 0.01)	0.19	(0.19 - 0.19)	0.01	(0.00 - 0.01)	0.71	(0.71 - 0.72)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.19	0.30	0.51	0.19	(0.19 - 0.19)	0.01	(0.01 - 0.01)	0.28	(0.28 - 0.29)	0.01	(0.00 - 0.01)	0.50	(0.50 - 0.50)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.18	0.37	0.45	0.18	(0.18 - 0.18)	0.01	(0.01 - 0.02)	0.35	(0.35 - 0.36)	0.01	(0.00 - 0.01)	0.44	(0.44 - 0.45)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.19	0.24	0.57	0.19	(0.19 - 0.19)	0.01	(0.01 - 0.01)	0.22	(0.22 - 0.23)	0.01	(0.00 - 0.01)	0.56	(0.56 - 0.57)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.06	0.35	0.59	0.06	(0.06 - 0.06)	0.01	(0.00 - 0.01)	0.34	(0.33 - 0.34)	0.01	(0.00 - 0.01)	0.58	(0.58 - 0.58)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.14	0.30	0.56	0.14	(0.14 - 0.14)	0.01	(0.01 - 0.01)	0.28	(0.28 - 0.28)	0.01	(0.00 - 0.01)	0.55	(0.55 - 0.56)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.32	0.38	0.30	0.31	(0.31 - 0.32)	0.02	(0.01 - 0.02)	0.36	(0.36 - 0.37)	0.01	(0.00 - 0.01)	0.29	(0.29 - 0.30)	0.00	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.04	0.25	0.71	0.04	(0.04 - 0.04)	0.00	(0.00 - 0.01)	0.24	(0.23 - 0.24)	0.01	(0.00 - 0.01)	0.70	(0.70 - 0.71)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.02	0.25	0.73	0.02	(0.01 - 0.02)	0.00	(0.00 - 0.00)	0.24	(0.24 - 0.25)	0.01	(0.00 - 0.01)	0.73	(0.72 - 0.73)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.05	0.45	0.00	0.05	(0.05 - 0.05)	0.01	(0.00 - 0.01)	0.44	(0.43 - 0.44)	0.01	(0.01 - 0.01)	0.49	(0.49 - 0.50)	0.01	(0.00 - 0.01)	1.00	(1.00 - 1.00)
0.70	0.25	0.05	0.70	(0.70 - 0.70)	0.02	(0.02 - 0.03)	0.23	(0.22 - 0.23)	0.00	(0.00 - 0.00)	0.05	(0.05 - 0.05)	0.00	(0.00 - 0.00)	1.00	(1.00 - 1.00)
0.25	0.70	0.05	0.25	(0.25 - 0.25)	0.02	(0.01 - 0.02)	0.68	(0.68 - 0.68)	0.01	(0.00 - 0.01)	0.05	(0.04 - 0.05)	0.00	(0.00 - 0.00)	1.00	(1.00 - 1.00)
0.60	0.05	0.35	0.60	(0.60 - 0.60)	0.01	(0.01 - 0.01)	0.04	(0.04 - 0.04)	0.00	(0.00 - 0.00)	0.35	(0.35 - 0.35)	0.00	(0.00 - 0.00)	1.00	(1.00 - 1.00)

Table 2b. Classification simulation analysis: Accuracy of individual assignments to each candidate source population with a critical likelihood ratio of 10.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).											
AMER	NACH	UYAK	Correctly assigned to American River		Incorrectly assigned to American River		Correctly assigned to Naches River		Incorrectly assigned to Naches River		Correctly assigned to upper Yakima River		Incorrectly assigned to upper Yakima River		Assigned	
0.02	**0.06**	**0.92**	0.01	(0.01 - 0.02)	0.00	(0.00 - 0.00)	0.04	(0.04 - 0.05)	0.00	(0.00 - 0.00)	0.91	(0.91 - 0.92)	0.00	(0.00 - 0.00)	0.97	(0.97 - 0.98)
0.07	**0.09**	**0.84**	0.07	(0.07 - 0.07)	0.00	(0.00 - 0.00)	0.07	(0.06 - 0.07)	0.00	(0.00 - 0.00)	0.82	(0.82 - 0.83)	0.00	(0.00 - 0.00)	0.96	(0.96 - 0.97)
0.11	**0.22**	**0.68**	0.10	(0.10 - 0.10)	0.00	(0.00 - 0.00)	0.17	(0.16 - 0.18)	0.00	(0.00 - 0.00)	0.66	(0.66 - 0.66)	0.00	(0.00 - 0.00)	0.94	(0.93 - 0.94)
0.10	**0.19**	**0.71**	0.09	(0.09 - 0.09)	0.00	(0.00 - 0.00)	0.15	(0.14 - 0.16)	0.00	(0.00 - 0.00)	0.69	(0.69 - 0.70)	0.00	(0.00 - 0.00)	0.94	(0.93 - 0.95)
0.18	**0.33**	**0.49**	0.17	(0.17 - 0.18)	0.00	(0.00 - 0.00)	0.27	(0.26 - 0.28)	0.00	(0.00 - 0.00)	0.47	(0.46 - 0.47)	0.00	(0.00 - 0.00)	0.91	(0.90 - 0.92)
0.16	**0.33**	**0.51**	0.15	(0.15 - 0.16)	0.00	(0.00 - 0.00)	0.27	(0.26 - 0.28)	0.00	(0.00 - 0.00)	0.49	(0.48 - 0.50)	0.00	(0.00 - 0.00)	0.92	(0.91 - 0.92)
0.25	**0.40**	**0.36**	0.24	(0.23 - 0.24)	0.00	(0.00 - 0.00)	0.33	(0.32 - 0.34)	0.00	(0.00 - 0.00)	0.33	(0.33 - 0.34)	0.00	(0.00 - 0.00)	0.90	(0.90 - 0.92)
0.14	**0.26**	**0.61**	0.13	(0.13 - 0.13)	0.00	(0.00 - 0.00)	0.21	(0.20 - 0.22)	0.00	(0.00 - 0.00)	0.58	(0.58 - 0.59)	0.00	(0.00 - 0.00)	0.93	(0.92 - 0.93)
0.12	**0.26**	**0.62**	0.11	(0.11 - 0.11)	0.00	(0.00 - 0.00)	0.21	(0.20 - 0.22)	0.00	(0.00 - 0.00)	0.60	(0.60 - 0.61)	0.00	(0.00 - 0.00)	0.92	(0.92 - 0.93)
0.14	**0.24**	**0.62**	0.13	(0.13 - 0.14)	0.00	(0.00 - 0.00)	0.19	(0.18 - 0.19)	0.00	(0.00 - 0.00)	0.60	(0.60 - 0.61)	0.00	(0.00 - 0.00)	0.93	(0.92 - 0.94)
0.08	**0.20**	**0.72**	0.08	(0.07 - 0.08)	0.00	(0.00 - 0.00)	0.16	(0.15 - 0.17)	0.00	(0.00 - 0.00)	0.70	(0.70 - 0.70)	0.00	(0.00 - 0.00)	0.94	(0.93 - 0.95)
0.19	**0.30**	**0.51**	0.18	(0.18 - 0.19)	0.00	(0.00 - 0.00)	0.24	(0.24 - 0.25)	0.00	(0.00 - 0.00)	0.49	(0.48 - 0.49)	0.00	(0.00 - 0.00)	0.92	(0.91 - 0.93)
0.18	**0.37**	**0.45**	0.17	(0.17 - 0.17)	0.00	(0.00 - 0.00)	0.31	(0.30 - 0.32)	0.00	(0.00 - 0.00)	0.42	(0.42 - 0.43)	0.00	(0.00 - 0.00)	0.91	(0.90 - 0.92)
0.19	**0.24**	**0.57**	0.18	(0.18 - 0.19)	0.00	(0.00 - 0.00)	0.19	(0.18 - 0.19)	0.00	(0.00 - 0.00)	0.55	(0.55 - 0.55)	0.00	(0.00 - 0.00)	0.93	(0.92 - 0.93)
0.06	**0.35**	**0.59**	0.06	(0.06 - 0.06)	0.00	(0.00 - 0.00)	0.30	(0.29 - 0.31)	0.00	(0.00 - 0.00)	0.56	(0.55 - 0.57)	0.00	(0.00 - 0.00)	0.92	(0.91 - 0.93)
0.14	**0.30**	**0.56**	0.14	(0.13 - 0.14)	0.00	(0.00 - 0.00)	0.24	(0.23 - 0.25)	0.00	(0.00 - 0.00)	0.54	(0.53 - 0.54)	0.00	(0.00 - 0.00)	0.92	(0.91 - 0.93)
0.32	**0.38**	**0.30**	0.31	(0.30 - 0.31)	0.00	(0.00 - 0.01)	0.31	(0.30 - 0.32)	0.00	(0.00 - 0.00)	0.28	(0.28 - 0.28)	0.00	(0.00 - 0.00)	0.90	(0.89 - 0.91)
0.04	**0.25**	**0.71**	0.04	(0.04 - 0.04)	0.00	(0.00 - 0.00)	0.21	(0.20 - 0.22)	0.00	(0.00 - 0.00)	0.69	(0.68 - 0.69)	0.00	(0.00 - 0.00)	0.94	(0.93 - 0.94)
0.02	**0.25**	**0.73**	0.01	(0.01 - 0.02)	0.00	(0.00 - 0.00)	0.22	(0.22 - 0.23)	0.00	(0.00 - 0.00)	0.71	(0.70 - 0.71)	0.00	(0.00 - 0.00)	0.94	(0.94 - 0.95)
0.05	**0.45**	**0.00**	0.04	(0.04 - 0.05)	0.00	(0.00 - 0.00)	0.40	(0.40 - 0.41)	0.00	(0.00 - 0.00)	0.47	(0.47 - 0.48)	0.00	(0.00 - 0.00)	0.92	(0.91 - 0.93)
0.70	**0.25**	**0.05**	0.69	(0.69 - 0.69)	0.01	(0.00 - 0.01)	0.19	(0.18 - 0.19)	0.00	(0.00 - 0.00)	0.04	(0.04 - 0.05)	0.00	(0.00 - 0.00)	0.93	(0.92 - 0.94)
0.25	**0.70**	**0.05**	0.23	(0.23 - 0.24)	0.00	(0.00 - 0.00)	0.63	(0.62 - 0.64)	0.00	(0.00 - 0.00)	0.04	(0.04 - 0.04)	0.00	(0.00 - 0.00)	0.91	(0.90 - 0.91)
0.60	**0.05**	**0.35**	0.60	(0.60 - 0.60)	0.00	(0.00 - 0.01)	0.03	(0.02 - 0.03)	0.00	(0.00 - 0.00)	0.34	(0.34 - 0.35)	0.00	(0.00 - 0.00)	0.97	(0.97 - 0.98)

Table 2c. Classification simulation analysis: Accuracy of individual assignments to each candidate source population with a critical likelihood ratio of 100.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).											
AMER	NACH	UYAK	Correctly assigned to American River		Incorrectly assigned to American River		Correctly assigned to Naches River		Incorrectly assigned to Naches River		Correctly assigned to upper Yakima River		Incorrectly assigned to upper Yakima River	Assigned
0.02	**0.06**	**0.92**	0.01	(0.01 - 0.01)	0.00	(0.00 - 0.00)	0.02	(0.02 - 0.03)	0.00	(0.00 - 0.00)	0.89	(0.89 - 0.90)	0.00 (0.00 - 0.00)	0.93 (0.93 - 0.94)
0.07	**0.09**	**0.84**	0.07	(0.06 - 0.07)	0.00	(0.00 - 0.00)	0.03	(0.02 - 0.04)	0.00	(0.00 - 0.00)	0.80	(0.80 - 0.81)	0.00 (0.00 - 0.00)	0.90 (0.89 - 0.91)
0.11	**0.22**	**0.68**	0.09	(0.09 - 0.10)	0.00	(0.00 - 0.00)	0.11	(0.09 - 0.11)	0.00	(0.00 - 0.00)	0.63	(0.62 - 0.63)	0.00 (0.00 - 0.00)	0.82 (0.81 - 0.83)
0.10	**0.19**	**0.71**	0.08	(0.08 - 0.09)	0.00	(0.00 - 0.00)	0.09	(0.08 - 0.10)	0.00	(0.00 - 0.00)	0.66	(0.65 - 0.67)	0.00 (0.00 - 0.00)	0.83 (0.82 - 0.84)
0.18	**0.33**	**0.49**	0.15	(0.15 - 0.16)	0.00	(0.00 - 0.00)	0.18	(0.17 - 0.19)	0.00	(0.00 - 0.00)	0.43	(0.42 - 0.44)	0.00 (0.00 - 0.00)	0.77 (0.75 - 0.78)
0.16	**0.33**	**0.51**	0.14	(0.13 - 0.14)	0.00	(0.00 - 0.00)	0.18	(0.17 - 0.19)	0.00	(0.00 - 0.00)	0.45	(0.45 - 0.46)	0.00 (0.00 - 0.00)	0.77 (0.76 - 0.78)
0.25	**0.40**	**0.36**	0.21	(0.21 - 0.22)	0.00	(0.00 - 0.00)	0.22	(0.21 - 0.24)	0.00	(0.00 - 0.00)	0.30	(0.29 - 0.31)	0.00 (0.00 - 0.00)	0.74 (0.72 - 0.75)
0.14	**0.26**	**0.61**	0.12	(0.11 - 0.12)	0.00	(0.00 - 0.00)	0.13	(0.12 - 0.14)	0.00	(0.00 - 0.00)	0.55	(0.54 - 0.56)	0.00 (0.00 - 0.00)	0.80 (0.79 - 0.81)
0.12	**0.26**	**0.62**	0.10	(0.09 - 0.10)	0.00	(0.00 - 0.00)	0.13	(0.12 - 0.14)	0.00	(0.00 - 0.00)	0.57	(0.56 - 0.57)	0.00 (0.00 - 0.00)	0.80 (0.79 - 0.81)
0.14	**0.24**	**0.62**	0.12	(0.12 - 0.13)	0.00	(0.00 - 0.00)	0.11	(0.11 - 0.12)	0.00	(0.00 - 0.00)	0.57	(0.56 - 0.57)	0.00 (0.00 - 0.00)	0.81 (0.79 - 0.81)
0.08	**0.20**	**0.72**	0.07	(0.06 - 0.07)	0.00	(0.00 - 0.00)	0.10	(0.09 - 0.11)	0.00	(0.00 - 0.00)	0.67	(0.66 - 0.67)	0.00 (0.00 - 0.00)	0.83 (0.82 - 0.84)
0.19	**0.30**	**0.51**	0.17	(0.16 - 0.17)	0.00	(0.00 - 0.00)	0.16	(0.14 - 0.17)	0.00	(0.00 - 0.00)	0.45	(0.44 - 0.46)	0.00 (0.00 - 0.00)	0.78 (0.76 - 0.79)
0.18	**0.37**	**0.45**	0.15	(0.15 - 0.16)	0.00	(0.00 - 0.00)	0.21	(0.20 - 0.23)	0.00	(0.00 - 0.00)	0.39	(0.38 - 0.40)	0.00 (0.00 - 0.00)	0.75 (0.74 - 0.76)
0.19	**0.24**	**0.57**	0.17	(0.17 - 0.17)	0.00	(0.00 - 0.00)	0.11	(0.11 - 0.12)	0.00	(0.00 - 0.00)	0.52	(0.51 - 0.53)	0.00 (0.00 - 0.00)	0.80 (0.79 - 0.81)
0.06	**0.35**	**0.59**	0.05	(0.04 - 0.05)	0.00	(0.00 - 0.00)	0.22	(0.21 - 0.23)	0.00	(0.00 - 0.00)	0.52	(0.51 - 0.52)	0.00 (0.00 - 0.00)	0.78 (0.77 - 0.79)
0.14	**0.30**	**0.56**	0.12	(0.12 - 0.13)	0.00	(0.00 - 0.00)	0.16	(0.15 - 0.17)	0.00	(0.00 - 0.00)	0.50	(0.49 - 0.51)	0.00 (0.00 - 0.00)	0.78 (0.77 - 0.79)
0.32	**0.38**	**0.30**	0.28	(0.27 - 0.29)	0.00	(0.00 - 0.00)	0.21	(0.20 - 0.22)	0.00	(0.00 - 0.00)	0.25	(0.25 - 0.26)	0.00 (0.00 - 0.00)	0.74 (0.73 - 0.76)
0.04	**0.25**	**0.71**	0.03	(0.03 - 0.03)	0.00	(0.00 - 0.00)	0.15	(0.14 - 0.16)	0.00	(0.00 - 0.00)	0.64	(0.64 - 0.65)	0.00 (0.00 - 0.00)	0.82 (0.82 - 0.83)
0.02	**0.25**	**0.73**	0.01	(0.01 - 0.01)	0.00	(0.00 - 0.00)	0.17	(0.16 - 0.18)	0.00	(0.00 - 0.00)	0.66	(0.65 - 0.67)	0.00 (0.00 - 0.00)	0.84 (0.83 - 0.85)
0.05	**0.45**	**0.00**	0.03	(0.03 - 0.04)	0.00	(0.00 - 0.00)	0.32	(0.30 - 0.33)	0.00	(0.00 - 0.00)	0.42	(0.42 - 0.43)	0.00 (0.00 - 0.00)	0.77 (0.76 - 0.79)
0.70	**0.25**	**0.05**	0.67	(0.66 - 0.67)	0.00	(0.00 - 0.00)	0.12	(0.11 - 0.13)	0.00	(0.00 - 0.00)	0.04	(0.03 - 0.04)	0.00 (0.00 - 0.00)	0.82 (0.82 - 0.83)
0.25	**0.70**	**0.05**	0.20	(0.19 - 0.21)	0.00	(0.00 - 0.00)	0.52	(0.51 - 0.53)	0.00	(0.00 - 0.00)	0.03	(0.03 - 0.03)	0.00 (0.00 - 0.00)	0.75 (0.73 - 0.76)
0.60	**0.05**	**0.35**	0.60	(0.59 - 0.60)	0.00	(0.00 - 0.00)	0.01	(0.00 - 0.01)	0.00	(0.00 - 0.00)	0.34	(0.33 - 0.34)	0.00 (0.00 - 0.00)	0.94 (0.94 - 0.95)

Table 2d. Classification simulation analysis: Accuracy of individual assignments to each candidate source population with a critical likelihood ratio of 1000.

