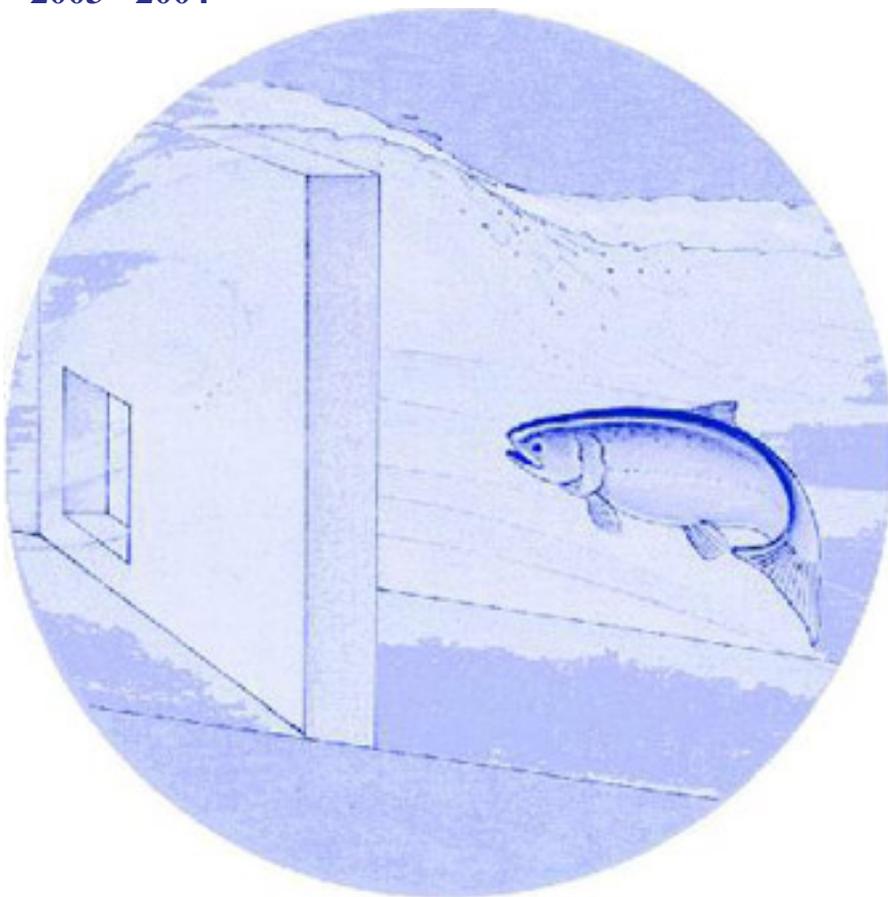


Yakima Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation Report 1 of 7

Annual Report
2003 - 2004



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Yakima/Klickitat Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2003

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Executive Summary

Genetic work for 2003, as in previous years, was quite diverse.

- In chapter 1 we report on the use of DNA microsatellite markers to sex spring chinook collected at Roza. We have learned through comparison of sex determinations at Roza and then at CESRF that sexing green fish on the basis of morphology is somewhat inaccurate, and accurate sexing of fish at Roza is needed to estimate sex ratios of fish on the spawning grounds. Using DNA microsatellite markers, sexing accuracy was high, but not perfect.
- In chapter 2 we report on new genetic risk concepts currently being developed and their implications for the YKFP spring chinook program. The impact on domestication of gene flow between the natural and hatchery spawning components is now much better understood. It is now possible to compare the risk of different hatchery programs much more quantitatively in the past. Thus, we can now make good predictions of how much less domesticating the Yakima spring chinook supplementation effort is than other programs.
- In chapter 3 we present the initial results of morphological comparisons of adult 1) hatchery-origin Upper Yakima spring chinook, 2) natural-origin U. Yakima spring chinook, and 3) Naches spring chinook. Canonical variate analysis allowed both sexes of the three groups to be classified correctly with over accuracy. The differences are subtle, but hatchery-origin fish appear to be someone thinner than natural-origin fish. This is consistent with observations of hatchery vs wild morphology in coho.
- In chapter 4 we describe the ongoing work to refine the Domestication Research/Monitoring Plan. Work for last year included analysis of the impact of HC line precocious males spawning in the wild, development of a misting incubation system for off-site incubation of Naches eggs, and refinement of some aspects of experimental design. The misting incubation system has broad applicability outside the project. The most recent version of the domestication monitoring plan is included as an appendix.
- In chapter 5 we present a final report on computer simulations of factorial mating designs. Using three different schemes for combining breeding values of fish, we found that full factorial mating offers a substantial increase in effective size over single-pair mating. Although full factorial mating may be too difficult logistically, but a significant proportion of the full factorial mating advantage can be obtained by using 2x2 partial factorials. We have developed a method that allows us to determine the relative effective size advantage of mixed partial factorial designs.
- In chapter 6 we report on an analysis of stock origin of smolts collected at Chandler. The 702 Chinook salmon smolts collected at the Chandler trap in 2003 were screened at 12 microsatellite DNA loci. A new Yakima basin baseline, consisting of spring chinook from the upper Yakima, Naches, and American River populations and fall chinook from the Marion Drain and lower Yakima populations, was created for these same 12 loci. DNA template problems with the tissue collections from the Naches, and American River populations prompted the omission of four loci prior to analysis.

The results indicated: 80% Naches spring, 13% American River spring, 7% upper Yakima spring, and less than 1% for the two fall populations combined. The estimated stock proportions in the 2003 Chandler collection differed substantially from those for the 2002 collection. The temporal pattern of sampling in both Chandler smolt collections was not proportional to the observed outmigration in each year, suggesting that both of these estimates should be regarded with caution. Strengthening of the baseline data set will be a high priority for future work with Chandler smolts.