Simulated Mixture proportions			Mean proportion from 500 iterations (5 - 95 percentile interval from 500 iterations).											
AMER	NACH	UYAK	Correctly assigned to American River		Incorrectly assigned to American River		Correctly assigned to Naches River		Incorrectly assigned to Naches River		Correctly assigned to upper Yakima River		Incorrectly assigned to upper Yakima River		Assigned	
0.02	**0.06**	**0.92**	0.01	(0.01 - 0.01)	0.00	(0.00 - 0.00)	0.00	(0.00 - 0.01)	0.00	(0.00 - 0.00)	0.88	(0.87 - 0.91)	0.00	(0.00 - 0.01)	0.91	(0.89 - 0.93)
0.07	**0.09**	**0.84**	0.06	(0.06 - 0.07)	0.00	(0.00 - 0.01)	0.00	(0.00 - 0.01)	0.00	(0.00 - 0.00)	0.80	(0.78 - 0.82)	0.01	(0.00 - 0.01)	0.87	(0.85 - 0.91)
0.11	**0.22**	**0.68**	0.08	(0.08 - 0.09)	0.00	(0.00 - 0.00)	0.03	(0.02 - 0.04)	0.00	(0.00 - 0.00)	0.60	(0.58 - 0.61)	0.00	(0.00 - 0.00)	0.71	(0.70 - 0.72)
0.10	**0.19**	**0.71**	0.08	(0.07 - 0.08)	0.00	(0.00 - 0.00)	0.02	(0.01 - 0.03)	0.00	(0.00 - 0.00)	0.64	(0.62 - 0.66)	0.00	(0.00 - 0.00)	0.74	(0.72 - 0.75)
0.18	**0.33**	**0.49**	0.14	(0.13 - 0.14)	0.00	(0.00 - 0.00)	0.06	(0.04 - 0.07)	0.00	(0.00 - 0.00)	0.40	(0.38 - 0.41)	0.00	(0.00 - 0.00)	0.59	(0.58 - 0.60)
0.16	**0.33**	**0.51**	0.12	(0.11 - 0.12)	0.00	(0.00 - 0.00)	0.06	(0.05 - 0.07)	0.00	(0.00 - 0.00)	0.41	(0.40 - 0.42)	0.00	(0.00 - 0.00)	0.59	(0.58 - 0.60)
0.25	**0.40**	**0.36**	0.18	(0.17 - 0.19)	0.00	(0.00 - 0.00)	0.08	(0.06 - 0.10)	0.00	(0.00 - 0.00)	0.27	(0.25 - 0.28)	0.00	(0.00 - 0.00)	0.53	(0.52 - 0.54)
0.14	**0.26**	**0.61**	0.10	(0.10 - 0.11)	0.00	(0.00 - 0.00)	0.04	(0.03 - 0.05)	0.00	(0.00 - 0.00)	0.52	(0.51 - 0.53)	0.00	(0.00 - 0.00)	0.66	(0.65 - 0.67)
0.12	**0.26**	**0.62**	0.09	(0.08 - 0.09)	0.00	(0.00 - 0.00)	0.04	(0.03 - 0.04)	0.00	(0.00 - 0.00)	0.53	(0.52 - 0.55)	0.00	(0.00 - 0.00)	0.66	(0.65 - 0.67)
0.14	**0.24**	**0.62**	0.11	(0.10 - 0.12)	0.00	(0.00 - 0.00)	0.03	(0.02 - 0.04)	0.00	(0.00 - 0.00)	0.54	(0.52 - 0.55)	0.00	(0.00 - 0.00)	0.68	(0.66 - 0.69)
0.08	**0.20**	**0.72**	0.06	(0.05 - 0.07)	0.00	(0.00 - 0.00)	0.03	(0.02 - 0.03)	0.00	(0.00 - 0.00)	0.64	(0.62 - 0.65)	0.00	(0.00 - 0.00)	0.72	(0.71 - 0.74)
0.19	**0.30**	**0.51**	0.15	(0.14 - 0.16)	0.00	(0.00 - 0.00)	0.04	(0.03 - 0.06)	0.00	(0.00 - 0.00)	0.42	(0.41 - 0.43)	0.00	(0.00 - 0.00)	0.61	(0.61 - 0.63)
0.18	**0.37**	**0.45**	0.13	(0.12 - 0.14)	0.00	(0.00 - 0.00)	0.08	(0.06 - 0.09)	0.00	(0.00 - 0.00)	0.34	(0.33 - 0.36)	0.00	(0.00 - 0.00)	0.55	(0.54 - 0.57)
0.19	**0.24**	**0.57**	0.16	(0.15 - 0.17)	0.00	(0.00 - 0.00)	0.03	(0.02 - 0.04)	0.00	(0.00 - 0.00)	0.50	(0.48 - 0.51)	0.00	(0.00 - 0.00)	0.68	(0.67 - 0.70)
0.06	**0.35**	**0.59**	0.03	(0.03 - 0.04)	0.00	(0.00 - 0.00)	0.10	(0.08 - 0.11)	0.00	(0.00 - 0.00)	0.46	(0.45 - 0.47)	0.00	(0.00 - 0.00)	0.59	(0.58 - 0.60)
0.14	**0.30**	**0.56**	0.11	(0.10 - 0.12)	0.00	(0.00 - 0.00)	0.05	(0.04 - 0.06)	0.00	(0.00 - 0.00)	0.47	(0.45 - 0.48)	0.00	(0.00 - 0.00)	0.62	(0.61 - 0.63)
0.32	**0.38**	**0.30**	0.25	(0.24 - 0.26)	0.00	(0.00 - 0.00)	0.07	(0.06 - 0.08)	0.00	(0.00 - 0.00)	0.22	(0.21 - 0.24)	0.00	(0.00 - 0.00)	0.54	(0.53 - 0.56)
0.04	**0.25**	**0.71**	0.02	(0.02 - 0.03)	0.00	(0.00 - 0.00)	0.06	(0.04 - 0.07)	0.00	(0.00 - 0.00)	0.59	(0.58 - 0.61)	0.00	(0.00 - 0.00)	0.67	(0.66 - 0.68)
0.02	**0.25**	**0.73**	0.01	(0.00 - 0.01)	0.00	(0.00 - 0.00)	0.09	(0.07 - 0.10)	0.00	(0.00 - 0.00)	0.59	(0.57 - 0.61)	0.00	(0.00 - 0.00)	0.68	(0.67 - 0.70)
0.05	**0.45**	**0.50**	0.02	(0.02 - 0.03)	0.00	(0.00 - 0.00)	0.18	(0.15 - 0.19)	0.00	(0.00 - 0.00)	0.36	(0.34 - 0.37)	0.00	(0.00 - 0.00)	0.55	(0.54 - 0.57)
0.70	**0.25**	**0.00**	0.63	(0.62 - 0.65)	0.00	(0.00 - 0.00)	0.04	(0.03 - 0.05)	0.00	(0.00 - 0.00)	0.03	(0.03 - 0.04)	0.00	(0.00 - 0.00)	0.70	(0.69 - 0.71)
0.25	**0.70**	**0.05**	0.14	(0.13 - 0.15)	0.00	(0.00 - 0.00)	0.34	(0.32 - 0.36)	0.00	(0.00 - 0.00)	0.02	(0.02 - 0.02)	0.00	(0.00 - 0.00)	0.50	(0.49 - 0.52)
0.60	**0.05**	**0.35**	0.59	(0.59 - 0.60)	0.01	(0.00 - 0.01)	0.00	(0.00 - 0.00)	0.00	(0.00 - 0.00)	0.33	(0.33 - 0.34)	0.00	(0.00 - 0.00)	0.94	(0.92 - 0.95)

Chapter 3

Gamete Cryopreservation Activities

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Introduction

Cryopreservation of semen from presupplementation Upper Yakima River spring chinook is part of the design for evaluating domestication related to supplementation activities in the Yakima spring chinook program (Chapter 4 in this report and Busack et al. 2002). The basic idea is that at some point x generations in the future, the cryopreserved semen can be used, if desired, to generate offspring that can be compared with other offspring sired by contemporary males. The differences in performance between the two groups will be a reflection of genetic change in the population over the x generations of selection. The test will not be definitive for domestication effects, as there may have been other genetic changes taking place, such as drift. It will not be as powerful as the control line approach that we are implementing, but possibly could be used in concert with the control line approach to increase power.

Detailed plans for implementation of this approach have not yet been developed. The most powerful approach would be to split a females egg lot, fertilizing half with cryopreserved semen and the other half with semen from a contemporary male, but more precise power calculations to determine how many females to involve have yet to be done. Sizing the experiment must consider the disposition of the fish to be produced, the impact of this experiment on other aspects of production and evaluation in terms of space allocation, and the expected 50% loss of eggs due to poor fertility of cryopreserved sperm .(Scheerer and Thorgaard 1989) It is conceivable that the experiment will be considered only as a backup approach, or used off-site.

Cryopreservation of semen is a wise thing to do just from the standpoint of gene banking. The cryopreserved material will soon be the only remaining germ plasm from the Upper Yakima River population as it existed before supplementation. As such it may later be used in restoration or currently unplanned research to assist conservation of this or another salmon population (Cloud et al. 1990).

We began cryopreserving semen in 2001 with the intent of sampling bona fide "wild"males as long as possible, with a goal of 200 males. Bona fide wild males are guaranteed to appear as four year olds only through 2002. In 2003, there is a slight possibility of 4-year olds appearing that were sired by precocious males from the 1997 brood year, although 5-year olds will still be guaranteed wild. We will take additional samples in 2003, assuming the proportion of fish sired by precocious males is negligible.

In 2004, however, 4-year old males sired by jacks from the 1997 brood year will begin appearing. Any sampling done in 2004 will be of 5-year olds only, and cryopreservation will cease after 2004.

Methods

All cryogenic activities were carried out in the walk-in refrigerator at the Cle Elum Supplementation and Research Facility (CESRF). Labeled bags of surplus semen were carried in from the fertilization room, mixed 1:3 with extender and vacuum-pumped from a bubbler into plastic 0.5 ml straws, which were then sealed with latex powder. Typically we filled 40 straws/male, less if less semen was available. For the most part we used the DMSO-glucose-egg yolk extender described by Wheeler and Thorgaard (1991) , but in 2002 we also used the extender of Erdahl (1982). Straws were placed in groups of 5 into plastic goblets, which were clipped into pairs onto metal canes. Canes were laid on a rack over liquid nitrogen for initial freezing for at least 10 min. After freezing, the canes were placed into canisters and immersed in liquid nitrogen in a large Dewar cryo flask. Bubbler, pump, straws, sealing powder and canes were all obtained from IMV International (Maple Grove, MN).

Samples were stored in the walk-in freezer at CESRF, with nitrogen being added as required. Samples were transported in the Dewar flask to Washington State University, where they were placed in long-term storage in the BPA-funded Nez Perce Gene Bank facility in Heald Hall.

Results

Tables 1 and 2 present the males used, dates spawned, and amount of material cryopreserved per male. Fifty-seven males were sampled in 2001, and 91 males were sampled in 2002. These

Table 1. Summary of spring chinook cryopreservation activities at CESRF in 2001

Spawning Date	Male #	Cannister Number	Total Canes	Total Goblets (2/cane)	Total Straws (5/goblet)
9/17/01	48	5	4	8	40
9/17/01	49	5	4	8	40
9/17/01	50	5	4	8	40
9/17/01	51	5	3	6	30
9/17/01	51	6	1	2	10
9/17/01	52	5	3	6	30
9/17/01	52	6	1	2	10
9/17/01	53	6	2	4	20
9/17/01	55	6	4	8	40
9/17/01	56	6	4	8	40
9/17/01	57	6	3	6	30
9/17/01	58	2	3	6	30
9/17/01	58	6	1	2	10

9/17/01	59	2	4	8	40
9/17/01	60	2	4	8	40
9/17/01	61	2	4	8	40
9/17/01	62	1	4	8	40
9/17/01	63	2	3	6	30
9/17/01	64	2	1	2	10
9/17/01	64	6	3	6	30
9/17/01	65	2	2	4	20
9/17/01	65	5	1	2	10
9/17/01	65	6	2	4	20
9/17/01	68	5	1	2	10
9/17/01	68	6	2	4	20
9/17/01	69	5	2	4	20
9/17/01	69	6	2	4	20
9/17/01	70	1	4	8	40
9/17/01	71	1	4	8	40
9/17/01	72	1	4	8	40
9/17/01	74	1	4	8	40
9/17/01	75	1	3	6	30
9/24/01	77	4	2	4	20
9/24/01	78	4	2	4	20
9/24/01	79	4	2	4	20
9/24/01	80	4	2	4	20
9/24/01	81	4	2	4	20
9/24/01	82	4	2	4	20
9/24/01	83	4	2	4	20
9/24/01	84	4	2	4	20
9/24/01	85	3	2	4	20
9/24/01	86	3	2	4	20
9/24/01	87	3	2	4	20
9/24/01	88	3	2	4	20
9/24/01	89	3	1	2	10
9/24/01	89	4	1	2	10
9/24/01	90	4	2	4	20
9/24/01	92	4	2	4	20
9/24/01	93	4	2	4	20
9/24/01	94	6	2	4	20
9/24/01	95	4	2	4	20
9/24/01	96	1	2	4	20
9/24/01	99	4	2	4	20
9/24/01	100	3	2	4	20
9/24/01	101	2	2	4	20
9/24/01	101	6	1	2	10
9/24/01	102	2	2	4	20
9/24/01	103	2	2	4	20
9/24/01	104	2	2	4	20
9/24/01	105	3	2	4	20
9/24/01	106	3	2	4	20
9/24/01	107	3	2	4	20
9/24/01	109	3	2	4	20
9/24/01	110	3	2	4	20

9/24/01	111	3	2	4	20
9/24/01	115	3	2	4	20
9/24/01	116	3	2	4	20

Table 2. Summary of spring chinook cryopreservation activities at CESRF in 2002

Spawning Date	Male #	Cannister Number	Total Canes	Total Goblets (2/cane)	Total Straws (5/goblet)	Notes
09/10/2002	7	1	2	4	20	
09/10/2002	8	1	2	4	20	
09/10/2002	9	1	2	4	20	
09/10/2002	10	1	2	4	20	
09/10/2002	11	2	2	4	20	
09/10/2002	12	2	2	4	20	
09/17/2002	17	2	2	4	20	
09/17/2002	18	2	2	4	20	
09/17/2002	20	3	2	4	20	
09/17/2002	21	3	2	4	20	
09/17/2002	22	3	2	4	20	
09/17/2002	26	4	2	4	20	
09/17/2002	27	4	2	4	20	
09/17/2002	28	4	2	4	20	
09/17/2002	29	4	2	4	20	
09/17/2002	30	4	2	4	20	
09/17/2002	31	5	2	8	40	
09/17/2002	33	5	2	4	20	
09/17/2002	35	5	2	4	20	
09/17/2002	36	5	2	4	20	
09/17/2002	37	6	2	4	20	
09/17/2002	38	6	1	2	10	
09/24/2002	50	6	2	4	20	
09/24/2002	52	6	2	4	20	
09/24/2002	53	6	2	4	20	
09/24/2002	54	1	2	4	20	
09/24/2002	58	1	2	4	20	
09/24/2002	59	1	2	4	20	
09/24/2002	60	1	2	4	20	
09/24/2002	61	1	2	4	20	
09/24/2002	62	1	2	4	20	
09/24/2002	63	1	2	4	20	
09/24/2002	64	1	2	4	20	
09/24/2002	65	1	2	4	20	
09/24/2002	66	1	2	4	20	
09/24/2002	67	1	2	4	20	
09/24/2002	68	1	2	4	20	
09/24/2002	69	1	2	4	20	
09/24/2002	70	2	2	4	20	
09/24/2002	71	2	2	4	20	
09/24/2002	72	2	2	4	20	
09/24/2002	73	2	2	4	20	
09/24/2002	74	2	2	4	20	

09/24/2002	77	2	2	4	20	
09/24/2002	78	2	2	4	20	
09/24/2002	79	2	2	4	20	
09/24/2002	81	2	2	4	20	
09/24/2002	82	2	2	4	20	
09/24/2002	83	2	2	4	20	
09/24/2002	85	2	2	4	20	
09/24/2002	86	2	2	4	20	
09/24/2002	87	3	2	4	20	
09/24/2002	88	3	2	4	20	
09/24/2002	89	3	2	4	20	
10/01/2002	132	3	2	4	20	
10/01/2002	133	3	1	2	10	
10/01/2002	134	3	2	4	20	
10/01/2002	135	3	2	4	20	
10/01/2002	136	3	2	4	20	
10/01/2002	137	3	2	4	20	
10/01/2002	138	3	2	4	20	
10/01/2002	139	3	2	4	20	
10/01/2002	140	3	2	4	20	
10/01/2002	141	3	2	4	20	
10/01/2002	142	4	2	4	20	
10/01/2002	143	4	2	4	20	
10/01/2002	145	4	2	4	20	
10/01/2002	146	4	2	4	20	
10/01/2002	147	4	2	4	20	
10/01/2002	149	4	2	4	20	
10/01/2002	150	4	2	4	20	
10/01/2002	151	4	2	4	20	
10/01/2002	152	4	2	4	20	
10/01/2002	153	4	2	4	20	
10/01/2002	157	4	2	4	20	
10/01/2002	160	4	2	4	20	
10/01/2002	166	4	2	4	20	
10/01/2002	167	4	2	4	20	
10/08/2002	170	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	171	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	172	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	173	5	1	2	10	
10/08/2002	174	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	175	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	176	5	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	177	6	2	4	20	
10/08/2002	178	6	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	180	6	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	181	6	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	182	6	2	4	20	Equal amount stored using Erdahl extender
10/08/2002	183	6	2	4	20	Equal amount stored using Erdahl extender

numbers represent 32% of the males spawned in 2001 and 53% of the males spawned in 2002. Table 3 shows the distribution of cryopreservation effort over the course of the spawning season. In 2001 we did cryopreservation on only two days, in the middle of the season, but achieved a high sampling rate on those two days. In contrast, in 2002, we did cryopreservation on five days that fairly well spanned the spawning season. In addition,

on the days we did cryopreservation we sampled all or nearly all the males spawned, so the samples are a good representation of the 2002 male spawning population.

Table 3. Males sampled for cryopreservation 2001-2002 compared to total males spawned

	Spawn Date	Males Spawned	Males Cryopreserved	Percentage Cryopreserved
2001				
	09/04/2001	18		0%
	09/10/2001	25		0%
	09/12/2001	13		0%
	09/17/2001	29	24	83%
	09/19/2001	10		0%
	09/24/2001	52	33	63%
	01/01/2001	26		0%
	10/08/2001	3		0%
2002				
	09/04/2002	3		0%
	09/10/2002	6	6	100%
	09/11/2002	12		0%
	09/17/2002	21	16	76%
	09/19/2002	10		0%
	09/24/2002	35	32	91%
	09/25/2002	34		0%
	10/01/2002	32	24	75%
	10/08/2002	17	13	76%

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Chapter 4

Domestication Research/Monitoring Design

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Prefatory Note: Domestication monitoring design is still undergoing considerable discussion and refinement as a result of the project's consultation with the Independent Scientific Review Panel (ISRP). The material presented in this report is a summary of this process up until the end of the contract period. Additional information on the development of the design can be found in our 2001 report (Busack et al. 2002). Note that although only three authors are listed, the design is the result of a great deal of input both by the other members of the Monitoring Implementation Planning Team and the Science/Technical Advisory Committee.

Introduction

At the end of the 2001 contract period, the preferred design for the domestication study was as follows, taken directly from Busack et al.(2002):

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.

This design was presented to the ISRP in February 2002 as part of the project's ongoing consultation with them. In their June response to our February submittal, the ISRP again challenged us to establish an internal wild control line. ISRP concerns were further clarified in a discussion between ISRP chair Rick Williams and Craig Busack. In response to the formal communication and ensuing discussion with the ISRP we reevaluated our position with regard to wild control lines. We remained convinced that a wild control line within the Upper Yakima population was unworkable for all the reasons we had previously presented. However, we also reevaluated the feasibility of using the two populations in the Naches arm of the Yakima basin as a wild control line, and reevaluated as well the possibilities for other wild control populations in the Columbia Basin. We concluded that the two Naches populations (Naches River and American River) collectively could serve reasonably well as a wild control for the study of domestication under supplementation, and we revised our design accordingly. On the evening of July 15 we presented our position regarding an internal wild control line, and our proposal to use the Naches basin fish as a wild control to several ISRP members at NWPPC headquarters in Portland.

Use of Naches Basin Spring Chinook as Wild Control for Supplementation

A genetic survey of the Yakima basin beginning in 1989 found that there were three distinct spring chinook populations in the Yakima basin: Upper Yakima, Naches, and American (Busack and Marshall 1991). The Naches population spawns in the upper Naches basin except the American River. The American River population spawns only in the American River. The Upper Yakima population spawns in the upper Yakima basin upstream of the Yakima canyon, but mainly in the area between Easton and Cle Elum.

The Naches populations differ from the Upper Yakima population in several aspects of life history. First, they mature later. Five-year olds are not very common in the Upper Yakima population, but are common in the Naches populations (Fig. 1).

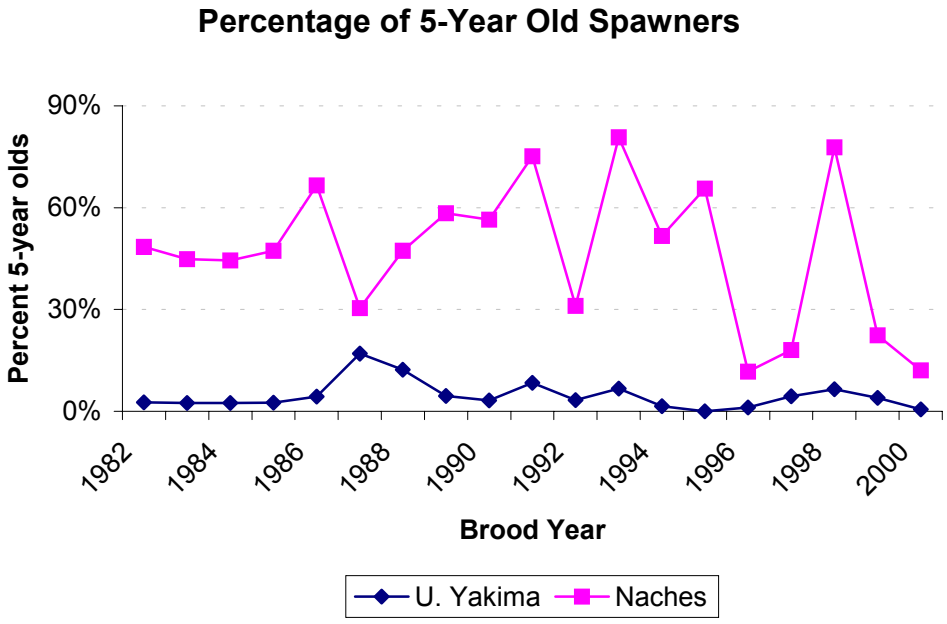

Fig. 1. Percentage of 5-year old spawners in Yakima basin spring chinook populations. Data and figure provided by B. Watson, YN.

Five year-olds are the most numerous age class in the American River population and they tend to be larger at age than Upper Yakima spawners (Knudsen 1991; Knudsen et al. in prep.).

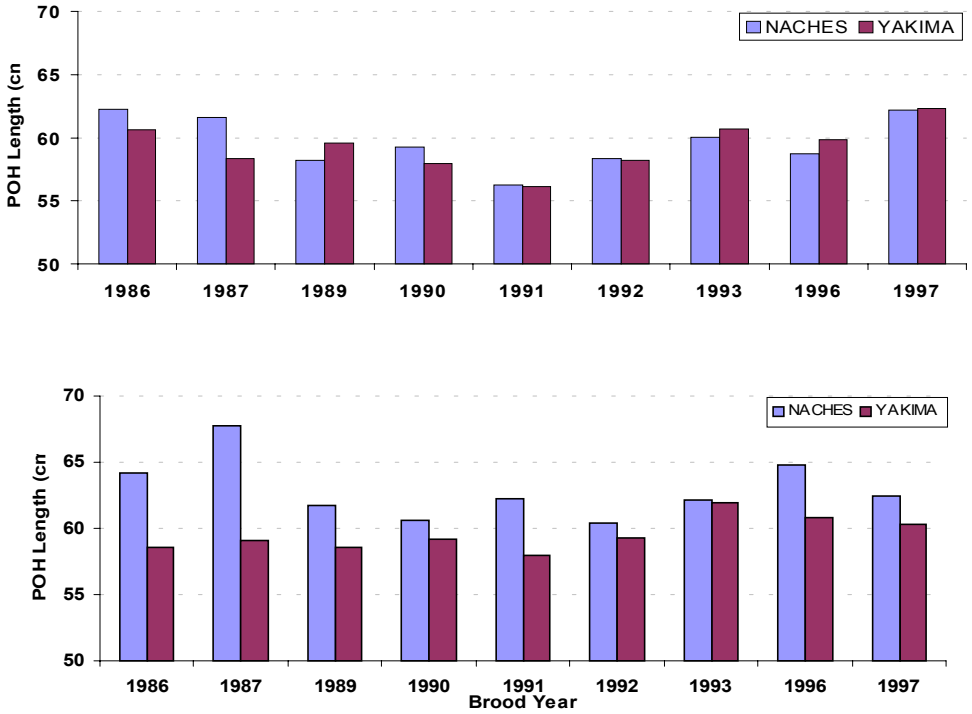

Figure 2. Comparison of mean Naches and upper Yakima River age-4 A) male and B) female post-orbital hypural plate (POH) lengths showing that Naches fish are generally larger at age over years.

Second, they tend to be larger than Upper Yakima fish. Fig. 2 compares the lengths of 4-year olds of both sexes in the three populations; a similar pattern holds for 5-year olds.

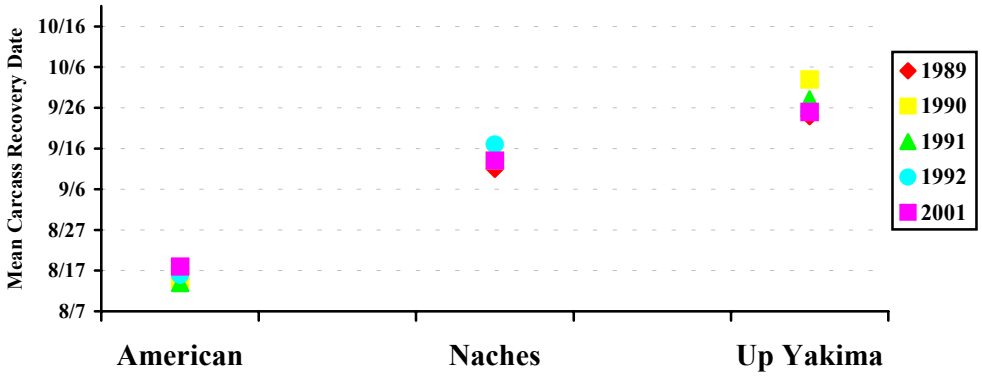

Figure 3. Mean carcass recovery date for America River, Naches system and upper Yakima River populations for the years 1989-1992 and 2001. Data provided by YN.

Third, they spawn considerably earlier than the Upper Yakima population (Fig. 3). The mean spawning date for Naches fish is 10 days earlier than that of the Upper Yakima population, and the mean spawning date of the American River population is five weeks earlier than that of the Upper Yakima population, probably a reflection of colder water in the Naches arm of the basin. Despite all these life history differences, the Naches populations' escapements track those of the Upper Yakima population surprisingly well (Fig. 5). As would be expected, escapements of 5-year olds do not track as well ($r = .62$), but the correlation between the Naches populations and Upper Yakima for 4-year olds ($r = .92$) is quite high. It is even higher ($r = .96$) between the 4-year olds in the Naches population (exclusive of American River) and the Upper Yakima 4-year olds.

In view of these observations, how suitable are the Naches populations for use as wild control lines? To answer this we evaluated the Naches populations collectively by the following four criteria for control lines (Busack et al. 2002):

1. The candidate 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 candidate 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 candidate control population and experimental populations must be reproductively isolated to the extent that they do not affect each other's performance. This close demographic tracking suggests the Naches populations collectively will be suitable as a wild control line for the supplementation effort.
4. The candidate control population has to be adequately accessible so that the differences between it and the experimental population can be measured (i.e., you must be able to monitor it and sample it).

As we have seen there are several life history differences between the Naches populations and the Upper Yakima population, and it is these differences which have made them not seem desirable as a control group in the past. However, in monitoring genetic change due to domestication, it is not differences between lines in any one year we are interested in so much as changes in these differences over time. For example, it does not really matter that the Naches fish are larger at age than the Upper Yakima fish. If domestication causes a change in size, we would expect the supplemented Upper Yakima population to change in size, but the Naches fish to not change in size. The difference between the two lines should change over time. More important is the genetic background of the control and experimental lines. It should be similar enough so that any environmental trends affecting traits of interest should affect both lines equally. Thus, we would hope that if Naches fish are a good control for Upper Yakima, some environmental trend causing fish size (for example) would affect both populations equally. The only measure we have of genetic background similarity is from allozyme markers. The three populations are genetically distinct, but more similar to each other than they are to any other chinook in the region (Marshall et al. 1995, Fig. 1). In addition, like the Upper Yakima, the Naches populations have been subjected to very little hatchery influence prior to the beginning of the supplementation effort. Thus, despite the life history differences, we concluded the Naches populations are genetically close enough to serve as a wild control line.

We felt the high correlation in escapements between the Naches and the Upper Yakima populations is good evidence of adequate environmental homogeneity, satisfying criterion 2.

We have no direct measure of reproductive isolation, but feel the genetic differences observed between the Upper Yakima and Naches populations that there is little straying between them. One concern in this project was that once supplemented, the Upper Yakima population would stray into the Naches basin in unacceptable numbers. Surveys during 2001, the first adult return year and a year of very large hatchery returns, no Upper Yakima hatchery-origin fish were found on Naches or American river spawning grounds. We therefore felt that criterion 3 was satisfied.

Criterion 4 deals with access to the control line. For the Naches fish to serve as a convincing wild control line we must be able to measure differences between them and the supplemented line at a sufficiently large number of traits of interest. We determined that Naches fish can be used for most adult traits and about half the juvenile traits in our original design, provided we could survey the spawning grounds, collect 10 pairs per year to take into the CESRF for research, and sample an additional number (max of 140) at a trap. Spawning ground surveys are already routinely done. Fish could be sampled and collected at a trap at Cowiche Dam on the lower Naches River. The trap is currently usable, but not very efficient at high flows. Some modifications to the trap to increase efficiency and thus increase our ability to take random samples are highly desirable. The Naches populations thus met criterion 4.

The issue of accessibility brought up the issue of to what extent the two Naches populations could be used separately rather than collectively. They appear to migrate upstream over the same time period past Cowiche Dam (Hockersmith et al. 1994), so they will be commingled except on the spawning grounds. We had not yet fully evaluated our ability to assign fish to population by DNA, but based on our experiences with attempting this with a Dungeness River pink salmon hatchery program we would expect this to be feasible. However, it was unclear that this would be worth the expense, logistical difficulty, and added interference with the upstream migration of the fish. Thus, for the foreseeable future with the exception of the spawning grounds we would use the two populations collectively as a single wild control line.

In consideration of all the above discussion, we concluded that the Naches populations collectively would be an adequate wild control line for the evaluation of the Upper Yakima supplementation program, and presented this option to the ISRP to the July meeting. The ISRP was very receptive to the basic design, so we submitted yet another revised design.

Summary of July 2002 Revised Design for Domestication Selection Monitoring

The revised design consisted of comparing three lines- a wild control line, a supplemented line, and a hatchery control line annually or nearly annually for 13 adult and 12 juvenile traits.

A. Wild control line (WC): composite of Naches and American river stocks. These stocks will not be supplemented during the study. This elevation of the use of the Naches fish from an "as appropriate" to a full wild control was the essential difference between this design and the February design.

B. Supplemented line (S): the Upper Yakima population, supplemented annually by production from 16 raceways at CESRF and associated acclimation sites at Jack Creek, Easton, and Clark Flat. Broodstock collected randomly throughout run. Broodstock consists of 100% natural origin fish. all other aspects of the program are as already described in numerous project documents.

C. Hatchery control line (HC): a subline of the Upper Yakima population, to be founded from returning hatchery fish, collected from throughout the run, in 2002. Two of the 18 CESRF raceways (randomly chosen each year) will be dedicated to rearing of this line. These fish will be the offspring of a minimum of 30 pairs of fish, which should provide the H line an effective size of at least 100 per generation. HC fish will be reared and released exactly as will their supplementation line (S) counterparts. No HC fish will be allowed to spawn in the wild; any returnees in excess of broodstock needs will be removed at the adult collection facility at Roza Dam.

By comparing the supplemented line to both controls, we will address two key questions: 1) how much domestication is incurred by a population undergoing YKFP-style supplementation ?; 2) how much less domestication is incurred under YKFP-style

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

In the February design, one of the major elements was the cryopreservation of sperm from approximately 200 presupplementation males to be used in test matings some time in the future to evaluate divergence of the supplemented line from its presupplementation state. This design concept has a number of issues associated with it, but provides the potential for an internal quasi-wild control in the absence of a formal wild control line. Now that the design will include a wild control line the case for the cryopreservation approach was considerably weaker, but it may still be desirable to do this type of work at some level at some time in the future. Therefore we will continue to collect and freeze semen for this potential use. Storing sperm from the presupplementation population is a worthwhile gene-banking exercise anyway, and the cost is very low.

Along with the basic description of the overall design, at the request of the ISRP we included detailed information on how we intended to approach measurement and analysis of each trait. Until this point we had included only a list of traits.

From the July 2002 Design to Present

The ISRP responded to the formal July design favorably. They did have numerous comments and suggestions, but the design was approved. There were many comments, all of which we attempted to address. Major comments were in four areas:

1. Wild control line. They felt using the composite Naches/American population would not result in a sufficiently rigorous comparison. They also felt that 10 pairs per year was too small a sample for evaluation of reproductive traits.

2. Hatchery control line. The ISRP felt that the line proposed would have too low an effective size, and thus would be subject to genetic drift.

3. Elaborate and expensive experimentation. The ISRP was concerned in particular about a detailed comparison among lines of the tendency to produce precocious males, arguing that this subject was basically a whole line of research, and one that should be approached differently. They also were concerned about the value of an expensive proposal to measure survival of the fry from different lines in a seminatural environment (the hatchery slough at CESRF), arguing that this would not approximate a natural habitat.

4. Importance of some traits. The ISRP commented that some traits were not really traits, but rather measures of aspects of fish culture.

Our responses to categories 3 and 4 were quick and simple. The study of production of precocious males was deemed too ambitious and too expensive and dropped. We disagree with the ISRP about the value of the survival study. The environment is a good habitat, and this trait was one of very few traits on juveniles that would be measured in the natural rather than hatchery environment. We felt it was an important complement to the work in the hatchery environment. However, it too was too expensive, at least at present. On the issue of whether all our traits were in fact traits, we maintain that they are. There are some that perhaps would not have been measured if they were not measured in the course of routine operations (i.e., we would not design a specific experiment around them), but they are all worth considering.

The other two categories of comments cause a considerable amount of discussion within the project. We agreed with the ISRP from the start that using the Naches and American populations in composite for many traits was indeed suboptimal, so we evaluated methods for separating them. The only reliable method was to trap them at Cowiche and classify them to stock using DNA. After much discussion, including concerns for minimizing the impact of sampling on the population, we decided to sample partially spent spawners on the spawning grounds, and return unneeded eggs to the river. We set the minimum sample size at 10 pairs to minimize impact to the population, but have conducted power analysis (Busack and Knudsen, chapter 5 of this report) and concluded that the 10-pair minimum does provided adequate power over several generations. Clearly, taking a larger sample size when possible will improve the power situation dramatically.

Size of the hatchery control line was the other big ISRP issue. Again, clearly a larger line would be better experimentally in terms of power (Busack and Knudsen, chapter 5 of this report) and in terms of genetic drift. Appropriate sizing of the HC line requires balancing several concerns: power, genetic drift, allocation of hatchery resources, disease concerns, sustainability, wasting of gametes and creating surplus adults. All these issues have been the subject of considerable discussion. It is not clear that the discussion has been concluded, but at this point it appears that about 35 pairs is the best compromise. This should provide enough fish for the two raceways, provide enough buffer for BKD incidence (high-titer females whose entire egg lots will have to be dumped), adequate

power, and adequate effective size over the course of the study. We estimate that the per-generation effective size should be at least 100, well over the minimum of 50 recommended by Roff (1997). Drift will eventually become a significant proportion of the difference between the HC and other lines, but will not be expected to over 3-4 generations.

An issue that came up during the discussion of the HC line is the possible bias caused by precocious HC males spawning in the wild with S females. Some power analysis of this situation has been done (Busack and Knudsen, chapter 6 of this report), but the issue is still unresolved. The key factor is the number of precocious HC fish to expect on the spawning grounds (Pearsons et al. 2003) and their reproductive success (Schroder et al. 2003).

The current version of the domestication research/monitoring plan is attached as an appendix.

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Appendix

Draft Design for Domestication Monitoring in the Yakima Spring Chinook Program

DRAFT
Design for Domestication Monitoring in the Yakima Spring Chinook Program

Yakima/Klickitat Fisheries Project Monitoring Implementation Planning Team

April 28, 2003

Introduction

We propose to evaluate to evaluate the domesticating effects of supplementation, and compare the intensity of domestication incurred under supplementation as practiced in the YKFP spring chinook program at the Cle Elum Supplementation Research Facility (CESRF) to that incurred under a more conventional regime of continuous hatchery culture. The primary design consists of comparing three lines- a wild control line, a supplemented line, and a hatchery control line- for 13 adult and 17 juvenile traits. Traits vary in frequency of evaluation from annually to once per generation. Details on the traits are presented in the Trait, Protocol and Analysis Overview section. The YKFP spring chinook supplementation program began with broodstock collection in 1997. The first adult (4-year olds) return was in 2001. The formal domestication research effort began in the fall of 2002, although data for evaluation of many of the traits began in 1997.

Experimental Lines and General Hypotheses

A. *Supplementation line (S):* the Upper Yakima spring chinook population, supplemented annually by production from 16 raceways at CESRF and associated acclimation sites at Jack Creek, Easton, and Clark Flat. Broodstock collection is at the Roza Adult Monitoring Facility (RAMF) at Roza Dam (Fig. 1). In contrast to most hatchery programs, broodstock are collected randomly throughout run, and consist of 100% natural origin fish. Other aspects of the program are as already described in numerous project documents.

B. *Wild control line (WC):* Naches River spring chinook. The Naches River spring chinook occur in the Naches arm of the Yakima basin (Fig. 1). Because they will not be supplemented during the study, they are available as wild control lines. We have determined that Naches fish can be used for 10 of 13 adult traits and 9 of 15 juvenile traits in our design, provided we can adequately sample fish on the spawning grounds, and collect gametes from a minimum of 10 pairs per year for research. Spawning ground surveys are already routinely done. We anticipate that in the future we may also be able to sample fish can be sampled and collected at a trap at the Cowiche Dam on the lower Naches River (Fig. 1). This trap is designed to collect coho salmon, so some modifications to the trap or the dam itself may have to be made to facilitate the efficient capture of chinook.

To minimize impacts to the control population, collection of gametes from the Naches population will be minimal, semen and partial egg lots from 10-30 pairs per year, depending on run size. Gametes will be used for evaluation of some adult traits, but mainly for production of juveniles for research. Ideally this research will be done at CESRF, but because of disease considerations it may have to be done offsite.

C. *Hatchery control line (HC):* a subline of the Upper Yakima population, to be founded from returning hatchery fish, collected from throughout the run, in 2002. Two of the 18 CESRF raceways (randomly chosen each year) will be dedicated to rearing of this line. These fish will be the offspring of a minimum of 36 pairs of fish, which should provide the H line an effective size of at least 100 per generation. A larger line of HC fish was deemed to be politically untenable because of the large number of fish that would potentially have to be removed at Roza Dam. Although larger effective size would be preferable, but this is far larger than the minimum of 50 for quantitative genetic studies deemed to be adequate by Roff (1997). Because the number of fish used to found the HC line is relatively small, the decision was made to have a single line to avoid the possibility of smaller replicate lines going extinct. HC fish will be reared and released exactly as will their supplementation line (S) counterparts. No HC fish will be allowed to spawn in the wild; any returnees in excess of broodstock needs will be removed at the Roza adult monitoring facility (RAMF, Fig. 1).

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

One critical issue regarding this design that is still under discussion is "leakage" from the H line into the S line through precocious males from the H line spawning in the wild with S-line females. If this occurs at an appreciable rate, the effect will be to increase the amount of domestication incurred by the S line. This issue raises two concerns. First, it will bias the H-S and S-W comparisons, making the supplementation treatment appear more domesticating than it is. Second, the S line will undergo more domestication than it should for the lifespan of the H line, a conservation concern. Assuming that adequate monitoring can be done of the reproductive success of H-line precocials, the first issue can be dealt with, but not the second. Work is currently underway to evaluate this risk from a variety of angles, including measures for reducing production of precocious fish.

We also intend to cryopreserve the sperm of approximately 200 presupplementation Upper Yakima males. This will give us the potential to evaluate divergence of the supplementation line from its presupplementation state. This design concept has a number of issues associated with it, but it may be desirable to do this type of work at some level at some time in the future. Storing sperm from the presupplementation population is a worthwhile gene-banking exercise anyway, and the cost is very low.

Trait, Protocol, and Analysis Overview

The following pages provide details in a standard format, one trait at a time, on the 13 adult and 17 juvenile traits we intend to evaluate with this design. Most traits will be evaluated annually in order to maximize power, but some may be done less frequently due to logistical limitations. Protocols may vary from year to year to allow collection of key baseline information some years, and experimental data in others. For many traits it is important to distinguish between S line fish of hatchery-origin and those of natural origin: we call these two "sublines" SH and SN in the write-ups. This distinction is made to allow a cleaner measure of genetic differences. Consider nearly any comparison of HC and S fish. Part of the difference in performance between SN and HC fish will be genetic, but part may also be phenotypic, due to the effect of being reared in a hatchery. If HC fish are compared to SH fish, because they share the phenotypic effect of hatchery rearing, the performance difference will be exclusively genetic. It is important to keep in mind when reading the write-ups, however, that although we call SN and SH lines in describing experimental designs, they differ only in their rearing history. Any given pair of SN and SH fish can have the same grandparents. The SN and SH designations are also used to designate test groups for juvenile traits, but in this case the designations refer to the *parents* of the juveniles being evaluated.

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 13 adult traits and 15 juvenile traits to be evaluated. One juvenile trait proposed earlier has been dropped, but to prevent confusion we did not renumber the other traits: thus there is no trait J7. The number of traits can be misleading. Many of the traits are measured on the same fish with no difference in protocol except for the measurement. Thus, the "effective" number of traits in terms of logistics and cost is considerably lower. The best example of this is the set of traits A7-A9, which are all measurements of reproductive traits on the same fish. We list the measurements as separate traits because we consider them all important, and because we want to insure they are all done. Some traits require considerable effort and cost, whereas others will be measured in the course of ordinary fish culture operations. Our guiding philosophy was to take advantage of the opportunities offered by the CESRF and other facilities in the basin to measure as many traits relevant to domestication as feasible while minimizing impacts to the supplementation effort and the wild control population.

The individual trait write-ups are in general not complete. The format is still in flux, with several new fields having only recently been added (justification, start, frequency). These will be completed in the next year as protocols are solidified and power analyses are completed. The write-ups reflect the discussion at the last comprehensive evaluation of the plan by MIPT, on 11/07/2002.

Trait	***Revised***	***11/07/02***
A1. Adult Recruits/Adult-Adult Survival		
Justification		
Supplementation success is ultimately measured as the increase in natural origin recruits produced by the population. Measuring adult-adult survival is measure of population fitness, the overall trait of key interest in domestication.		
Location(s)		
Roza and Prosser Dams, Upper Yakima, Naches, American spawning ground		
Start Date		
2002		
Frequency		
Annually		
*Lines Compared***:**		
WC,HC,S (SN and SH)		
Protocol		
At Prosser all adults from all populations in the basin are counted and classified as hatchery or natural, resulting in counts for hatchery origin (HC+SH) and natural origin (SN + America + Naches(WC)). At Roza SH, SN, and HC are counted and sampled for sex and age. An estimate of Naches + American abundance will be made by comparing Prosser and Roza counts after adjustment for harvest and incidental in-river mortality. Redd counts will be obtained from spawning ground surveys on the Naches and the American. Final Naches adult counts will be calculated as the product of the Naches+ American escapement and the Naches proportion of the Naches+American redd counts. Additional adjustments may be made to correct for fish/redd and sex ratio on the spawning grounds. Adult-adult survival by brood year can be estimated for WC, HC, SH, and S natural spawners (mix of SN and SH spawning in wild).		
Expectations/Hypotheses		
If domestication does not occur, differences in survival among all four groups will remain constant over time. Conversely, if domestication does occur we would expect HC and SH survivals to increase over time. Furthermore, HC survival should increase at a greater rate than SH. In addition, the survival of S fish spawning in the wild will decrease.		
Analytical/Statistical Methods and Issues		
Within brood years no statistical analysis will be done, as no variance estimates will be available. Over brood years analysis of covariance will be used to evaluate differences in trends. Trend analysis will take into account year-to-year environmental fluctuations and temporal autocorrelations.		
Power Analysis Completed?		
No.		
New Effort Required		
None. All required activities are already being done.		