- Two additional important pieces of genetic work were done as part of the reproductive success work (see Schroder's companion report). Both are described here but are not treated elsewhere in the report (they will be treated in later reports by Schroder et al). The first piece is a reanalysis by Sewall Young (WDFW) of approximately 2000 juveniles resulting from the 2001 spawning to determine the parentage of 80 progeny that could not be assigned to parents. The conclusion was that three unrecognized precocious males sired these fish.
- The other important piece of work related to the reproductive success study was a pedigree analysis, by Todd Kassler and Sewall Young (WDFW), of juveniles from the 2002 spawning. All 107 known potential parents (females, adult males, jacks, and precocial males) in the CESRF spawning channel were genotyped at 14 loci (Ocl-1, Ogo-2, Ogo-4, Omm-1135, Omm-1142, Omy-1001, One-8, Ots-1, Ots-2M, Ots-3M, Ots-100, Ots-101, Ots-107, and Ssa-197). The collections of emigrating fry from sections #1 (N = 4,637) and #2 (3,982) were each proportionally subsampled (by week) to select approximately 1,500 fry from each section for genotyping and pedigree analysis. The resulting sets of fry (total of over 3,000) were screened at the same 14 loci screened in the potential parents. Final genotypes at 13 loci were obtained for these fry. We used the program CERVUS to assign individual fry to their most likely female and male parent. Initial parental assignments for approximately 1,500 fry from section #1 and approximately 1,100 fry from section #2 were completed. Final parental assignments will be completed after reruns of selected fish, probable collection of genotype data at one or a few additional loci, and re-analysis using CERVUS (and other methods to detect un-typed male parents ["sneaker precocious males"]). This final step will allow the relative reproductive success of such "sneaker precocious males" to be estimated and provide a more complete picture of the relative reproductive success for all potential parents (and treatment groups) in the experimental stream, not just for the known potential parents. This is an important step, given that Young identified reproductive contributions by previously unrecognized and un-sampled precocious males in the experimental channel in both of the two previous years using DNA analysis.

Table of Contents

1. DNA-Based Gender Determination of Hatchery-Origin Chinook Salmon Passing Roza Dam (Yakima River) in 2002 and 2003.....	6
2. Recent Developments in Genetic Risk Assessment of Integrated Hatchery Programs: Application to Upper Yakima Spring Chinook.....	53
3. Initial Morphometric Analysis of Upper Yakima and Naches Adult Spring Chinook: Progress on Trait A11 of Domestication Monitoring Plan	61
4. YKFP Spring Chinook Domestication Monitoring Plan Development.....	78
5. Modeling the Effective Size Advantage of Factorial Mating in Hatcheries.....	122
6. Year 2003 Chandler Chinook Smolt Stock-of-Origin Assignments	137

Chapter 1

DNA-Based Gender Determination of Hatchery-Origin Chinook Salmon Passing Roza Dam (Yakima River) in 2002 and 2003

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Introduction

DNA-based gender identification of Chinook salmon is part of an integrated suite of tasks, which are included in the Yakima/Klickitat Fisheries Project. Washington Department of Fish and Wildlife scientists have lead the development and refinement of detailed monitoring plans stating major objectives, experimental hypotheses, risk containment measures, and specific field protocols to guide evaluation of supplementation success in the Yakima/Klickitat Fisheries Project. Scientists have conducted natural production, genetic, reproductive success, and ecological interactions monitoring as specified in the Yakima Fisheries Project Spring Chinook Monitoring Plan (Busack et al. 1997)(DOE/BP-64878-1).

The objective described in this report is to identify the gender of Chinook salmon using sex-linked molecular markers. These markers are linked to the Y chromosome (normally found in males) and enable the identification of gender at the nucleotide level (Devlin et al. 1991, Du et al. 1993, Devlin et al. 1994, Forbes et al. 1994, Clifton and Rodriguez 1997, Devlin et al. 2002, and Brunelli and Thorgaard unpublished). However, it is notable that Nagler et al. (2001) found 84% of phenotypically sexed females in the Hanford Reach of the Columbia River had a Y chromosome linked DNA marker. They suggested that this is potentially the result of a sex reversal that occurred in Chinook from temperature or chemical fluctuations. Presence of the Y chromosome linked marker in females may also be the result of the Y chromosome sequences moving to the X chromosome or to an autosome (Brunelli and Thorgaard unpublished).

Material and Methods

Polymerase chain reaction (PCR) was used to amplify two sets of sex specific markers (Clifton and Rodriguez 1997, Brunelli and Thorgaard unpublished) that exist in distinct locations on the Y chromosome of Chinook salmon (Brunelli and Thorgaard unpublished).

Known-gender Chinook salmon were analyzed first to determine the reliability of the two sets of markers to identify gender. Samples of unknown gender collected in 2002 and 2003 were then analyzed to determine the sex of each fish and, ultimately, the ratio of male and female Chinook salmon passing at Roza Dam on the Yakima River. The gender of these unknown samples was also determined by assessing external morphological features of each live adult as it was handled and passed at the dam. After the DNA-based gender identification was completed, the morphological and genetic based gender determinations were compared.

Collections of Known Gender

Three collections (94EJ, 01DC, and 01IK) of Chinook salmon were analyzed to determine how well gender markers could identify known male and female samples. Two collections, 01DC (Warm Springs, mixture of hatchery and natural-origin adults) and 01IK (Cle Elum spawning channel, natural origin adults), are spring-run Chinook and one collection, 94EJ (Methow River, mixture of hatchery and natural-origin adults) is a summer-run population. Each collection was analyzed independently and then the accuracy of the DNA-based gender determinations was assessed by comparison with the actual determinations based on macroscopic examination of the gonads.

Collections of Unknown Gender

Fin-clip tissue samples were collected from Chinook salmon as they were passed at Roza Dam on the Yakima River in 2002 (N = 280; collection 02BL) and 2003 (N = 280; collections 03BY, 03BZ, and 03CA). The tissue samples were preserved in 100% ethanol and stored in pre-labeled vials.

DNA Extraction Methods

Genomic DNA was extracted by digesting a small piece of fin tissue in a 5% chelex (BioRad Chelex 100 resin) solution containing 0.4 mg proteinase K (Sigma). Following digestion at 65°C for 180 min, the samples were heated for 10 minutes at 95°C to denature proteins. The DNA extracts were stored at 5°C until all analyses had been completed.

PCR and Gel Methods

Two sets of gender markers (Clifton and Rodriguez 1997, and Brunelli and Thorgaard unpublished) were used to analyze both the known and unknown samples. Analysis of the markers described by Clifton and Rodriguez (1997) utilized primers p551 and p559 that were sequenced from a sex specific marker (OT-24) that amplifies a 950 base pair fragment in males while no fragment is amplified in females. A second pair of primers (p709 and p710) derived from HSP30 (425 base pairs) is monomorphic in Chinook. The p709 and p710 primers were multiplexed with the OT-24 primers to be used as a control to determine that there had not been a false identification as a female because the PCR amplification was not successful.