Trait	***Revised***	***11/07/02***
A2. Age composition by sex		
Justification		
Location(s)		
RAMF, CESRF, Naches spawning grounds		
Start Date		
2002		
Frequency		
Annually		
Lines Compared:		
WC,HC,S (SN and SH)		
Protocol		
Requires sex and age determination of adequate samples of fish. For all fish used in the hatchery (SN and HC for production, few SH for research) and for those sampled on the spawning grounds as carcasses (WC), sex can be determined visually. Sex determination based on visual inspection of green fish is not reliable (e.g.,30% of the fish classified at Roza as males are females) so sex determination based on DNA will be used on most SH, and HC fish. Age will be determined on all fish by scale analysis. Minimum target sample size is 140 for WC and 200 for SH (this analysis will not be needed on SN or HC fish because they will all be sexed at spawning or removal). This will provide estimates of age composition with multinomial confidence intervals of $\pm$10% or less at α=0.05 (Thompson 1987).		
Expectations/Hypotheses		
Hatchery fish tend to return at younger ages than naturally produced fish, so younger age structures would be expected for HC and SH relative to naturally produced fish, and these differences may be only phenotypic. If domestication does not occur, differences in age structure among all four groups will remain constant over time. If domestication does occur we would expect age structure to decrease (Reisenbichler and Rubin 1999). Because HC should be most domesticated, its age structure should decrease more, but age structure of S should decrease as well.		
Analytical/Statistical Methods and Issues		
Within years multinomial contingency tests will be used to compare age structures. Comparison of HC and SH will be especially informative for determining genetic effects. Over years analysis of covariance will be used to evaluate differences in trends. Analysis will be complicated by the fact that age structure is in part a reflection of the genetic composition of the population, but can be strongly influenced by environmental fluctuations in brood-year survival.		
Power Analysis Completed?		
No.		
New Effort Required		
DNA sexing of ~200 fish at $10/fish estimated maximum. other activities already in place.		

Trait	***Revised***	***11/07/02***
A3. Size-at-age by sex		
Justification		
Location(s)		
RAMF, CESRF, and Naches spawning grounds		
Start Date		
Frequency		
Annually		
Lines Compared:		
WC,HC,S (SN and SH)		
Protocol		
Protocol same as for trait A2 (same fish) but with post-orbital hypural (POH) lengths measured		
Expectations/Hypotheses		
For unknown reasons, hatchery fish have been observed on several occasions to be smaller than naturally produced fish of the same age; e.g.,2001 returnees to Cle Elum were ~2 cm shorter than naturally produced fish (see also Gallinat et al. 2001, Fresh et al. in press), so smaller sizes would not be surprising in HC and SH relative to naturally produced fish, but these differences may be only phenotypic. If domestication does not occur, sizes of all four groups will remain constant over time. Assuming that the smaller size observed in hatchery fish is in part a result of domestication, size can be expected to decline as domestication proceeds. Thus the size of the WC fish should remain constant, and the size of S and HC should decline, with HC fish declining most.		
Analytical/Statistical Methods and Issues		
Within years, analysis of variance will be used to compare mean POH lengths. Comparison of HC and SH will be especially informative for determining genetic effects. Over years analysis of covariance will be used to evaluate differences in trends.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
No new effort required beyond trait A2 except slight additional labor for measuring fish.		

Trait	***Revised***	***11/08/02***
A4. Sex ratio at age		
Justification		
Location(s)		
RAMF, CESRF, and Naches spawning grounds		
Start Date		
Frequency		
Lines Compared:		
WC,HC,S (SN and SH)		
Protocol		
Protocol same as for trait A2 (same fish).		
Expectations/Hypotheses		
If domestication does not occur we would expect to see no changes in the sex ratios of fish maturing at different ages. If domestication does occur we anticipate that the HC line will produce fewer precocial males. Consequently, greater proportions of males will exist in the later maturing age classes (e.g. 4- and 5-yr olds) in the HC line.		
Analytical/Statistical Methods and Issues		
Within years, binomial test of proportions will be used. Over years analysis of covariance will be used to evaluate differences in trends. Cowiche trap may not yield unbiased samples.		
Power Analysis Completed?		
New Effort Required		
No new effort required beyond trait A2.		

Trait	***Revised***	***11/08/02***
A5. Migration timing to trap		
Justification		
Location(s)		
RAMF		
Start Date		
Frequency		
Lines Compared:		
HC,S (SN and SH). WC is not included because they do not go to RAMF, and there is no comparable equivalent site in the Naches basin.		
Protocol		
Sampling fish passing for marks and recording origin and date of passage.		
Expectations/Hypotheses		
No expectations on how this trait will change, but data will already be available.		
Analytical/Statistical Methods and Issues		
Within years, Kolmogorov-Smirnov test will be used on cumulative passage distributions. Over years analysis of covariance will be used on median arrival date.		
Power Analysis Completed?		
New Effort Required		
No new effort required.		

Trait	***Revised***	***11/08/02***
A6. Spawning timing		
Justification		
Location(s)		
CESRF, Upper Yakima and Naches spawning grounds		
Start Date		
2002		
Frequency		
Annual		
*Lines Compared***:**		
WC, HC,S (SN and SH)		
Protocol		
Monitoring this trait has two components: 1) comparing S -and WC temporal trends in redd count and carcass recovery distributions from weekly spawning ground surveys; and 2) comparing SH with HC spawn timing distributions in the hatchery.		
Expectations/Hypotheses		
Our expectation is that time of maturation will not change. Changes in spawning timing have been commonplace in hatchery operations, but this is likely tightly linked to taking eggs from the first part of the run. In this project we have made a concerted effort to take eggs in a representative fashion throughout the spawning season. Thus we do not expect to see a change in the time of spawning.		
Analytical/Statistical Methods and Issues		
Within years we will compare the temporal distributions of HC with SN spawners for each sex separately by using the non-parametric Kolmogorov-Smirnov test. Within-year analyses of WC and S fish will not be done, but median spawning/recovery dates for each of these populations will be calculated. Over years analyses of covariance will be used on median spawning dates by sex. Two of these analyses (one for each sex) will examine temporal changes in the HC and SN fish while two others (if possible one for each sex) will examine similar trends in WC and S fish. Naches information will not be very precise.		
Power Analysis Completed?		
New Effort Required		
No new effort required. Weekly spawning ground surveys covering the entire spawning season are already being done for other purposes.		

Trait	***Revised***	***11/08/02***
A7. Fecundity		
Justification		
Location(s)		
CESRF		
Start Date		
2002		
Frequency		
Annual		
Lines Compared:		
HC,S (SN and SH)		
Protocol		
Enumerate eggs from HC, SH, and SN females. Requires holding SH origin females (a minimum of 30) to maturity at hatchery. Fecundity samples from SN and HC females will be taken from fish being held for S and H line broodstock. WC is not included because we intend to collect partially spawned females and thus will not be able to get total eggs counts.		
Expectations/Hypotheses		
If domestication does not occur fecundity will remain constant. However, Fleming and Gross (1989, 1992) predicted that under hatchery culture fecundity will decrease, at least for coho salmon. Thus, we would expect fecundity to decrease in S and HC, and the decrease should be greater in HC.		
Analytical/Statistical Methods and Issues		
Within years, analysis of covariance will be used to compare body traits vs. fecundity within age classes. Analysis of variance will be used within years to compare absolute fecundities within age classes. Over years analysis of covariance will be used on mean fecundity by age to detect trend differences among groups. Naches females, because there will be so few of them, should represent a variety of sizes.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
Activities already in place.		

Trait	***Revised***	***11/08/02***
A8. Egg size		
Justification		
Location(s)		
CESRF, Naches spawning grounds		
Start Date		
Frequency		
Lines Compared:		
WC, HC,S (SN and SH)		
Protocol		
Measure size of eggs from WC, HC,SH, and SN females. Same fish used for trait A7. Requires holding some SH origin females (a minimum of 30) to maturity at hatchery in addition to the SN females that will be held for S broodstock and the HC females that will be used for HC broodstock. Also requires sampling eggs from a minimum of 10 Naches females on spawing grounds.		
Expectations/Hypotheses		
If domestication does not occur egg size will not change. However, Fleming and Gross(1989, 1992) and Petersson et al. (1996) observed that under hatchery culture coho egg size increased. Thus, we would expect egg size to increase in S and HC, and the increase should be greater in HC. However, Jonsson et al. (1996) found that wild Atlantic salmon females had larger eggs than hatchery origin females.		
Analytical/Statistical Methods and Issues		
Within years, analysis of covariance will be used to compare body traits vs. egg size within age classes. Analysis of variance will be used within years to compare absolute fecundities within age classes. Over years analysis of covariance will be used on mean egg size by age to detect trend differences between groups. Naches females, because there will be so few of them, should represent a variety of sizes.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
No new effort over that required for trait A7. Other activities already in place.		

Trait	***Revised***	***11/08/02***
A9. Reproductive effort		
Justification		
Location(s)		
CESRF		
Start Date		
Frequency		
Lines Compared:		
HC,S (SN and SH)		
Protocol		
Measure weight of testes and ovaries from HC,SH, and SN fish, and compare to fish weight. Same females used for traits A7 and A8. Requires holding some SH origin males and females (a minimum of 30 pairs of SH) to maturity at hatchery in addition to the SN fish that will be held for S broodstock and the HC fish that will be used for HC broodstock. WC will not be included because we will be collecting partially spawned WC females, and thus will not be able to measure the total gametic weight.		
Expectations/Hypotheses		
If domestication does not occur we no changes in reproductive effort will occur. However, Fleming and Gross (1989,1992) and Jonsson et al. (1996) observed that under hatchery culture reproductive effort will increase. Thus, we would expect reproductive effort to increase in S and HC, and the increase should be greater in HC.		
Analytical/Statistical Methods and Issues		
Within years, analysis of covariance will be used to compare body traits vs. reproductive effort within age classes. Analysis of variance will be used within years to compare absolute fecundities within age classes. Over years analysis of covariance will be used on mean reproductive effort by age to detect trend differences between groups. Naches females, because there will be so few of them, should represent a variety of sizes.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
No new effort over that required for traits A7 and A8. Other activities already in place.		

Trait	***Revised***	***11/08/02***
A10. Male and female fertility		
Justification		
Location(s)		
CESRF		
Start Date		
Frequency		
Lines Compared:		
WC, HC,S (SN and SH)		
Protocol		
Estimate fertility of WC, HC, SH, and SN fish by doing *inter se* (within line) test crosses using 2 x 2 or 3 x 3 factorial mating designs. Same fish used for trait A9. Requires holding some SH origin males and females (a minimum of 30 pairs) to maturity at hatchery in addition to the SN fish that will be held for S broodstock and the HC fish that will be used for HC broodstock. Will also require sampling gametes from a minimum of 10 pairs of Naches fish on spawning grounds. About 400 eggs will be used to create each family. Therefore, 800 eggs per female would be used in the 2 x 2 crosses and 1,200 in the 3 x 3 crosses. Each family of approximately 400 eggs will be incubated in its own isolette. If male or female gamete quality is poor, it is readily discerned by this approach since it allows both males and females to produce zygotes with multiple mates.		
Expectations/Hypotheses		
If domestication does not occur fertility will remain constant. However, under hatchery culture selection for fertility may be relaxed considerably, especially in males. If so, fertility could decrease in the S and HC lines, and should decrease more in the HC line.		
Analytical/Statistical Methods and Issues		
Within years, analysis of variance will be used to compare fertility of individual animals within groups. Over years analysis of covariance will be used on mean fertility to detect trend differences between groups.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
No new effort required over that already in place for reproductive success studies except trapping and transporting Naches fish, as already mentioned.		

Trait	***Revised***	***11/08/02***
A11. Adult morphology at spawning		
Justification		
Location(s)		
CESRF and possibly some effort on Naches spawning grounds		
Start Date		
Frequency		
Lines Compared:		
WC, HC,S (SN and SH)		
Protocol		
Collect digitized measurement data from lateral image landmarks on photos of adults. Develop orthogonal variables with which to compare WC, HC, SH, and SN fish. Same fish used for traits A7- A10. Requires holding some SH origin males and females (about 30 pairs) to maturity at hatchery in addition to the SN fish that will be held for S broodstock and the HC fish that will be used for HC broodstock. Data on Naches fish will be collected from carcasses on spawning grounds.		
Expectations/Hypotheses		
If domestication does not occur no changes in morphology will occur. If domestication does occur, we expect secondary sexual characteristics in both sexes to become less pronounced; e.g., reduced kype length, reduced body depth, less fusiform body shape, smaller adipose fins (Fleming and Gross 1992, Berejikian et al. 1997, Petersson et al. 1996, Webb et al. 1991, Petersson and Jarvi 1993, Hard et al. 2000). We would thus expect these types of changes in the S and HC lines, with greater changes in the HC line.		
Analytical/Statistical Methods and Issues		
Multivariate analysis of variance of digitized orthogonal shape variables generated by Procrustean distance methods, and other methods described by Hard et al. (2000). Methods will be applied within years and across years (to measure trends). Hard has agreed to collaborate in this effort.		
Power Analysis Completed?		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
Photos are already being routinely taken, but about two weeks/year technician help will be needed to digitize photos. If photos are to be taken on Naches spawning grounds, additional help will be needed.		

Trait	***Revised***
A12. Adult spawning behavior	
Justification	
Location(s)	
Cle Elum experimental spawning channel	
Start Date	
Frequency	
Lines Compared:	
SN,SH,HC	
Protocol	
Small numbers of SN,SH, and HC adults will be tagged and placed into sections of the channel, and scan and focused behavioral observations will be made on fish as they spawn. Traits observed will be chosen from among those used by Schroder (1981) and Berejikian et al. (1997): e.g., dominance relationships, nuptial coloration, number of spawnings, redd location. This information will be coupled with measurements of reproductive success (see trait A13). To get a full perspective on these behaviors, the observations need to be done with groups isolated from each other and with groups in competition. WC will not be included because our plan is to collect only partially spawned fish. Because the Naches population spawns earlier than the U. Yakima population, partially spawned fish, if available, would not be at all at the same reproductive starting point as S and HC fish for channel studies.	
Expectations/Hypotheses	
We expect to see differences in behavior between hatchery origin and wild origin fish due to hatchery rearing (e.g., Fleming and Gross 1992, 1993; Berejikian et al. 1997; Webb et al. 1991; Lura et al. 1992; Petersson and Jarvi 1997). The magnitude of this effect will be determined by comparing SH and SN. If domestication does not occur we will not see genetic changes in behavior, so we would expect the behavior of SH and HC to be comparable over time. If domestication does occur, we expect behavior to change in both the S and HC lines, but effects should be more pronounced in HC. Comparisons of SH and HC will provide a measure of genetic change caused by the difference in selective intensity between the hatchery-only and supplementation regimens. Use of WC is not possible because of the difference in spawning timing and concerns over the impact of removing additional fish beyond those used for other traits for this purpose from the population. Behavior changes expected under domestication are reduced dominance, greater expression of subdominant color patterns, reduced number of spawnings, suboptimal redd locations and incomplete redds.	
Analytical/Statistical Methods and Issues	
Within years, non-parametric analysis of variance will be used to test differences between groups. Over years trend analysis will be done to evaluate line divergence.	
Power Analysis Completed?	
New Effort Required	
Some additional channel supplies and maintenance will be needed.	

Trait	***Revised***
A13. Adult spawning success	
Justification	
Location(s)	
Cle Elum experimental spawning channel	
Start Date	
Frequency	
Lines Compared:	
SN,SH,HC	
Protocol	
Small numbers of SN,SH, and HC adults will be tagged and DNA sampled, and placed into sections of the channel, and allowed to spawn (same fish as in trait A12). Reproductive success will be measured by pedigree analysis using DNA microsatellites of juveniles exiting the channel (Berejikian et al. 2001; Schroder et al. in preparation). Carcasses will also be checked for gonad depletion and egg retention. WC will not be included because our plan is to collect only partially spawned fish. Because the Naches population spawns earlier than the U. Yakima population, partially spawned fish, if available, would not be at all at the same reproductive starting point as S and HC fish for channel studies.	
Expectations/Hypotheses	
We expect to see a reduction in reproductive success in hatchery origin fish relative to wild origin fish due to hatchery rearing (e.g., Fleming and Gross 1992, 1993; Berejikian et al. 1997, 2001; Webb et al. 1991; Lura et al. 1992; Petersson and Jarvi 1996). The magnitude of this effect will be determined by comparing SH and SN. If domestication does not occur no changes no genetic changes in reproductive success will occur therefore the reproductive success of SH and HC individuals will be comparable over time. If domestication does occur, we expect reproductive success to decline in both the S and HC lines, but effects should be more pronounced in HC. Comparisons of SH and HC will provide a measure of genetic change caused by the difference in selective intensity between the hatchery-only and supplementation regimens. Use of WC is not possible because of the difference in spawning timing and concerns over the impact of removing additional fish beyond those used for other traits for this purpose from the population.	
Analytical/Statistical Methods and Issues	
Males and females will be analyzed separately. Males: Because progeny per male will probably not be normally distributed, in competition situations we will use a nonparametric analysis of variance to examine differences in reproductive success of different lines. Females: Fecundity is normally distributed, so we will use analysis of variance to examine differences between groups in percentage of potential egg deposition (absolute fecundity based on body size) producing fry. We will also evaluate percentage of actual eggs deposited (fecundity – retained eggs) producing fry by analysis of variance (parametric or nonparametric), depending on behavior of variables. In addition, we will use multiple regression analyses to examine the importance of various adult behavioral and phenotypic traits, e.g. percentage of the time dominant, percentage of time in dominant nuptial color morph, number of times observed spawning, relative body size. Over years trend analysis will be done to evaluate line divergence.	
Power Analysis Completed?	
New Effort Required	
Some additional channel supplies and maintenance will be needed.	

Trait	***Revised***	***4/24/03***
J1. Emergence timing		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility incubation room		
Start Date		
Frequency		
Lines Compared:		
WC,SN,SH,HC		
Protocol		
Compare emergence timing of fish from different lines produced by *inter se* matings (same matings in trait A10). Eggs will be housed in 100-egg upwelling incubation chambers that allow fish to volitionally exit. Number of fish exiting will be noted daily. Eggs used will be those from the studies of adult reproductive traits.		
Expectations/Hypotheses		
If domestication does not occur, we would expect no changes in emergence timing or duration of emergence. If domestication does occur, we would expect duration of emergence to be compressed due to the more homogeneous environment presented by the hatchery, however, other investigators have not examined this trait. Thus, duration would be reduced in HC and SH, but more so in HC. If egg size increases as a result of domestication (see trait A8), then time to emergence will increase in SH and HC, with HC showing a greater increase.		
Analytical/Statistical Methods and Issues		
Two within-year analyses will be performed: 1) a nonparametric or parametric analysis of variance will be used to compare duration of emergence. If egg size and duration are correlated, then analysis of covariance will be used to correct for this factor; 2) analysis of covariance will be used to compare median date of emergence among lines. Over years, analysis of covariance will be used to examine differences in trends in these two variables.		
Power Analysis		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
Activities are already budgeted.		

Trait	***Revised***	*4/24/03*
J2. K_D at emergence		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility incubation room		
Start Date		
Frequency		
Lines Compared:		
WC,SN,SH,HC		
Protocol		
Compare developmental condition at emergence (K_D, Bams 1970) of fish from different lines produced by *inter se* matings (same fish as in J1). Eggs will be housed in 100-egg upwelling incubation chambers that allow fish to volitionally exit. K_D will be measured daily on fish as they exit. Eggs used will be those from the studies of adult reproductive traits.		
Expectations/Hypotheses		
If domestication does not occur, we would expect no changes in K_D. If domestication does occur, and egg size increases as a result, we would expect K_D to increase. Thus, K_D would increase in SH and HC, but more so in HC.		
Analytical/Statistical Methods and Issues		
Within years analysis of covariance (with egg size as covariate) will be used to compare slopes and adjusted means among groups. Over years, analysis of covariance will be used to examine differences in trends in these two variables.		
Power Analysis		
Some work done. See Busack and Knudsen (2003a,b).		
New Effort Required		
Could require an extra month of tech time over what has already been budgeted.		

Trait	***Revised***
J3. Egg-fry survival	
Justification	
Location(s)	
Cle Elum Supplementation and Research Facility incubation room	
Start Date	
Frequency	
Lines Compared:	
WC,SN,SH,HC	
Protocol	
Compare egg-to-fry survival of fish from different lines produced by *inter se* matings (same matings in trait A10). Eggs will be housed in 400-egg isolettes (see trait A10). At the eyed-egg stage mortalities in each isolette will be counted. Then 100 live eggs from each female will be placed into the upwelling chambers described in J-1 and 2. The remaining eggs will be returned to their isolettes and mortality will be assessed at yolk absorption. In addition, mortality will be assessed in the upwelling chambers after emergence has been completed.	
Expectations/Hypotheses	
If domestication does not occur, we would expect no changes in egg-to-fry survival. If domestication does occur, we would expect survival of HC fish to increase over time (Reisenbichler and McIntyre 1977). Survival of SH fish should also increase but not as rapidly as HC and SN fish will show an even smaller increase. WC egg-to-fry survival values should not exhibit a temporal trend.	
Analytical/Statistical Methods and Issues	
Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent survival in each isolette. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in survival diverge over time.	
Power Analysis Completed?	
New Effort Required	
Will require one additional week of technician time to inspect upwelling incubation chambers and isolettes for mortalities	

Trait	***Revised***
J4. Occurrence of developmental abnormalities	
Justification	
Location(s)	
Cle Elum Supplementation and Research Facility incubation room	
Start Date	
Frequency	
Lines Compared:	
WC,SN,SH,HC	
Protocol	
Compare the percentage of abnormally appearing alevins originating from each line using the progeny produced from the *inter se* matings (same matings in trait A10). Eggs will be housed in 400-egg isolettes (see trait A10). After yolk absorption abnormal appearing alevins in each isolette will be counted.	
Expectations/Hypotheses	
If domestication does not occur, we would expect no changes in the occurrence of abnormal fry. If domestication does occur, we would expect a higher incidence of abnormalities to be expressed in the HC line. This expectation is based on the premise that genetic diversity in the HC line will decrease over time increasing the likelihood of inbreeding (Kincaid 1976). The proportion of abnormal offspring present in the SH and SN line is also expected to increase but at a lower rate than that expressed by the HC population. No temporal trend in the incidence of abnormal appearing alevins is expected to manifest itself in the WC line.	
Analytical/Statistical Methods and Issues	
Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent abnormalities in each isolette. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in percent abnormalities diverge over time.	
Power Analysis Completed?	
New Effort Required	
No additional effort will be required (work will be covered under J-2 and 3).	