Brunelli and Thorgaard identified a primer sequence OTY2-WSU (unpublished data) that allowed sex identification of male and female Chinook and other Pacific salmon spp. A fragment of approximately 287 base pairs amplifies in males while females do not amplify any sex-specific products. A second set of primers amplifying the glyceraldehyde-3-phosphate dehydrogenase (GAPDH forward and GAPDH reverse) gene (approx. 750 base pairs) was used as a control to determine that there had not been a false identification as a female because the PCR amplification was not successful.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.1 µM of each oligonucleotide primer, and 0.5 units *Taq* polymerase (Promega). Amplification was performed using an MJ Research PTC-200 thermocycler. The thermal profile for the OT-24 primers (Clifton and Rodriguez 1997) was as follows: an initial denaturation step of 3 minutes at 94°C; 35 cycles of 15 seconds at 94°C, 30 seconds at 48°C, and 1 minute at 72°C; plus a final

extension step at 70°C for 30 minutes, followed by a final indefinite holding step at 4°C. The thermal profile for the OTY-2 primers (Brunelli and Thorgaard unpublished) was as follows: an initial denaturation step of 3 minutes at 94°C; 35 cycles of 15 seconds at 94°C, 30 seconds at 63°C, and 1 minute at 72°C; plus a final extension step at 70°C for 30 minutes, followed by a final indefinite holding step at 4°C.

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

Scoring Methods

Independent gender determinations were given for all known and unknown samples by three researchers. Accuracy for the known gender samples was calculated for all three independent gender determinations and then a cumulative gender was determined using several criteria. The cumulative determination included samples that were assigned the same gender by two or three of the researchers. Samples that were not given the same gender determination by two or three researchers were excluded from the analysis. When the gender determination varied for the unknown samples among the three researchers they were reanalyzed.

Additional Tests Performed Using Clifton and Rodriguez Markers

Tests were performed using old versus new DNA extracts (time between extraction and testing was approximately six months for some experiments and only a few days for others) to determine if the deterioration of DNA extracts would result in an inability to correctly identify gender. Six males and six females were run and scored four times each for both sets of new and old extracts.

A second set of tests was performed using 10 individuals (five males and females) and PCR cocktail mix to determine if 10 independent PCR amplifications would result in the same gender identification each of the 10 times.

A test of multiple PCR amplifications was conducted on 96 of the unknown samples from 2002 to determine if the gender identifications would be the same when amplified on two different occasions.

Results

Analysis of Known-Sex Samples

The analysis of the known-sex samples (Table 1) resulted in accuracy ranging between 85.3% - 100.0% for the OT-24 markers and 74.5% - 98.5% for the OTY2-WSU markers. The 01DC collection (Warm Springs) had the lowest accuracy using both techniques (74.5% and 85.3%), and the lowest cumulative percentage of scored individuals (49.0% and 70.8% of the samples). Assessing the accuracy for the other two collections only, the range was between 92.1% and 100.0% for both techniques.

Analysis of Unknown Samples

Analysis of the 2002 and 2003 samples of unknown gender (total of 560 fish) resulted in agreement among three independent scorers and between both methods with the exception of a few individuals. There were a total of 11 individuals scored with only the OT-24 primers and 14 individuals scored only with the OTY-2WSU primers. All other individuals were scored the same by both methods and by at least two of three scorers. A combined data set was generated and the final results of all the combined scores are in Tables 2 and 3.

Comparison of Unknown Samples Sexed by Morphology and Genetics

The comparison of sex identifications based on morphology (sexed at the time the live fish were handled and passed at the dam) and genetics resulted in 38 of 280 (13.6%) individuals in the 2002 collection that had different gender determinations (Appendix 1). Thirty of the 38 (78.9%) differences were identified as females based on morphological

characteristics and males by genetic analysis. The remaining eight were identified as males using morphological characteristics and females by genetic analysis.

Analysis of the unknown samples from 2003 resulted in 24 of 280 (8.6%) individuals that were sexed differently between the morphological and genetic analysis (Appendix 2). Twenty-two of the 24 differences (91.7%) were for fish identified as females by morphological characteristics but males by the genetic analysis while only one sample was identified as a male by morphology but as female by genetic analysis. The remaining sample was identified as a jack (small mature male) by morphology but as a female by the genetic analysis. All other jacks and precocials were identified as males by the genetic analysis.

Additional Tests Performed Using Clifton and Rodriguez Markers

Amplifications of old and new DNA extracts among four amplifications (96 total samples) resulted in two individuals being scored incorrectly. One individual was incorrectly identified in all four amplifications of the old extracts while the other individual was incorrectly scored in only one of the four amplifications of the old extracts. In both cases, the incorrect identification was as a female instead of a male.

The test of 10 PCR amplifications for the same five males and females resulted in correct identification of both sexes in all cases.

Analyses of the 96 unknown samples amplified on two different occasions resulted in one male individual that was scored incorrectly as a female, however the banding pattern for that sample was faint and this could explain the lack of amplification (detection) of the male sex band. Comparison of results for both techniques would have resulted in this individual being excluded or re-analyzed if there was any inconsistency of scores, therefore, the change in score would have been identified when using the second technique.

Discussion

Gender identification using genetic techniques has the potential of determining gender of live pre-spawned salmon that are difficult to identify morphologically. This analysis assessed Chinook salmon samples that were identified as males or females using

morphological characteristics and then by genetic markers to determine if the morphological and genetic assessment were in agreement.

The first step in using genetic analysis was to assess the accuracy of the two different genetic markers by analyzing Chinook salmon of known gender. Genetic differentiation of Chinook salmon can exist among different seasonal runs or because of isolation through geographical separation. We wanted to determine if any genetic differences among runs resulted in the inability of the sex markers to correctly identify gender. We tested this hypothesis by using Chinook salmon from a spring and summer run that are close in geographic proximity (Columbia River basin tributaries). Three collections of Chinook salmon were analyzed and the accuracy of both methods for two of the three collections was over 92%. The accuracy using the third collection was lower than 85%, and the overall number of samples that could be scored was approximately 50%. The poor performance of both DNA methods of gender identification for these samples (Warm Springs) could be due to poor quality of the DNA extracts, poor quality of the tissue samples, or a genetic difference in this stock that made both tests unreliable. High accuracies for the other two collections suggest the methods we used are good for identifying gender and that any genetic differences in run timing of Chinook do not impede gender determination. We chose collections that were all in geographic proximity; therefore we were unable to tell if geographic separation of samples would affect the ability of the genetic analysis to identify gender. An accuracy of less than 100% of our known samples may have been the result of samples that had been incorrectly identified to gender at the time of collection.

Two independent genetic methods were used to determine if they would reveal the same gender identification. There was consistent agreement between the gender identifications by both methods even when the identification was different than expected based on external morphology. Similar results by both methods support the possibility of samples that were incorrectly identified at time of collection. Agreement between the two sets of sex-linked markers on different physical parts of the Y chromosome also suggests there has not been any sex reversal in these Chinook salmon. If sequences of the Y chromosome had moved to the X chromosome then it would seem likely that there would not be complete agreement between the gender identifications of the two methods.

Analysis of the unknown samples from 2002 and 2003 were in agreement among the scorers and methods used. The only exceptions occurred when a sample was not scored using one method because the banding was faint. A score for those individuals were included because the banding seen by the other method was strong and positive gender identification could be given. Additional re-runs could have been done, but we felt it was unnecessary given the high degree of accuracy from all other samples.

Assessment of the morphological and genetic identifications in both years revealed over 11% (62/560) of the samples were identified differently. Most of these differences (over 80%) occurred when an individual was identified as a female by morphological characteristics and male by genetic analysis. This was not surprising considering the morphology of a female and small male are similar, and therefore difficult to distinguish.

Given that approximately 89% of the unknown samples were correctly identified to gender by both the morphological assessment and genetic analysis it would again suggest that there had been very little if any sex reversal in the samples that were analyzed. If sex reversal had occurred because of temperature or chemical fluctuations, then the expectation would be that many of the samples would have been identified incorrectly as Nagler et al. (2001) reported.

Considering we don't have an absolutely unambiguous method to identify gender of live unknowns that are only briefly handled and observed as they are passed at the dam, we wanted to assess the accuracy of the genetic analysis. The morphological determination of gender was done at the time of fish passage, and therefore we were unable to determine a level of confidence in that assessment. We did, however, conduct several tests to determine if there were any variations in the genetic analysis process that would result in a mis-identification of gender.

One possibility was that poor quality of extracted DNA would not allow the correct identification of gender. The small number of males that were scored incorrectly as females suggests that the regions of DNA amplified by the sex markers had degraded, and therefore did not amplify in the old extractions. Only two individuals were not correctly identified, therefore it appears the use of old extractions is not detrimental to

gender identification. However, using the best tissue and extracts provides the most reliable results.

Secondly, a test using the same PCR cocktail mix in 10 PCR amplifications of five males and females was performed to determine if the primers would anneal the same. The same PCR cocktail mix was used for all amplifications so we could eliminate the potential of chemical differences among the amplifications. We found no differences in gender identifications of either male or females among the 10 amplifications providing confidence that the sex markers were annealing consistently when amplified multiple times.

A test of repeatability was also performed on the unknown samples to determine if the same gender score was assigned to all individuals when the analysis was performed at different times. This analysis revealed the same scores for all but one male, providing confidence that the sex markers amplified consistently. The one male individual that was scored incorrectly did not amplify very well; therefore it was questioned as a good score. Comparison of results for both techniques would have resulted in this individual being excluded or re-analyzed.

Conclusions

Our investigations using these two different DNA markers for gender determination in Chinook suggested high, but not 100% accuracy. Using these DNA markers to determine the sex ratios of hatchery-origin Chinook passing Roza Dam we estimated 83 males:197 females in 2002 and 182 males:98 females in 2003.

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Table 1. Estimated accuracy of the DNA-based gender determinations using three collections of known-sex Chinook using the two different DNA methods.

	Clifton - Rodriguez Markers			Brunelli - Thorgaard Markers				
	94EJ - Methow River (SU)			94EJ - Methow River (SU)				
	Score 1	Score 2	Score 3	Cumulative	Score 1	Score 2	Score 3	Cumulative
# Scored / Total # Analyzed	88 / 88	88 / 88	88 / 88	88 / 88	75 / 88	82 / 88	69 / 88	76 / 88
# Scored Correctly / # Scored	88 / 88	88 / 88	88 / 88	88 / 88	69 / 75	75 / 82	69 / 69	70 / 76
% Scored Correctly	100.0%	100.0%	100.0%	100.0%	92.0%	91.5%	100.0%	92.1%
	01IK - Cle Elum Parents (SP)			01IK - Cle Elum Parents (SP)				
	Score 1	Score 2	Score 3	Cumulative	Score 1	Score 2	Score 3	Cumulative
# Scored / Total # Analyzed	80 / 96	86 / 100	85 / 100	84 / 100	62 / 96	72 / 100	69 / 100	68 / 100
# Scored Correctly / # Scored	75 / 80	78 / 86	79 / 85	78 / 84	62 / 62	70 / 72	68 / 69	67 / 68
% Scored Correctly	93.8%	90.7%	92.9%	92.9%	100.0%	97.2%	98.6%	98.5%
	01DC - Warm Springs (SP)			01DC - Warm Springs (SP)				
	Score 1	Score 2	Score 3	Cumulative	Score 1	Score 2	Score 3	Cumulative
# Scored / Total # Analyzed	65 / 96	74 / 96	73 / 96	68 / 96	51 / 96	67 / 96	49 / 96	47 / 96
# Scored Correctly / # Scored	55 / 65	61 / 74	61 / 73	58 / 68	43 / 51	48 / 67	35 / 49	35 / 47
% Scored Correctly	84.6%	82.4%	83.6%	85.3%	84.3%	71.6%	71.4%	74.5%

Table 2. Gender determination for Chinook salmon passed at Roza Dam in 2002. Three independent gender identifications and a final consensus gender determination are shown. [methods: 'C - R' = Clifton & Rodriguez; 'B - T' = Brunelli & Thorgaard]

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method			
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3	
1	02BL 01	M	M	M	M	M	M	M	25	02BL 105	M	M	M	M	M	M	M	M
2	02BL 06	M	M	M	M	M	M	M	26	02BL 109	F	M	F	F	F	F	F	F
3	02BL 10	M	M	M	M	M	M	M	27	02BL 114	F	M	F	F	F	F	F	F
4	02BL 14	M	M	M	M	M	M	M	28	02BL 118	M	M	M	M	M	M	M	M
5	02BL 19	M	M	M	M	M	M	M	29	02BL 123	F	M	F	F	F	F	F	F
6	02BL 24	F	F	F	F	F	F	F	30	02BL 127	M	M	M	M	M	M	M	M
7	02BL 28	F	F	F	F	F	F	F	31	02BL 132	F	M	F	F	F	F	X	X
8	02BL 33	F	F	F	F	F	F	F	32	02BL 136	F	M	F	F	F	F	X	F?
9	02BL 37	F	F	F	F	F	F	F	33	02BL 140	F	M	F	F	F	F	F	F
10	02BL 41	M	M	M	M	M	M	M	34	02BL 144	F	M	M	M	M	M	F	F
11	02BL 46	M	M	M	M	M	M	M	35	02BL 148	F	M	M	M	M	M	F	F
12	02BL 50	F	F	F	F	F	F	F	36	02BL 152	F	M	F	F	F	F	F	F
13	02BL 54	F	F	F	F	F	F	F	37	02BL 157	F	M	F	F	F	F	F	F
14	02BL 58	F	F	F	F	F	F	F	38	02BL 162	M	M	M	M	M	M	M	M

Table 2. Continued. [methods: 'C - R' = Clifton &Rodriquez; 'B - T' = Brunelli & Thorgaard]