Trait	***Revised***	***4/28/03***
J5. Fry-smolt survival in a hatchery environment		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility		
Start Date		
Frequency		
Lines Compared:		
SH,HC		
Protocol		
Compare the fry-to-smolt survival of fish from the SH and HC lines while being reared in a hatchery environment. HC and SH fish will be reared in separate raceways under comparable conditions (loading densities, feeding rates, water temperatures, flows, etc.). Mortalities will be counted throughout the entire rearing period until volitional release begins. These fish will be cultured in regular production raceways under standard cultural conditions. This comparison will not include WC because there is no intention to raise WC to the smolt stage. Raising WC fish to the smolt stage would require additional hatchery facilities and these fish would have to be sacrificed rather than be released. Also, taking enough eggs to have enough WC fry to fill a raceway to the same density as for the S and HC fish would have an unacceptably high impact on the Naches population.		
Expectations/Hypotheses		
If domestication does not occur, we would expect mortality rates to be comparable in the HC and SH lines. If domestication does occur, we would expect HC fish to have lower mortality rates during the rearing period (Reisenbichler and McIntyre 1977).		
Analytical/Statistical Methods and Issues		
Within years analysis will be conducted by using a one-way ANOVA. The random variable will be percent mortality experienced over the entire rearing period by raceway. The arc-sin transformation will be used to normalize the data. Analysis of covariance will be used to ascertain if trends in mortalities diverge over time. Since at present there are only two HC raceways within-year tests will not be statistically robust. However, over time replicates will take place increasing the power of this evaluation.		
Power Analysis Completed?		
New Effort Required		
Routine hatchery operations will monitor mortalities		

Trait	***Revised***	***4/28/03***
J6. Juvenile morphology at release		
Justification		
Location(s)		
HC Acclimation site		
Start Date		
Frequency		
Lines Compared:		
HC, SH		
Protocol		
Collect digitized measurement data from lateral image landmarks on photos of juveniles photographed just prior to release from acclimation site. Develop orthogonal variables with which to compare HC and SH fish. Each raceway will have 50 fish photographed for a total of 100 HC and 200 SH fish. WC fish will not be included for reasons outlined under J5.		
Expectations/Hypotheses		
If domestication does not occur no changes in morphology will occur. If domestication does occur, SH and HC morphology will diverge. We do not have an expected direction of divergence in form.		
Analytical/Statistical Methods and Issues		
Multivariate analysis of variance of digitized orthogonal shape variables generated by Procrustean distance methods, and other methods described by Hard et al. (2000). Methods will be applied within years and across years (to measure trends). Hard has agreed to collaborate in this effort.		
Power Analysis Completed?		
New Effort Required		
Photos have been taken on SH releases in 1999. One week/year of technician time to sample and photograph juveniles and organize digital files. About two weeks/year technician help will be needed to digitize photos.		

Trait	***Revised***	***4/28/03***
J8. Smolt-to-smolt survival a) from acclimation sites and upper basin to Chandler b) from Chandler to McNary and John Day dams		
Justification		
Location(s)		
From Acclimation sites and Upper basin to Chandler, McNary and John Day dams		
Start Date		
Frequency		
Lines Compared:		
a)SN,SH,HC b)SN,SH, HC, WC		
Protocol		
a) A sub-sample of SN, SH, and HC fish will receive PIT tags at Roza. Survival rate comparisons of SN, SH, and HC fish will only occur among individuals that passed through the Roza juvenile trap during the same time period. HC and SH survival comparisons will include all PIT tagged fish. WC will not be included here because they do not occur in the monitoring area. b) Additional fish will be tagged at Chandler, including Naches and American fish (identified by DNA microsatellites) Comparisons of survival rates among these fish will be based on PIT tag recoveries at monitoring sites located at McNary, John Day, and any other suitably equipped downstream sites.		
Expectations/Hypotheses		
If domestication does not occur, we would expect smolt-to-smolt survivals between the HC, SH lines to be comparable. SN fish are expected to survive at higher rates. This phenomenon has been observed in many other salmonid populations. If domestication does occur, we would expect SH smolts to survive at higher rates than HC individuals but not as well as SN fish. The comparisons involving SN need to be interpreted carefully, because they include only SN fish that are spring smolts. Winter migrants, another major life history, will not be included.		
Analytical/Statistical Methods and Issues		
Within-year analyses will be performed by using logistic regression analysis. Analysis of covariance will be used to ascertain if trends in survival diverge over time.		
Power Analysis Completed?		
New Effort Required		
An increase in PIT tagging effort may be required.		

Trait	***Revised***	***11/08/03***
J9. Natural Smolt Production		
Justification		
Location(s)		
Chandler Smolt Facility.		
Start Date		
Frequency		
Lines Compared:		
WC, SN, SH, HC		
Protocol		
Outmigrating smolts made up of a mixture of WC, SN, SH and HC origin fish are sub-sampled as they pass downstream through the Chandler facility. DNA methods will be applied to all unmarked smolts and used to estimate the proportion of each naturally reproducing population: American River, Naches system (WC) or upper Yakima (SN). Marked fish will be assigned to HC and SH lines based on their respective marks. Three temporal samples will be collected at Chandler representing approximately the early third, middle third, and latter third of the total spring chinook outmigration. Total smolt passage numbers are also estimated during these temporal periods and allocated to each population based on the results of the DNA analyses and mark recoveries. These estimates are summed across periods to get indices of total smolt production for WC, SN, SH and HC populations.		
Expectations/Hypotheses		
If domestication does not occur, we would expect SN, HC and SH fish to have equivalent rates of productivity. If domestication does occur, we would expect SN-origin to have the highest productivity and SH-origin fish to have higher productivity than HC individuals. The WC smolt productivity is unknown relative to the other lines. Its primary use is as a wild control benchmark against which the trends observed over time in the upper Yakima lines will compared.		
Analytical/Statistical Methods and Issues		
Within year analysis will consist of the total number of smolts produced each year by population with confidence intervals.		
Power Analysis Completed?		
New Effort Required		
No new effort required		

Trait	***Revised***	***4/28/03***
J10. Smolt-to-adult survival		
Justification		
Location(s)		
From one acclimation site to RAMF		
Start Date		
Frequency		
Lines Compared:		
SH,HC		
Protocol		
Prior to release, every SH and HC fish will be tagged so that its origin can be identified. An estimate of the number of smolts leaving each raceway will be made via continuous PIT tag monitoring. The numbers of adult fish produced from each raceway returning to Roza will be recorded by inspecting fish for tags and marks. Scale samples will be taken to assign an age to each returning adult. The survival of fish by age class will be calculated for each raceway by broodyear. This will be done by dividing the number of 3, 4, or 5 year-olds originating from a raceway/broodyear combination by the total number of fish released from that raceway. WC fish will not be included for reasons outlined under J5.		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC and SH fish to have equivalent survival rates. If domestication does occur, we would expect SH-origin fish to have higher survivals than HC individuals.		
Analytical/Statistical Methods and Issues		
Within brood year analysis a two-way ANOVA estimating origin, age and interaction effects will be performed. Analysis of covariance will be used to ascertain if trends in survival by age in HC and SH fish diverge over time.		
Power Analysis Completed?		
New Effort Required		
No new effort required		

Trait	***Revised***	***4/28/03***
J11. Smolt out-migration timing		
Justification		
Location(s)		
From one acclimation site to downstream monitoring sites		
Start Date		
Frequency		
Lines Compared:		
SN,SH,HC		
Protocol		
Two comparisons of migration timing will be made. In the first a sub-sample of SN, SH, and HC fish will receive PIT tags as they are collected at the Roza juvenile trap and migration rate comparisons will then be made between SN, SH, and HC fish. In this case, only individuals that passed through the Roza juvenile trap during the same time period are compared. In the second comparison HC and SH migration comparisons will be made that include all PIT tagged fish released from the acclimation site. Comparisons of migration timing among these fish will be based on PIT tag recoveries at monitoring sites located throughout the Columbia Basin. WC fish will not be included for reasons outlined under J5.		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC and SH fish to have similar migration timing. In the first comparison SN individuals are expected to have equivalent migration rates to HC and SH fish because all of these fish are actively migrating smolts. If domestication does occur, we are uncertain what effect if any it will have on migration timing. The reason we are investigating this trait is that it has profound effects on smolt-to-adult survival.		
Analytical/Statistical Methods and Issues		
Within year analysis will use Kolmogrov-Smirnov tests. Analysis of covariance will be used to ascertain if genetically based trends in median out-migration timing occur in HC and SH fish. SN data will not be included in this analysis.		
Power Analysis Completed?		
New Effort Required		
No new effort required		

Trait	***Revised***	***4/28/03***
J12. Food conversion efficiency		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility and smolt acclimation sites		
Start Date		
Frequency		
Lines Compared:		
SH,HC		
Protocol		
This trait is a surrogate for growth rate. HC and SH fish will experience normal hatchery rearing procedures, which includes being fed at a rate based on size. The quantity of food supplied to each raceway from ponding to release will be recorded. Two random samples of fish will be removed from each raceway, one at the time of tagging (after 8 months of rearing) and another just prior to release (approximately 12 months of rearing). Individual weights will be taken on 200 fish from each raceway. The weight data will be used to estimate the biomass of fish in each raceway at the time of sampling. Food conversion efficiencies will be determined by dividing total biomass of fish by total weight of food delivered to a raceway. WC fish will not be included for reasons outlined under J5.		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC and SH fish to have equivalent food conversion rates at tagging and again just prior to release. If domestication does occur, we would expect HC fish to have greater food conversion efficiencies than SH fish (Reisenbichler, pers. comm.).		
Analytical/Statistical Methods and Issues		
Within year analyses will use one-way ANOVAs (per sample period) to examine food conversion rates in HC and SH raceways. A single within year analysis will have low power because there are only two HC raceways. However, by analyzing multiple years with two-way ANOVAs power will be increased, allowing us to examine year and treatment effects. Within-year analyses of conversion rate will be done by two-way fixed treatment ANOVAs estimating origin, raceway, and interaction effects. In addition, analysis of covariance will be used to ascertain if trends in food conversion in these two groups diverge over time		
Power Analysis Completed?		
New Effort Required		
Some additional labor for weighing fish may be needed.		

Trait	***Revised***	***4/28/03***
J13. Juvenile Length-Weight Relationships		
Justification		
Location(s)		
CESRF and smolt acclimation sites		
Start Date		
Frequency		
Lines Compared:		
SH,HC		
Protocol		
HC and SH fish will experience normal hatchery rearing procedures. Two random samples of fish will be removed from each raceway, one at the time of tagging (after 8 months of rearing) and another just prior to release (approximately 12 months of rearing). Individual lengths and weights will be taken on 200 fish from each raceway. WC fish will not be included for reasons outlined under J5.		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC and SH fish to have equivalent length/weight relationships at tagging and again just prior to release. If domestication does occur, we would expect HC fish to have steeper slopes (greater biomass increase per unit length) than SH fish.		
Analytical/Statistical Methods and Issues		
Within year analyses will compare (log length/log weight) relationships using ANCOVA. In addition, analysis of covariance will be used to ascertain if trends in mean length and weight in these two groups diverge over time		
Power Analysis Completed?		
New Effort Required		
Four days of sampling time are needed at the time of release to collect weight and length data.		

Trait	***Revised***	***4/28/03***
J14. Agonistic-competitive behavior		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility		
Start Date		
Frequency		
Lines Compared:		
WC,SN,SH,HC		
Protocol		
Juvenile fish produced from the crosses used in J3 will be test subjects. In this behavioral assay, three population comparisons will be made: HC vs. SN, HC vs. SH, HC vs. WC, and SN vs. WC (time permitting). Size-matched pairs of fish (each fish represents a different population) will be simultaneously introduced into tanks possessing one piece of cover. A single tube used to introduce food into each tank will be located adjacent to the cover. Dominance will be determined by quantifying which fish obtains the most food, dominates the majority of the social interactions occurring between the two fish, and spends the majority of the time adjacent to the food tube and cover. Fish will remain in a tank until a clear dominance relationship between them has been established. Trials will conducted for 7 days. If after 7 days this relationship is not clear, the fish will be removed and replaced by another size-matched pair. Number of trials will be determined by power analysis (see below)		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC, SH, and SN fish to have equivalent levels of aggression and dominance. How aggressive WC fish may be is unknown, but their behavior is not expected to change over time and therefore they will act as a valuable reference. If domestication does occur, we would expect HC-origin fish to be the most aggressive, SH-origin fish would follow and the least aggressive of the Upper Yakima groups would be the SN population.		
Analytical/Statistical Methods and Issues		
Within a year three to four separate Chi-square analyses will be performed, comparing HC fish with individuals from each of the other three populations, and comparing SN to WC. Analysis of covariance will be used to ascertain if trends in dominance among the three to four pairwise comparisons diverge over time.		
Power Analysis Completed?		
Some Analysis Completed? (see next page)		
New Effort Required		
Tanks to conduct this assay are currently being fabricated and installed; staff time to conduct these assays will be required.		

Trait J4 (continued)

Preliminary Power Analysis for Trait J14

Power to detect to reject a 50:50 null hypothesis with various true proportions			
	True Proportions		
Number of trials	60:40	70:30	80:20
25	.16	.50	.89
50	.33	.86	1.00
100	.54	.99	1.00
150	.72	1.00	1.00
200	.83	1.00	1.00
250	.89	1.00	1.00

Trait	***Revised***	***4/28/03***
J15. Predator avoidance		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility		
Start Date		
Frequency		
Lines Compared:		
WC,SN,SH,HC		
Protocol		
To avoid pseudo-replication, multiple arenas possessing different individual fish predators will be established. Two predation bioassay approaches will be tested to determine which approach is the most powerful. In the first one, 50 size-matched fish from each line will be simultaneously liberated into an arena containing 3 rainbow trout and 3 torrent sculpin predators. Prior to introduction, fish from each line will be differentially marked or tagged. After a proscribed period of time has elapsed (e.g., 4 days) or approximately 50% of the introduced fish have been eaten, survivors will be removed from each arena and enumerated. In the second assay, only individuals from the same line will be released into the same predator-filled arenas, but the number of fish will be the same (200). The number of fish remaining after a fixed amount of time (e.g., 4 days) will be recorded. We will determine which assay is the most powerful and then continue with the best approach. This assay is being performed to determine if innate anti-predator behaviors differ among the lines.		
Expectations/Hypotheses		
If domestication does not occur, we would expect fish from all lines to survive at equal rates. In addition, the expression and use of innate anti-predator behaviors should remain constant within a line over time. If domestication does occur, we would expect WC fish to have the highest survival rates followed by SN, SH, and HC individuals in that order.		
Analytical/Statistical Methods and Issues		
Within year analysis for bioassay one will use two-way ANOVAs. These tests will tell us whether survival has been affected by line origin, arena, and if interactions exist between arenas and fish origin. Within year analysis for bioassay two will use non-parametric analysis of variance where the random variable will be the survival rate Analysis of covariance will be used to determine if trends in survival are manifested over time in both assays.		
Power Analysis Completed?		
New Effort Required		
Establishment and stocking of the arenas plus labor to conduct predation trials.		

Trait	***Revised***	***4/28/03***
J16. Incidence of precocialism in production raceways		
Justification		
Location(s)		
One smolt acclimation site		
Start Date		
Frequency		
Lines Compared:		
SH,HC		
Protocol		
Just prior to release, two hundred fish from the six raceways located at an acclimation site will be examined to determine the percentage of the males that are precocial. One acclimation site is being used because there are only two raceways of HC fish. Additionally, by using one acclimation site the environmental conditions the fish experience will be standardized. WC fish will not be included as none will be reared in raceways, for reasons mentioned earlier.		
Expectations/Hypotheses		
If domestication does not occur, we would expect HC and SH fish to have equivalent rates of precocial development. If domestication does occur, we would expect HC-origin fish to have a lower incidence of precocialism.		
Analytical/Statistical Methods and Issues		
Within year analysis will use one-way ANOVAs. Analysis of covariance will be used to ascertain if trends in the production of precocial males in these two lines diverge over time		
Power Analysis Completed?		
New Effort Required		
Labor will be needed to collect and sex each sampled fish, a task that will take approximately two days to complete.		

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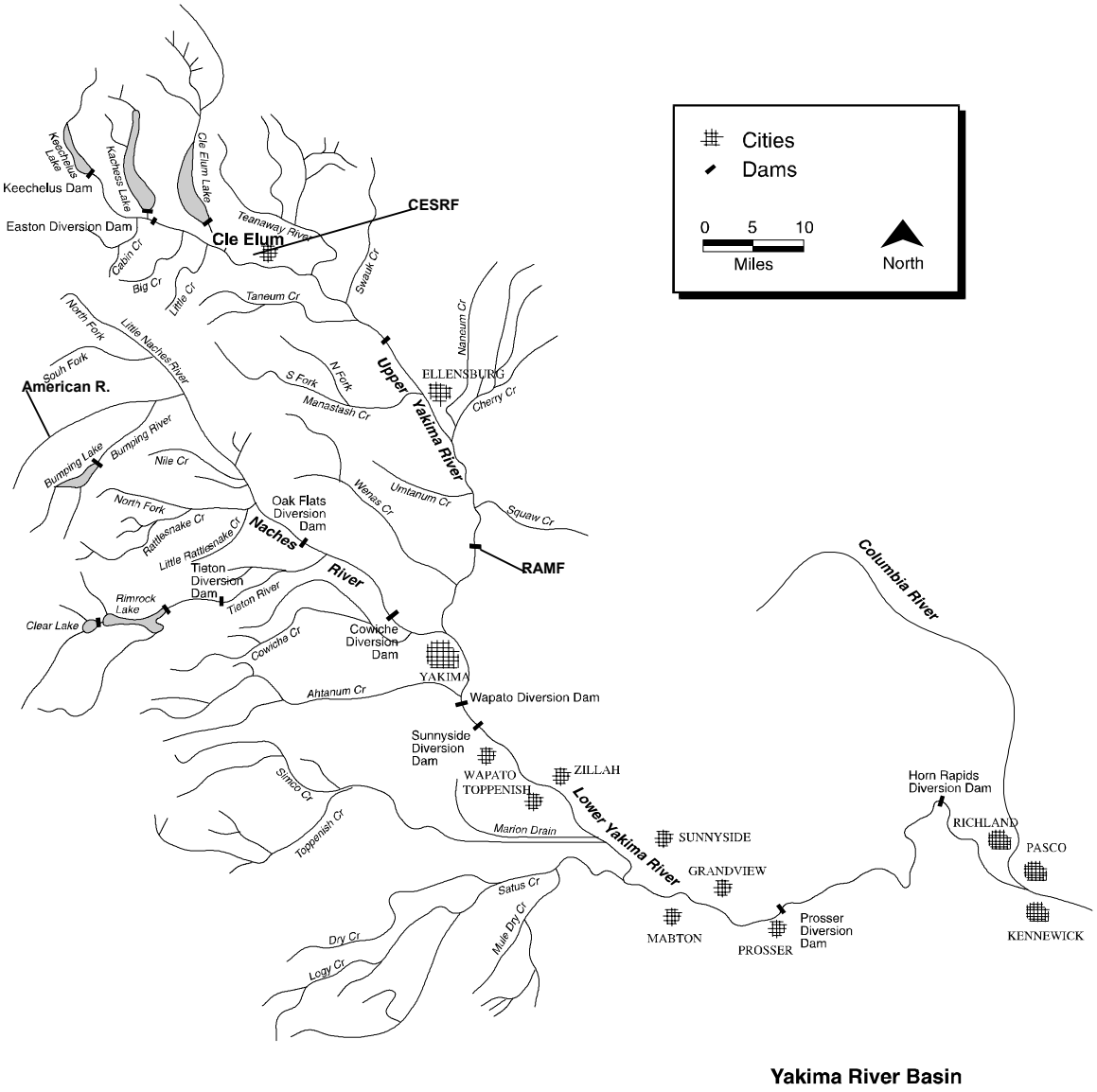


Yakima River Basin

Figure 1. Map of Yakima basin.

Chapter 5

Power Analysis of the YKFP Spring Chinook Domestication Research/Monitoring Design. A. Traits Involving Comparisons of Individual Adults from the WC, S, and HC Lines

Craig Busack, WDFW
Curt Knudsen, Oncorh Consulting

Introduction

A statistical test always involves testing a null hypothesis (H_o) no effect against an alternate hypothesis (H_A) of an effect occurring. The true situation is that the effect either did occur or did not occur, and the test is employed to learn which is the true situation. There are two types of errors that can occur in the course of applying a test, as diagrammed in the table below:

Table 1. Possible Decisions Resulting from a Statistical Test

	True Situation	
Test Result	H_O True	H_O False
Accept H_O	*Correct Decision*	Type 2 Error
Reject H_O	Type 1 Error	*Correct Decision*

The probability of a Type 1 error, rejecting the null hypothesis when it is true, is usually denoted as ". This topic is well covered in most basic statistics courses, and as a result, the error is well managed by specifying the acceptable level of " in the test. Typically, " is set at 0.05 or less.