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
49	02BL 211	F	F	F	F	F	F	F	73	02BL 316	M	M	M	M	M	M	M
50	02BL 215	F	F	F	F	F	F	F	74	02BL 321	F	F	F	F	F	F	F
51	02BL 219	F	F	F	F	F	F	F	75	02BL 325	M	M	M	M	M	M	M
52	02BL 223	F	F	F	F	F	F	F	76	02BL 330	F	F	F	F	F	F	F
53	02BL 227	F	F	F	F	F	F	F	77	02BL 335	F	F	F	F	F	F	F
54	02BL 232	F	F	F	F	F	F	F	78	02BL 339	F	F	F	F	F	F	F
55	02BL 236	M	M	M	M	M	M	M	79	02BL 344	M	M	M	M	M	M	M
56	02BL 240	F	F	F	F	F	F	F	80	02BL 349	F	F	F	F	F	F	F
57	02BL 245	F	F	F	F	F	F	F	81	02BL 354	F	F	F	F	F	F	F
58	02BL 249	F	F	F	F	F	F	F	82	02BL 358	M	M	M	M	M	M	M
59	02BL 254	F	F	F	F	F	F	F	83	02BL 363	F	F	F	F	F	F	F
60	02BL 258	F	F	F	F	F	F	F	84	02BL 367	F	F	F	F	F	F	F
61	02BL 263	F	F	F	F	F	F	F	85	02BL 371	F	F	F	F	F	F	F
62	02BL 267	F	F	F	F	F	F	F	86	02BL 376	M	M	M	M	M	M	M

Table 2. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
97	02BL 423	M	M	M	M	M	M	M	121	02BL 529	F	F	F	F	F	F	
98	02BL 427	M	M	M	M	M	M	M	122	02BL 534	F	F	F	F	F	F	
99	02BL 431	M	M	M	M	M	M	M	123	02BL 539	F	F	F	F	F	F	
100	02BL 435	F	F	F	F	F	F	F	124	02BL 543	F	F	F	F	F	F	
101	02BL 440	F	F	F	F	F	F	F	125	02BL 548	F	F	F	F	F	F	
102	02BL 444	M	M	M	M	M	M	M	126	02BL 552	F	F	F	F	F	F	
103	02BL 449	F	F	F	F	F	F	F	127	02BL 557	M	M	M	M	M	M?	
104	02BL 453	F	F	F	F	F	F	F	128	02BL 561	M	M	M	M	M	M?	
105	02BL 458	M	M	M	M	M	M	M	129	02BL 566	M	M	M	M	M	M	
106	02BL 462	F	F	F	F	F	F	F	130	02BL 570	F	F	F	F	F	F	
107	02BL 467	F	F	F	F	F	F	F	131	02BL 575	M	M	M	M	M	M	
108	02BL 471	F	F	F	F	F	F	F	132	02BL 578	M	M	M	M	M	M	
109	02BL 476	F	F	F	F	F	F	F	133	02BL 584	M	M	M	M	M	M	
110	02BL 480	F	F	F	F	F	F	F	134	02BL 588	F	F	F	F	F	F	

Table 2. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
111 02BL 485		F	F	F	F	M?	M?	X	135 02BL 593	F	F	F	F	F	F	F	
112 02BL 489		F	F	F	F	F	F	X	136 02BL 597	F	F	F	F	F	F	F	
113 02BL 494		F	F	F	F	F	F	F	137 02BL 602	F	F	F	F	F	X	F	
114 02BL 498		F	F	F	F	F	F	F	138 02BL 607	F	F	F	F	F	F	F	
115 02BL 503		F	F	F	F	F	F	F	139 02BL 611	F	F	F	F	F	F	F	
116 02BL 507		M	M	M	M	M	M	M	140 02BL 615	F	F	F	F	F	F	F	
117 02BL 512		F	F	F	F	F	F	F	141 02BL 620	F	F	F	F	F	F	F	
118 02BL 517		F	F	F	F	F	F	F	142 02BL 624	F	F	F	F	F	F	F	
119 02BL 521		M	M	M	M	M	M	M	143 02BL 629	M	M	M	M	M	M	M	
120 02BL 525		M	M	M	M	M	M	M	144 02BL 633	F	F	F	F	F	F	F	

Table 2. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
159 02BL 701		F	F	F	F	F	F	X	183 02BL 809	M	M	M	M	M	M	M	
160 02BL 705		F	F	F	F	F	F	X	184 02BL 813	F	F	F	F	F	F	X	
161 02BL 710		M	M	M	M	M	M	M	185 02BL 817	M	M	M	M	M	M	M	
162 02BL 714		M	M	M	M	M	M	M	186 02BL 822	F	F	F	F	F	F	F	
163 02BL 720		F	F	F	F	F	F	F	187 02BL 827	F	F	F	F	F	F	F	
164 02BL 723		F	F	F	F	F	F	F	188 02BL 831	F	F	F	F	F	F	F	
165 02BL 728		F	F	F	F	F	F	F	189 02BL 836	F	F	F	F	F	F	F	
166 02BL 732		M	M	M	M	M	M	M	190 02BL 841	M	M	M	M	M	M	M	
167 02BL 736		F	F	F	F	F	F	F	191 02BL 845	M	M	M	M	M	M	M?	
168 02BL 741		F	F	F	F	F	F	F	192 02BL 849	F	F	F	F	F	F	X	

Table 2. Continued.																	
Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
207	02BL 917	M	X	X	X	M	M	M	231	02BL 1025	F	F	F	F	F	F	F
208	02BL 921	F	X	X	X	F	F	F	232	02BL 1029	M	M	M	M	M	M	M
209	02BL 926	F	F	F	F	F	F	F	233	02BL 1034	F	F	F	F	F	F	F
210	02BL 930	F	F	F	F	F	F	F	234	02BL 1038	M	M	M	M	M	M	M
211	02BL 935	F	F	F	F	F	F	F	235	02BL 1042	F	F	F	F	F	F	F
212	02BL 939	F	F	F	F	F	F	F	236	02BL 1046	F	F	F	F	F	F	F
213	02BL 944	F	F	F	F	F	F	F	237	02BL 1050	F	F	F	F	F	F	F
214	02BL 948	M	M	M	M	M	M	M	238	02BL 1054	M	M	M	M	M	M	M
215	02BL 953	F	F	F	F	F	F	F	239	02BL 1058	F	X	F	X	F	F	F
216	02BL 957	F	F	F	F	F	F	F	240	02BL 1063	F	X	F?	X	F	F	F

Table 3. Gender determination for Chinook salmon passed at Roza Dam in 2003. Three independent gender identifications and a final consensus gender determination are shown. [methods: 'C - R' = Clifton & Rodriguez; 'B - T' = Brunelli & Thorgaard]