The probability of a Type 2 error, accepting the null hypothesis when it is untrue, is usually denoted as $. The probability of rejecting the null hypothesis when it is untrue is therefore 1- $. This quantity, called power, is the probability of detecting an effect. Calculating power is less straightforward than dealing with type 1 error, and thus is not covered well in basic statistics courses. As a result, it has been greatly underemphasized in research. This is extremely unfortunate because power is very important. Findings of no effect have little meaning if studies have no reasonable chance to find an effect. Countless numbers of papers have been published reporting no effect without reporting how likely it is that the study could have detected an effect. Not only can underpowered studies be misleading, leading to incorrect decisions, but they can waster staggering amounts of money and can have unwarranted impacts in study areas. The historical lack of attention to statistical power in fisheries research was pointed out by Peterman (1989). The situation has improved considerably since then, and numerous power analysis

software packages are now available. In the YKFP we have been very conscious of experimental power from the very beginning. For example, the final spring chinook supplementation design was based on a power analysis effort by Hoffmann et al. (1994).

The specific issue dealt with in this report is power of the planned spring chinook domestication study, which involves a variety of comparisons of three lines of fish: wild control (WC [Naches stock]), supplemented (S [Upper Yakima stock]), and hatchery control (HC [Upper Yakima stock subjected to continuous hatchery culture). There are two major aspects to be considered in this design: hatchery oriented and non-hatchery oriented. In each case we have designed monitoring measures that deal with adult and with juvenile fish. A single power analysis cannot cover all the traits. In this report we deal only with adult traits measured in the hatchery environment. Further work will deal with the other categories of traits.

Specifics of this Analysis

This report presents power analyses for normally or log-normally distributed traits that will be measured on individual adults at CESRF. In each case we assume that two comparisons are of interest: S vs HC and S vs WC. A summary of traits and comparisons is presented in the table below:

Table 2. Traits amenable to this power analysis design. Note that comparisons using WC will not be done from traits A7 and A9. This is because of the decision to capture partially spawned Naches fish rather than trap green fish. Fecundity and reproductive effort can be measured only on fish that have not spawned previous to capture. Asterisks denote traits in which sample sizes can be augmented by data from the spawning grounds. CV's are 2001 values. Ranges in CV are a reflection of sex and age class differences.

#	Trait Name	Comparisons Possible		CV(%)
		S vs HC	S vs WC	
A3	Size at age by sex*	X	X	5.0-7.9
A7	Fecundity	X		13.5-21.0
A8	Egg Size	X	X	12.9-19.3
A9	Reproductive effort	X		9.6-15.5
A10	Male and female fertility	X	X	
A11	Adult morphology at spawning*	X	X	assumed same as size
J1	Emergence timing	X	X	
J2	K_D at emergence	X	X	3.1 (fry length), 12.1 (fry weight)
J3	Egg-fry survival	X	X	12.3
J4	Occurrence of developmental abnormalities	X	X	

The current design that uses small numbers of Naches fish (about 10 pairs/year) and a small HC line (30-50 pairs producing a release of 100,000 fish) for these traits. Questions have arisen about the sizing of both components. In the case of the Naches

stock, we have decided on 10 pairs/yr (possibly higher in large return years) to minimize impacts to that stock, but the ISRP has raised concerns about 10 pairs being enough for a meaningful analysis. The situation is similar with the HC line. Because the release is limited to two raceways of smolts (about 100,000 fish) we really need to spawn about 30 pairs. The ISRP would like us to spawn many more (100 pairs if we had them) for the sake of increasing effective population size, but this will result in the production of many thousands of surplus eggs. We have therefore argued for an effective size (about 100/generation) smaller than the ISRP wants, but at a size where we feel that genetic drift will not be a significant source of bias for several generations.

Materials and Methods

The approach we have taken here is to assume that in any trait for either of the two line comparisons, if domestication takes place, the two lines will diverge over the course of generations. We modeled this as one line changing relative to the other by a set generational effect due to domestication (g), specified as a proportion of current performance. For example, we might model that one line changes at a rate of 5% relative to the other. So while the performance of the one line stays constant (except for error), the other increases or decreases (doesn't matter which, but it always has to increase or always has to decrease) by 5% every generation. This situation is represented by the linear model

$Y_{ijk} = \mu(1+g_i)^j + e_{ijk}$, where g_I is the genetic effect for the ith line (0 for control and 0.05, etc. for "treatment" line), : is the base mean for the trait, j is the generation, and e is a normally distributed error term. Note that this model is not exactly linear, but close to it for small values of g. Note also that this model considers not separate years of sampling, but only generational sampling. This is because upon reflection we realized that the sample size per generation is the key thing; number per year is not important. Thus, 100 sampled in one year is equivalent to 25 each year for 4 years.

The divergence of the two lines should be detectable by doing a regression against time in each line, and then comparing the slopes of the two regressions. A significant test for differing slopes can be taken as significant divergence of the two lines

We simulated data collection over 6 generations, two different genetic effect levels (0.02 and 0.05), and three levels of coefficient of variation (0.1,0.2, and 0.5). We did this for four sampling scenarios: 40, 80, 120, and 200 control fish vs 600 S fish per generation. year). The sample size for S, 600, is quite conservative. The scenarios were intended to approximate the basic sampling design for the WC and HC lines (40 and 120 pairs/generation, respectively), and offer insights into how much greater power would be with expansions of these basic designs (80 and 200).

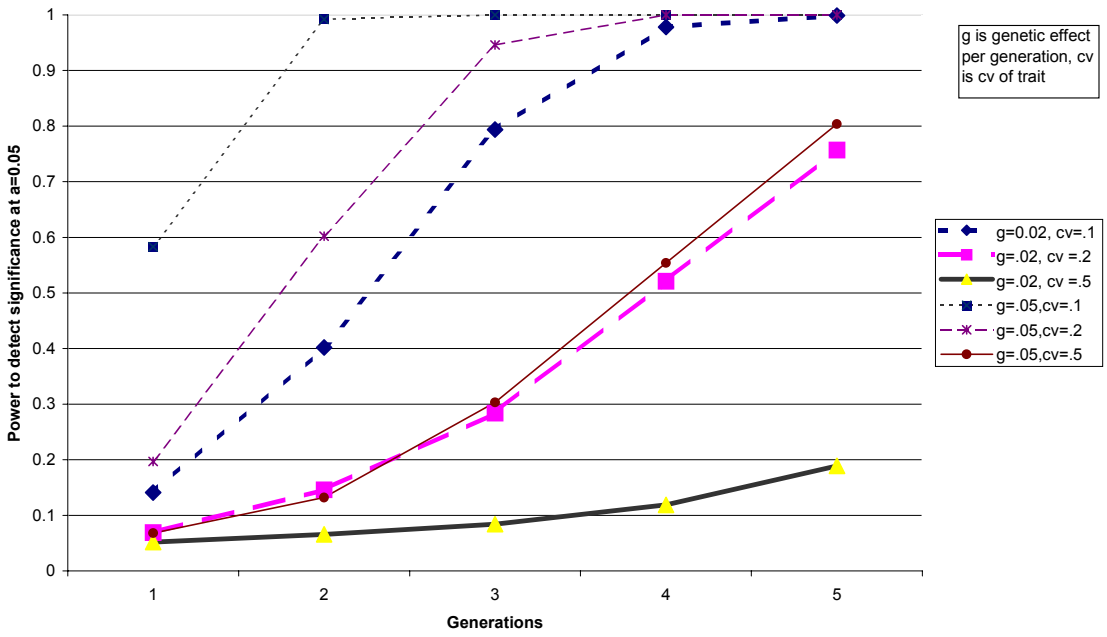
Each scenario was simulated 5000 times, in each generation from generation onwards, a comparison of slopes was done and checked for significance at "= 0.05. Power was calculated as the proportion of replicates that were significant.

Simulations were done using a FORTRAN 95 program written specifically for this purpose and compiled in Lahey-Fujitsu F95 5.5. Data were simulated using the RAN2 random number function and GASDEV normal deviate function of Press et al. (1986). Regression calculation and slope comparison logic was from Neter and Wasserman (1974). T-tests for significance of slope comparisons was done using functions BETAI, BETACF, and GAMMLN from Press et al. (1986). The source code, except for the Press et al. functions, is attached as an appendix.

Results and Discussion

Figure 1 shows results for the situation of 40 C vs 600S.

Fig. 1. Power to detect significant differences between a control and S lines, assuming 40 control and 600 S fish sampled per generation



High power (80% or more) is achieved with a 5% genetic effect in three generations with cv's of 20% or less. Because the maximum cv listed in Table 2 is 23%, this means for virtually all those traits. The same is true for genetic effects of 2% in low variability (CV=0.1) traits. For more variable traits with genetic effects of 2% it will take 5 generations to approach 80% power.

Fig. 2. Power to detect significant differences between a control and S lines, assuming 80 control and 600 S fish sampled per generation

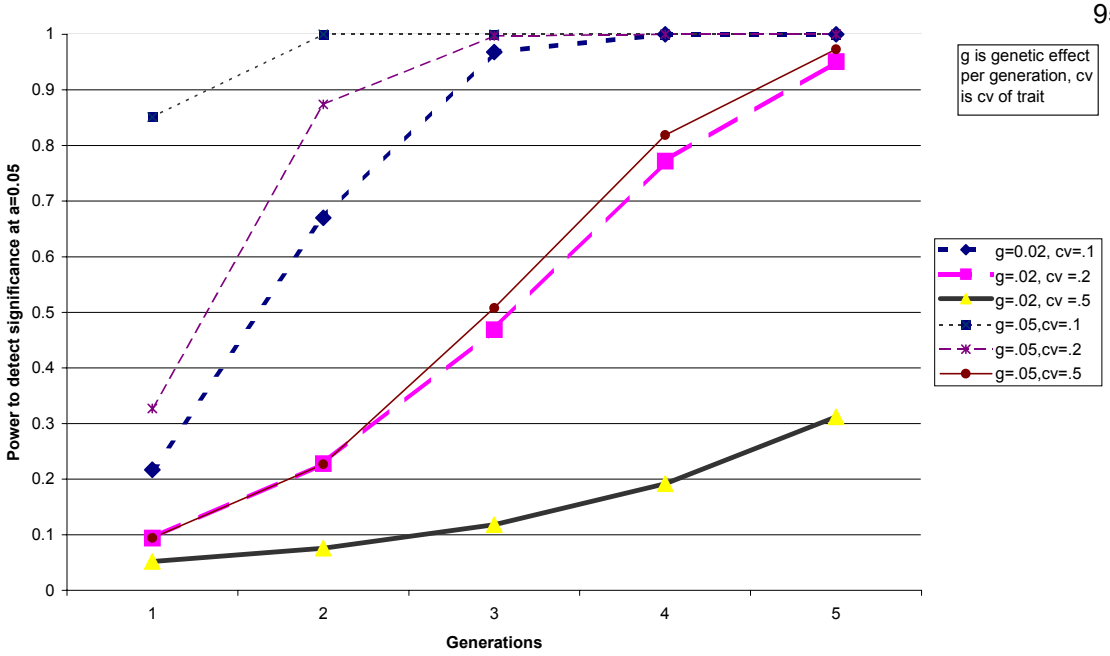


Fig.2 is a similar graph for 80 C vs 600 S comparisons. Here we assume that 600 S fish and 80 C fish will be available per generation (20 per year). This would be the situation if we doubled the sampling effort on WC fish. As you would expect from the larger sample sizes, power is somewhat better in this case than in the 40 vs 600 comparisons. 80% power is achievable in two generations for a genetic effect of 5% and traits with cv's of 20% or less, and by 4 generations for a genetic effect of 2% and traits with cv's of 20% or less.

Fig. 3. Power to detect significant differences between a control and S lines, assuming 120 control and 600 S fish sampled per generation

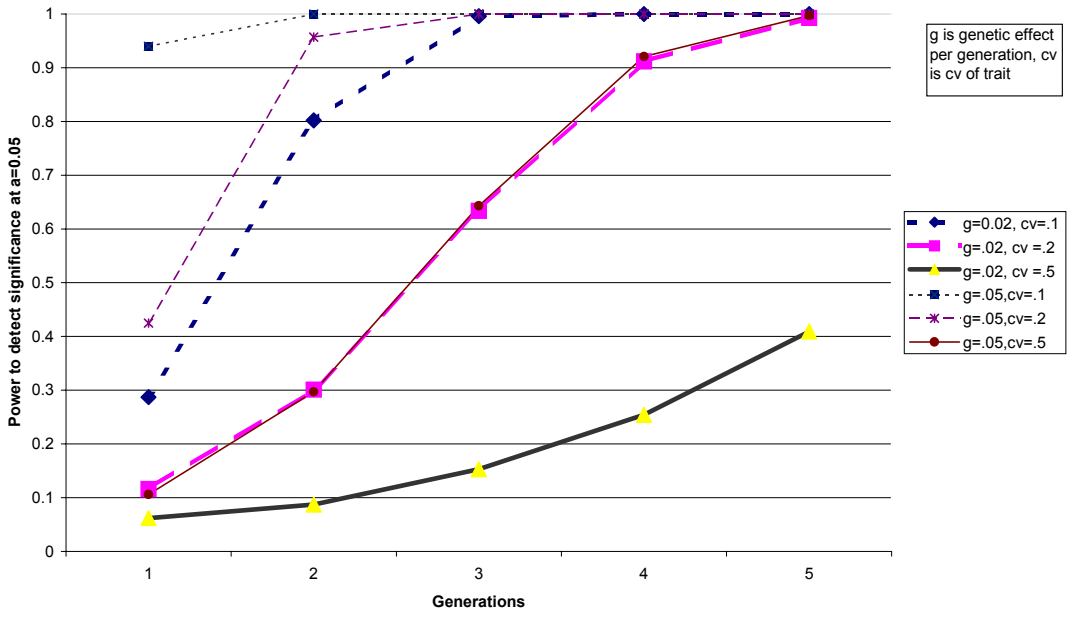
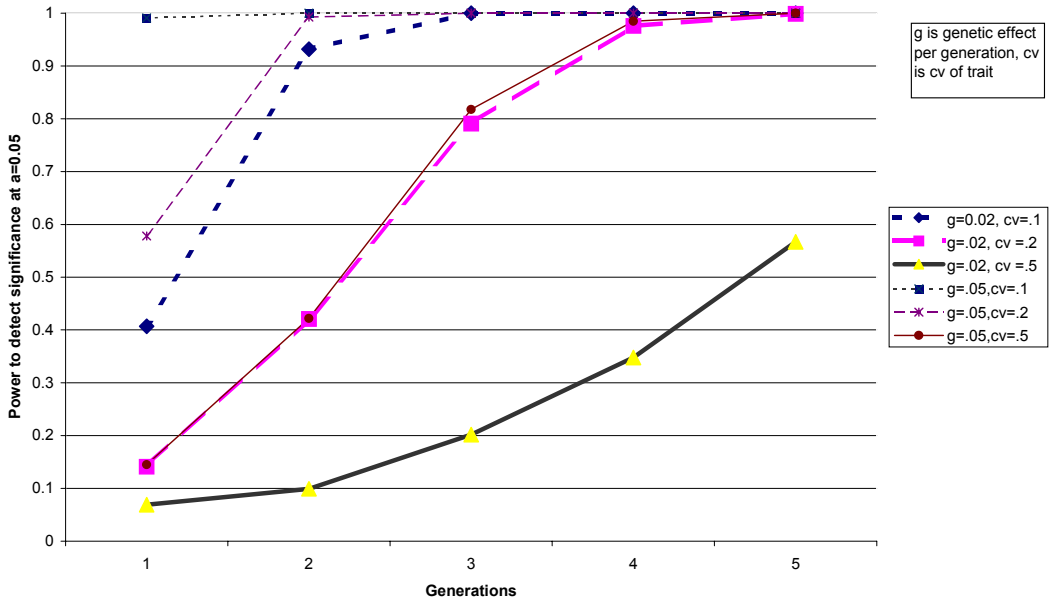


Fig.3 is a similar graph for 120 C vs 600 S comparisons, approximately the situation with the current plans for the HC line. As you would expect from the larger sample sizes, power is somewhat better in this case than in the 80 vs 600 comparisons. 80% power is

achievable in two generations for a genetic effect of 2% and traits with cv's of 20% or less.

Fig. 4. Power to detect significant differences between a control and S lines, assuming 200 control and 600 S fish sampled per generation



Finally, Fig. 4 shows the power for comparisons of 200 vs 600 fish. The most striking change from Fig. 3 is that the power for a genetic effect of 5% and a cv of 0.5 and for a an effect of 2% and a cv of 0.1 has now reached 80%.

The results are summarized below in Table 3 in terms of the number of generations it takes to reach high (80%) power. The shaded blocks represent the results for the

Table 3. Number of generations required to reach 80% power for traits of various coefficients of variation and control lines of various sizes in comparison with a supplemented line sample of 600 fish/generation.

Trait CV	Sample size for control line			
	40	80	120	200
Per-generation genetic effect of 2%				
0.1	3	3	2	2
0.2	5+	5	4	3
0.5	5+	5+	5+	5+
Per-generation genetic effect of 5%				
0.1	2	1	1	1
0.2	3	2	2	2
0.5	5	4	4	3

current plans for the WC and HC lines. If genetic effects are on the order of 2% per generation, power is not very high. Under the lowest cv modeled, 80% power will not be achieved until 3 generations for the WC vs S comparison and 2 generations for the HC vs

S comparison. Interestingly, making an attempt to increase sample size does not seem to help the power situation much. If the genetic is on the order of 5%, however, the situation changes considerably. Doubling the number of fish sampled in the WC line would result in achieving 80% power for traits of all cv's. Increasing the size of the HC line sampling to 200/generation would have this benefit only for traits with a cv of 0.5. This makes sense, because the sample size is already fairly large at 120.

The message from the simulations is clear. With the level of variation we have observed for these traits, we should be able to detect differences relatively quickly, three generations for WC vs S and two generations for HC vs S, so long as the genetic effect is 5% per generation. What size genetic effect to expect is unclear from the literature. Lynch and O'Hely(2001) suggest that fitness changes from domestication on the order of 2-5% per generation should be expected, but leave open the question of what to expect of component traits. Most traits we are investigating have not been the subject of rigorous per-trait study. [note: we need to beef this up with as many effect-size measurements as we can]. However, Reisenbichler and McIntyre(1977) found differences in juvenile survival after two generations of 20%, suggesting quite large effects. Similarly, Berejikian (1995) found that hatchery steelhead fry were 40% more susceptible to predation than wild fish after 4-5 generations, again suggesting a large effect.

Two final cautions are in order. First, this is not the only possible approach to power. Here power is based solely on being able to detect a difference in slope of performance trends over time. Another possible approach that might reveal even higher power for the HC vs S comparisons is a simple of analysis of variance or t-test, where once per generation the means are compared for significance. This works for the HC vs S comparison because at generation 0 the lines have the same mean, but is more complex for the WC vs S comparisons, where the lines initially will not have the same mean, and what is being looked for is a change in that difference. In this case the test would be evaluating whether the difference is significantly larger than what it was at the beginning of the study.

The second caution is that power does not tell the entire story. Power just tells you the probability that given there is a difference, that you will be able to conclude statistically that there is a difference. The magnitude of the difference and the amount of precision with which we can specify that difference is also important.

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Appendix: Program Source Code

```
!     Last change:  CAB  25 Nov 2002    1:43 pm
program multidompower
! dompower adapted for batch operation, varying numbers, cv's, and
genetic effects
TYPE rdata
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata),allocatable            :: ctrlpts(:)
TYPE (rdata),allocatable            :: dompts(:)
INTEGER                             :: n_iter, n_gens, n_control,
n_domest, iprint
REAL                                :: mean, cv(10), gen_effect(10),
dom_mean
INTEGER, ALLOCATABLE                :: n_counts(:,:,:)
INTEGER                             :: ig,ng,icv,ncv
open (4,FILE='mdp.in')
open (8,FILE='mdp.out')
READ(4,*)n_iter, n_gens, n_control, n_domest
READ(4,*)mean
READ(4,*)ng,(gen_effect(i),i=1,ng)
READ(4,*)ncv,(cv(i),i=1,ncv)

READ(4,*)iprint

ALLOCATE(ctrlpts(n_gens*n_control), dompts(n_gens*n_domest),
n_counts(ng,ncv,n_gens-1))
n_counts = 0
! Initialize Random Numbers
call random_seed
call random_number(xrand)
idum=-1*nint(xrand*10000.)
!idum = -741537
parameter_loop1: do ig = 1, ng
parameter_loop2: do icv=1, ncv
sd = cv(icv) * mean
iteration_loop: do iter = 1, n_iter
if(mod(iter,iprint)==0)print *,' Combination ', ig,' ',icv, ' of
',ng*ncv,': Iteration ', iter
do i_gen = 0, n_gens-1
    ! make controls
       do i = i_gen * n_control + 1, (i_gen + 1) * n_control
            ctrlpts(i)%x = REAL(i_gen)
            ctrlpts(i)%y = mean + gasdev(idum) * sd
       end do
   ! make doms
       IF(i_gen > 0) then
            dom_mean = dom_mean + gen_effect(ig) * dom_mean
       else
            dom_mean = mean
       endif
       do i = i_gen * n_domest + 1, (i_gen + 1) * n_domest
            dompts(i)%x = REAL(i_gen)
            dompts(i)%y = dom_mean + gasdev(idum) * sd
```

```
       end do
       IF(i_gen > 0) then
           call compare_slopes((i_gen+1)*n_control,
ctrlpts,(i_gen+1)*n_domest,dompts,prob)
           if (prob < 0.05) n_counts(ig,icv,i_gen) =
n_counts(ig,icv,i_gen) + 1
       endif
end do
ENDDO iteration_loop
ENDDO parameter_loop2
ENDDO parameter_loop1
WRITE(8,800)n_iter, n_control, n_domest
800 FORMAT(' Iterations:  ',i8,'   Control Fish:   ',i8, '   Treatment
Fish:   ', i8,//,&
           'Gen E',t10,'cv')
do i = 1, ng
   do j = 1, ncv
     PRINT*, gen_effect(i), cv(j),
(REAL(n_counts(i,j,k))/REAL(n_iter),k=1, n_gens-1)
    WRITE(8,801) gen_effect(i), cv(j),
(REAL(n_counts(i,j,k))/REAL(n_iter),k=1, n_gens-1)
    801 FORMAT(f5.3,3x, f5.3, 5x, 10f7.3)

   end do
end do
end program
!----------------------------------------------------------------------
subroutine compare_slopes(n1,datapts1,n2,datapts2,prob)
TYPE rdata
   sequence
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata)             :: datapts1(n1)
TYPE (rdata)             :: datapts2(n2)
REAL                     :: slope1, slope2, sse1,sse2,ssx1,ssx2,msef,
t, prob,df
call regress(n1,datapts1, slope1,sse1,ssx1)
call regress(n2,datapts2, slope2,sse2,ssx2)
df = REAL(n1+n2-4)
msef = (sse1 + sse2)/df
msef = msef * ((1/ssx1)+(1/ssx2))
sb  = SQRT(msef)

t = (slope1 - slope2)/sb
prob = betai(0.5*df, 0.5,df/(df+t**2))

end subroutine
!----------------------------------------------------------------------
subroutine regress(n,datapts,slope,sse, ssx)
TYPE rdata
   sequence
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata)             :: datapts(n)
INTEGER                  :: n
```

```
REAL           :: sumx,ssqx,sumy,ssqy,sumxy,ssx,ssy,ssxy,slope,ssr,sse
INTENT (IN) n, datapts
INTENT (OUT) slope, sse

sumx  = 0
ssqx  = 0
sumy  = 0
ssqy  = 0
sumxy  = 0
do i = 1, n
   sumx  = sumx  + datapts(i)%x
   ssqx  = ssqx  + datapts(i)%x * datapts(i)%x
   sumy  = sumy  + datapts(i)%y
   ssqy  = ssqy  + datapts(i)%y * datapts(i)%y
   sumxy = sumxy + datapts(i)%x * datapts(i)%y
end do
ssx = ssqx - (sumx**2)/REAL(n)
ssy = ssqy - (sumy**2)/REAL(n)
ssxy = sumxy - (sumx*sumy)/REAL(n)
slope = ssxy/ssx
ssr = slope*ssxy
sse = ssy - ssr
return
end subroutine
```

Chapter 6

Power Analysis of the YKFP Spring Chinook Domestication Research/Monitoring Design. B. Effect of Bias from Naturally Spawning Precocious Males from the HC Line on Comparisons of Individual Adults from the WC, S, and HC Lines

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Introduction

A statistical test always involves testing a null hypothesis (H_o) no effect against an alternate hypothesis (H_A) of an effect occurring. The true situation is that the effect either did occur or did not occur, and the test is employed to learn which is the true situation. There are two types of errors that can occur in the course of applying a test, as diagrammed in the table below:

Table 1. Possible Decisions Resulting from a Statistical Test

	True Situation	
Test Result	H_O True	H_O False
Accept H_O	*Correct Decision*	Type 2 Error
Reject H_O	Type 1 Error	*Correct Decision*

The probability of a Type 1 error, rejecting the null hypothesis when it is true, is usually denoted as ". This topic is well covered in most basic statistics courses, and as a result, the error is well managed by specifying the acceptable level of " in the test. Typically, " is set at 0.05 or less.

The probability of a Type 2 error, accepting the null hypothesis when it is untrue, is usually denoted as $. The probability of rejecting the null hypothesis when it is untrue is therefore 1- $. This quantity, called power, is the probability of detecting an effect. Calculating power is less straightforward than dealing with type 1 error, and thus is not covered well in basic statistics courses. As a result, it has been greatly underemphasized in research. This is extremely unfortunate because power is very important. Findings of no effect have little meaning if studies have no reasonable chance to find an effect. Countless numbers of papers have been published reporting no effect without reporting how likely it is that the study could have detected an effect. Not only can underpowered studies be misleading, leading to incorrect decisions, but they can waster staggering amounts of money and can have unwarranted impacts in study areas. The historical lack of attention to statistical power in fisheries research was pointed out by Peterman (1989).

The situation has improved considerably since then, and numerous power analysis software packages are now available. In the YKFP we have been very conscious of experimental power from the very beginning. For example, the final spring chinook supplementation design was based on a power analysis effort by Hoffmann et al. (1994).

In chapter 5 of this report we began dealing with power of the planned spring chinook domestication study, which involves a variety of comparisons of three lines of fish: wild control (WC [Naches stock]), supplemented (S [Upper Yakima stock]), and hatchery control (HC [Upper Yakima stock subjected to continuous hatchery culture). There are two major aspects to be considered in this design: hatchery oriented and non-hatchery oriented. In each case we have designed monitoring measures that deal with adult and with juvenile fish. A single power analysis cannot cover all the traits, so multiple reports will be generated. As in the other report, this report deals only with traits involving comparisons of individual adults from the WC, S, and HC lines, and of those traits, only with those adult traits measured in the CESRF hatchery environment. We assume traits are normally or log-normally distributed, and assume that two comparisons are of interest: S vs HC and S vs WC. A summary of traits and comparisons is presented in the table below:

Table 2. Traits amenable to this power analysis design. Note that comparisons using WC will not be done from traits A7 and A9. This is because of the decision to capture partially spawned Naches fish rather than trap green fish. Fecundity and reproductive effort can be measured only on fish that have not spawned previous to capture. Asterisks denote traits in which sample sizes can be augmented by data from the spawning grounds. CV's are 2001 values. Ranges in CV are a reflection of sex and age class differences.

#	Trait Name	Comparisons Possible		CV(%)
		S vs HC	S vs WC	
A3	Size at age by sex*	X	X	5.0-7.9
A7	Fecundity	X		13.5-21.0
A8	Egg Size	X	X	12.9-19.3
A9	Reproductive effort	X		9.6-15.5
A10	Male and female fertility	X	X	
A11	Adult morphology at spawning*	X	X	assumed same as size
J1	Emergence timing	X	X	
J2	K_D at emergence	X	X	3.1 (fry length), 12.1 (fry weight)
J3	Egg-fry survival	X	X	12.3
J4	Occurrence of developmental abnormalities	X	X	

In any situation where control lines are maintained over multiple generations, it is important to keep them genetically isolated. Crossbreeding between the lines can distort important differences, weakening the design of the experiment. Although there is some possibility of gene flow between the W and S lines, probably a natural occurrence, the big source of concern in this regard in this design is the possibility that precocious males

from HC line releases will spawn in the wild with S-line females. The expected genetic contribution of these spawning precocious HC-line males to the current cohort is given by:

$$gen_{prec} = \frac{0.5 * prop\_fert_{prec} * nat\_eggs * egg\_adult\_surv_{nat}}{nat\_eggs * egg\_adult\_surv_{nat} + hat\_eggs * egg\_adult\_surv_{hat}} \quad (1)$$

where $prop\_fert_{prec}$ is the proportion of naturally deposited eggs fertilized by precocious males; *nat_eggs* and *hat_eggs* are the total numbers of S-line eggs deposited in the natural and hatchery environments, respectively; and $egg\_adult\_surv_{nat}$ and $egg\_adult\_surv_{hat}$ are the egg-adult survival rates of natural- and hatchery- origin S-line eggs. This equation does not tell the long-term story, however. Because of the wild-only broodstock collection feature of the project, if the hatchery returns adults at a higher rate than the natural environment, the genetic contribution of the precocious males is magnified in the next hatchery cycle.

Calculating the genetic contribution under this regime requires a modeling exercise and this is currently underway. This exercise will involve using the latest information on abundance of precocious males on the spawning ground (Pearsons et al. 2003) and on their likely reproductive success (Schroder et al. 2003).

Modelling Statistical Power

The approach we have taken here is initially identical to that taken in chapter 5. We assume that in any trait for either of the two line comparisons, if domestication takes place, the two lines will diverge over the course of generations. We modeled this as one line changing relative to the other by a set generational effect due to domestication (g), specified as a proportion of current performance. For example, we might model that one line changes at a rate of 5% relative to the other. So while the performance of the one line stays constant (except for error), the other increases or decreases (doesn't matter which, but it always has to increase or always has to decrease) by 5% every generation. This situation is represented by the linear model

$Y_{ijk} = \mu(1+g_i)^j + e_{ijk}$, where g_I is the genetic effect for the ith line (0 for control and 0.05, etc. for "treatment" line), ： is the base mean for the trait, j is the generation, and e is a normally distributed error term. Note that this model is not exactly linear, but close to it for small values of g. Note also that this model considers not separate years of sampling, but only generational sampling. This is because upon reflection we realized that the sample size per generation is the key thing; number per year is not important. Thus, 100 sampled in one year is equivalent to 25 each year for 4 years.

The divergence of the two lines should be detectable by doing a regression against time in each line, and then comparing the slopes of the two regressions. A significant test for differing slopes can be taken as significant divergence of the two lines

We simulated data collection over 6 generations, two different genetic effect levels (0.02 and 0.05), and three levels of coefficient of variation (0.1,0.2, and 0.5). We did this for two sampling scenarios: 40 WC fish (equivalent to 10 pairs/yr) vs 600 S fish and 120 HC fish (equivalent to 30 pairs per year). The sample size for S, 600, is quite conservative.

Each scenario was simulated 5000 times, in each generation from generation onwards, a comparison of slopes was done and checked for significance at "= 0.05. Power was calculated as the proportion of replicates that were significant.

For evaluating bias due to the reproductive success of HC-line precocious males, this basic program was modified to create two additional programs. The first of these (HSBIASPOWER) considered bias affecting the H-S evaluation. Here, the S-line mean was changed each generation to reflect the introgression of HC-line genes. Thus, if the genetic contribution of HC-line precocious males was assumed to be a proportion x, then the S-line mean was given by:

$$S'\_mean = (1-x) * S\_mean + x * H\_mean$$

where *H_mean* is the mean of the HC line, and *S_mean* is the pre-introgression mean of the S line. Simulating this effect required introduction, obviously of a term for the percentage contribution of HC-line precocious males. Evaluating the impact of HC-line precocious males in W-S comparisons was considerably more complicated than the H-S comparisons. The HC line was not even a factor in the first W-S simulations, but now had to be included, and a new term introduced to represent how much more domestication was occurring in the HC line than the S line. The result was a another program (WSBIASPOWER). Simulations of the biased situations were run in exactly the same way as the unbiased cases.

Simulations were done in FORTRAN 95 code written specifically for this purpose and compiled in Lahey-Fujitsu F95 version 5.5. Data were simulated using the RAN2 random number function and GASDEV normal deviate function of Press et al. (1986). Regression calculation and slope comparison logic was from Neter and Wasserman (1974). T-tests for significance of slope comparisons was done using functions BETAI, BETACF, and GAMMLN from Press et al. (1986). The source code for, except for the Press et al. functions, is attached as an appendix.

Results and Discussion

a) HC-S Comparisons

Figures 1 and 2 are similar to Figure 3 of chapter 5 except that the 2% and 5% genetic contributions have been split into two graphs, and they contain additional lines showing the effect on power of HC-S comparisons of 2%, 5%, and 10% genetic contribution from the HC line. The effect of the precocious males will be to decrease the differences between the HC and S line, making differences harder to detect. The greater the contribution of the precocious males, the greater this effect. The figures bear this out.

For a trait displaying a cv of .2 for example, power at three generations drops from about 63% to about 61% for a 2% genetic impact of precocious males and to 54% percent for a 10% impact. Note also that bias increases with time.

The key difference between Figures 1 and 2 is the size of the domestication effect: 2% for Figure 1 and 5% for Figure 2. Power is considerably higher with the larger effect, and the proportionate impact of the bias due to precocious males is considerably smaller.

Fig. 1. Power to detect significant differences between the HC and S lines, assuming 120 HC and 600 S per generation, a genetic effect of 2%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%

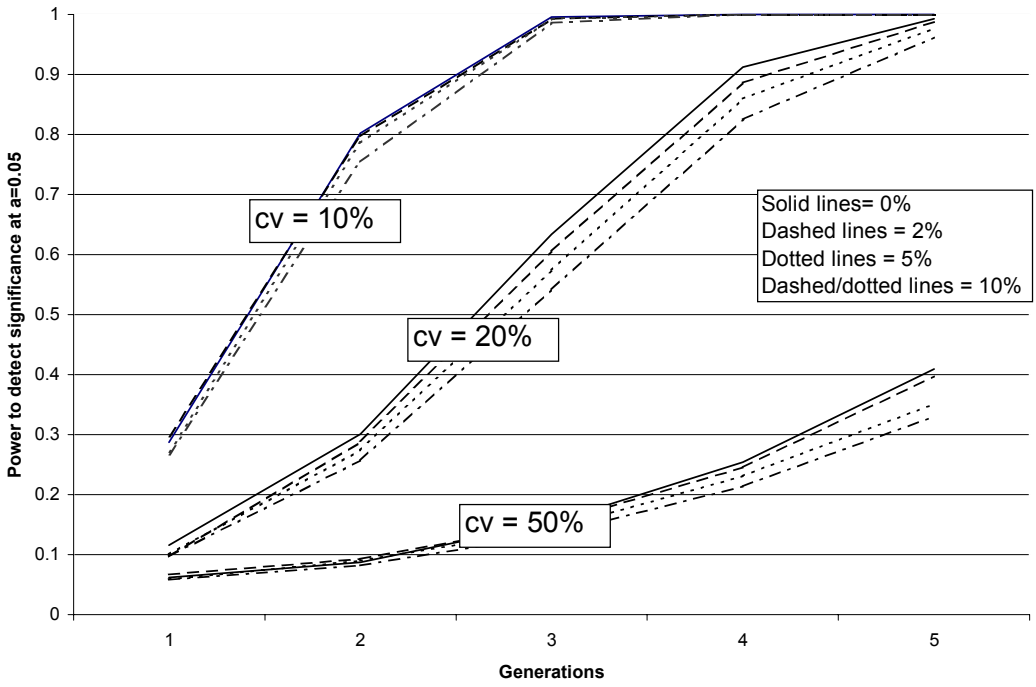
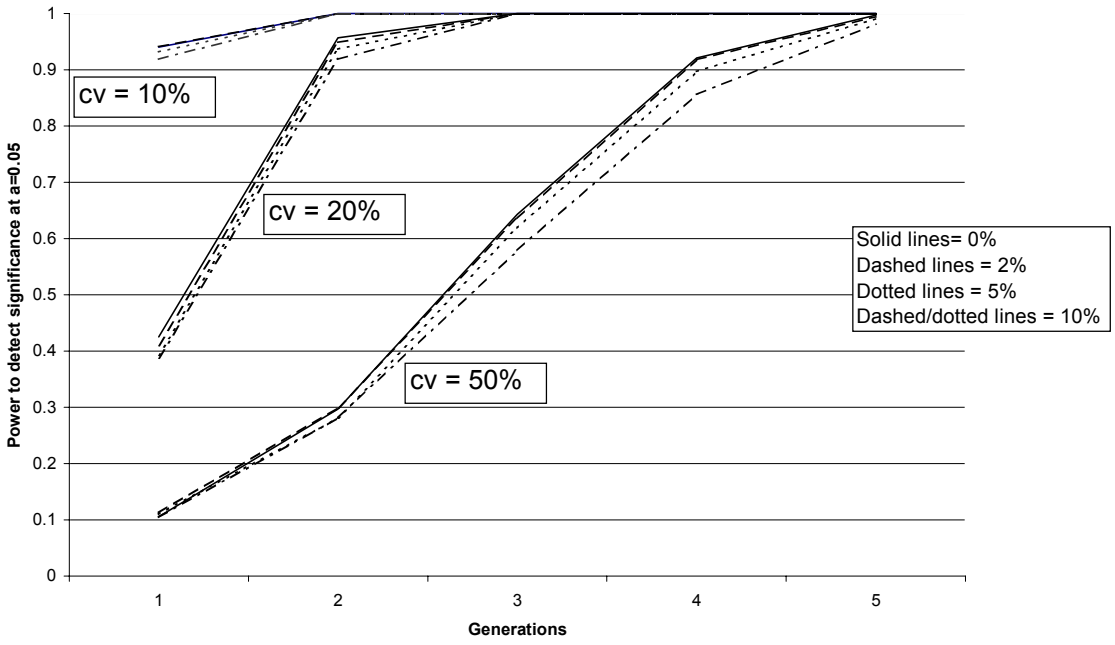


Fig. 2. Power to detect significant differences between the HC and S lines, assuming 120 HC and 600 S per generation, a genetic effect of 5%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%



With this large a domestication effect, traits with cv's of 0.5 display approximately the same power curve as traits with a cv of 0.2 under a domesticating effect of 2% (Figure 1). Here the power at three generations is 64% with no precocious effect, 64% with a 2% precocious impact, and 58% with a 10% impact. Clearly, the larger the domestication effect, the smaller the bias caused by a given level of impact from precocious males.

b) WC-S Comparisons

As already mentioned, modeling bias in the W-S comparisons is more complicated than the modeling for H-S comparisons. In the H-S comparisons it didn't matter how domesticated the S line was becoming, only how domesticated the HC line was becoming relative to the S line. In the W-S comparisons we have to keep track of domestication in both the S line and the HC line. So the simulations have to include not only the precocious effect, but also the rate of domestication of the S line and the HC line. Here we used HC-line domestication values relative to the S line of 1.5, 2, and 3. We also used S-line domestication effects of 1%, 2%, and 5%. We did not use 1% in earlier simulations but felt it was appropriate here because the effect of the HC-line precocious males in this case increases power. It is thus important to model how the precocious effect might increase the probability of a significant comparison when the "real" genetic effect of S-line domestication is very small. It is important to understand the relationship between the two domestication parameters. With an S-line effect of 2% per generation and a relative H-S domestication of 3, for example, the S line is changing due to domestication at 2% per generation and the HC line is changing at 6%.

Results are presented below in nine graphs, presented three to a page. The following table will be useful in navigating among them:

Table 3. Guide to figures presenting results of effects of precocious males on W-S comparisons. Absolute domestication of HC line is the product of the S-line domestication rate and the H-S relative rate.