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
1	03BY 1	F	?	?	M?	F	F	F?	25	03BY 54	F	F	F	F	F	F	F
2	03BY 3	M	M	M	M	M	M	M	26	03BY 56	M	M	M	M	M	M	M
3	03BY 4	M	M	M	M	M	M	M	27	03BY 57	M	M	M	M	M	M	M
4	03BY 7	M	M	M	M	M	M	M	28	03BY 60	M	M	M	M	M	M	M
5	03BY 9	F	F	F	F	F	F	F	29	03BY 63	F	F	F	F	F	F	F
6	03BY 11	M	M	M	M	M	M	M	30	03BY 65	M	M	M	M	M	M	M
7	03BY 12	F	F	F	F	F	F	F	31	03BY 66	F	F	F	F	F	F	F
8	03BY 15	F	F	F	F	F	F	F	32	03BY 69	F	F	F	F	F	F	F
9	03BY 18	F	F	F	F	F	F	F	33	03BY 72	F	F	F	F	F	F	F
10	03BY 20	M	M	M	M	M	M	M	34	03BY 74	M	M	M	M	M	M	M
11	03BY 21	F	F	F	F	F	F	F	35	03BY 75	F	F	F	F	F	F	F
12	03BY 24	F	F	F	F	F	F	F	36	03BY 78	F	F	F	F	F	F	F
13	03BY 27	F	F	F	F	F	F	F	37	03BY 81	F	F	F	F	F	F	F
14	03BY 29	F	X	X	X	X	X	X	38	03BY 83	F	F	F	F	F	F	F

Table 3. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
97	03BY 216	M	M	M	M	M	M	M	121	03BY 276	M	M	M	M	M	M	
98	03BY 218	M	M	M	M	M	M	M	122	03BY 279	F	F	F	F	F	F	
99	03BY 219	F	F	F	F	F	F	F	123	03BY 282	F	F	F	F	F	F	
100	03BY 222	M	M	M	M	M	M	M	124	03BY 285	M	M	M	M	M	M	
101	03BY 225	M	M	M	M	M	M	M	125	03BY 288	M	M	M	M	M	M	
102	03BY 227	F	F	F	F	F	F	F	126	03BY 291	M	M	M	M	M?	M	
103	03BY 228	F	F	F	F	F	F	F	127	03BY 294	F	F	F	F	F	F	
104	03BY 231	F	F	F	F	F	F	F	128	03BY 297	F	F	F	F	F	F	
105	03BY 234	F	F	F	F	F	F	F	129	03BY 300	F	F	F	F	F	F	
106	03BY 236	M	M	M	M	M	M	M	130	03BY 303	F	F	F	F	F	F	
107	03BY 237	M	M	M	M	M	M	M	131	03BY 306	F	M?	M?	M?	F	F	
108	03BY 240	F	X	F	F	F	F	F	132	03BY 309	M	M	M	M	M	M	
109	03BY 243	F	F	F	F	F	F	F	133	03BY 312	F	F	F	F	F	F	
110	03BY 246	M	M	M	M	M	M	M	134	03BY 315	M	M	M	M	M	M	

Table 3. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method						
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				
111	03BY 247	F	F	F	F	F	F	F	135	03BY 318	F	F	F	F	F	F	F	F	Score #1	Score #2	Score #3
112	03BY 249	F	F	F	F	F	F	F	136	03BY 321	F	F	F	F	F	F	F	F	F	F	F
113	03BY 252	F	F	F	F	F	F	F	137	03BY 324	F	F	F	F	F	F	F	F	F	F	F
114	03BY 255	F	F	F	F	F	F	F	138	03BY 327	F	F	F	F	F	F	F	F	F	F	F
115	03BY 258	M	M	M	M	M	M	M	139	03BY 330	F	M	M	M	M	M	M	M	F	F	F
116	03BY 261	F	F	F	F	F	F	F	140	03BY 333	F	F	F	F	F	F	F	F	F	F	F
117	03BY 264	F	F	F	F	F	F	F	141	03BY 336	F	F	F	F	F	F	F	F	F	F	F
118	03BY 267	F	F	F	F	F	F	F	142	03BY 339	F	F	F	F	F	F	F	F	F	F	F
119	03BY 270	M	M	M	M	M	M	M	143	03BY 342	F	M	M	M	M	M	M	M	F	F	F
120	03BY 273	M	M	M	M	M	M	M	144	03BY 345	F	M	M	M	M	M	M	M	F	F	F

Table 3. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
145	03BZ 2	M	M	M	M	M	M	M	169	03BZ 74	M	M	M	M	M	M	
146	03BZ 5	M	M	M	M	M	M	M	170	03BZ 77	M	M	M	M	M	M	
147	03BZ 8	M	M	M	M	M	M	M	171	03BZ 80	M	M	M	M	M	M	
148	03BZ 11	M	M	M	M	M	M	M	172	03BZ 83	M	M	M	M	M	M	
149	03BZ 14	M	M	M	M	M	M	M	173	03BZ 86	M	M	M	M	M	M	
150	03BZ 17	M	M	M	M	M	M	M	174	03BZ 89	M	M	M	M	M	M	
151	03BZ 20	M	M	M	M	M	M	M	175	03BZ 92	M	M	M	M	M	M	
152	03BZ 23	M	M	M	M	M	M	M	176	03BZ 95	M	M	M	M	M	M	
153	03BZ 26	M	M	M	M	M	M	M	177	03BZ 98	M	M	M	M	M	M	
154	03BZ 29	M	M	M	M	M	M	M	178	03BZ 101	M	M	M	M	M	M	
155	03BZ 32	M	M	M	M	M	M	M	179	03BZ 104	M	M	M	M	M	M	
156	03BZ 35	M	M	M	M	M	M	M	180	03BZ 107	M	M	M	M	M	M	
157	03BZ 38	M	M	M	M	M	M	M	181	03BZ 110	M	M	M	M	M	M	
158	03BZ 41	M	M	M	M	M	M	M	182	03BZ 113	M	M	M	M	M	M	

Table 3. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
159	03BZ 44	M	M	M	M	M	M	M	183	03BZ 116	M	M	M	M	M	M	M
160	03BZ 47	M	M	M	M	M	M	M	184	03BZ 119	M	M	M	M	M	M	M
161	03BZ 50	M	M	M	M	M	M	M	185	03BZ 122	M	M	M	M	M	M	M
162	03BZ 53	M	M	M	M	M	M	M	186	03BZ 125	M	M	M	M	M	M	M
163	03BZ 56	M	M	M	M	M	M	M	187	03BZ 128	M	M	M	M	M	M	M
164	03BZ 59	M	M	M	M	M	M	M	188	03BZ 131	M	M	M	M	M	M	M
165	03BZ 62	M	M	M	M	M	M	M	189	03BZ 134	M	M	M	M	M	M	M
166	03BZ 65	M	M	M	M	M	M	M	190	03BZ 137	M	M	M	M	M	M	M
167	03BZ 68	M	M	M	M	M	M	M	191	03BZ 140	M	M	M	M	M	M	M
168	03BZ 71	M	M	M	M	M	M	M	192	03BZ 143	M	M	M	M	M	M	M

Table 3. Continued.