Per-generation domestication of S line	Domestication of HC line relative to S line		
	1.5	2.0	3.0
1%	Fig. 3	Fig. 4	Fig. 5
2%	Fig. 6	Fig. 7	Fig. 8
5%	Fig. 9	Fig. 10	Fig. 11

Fig. 3. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 1%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 1.5 times that of S line

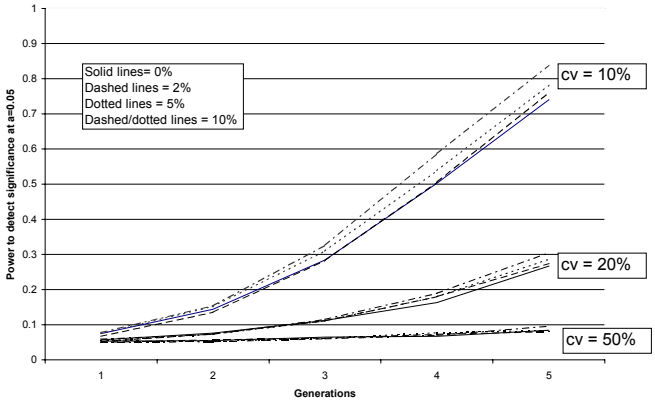


Fig. 4. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 1%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 2.0 times that of S line

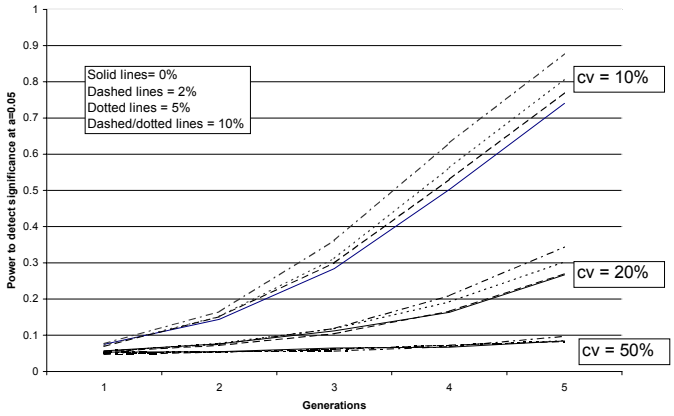


Fig. 5. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 1%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 3.0 times that of S line

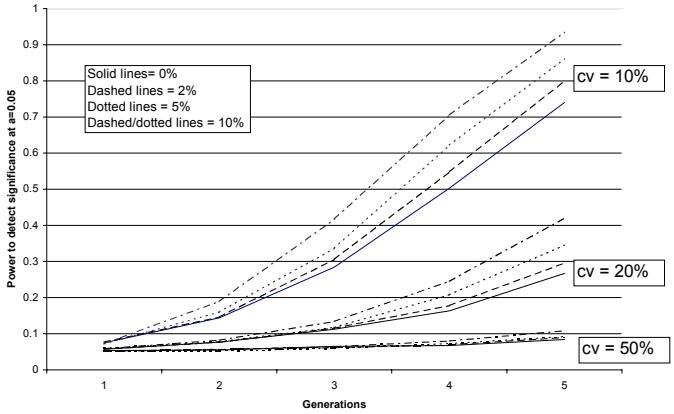


Fig. 6. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 2%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 1.5 times that of S line

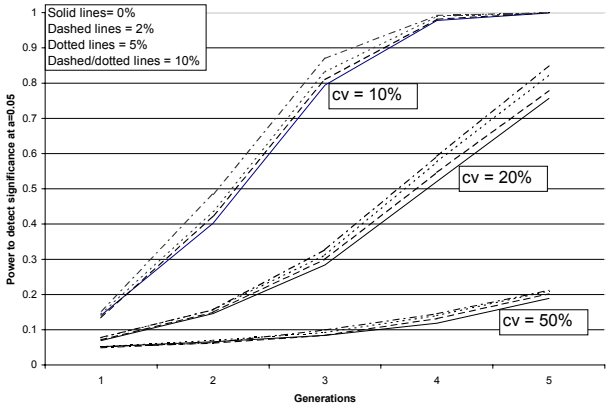


Fig. 7. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 2%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 2.0 times that of S line

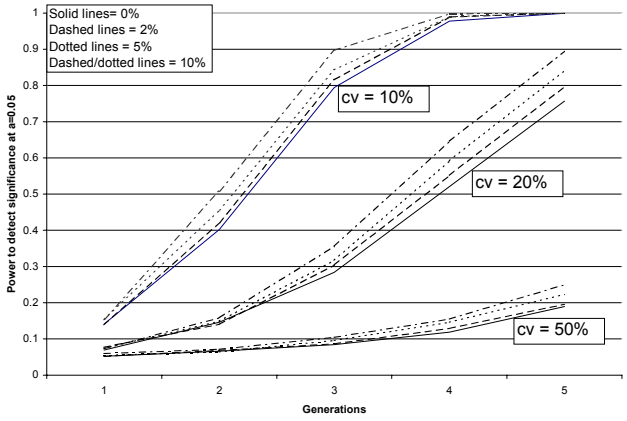


Fig. 8. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 2%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 3.0 times that of S line

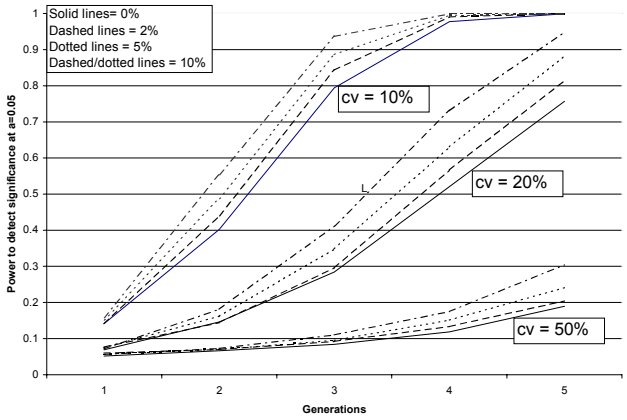


Fig. 9. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 5%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 1.5 times that of S line

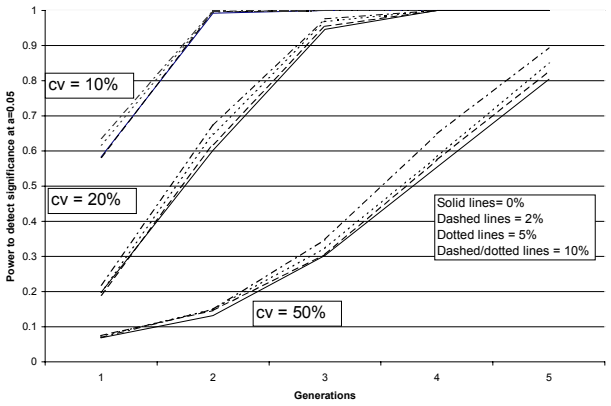


Fig. 10. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 5%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 2.0 times that of S line

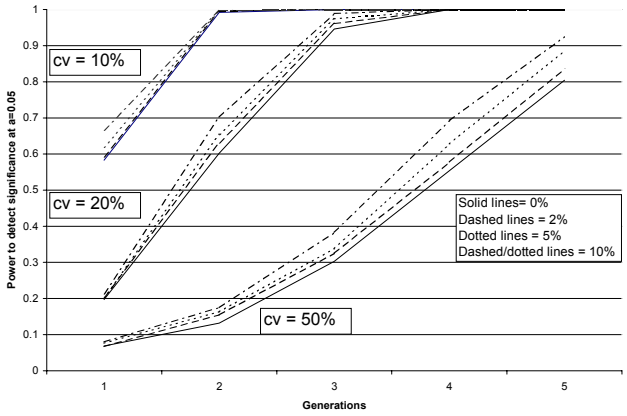
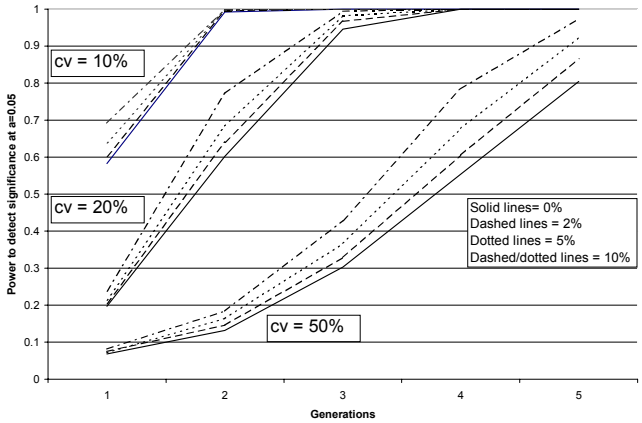


Fig. 11. Power to detect significant differences between the HC and S lines, assuming 40 W and 600 S per generation, a genetic effect of 5%, and contribution to S line from H-line of 0%, 2%, 5%, and 10%, and H line domestication 3.0 times that of S line



The figures clearly show that precocious impacts can bias results and this bias increases considerably as the magnitude of the precocious impact increases. Bias also increases with time, as in the HC-S analysis. Unlike the situation with the HC-S comparisons in which the bias decreases as the size of the S-line domestication effect increases, here the bias increases because as the S-line domestication effect increases, so does the HC-line effect. A 2% impact can raise power anywhere from an undetectable amount to about 5%. A 10% impact can raise power anywhere from about 2% to 20%. The cv of the trait has a large influence on the bias, with the bias being quite large for traits with cv's of 0.5.

Ultimately how this source of bias is viewed depends on your expectations of how much domestication will occur and how important it is to detect it, and on the trait you are examining. A situation of a 5% S-line domestication effect with an HC-line domestication effect twice or more as large would not be unexpected (Figs. 12, 13).

These simulations are only one way of looking at the precocious issue. Keep in mind that power is just the probability of detecting significant differences when differences exist. The situation without precocious males is one in which power is the probability of detecting true differences. With precocious males the situation is one of detecting effects that are either inflated or deflated by the effect of precocious males. Thus in Figure 11 we see that for a trait with a cv of 0.2 we have about a 60% chance of detecting a performance difference after two generations between the wild and S lines when the domestication effect is 5% per generation and there is no problem with precocious males. If precocious males have a 2% impact, the chance of detecting the difference is about 64%, but this is not the power of detecting that 5% domestication effect, but rather that 5% effect augmented by a 2% gene flow rate from the HC line. The HC line in turn is undergoing domestication at a rate of 15% per generation. So not only does power increase, but the measurement of the effect is biased. Possibly this analysis should be coupled with another one just of the biased measurements to give a full picture of the precocious issue. However, at this point, assuming there are no serious methodological errors in how we modeled the bias, it appears that anything more than an impact of a few percent by precocious males could be a serious bias concern.

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Appendix: Program Source Code

a) Program HSBIASPOWER

```
!     Last change:  CAB  10 Jan 2003   10:20 am
program hsbiaspower
! dompower adapted for batch operation, varying numbers, cv's, and
genetic effects
!multidompower modified to address issue of bias from h line precocials
interbreeding with S line
! effect is dealt with by making s mean go up by 0.5k% of the h line
each generation, where k is
! the proportion of eggs in line sired by H line fish (this means that
both wild and hatchery spawning need
! to be considered
TYPE rdata
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata),allocatable             :: ctrlpts(:)
TYPE (rdata),allocatable             :: dompts(:)
INTEGER                              :: n_iter, n_gens, n_control,
n_domest, iprint
REAL                                 :: mean, cv(10), gen_effect(10),
dom_mean,hperc(10), perc
INTEGER, ALLOCATABLE                 :: n_counts(:,:,:,:)
INTEGER                              :: ig,ng,icv,ncv, nhperc,ihperc
open (4,FILE='hsbias.in')
open (8,FILE='hsbias.out')
READ(4,*)n_iter, n_gens, n_control, n_domest
READ(4,*)mean
READ(4,*)ng,(gen_effect(i),i=1,ng)
READ(4,*)ncv,(cv(i),i=1,ncv)
READ(4,*)nhperc,(hperc(i),i=1, nhperc)

READ(4,*)iprint

ALLOCATE(ctrlpts(n_gens*n_control), dompts(n_gens*n_domest),
n_counts(ng,ncv,nhperc,n_gens-1))
n_counts = 0
! Initialize Random Numbers
call random_seed
call random_number(xrand)
idum=-1*nint(xrand*10000.)
!idum = -741537
parameter_loop1: do ig = 1, ng
parameter_loop2: do icv=1, ncv
parameter_loop3: do ihperc=1, nhperc
sd = cv(icv) * mean
perc = hperc(ihperc)
iteration_loop: do iter = 1, n_iter
if(mod(iter,iprint)==0)print *,' Combination ', ig,' ',icv, ' ',ihperc,
' of ',ng*ncv*nhperc,': Iteration ', iter
do i_gen = 0, n_gens-1

   ! make doms
```

```
        IF(i_gen > 0) then
             dom_mean = dom_mean + gen_effect(ig) * dom_mean
        else
             dom_mean = mean
        endif
        do i = i_gen * n_domest + 1, (i_gen + 1) * n_domest
             dompts(i)%x = REAL(i_gen)
             dompts(i)%y = dom_mean + gasdev(idum) * sd
        end do

   ! make controls
        IF(i_gen > 0) then
             ctrl_mean = ctrl_mean + 0.5 * perc * (dom_mean - ctrl_mean)
        else
             ctrl_mean = mean
        endif
        do i = i_gen * n_control + 1, (i_gen + 1) * n_control
             ctrlpts(i)%x = REAL(i_gen)
             ctrlpts(i)%y = ctrl_mean + gasdev(idum) * sd
        end do
        IF(i_gen > 0) then
            call compare_slopes((i_gen+1)*n_control,
ctrlpts,(i_gen+1)*n_domest,dompts,prob)
            if (prob < 0.05) n_counts(ig,icv,ihperc,i_gen) =
n_counts(ig,icv,ihperc,i_gen) + 1
        endif
IF(iter==1) WRITE(8,300)i_gen, gen_effect(ig), cv(icv),perc, ctrl_mean,
dom_mean
300 FORMAT(i6,3(2x,f5.3), 5x, f7.3,5x,f7.3)
end do
ENDDO iteration_loop
ENDDO parameter_loop3
ENDDO parameter_loop2
ENDDO parameter_loop1
WRITE(8,800)n_iter, n_control, n_domest
800 FORMAT(' Iterations:  ',i8,'   Control Fish:   ',i8, '   Treatment
Fish:   ', i8,//,&
            'Gen E',t10,'cv', t20,'perc')
do i = 1, ng
   do j = 1, ncv
      do k = 1, nhperc
               PRINT*, gen_effect(i),
cv(j),hperc(k),(REAL(n_counts(i,j,k,l))/REAL(n_iter),l=1, n_gens-1)
              WRITE(8,801) gen_effect(i), cv(j), hperc(k),
(REAL(n_counts(i,j,k,l))/REAL(n_iter),l=1, n_gens-1)
               801 FORMAT(f5.3,3x, f5.3,3x,f5.3, 5x, 10f7.3)
      ENDDO
   end do
end do
end program
!-----------------------------------------------------------------------
-----
subroutine compare_slopes(n1,datapts1,n2,datapts2,prob)
TYPE rdata
   sequence
   REAL x
   REAL y
```

```
END TYPE rdata
TYPE (rdata)             :: datapts1(n1)
TYPE (rdata)             :: datapts2(n2)
REAL                     :: slope1, slope2, sse1,sse2,ssx1,ssx2,msef,
t, prob,df
call regress(n1,datapts1, slope1,sse1,ssx1)
call regress(n2,datapts2, slope2,sse2,ssx2)
df = REAL(n1+n2-4)
msef = (sse1 + sse2)/df
msef = msef * ((1/ssx1)+(1/ssx2))
sb  = SQRT(msef)

t = (slope1 - slope2)/sb
prob = betai(0.5*df, 0.5,df/(df+t**2))

end subroutine
!-----------------------------------------------------------------------
---------
subroutine regress(n,datapts,slope,sse, ssx)
TYPE rdata
   sequence
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata)             :: datapts(n)
INTEGER                  :: n
REAL                     ::
sumx,ssqx,sumy,ssqy,sumxy,ssx,ssy,ssxy,slope,ssr,sse
INTENT (IN) n, datapts
INTENT (OUT) slope, sse

sumx  = 0
ssqx  = 0
sumy  = 0
ssqy  = 0
sumxy  = 0
do i = 1, n
   sumx  = sumx  + datapts(i)%x
   ssqx  = ssqx  + datapts(i)%x * datapts(i)%x
   sumy  = sumy  + datapts(i)%y
   ssqy  = ssqy  + datapts(i)%y * datapts(i)%y
   sumxy = sumxy + datapts(i)%x * datapts(i)%y
end do
ssx = ssqx - (sumx**2)/REAL(n)
ssy = ssqy - (sumy**2)/REAL(n)
ssxy = sumxy - (sumx*sumy)/REAL(n)
slope = ssxy/ssx
ssr = slope*ssxy
sse = ssy - ssr
return
end subroutine
!-----------------------------------------------------------------------
```

b) Program WSBIASPOWER

```
!     Last change:  CAB  15 Jan 2003   11:32 am
program wsbiaspower
! dompower adapted for batch operation, varying numbers, cv's, and
genetic effects
!multidompower modified to address issue of bias from h line precocials
interbreeding with S line
! effect is dealt with by making s mean go up by 0.5k% of the h line
each generation, where k is
! the proportion of eggs in line sired by H line fish (this means that
both wild and hatchery spawning need
! to be considered
! hsbais power adapted for divergence between wild and s lines; logic
is somewhat different from hsbias
! because need to include code for relative domestication of H and S
lines

TYPE rdata
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata),allocatable            :: ctrlpts(:)
TYPE (rdata),allocatable            :: dompts(:)
INTEGER                             :: n_iter, n_gens, n_control,
n_domest, iprint
REAL                                :: mean, cv(10), gen_effect(10),
dom_mean,hperc(10), perc, hf(10), h_factor, h_mean
INTEGER, ALLOCATABLE                :: n_counts(:,:,:,:,:)
INTEGER                             :: ig,ng,icv,ncv,
nhperc,ihperc,nhf, ihf, combo
open (4,FILE='wsbias.in')
open (8,FILE='wsbias.out')
READ(4,*)n_iter, n_gens, n_control, n_domest
READ(4,*)mean
READ(4,*)ng,(gen_effect(i),i=1,ng)
READ(4,*)ncv,(cv(i),i=1,ncv)
READ(4,*)nhf,(hf(i),i=1,nhf)
READ(4,*)nhperc,(hperc(i),i=1, nhperc)
READ(4,*)iprint
ALLOCATE(ctrlpts(n_gens*n_control), dompts(n_gens*n_domest),
n_counts(ng,ncv,nhf,nhperc,n_gens-1))
n_counts = 0
! Initialize Random Numbers
call random_seed
call random_number(xrand)
idum=-1*nint(xrand*10000.)
!idum = -741537
combo = 0
parameter_loop1: do ig      = 1, ng
parameter_loop2: do icv     = 1, ncv
parameter_loop3: do ihperc  = 1, nhperc
parameter_loop4: do ihf     = 1, nhf
sd = cv(icv) * mean
perc = hperc(ihperc)
h_factor = hf(ihf)
combo = combo + 1
```

```
iteration_loop: do iter = 1, n_iter
if(mod(iter,iprint)==0)print *,' Combination ', combo, ' of
',ng*ncv*nhperc*nhf,': Iteration ', iter
do i_gen = 0, n_gens-1
   ! make h-line mean
        IF(i_gen > 0) then
            h_mean = h_mean + gen_effect(ig) * h_factor  * h_mean
       else
            h_mean = mean
       endif
    ! make s-line fish
        IF(i_gen > 0) then
            dom_mean = dom_mean + gen_effect(ig) * dom_mean  + 0.5 *
perc * (h_mean - dom_mean)
       else
            dom_mean = mean
       endif
       do i = i_gen * n_domest + 1, (i_gen + 1) * n_domest
            dompts(i)%x = REAL(i_gen)
            dompts(i)%y = dom_mean + gasdev(idum) * sd
       end do

   ! make wild controls
         do i = i_gen * n_control + 1, (i_gen + 1) * n_control
            ctrlpts(i)%x = REAL(i_gen)
            ctrlpts(i)%y = mean + gasdev(idum) * sd
       end do
       IF(i_gen > 0) then
           call compare_slopes((i_gen+1)*n_control,
ctrlpts,(i_gen+1)*n_domest,dompts,prob)
           if (prob < 0.05) n_counts(ig,icv,ihf,ihperc,i_gen) =
n_counts(ig,icv,ihf,ihperc,i_gen) + 1
       endif
IF(iter==1) WRITE(8,300)i_gen, gen_effect(ig), cv(icv),h_factor, perc,
mean,  dom_mean, h_mean
300 FORMAT(i6,4(2x,f5.3),3( 5x, f7.3))
end do
ENDDO iteration_loop
ENDDO parameter_loop4
ENDDO parameter_loop3
ENDDO parameter_loop2
ENDDO parameter_loop1
WRITE(8,800)n_iter, n_control, n_domest
800 FORMAT(' Iterations:  ',i8,'   Control Fish:   ',i8, '   Treatment
Fish:   ', i8,//,&
           'Gen E',t10,'cv', t20,'hf', t30,'perc')
do i = 1, ng
   do j = 1, ncv
      do k = 1, nhf
         do l = 1, nhperc
              PRINT*, gen_effect(i),
cv(j),hf(k),hperc(l),(REAL(n_counts(i,j,k,l,m))/REAL(n_iter),m=1,
n_gens-1)
              WRITE(8,801) gen_effect(i), cv(j), hf(k),hperc(l),
(REAL(n_counts(i,j,k,l,m))/REAL(n_iter),m=1, n_gens-1)
              801 FORMAT(4(f5.3,3x), 2x, 10f7.3)
         ENDDO
```

```
      ENDDO
   end do
end do
end program
!---------------------------------------------------------------------
-----
subroutine compare_slopes(n1,datapts1,n2,datapts2,prob)
TYPE rdata
   sequence
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata)             :: datapts1(n1)
TYPE (rdata)             :: datapts2(n2)
REAL                     :: slope1, slope2, sse1,sse2,ssx1,ssx2,msef,
t, prob,df
call regress(n1,datapts1, slope1,sse1,ssx1)
call regress(n2,datapts2, slope2,sse2,ssx2)
df = REAL(n1+n2-4)
msef = (sse1 + sse2)/df
msef = msef * ((1/ssx1)+(1/ssx2))
sb  = SQRT(msef)

t = (slope1 - slope2)/sb
prob = betai(0.5*df, 0.5,df/(df+t**2))

end subroutine
!---------------------------------------------------------------------
---------
subroutine regress(n,datapts,slope,sse, ssx)
TYPE rdata
   sequence
   REAL x
   REAL y
END TYPE rdata
TYPE (rdata)             :: datapts(n)
INTEGER                  :: n
REAL                     ::
sumx,ssqx,sumy,ssqy,sumxy,ssx,ssy,ssxy,slope,ssr,sse
INTENT (IN) n, datapts
INTENT (OUT) slope, sse

sumx  = 0
ssqx  = 0
sumy  = 0
ssqy  = 0
sumxy  = 0
do i = 1, n
   sumx  = sumx  + datapts(i)%x
   ssqx  = ssqx  + datapts(i)%x * datapts(i)%x
   sumy  = sumy  + datapts(i)%y
   ssqy  = ssqy  + datapts(i)%y * datapts(i)%y
   sumxy = sumxy + datapts(i)%x * datapts(i)%y
end do
ssx = ssqx - (sumx**2)/REAL(n)
ssy = ssqy - (sumy**2)/REAL(n)
ssxy = sumxy - (sumx*sumy)/REAL(n)
```

```
slope = ssxy/ssx
ssr = slope*ssxy
sse = ssy - ssr
return
end subroutine
```