Sample #	Sample ID	Consensus	C - R Method			B - T Method			Sample #	Sample ID	Consensus	C - R Method			B - T Method		
			Score #1	Score #2	Score #3	Score #1	Score #2	Score #3				Score #1	Score #2	Score #3	Score #1	Score #2	Score #3
193	03BZ 146	M	M	M	M	M	M	M	217	03BZ 218	M	M	M	M	M	M	
194	03BZ 149	M	M	M	M	M	M	M	218	03BZ 221	M	M	M	M	M	M	
195	03BZ 152	M	M	M	M	M	M	M	219	03BZ 224	M	M	M	M	M	M	
196	03BZ 155	M	M	M	M	M	M	M	220	03BZ 227	M	M	M	M	M	M	
197	03BZ 158	M	M	M	M	M	M	M	221	03BZ 230	M	M	M	M	M	M	
198	03BZ 161	M	M	M	M	M	M	M	222	03BZ 233	M	M	M	M	M	M	
199	03BZ 164	M	M	M	M	M	M	M	223	03BZ 236	M	M	M	M	M	M	
200	03BZ 167	M	M	M	M	M	M	M	224	03BZ 239	M	M	M	M	M	M	
201	03BZ 170	M	M	M	M	M	M	M	225	03BZ 242	M	M	M	M	M	M	
202	03BZ 173	M	M	M	M	M	M	M	226	03BZ 245	M	M	M	M	M	M	
203	03BZ 176	M	M	M	M	M	M	M	227	03BZ 248	M	M	M	M	M	M	
204	03BZ 179	M	M	M	M	M	M	M	228	03BZ 251	M	M	M	M	M	M	
205	03BZ 182	M	M	M	M	M	M	M	229	03BZ 254	M	M	M	M	M	M	
206	03BZ 185	M	M	M	M	M	M	M	230	03BZ 257	M	M	M	M	M	M	

Appendix 2. Biological data and gender determination of Chinook salmon collected at Roza Dam in 2003 using morphological characteristics and genetic analysis.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
4/29/2003	03BY00001	F	F	78	67	6.1	4
5/1/2003	03BY00003	M	M	86	74	7.9	5
5/7/2003	03BY00004	M	F	88	73	8.2	5
5/9/2003	03BY00007	M	F	74	60	4.9	4
5/12/2003	03BY00009	F	F	71	61	4.3	4
5/12/2003	03BY00011	M	F	85	72	7.3	5
5/12/2003	03BY00012	F	F	83	71	7.4	5
5/12/2003	03BY00015	F	F	80	67	6.3	
5/12/2003	03BY00018	F	F	80	69	6.5	5
5/12/2003	03BY00020	M	M	101	82	11.8	5
5/13/2003	03BY00021	F	F	82	70	6	5
5/13/2003	03BY00024	F	F	85	73	7.9	5
5/13/2003	03BY00027	F	F	85	74	7	
5/13/2003	03BY00029	F	F	70	60	4.3	4
5/13/2003	03BY00030	F	F	78	68	6.1	5
5/13/2003	03BY00033	F	F	85	74	7.1	5
5/13/2003	03BY00036	F	F	85	72	7.4	5
5/13/2003	03BY00038	M	M	93	77	9.4	
5/14/2003	03BY00039	F	F	86	74	7.2	5
5/14/2003	03BY00042	F	F	68	57	4.3	4
5/14/2003	03BY00045	F	F	85	72	6.8	5
5/14/2003	03BY00047	F	F	79	67	6	5
5/14/2003	03BY00048	M	F	66	56	3.9	4
5/14/2003	03BY00051	M	F	70	58	4	4
5/15/2003	03BY00054	F	F	83	72	6.6	5
5/15/2003	03BY00056	M	F	70	60	4.4	4
5/15/2003	03BY00057	F	F	84	73	7	5
5/16/2003	03BY00060	F	F	82	66	5.8	5
5/16/2003	03BY00063	F	F	70	59	3.8	4
5/16/2003	03BY00065	F	F	82	70	6.5	4
5/16/2003	03BY00066	F	F	70	58	4.3	4
5/16/2003	03BY00069	M	M	86	71	6.9	4
5/16/2003	03BY00072	F	F	73	61	4.6	4
5/16/2003	03BY00074	F	F	70	58	4.4	4
5/16/2003	03BY00075	F	F	86	72	7.1	5
5/16/2003	03BY00078	M	F	74	62	4.8	4
5/16/2003	03BY00081	F	F	73	61	4.4	4
5/16/2003	03BY00083	F	F	84	71	7	5
5/16/2003	03BY00084	F	F	83	69	7.4	5
5/18/2003	03BY00087	M	F	69	58	3.8	4
5/18/2003	03BY00090	M	F	70	59	4.2	4
5/19/2003	03BY00092	M	M	85	68	7	5
5/20/2003	03BY00093	F	F	86	75	7.4	5
5/20/2003	03BY00096	F	F	81	71	5.8	
5/20/2003	03BY00099	M	M	84	70	6.9	4
5/20/2003	03BY00101	F	F	78	68	5.9	5

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/20/2003	03BY00102	F	F	88	75	8.2	5
5/20/2003	03BY00105	F	F	70	59	4	4
5/20/2003	03BY00108	F	F	79	68	5.4	5
5/20/2003	03BY00110	F	F	89	77	7.8	5
5/21/2003	03BY00111	M	M	91	75	8.5	5
5/21/2003	03BY00114	M	F	70	59	4.3	4
5/21/2003	03BY00117	F	F	81	68	6.1	5
5/21/2003	03BY00119	M	F	71	58	4.3	4
5/22/2003	03BY00120	F	F	68	58	3.6	4
5/22/2003	03BY00123	F	F	73.5	63	4.2	4
5/22/2003	03BY00126	M	M	90	75	8.3	5
5/22/2003	03BY00128	F	F	83	71	6.4	5
5/22/2003	03BY00129	M	M	92	78	8.4	
5/22/2003	03BY00132	F	F	69	59	4.1	4
5/22/2003	03BY00135	F	F	76	64	5.2	5
5/22/2003	03BY00137	F	F	80.5	68	5.6	5
5/22/2003	03BY00138	M	F	77	66	5.5	4
5/22/2003	03BY00141	F	F	72	62	4.4	4
5/22/2003	03BY00144	F	F	72	62	4.8	4
5/22/2003	03BY00146	F	F	69	59	4.1	4
5/22/2003	03BY00147	M	F	66	56	3.4	
5/23/2003	03BY00150	F	F	86	72	6.9	5
5/23/2003	03BY00153	F	F	74	62	4.7	4
5/23/2003	03BY00155	M	F	71.5	60	4.8	4
5/23/2003	03BY00156	F	M	86	71	7.2	5
5/23/2003	03BY00159	F	F	67.5	58	3.9	4
5/23/2003	03BY00162	F	F	80.5	69	6	5
5/24/2003	03BY00164	F	F	68	56	3.6	4
5/24/2003	03BY00165	M	M	68	56	3.6	4
5/24/2003	03BY00168	M	M	78	66	6.2	4
5/24/2003	03BY00171	M	M	91	67	8	5
5/24/2003	03BY00173	M	F	70	59	4.4	4
5/24/2003	03BY00174	F	F	69.5	59	4.2	4
5/24/2003	03BY00177	F	F	89	75	8.1	5
5/25/2003	03BY00180	F	F	74.5	63	5.5	4
5/25/2003	03BY00182	F	F	82	69	6.2	5
5/25/2003	03BY00183	M	M	75	61.5	4.6	
5/25/2003	03BY00186	F	F	74.5	63	4.9	4
5/26/2003	03BY00189	F	F	67	57	3.2	4
5/26/2003	03BY00191	F	F	85	73	6.9	5
5/26/2003	03BY00192	F	F	86	73	7.1	5
5/26/2003	03BY00195	M	M	85	71	5.9	5
5/27/2003	03BY00198	M	F	70	60	3.5	5
5/28/2003	03BY00200	F	F	83	71	6.5	5
5/28/2003	03BY00201	F	F	68	58	3.4	4
5/28/2003	03BY00204	F	F	71	61	4	5
5/28/2003	03BY00207	F	F	69	59	4.2	4
5/28/2003	03BY00209	F	F	86	73	7.1	5
5/28/2003	03BY00210	F	F	73	63	4.3	4
5/28/2003	03BY00213	M	F	74	63	4.5	4

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/28/2003	03BY00216	M	F	72	61	4.4	4
5/30/2003	03BY00218	M	M	83	69	6.4	5
5/30/2003	03BY00219	F	F	83	69	7	5
5/30/2003	03BY00222	M	M	70.5	59	4.3	4
5/30/2003	03BY00225	M	M	71	59	4.5	4
5/30/2003	03BY00227	F	F	75	63.5	5.3	4
5/30/2003	03BY00228	F	F	78.5	66	5.7	4
5/31/2003	03BY00231	F	F	86	73	6.4	5
6/1/2003	03BY00234	F	F	79	67	6.7	5
6/6/2003	03BY00236	M	F	70	60	4.2	5
6/7/2003	03BY00237	M	M	85	72	6.5	5
6/8/2003	03BY00240	F	F	69	59	3.9	4
6/12/2003	03BY00243	F	F	75	65	5.4	4
6/13/2003	03BY00246	M	M	75	62	4.7	4
6/13/2003	03BY00247	F	F	74	64	5	4
6/13/2003	03BY00249	F	F	66	54	3.3	4
6/13/2003	03BY00252	F	F	84	70.5	6.1	
6/14/2003	03BY00255	F	F	69	58	3.8	4
6/14/2003	03BY00258	M	M	91	75	8.5	5
6/14/2003	03BY00261	F	F	80	66	5.6	5
6/14/2003	03BY00264	F	F	78	65	5.9	4
6/15/2003	03BY00267	F	F	66	55	3	4
6/16/2003	03BY00270	M	M	77.5	64.5	5.4	4
6/18/2003	03BY00273	M	M	71	59	3.7	4
6/18/2003	03BY00276	M	M	77	65	4.8	4
6/19/2003	03BY00279	F	F	75	64	4.8	4
6/19/2003	03BY00282	F	F	77	67	5.4	4
6/19/2003	03BY00285	M	F	69	59	3.6	4
6/20/2003	03BY00288	M	M	80	65	5.5	4
6/21/2003	03BY00291	M	F	75	63	4.6	4
6/21/2003	03BY00294	F	F	78	66	5.7	5
6/24/2003	03BY00297	F	F	76	65	4.7	4
6/26/2003	03BY00300	F	F	80	68	5.2	5
6/28/2003	03BY00303	F	F	81	70	5.2	5
6/29/2003	03BY00306	F	F	81	68	5.5	5
7/8/2003	03BY00309	M	F	76	65	3.8	5
7/17/2003	03BY00312	F	F	67	55	3.3	4
7/30/2003	03BY00315	M	M	71	61	4.2	4
8/4/2003	03BY00318	F	F	91	77	7.9	5
8/5/2003	03BY00321	F	F	77	67	4.9	4
8/15/2003	03BY00324	F	F	70	60	3.5	4
8/24/2003	03BY00327	F	F	84	70	5.6	5
9/1/2003	03BY00330	F	F	75	62	4.5	4
9/5/2003	03BY00333	F	F	55	46	1.7	3
9/5/2003	03BY00336	F	F	82	70	5.3	5
9/5/2003	03BY00339	F	F	76	64	4.2	4
9/5/2003	03BY00342	F	F	72	61	3.8	4
9/8/2003	03BY00345	F	F	88	74	6.2	5
5/22/2003	03BZ00002	M	J	43	36	0.6	3
5/24/2003	03BZ00005	M	J	54	45	1.9	3

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/25/2003	03BZ00008	M	J	50.5	43	1.6	3
5/26/2003	03BZ00011	M	J	50	42	1.6	3
5/26/2003	03BZ00014	M	J	48	40	1.2	3
5/26/2003	03BZ00017	M	J	50	42	1.4	3
5/27/2003	03BZ00020	M	J	47	39	1.2	3
5/27/2003	03BZ00023	M	J	47	40	1.2	3
5/27/2003	03BZ00026	M	J	48.5	40.5	1.3	3
5/28/2003	03BZ00029	M	J	45	38	1	3
5/28/2003	03BZ00032	M	J	44	37	0.9	
5/28/2003	03BZ00035	M	J	50	42	1.6	3
5/29/2003	03BZ00038	M	J	49	41	1.5	3
5/30/2003	03BZ00041	M	J	49	41	1.5	3
5/30/2003	03BZ00044	M	J	50	43	1.6	
5/30/2003	03BZ00047	M	J	46.5	39	1.2	3
5/31/2003	03BZ00050	M	J	47	39	1.3	3
6/1/2003	03BZ00053	M	J	51	43	1.7	3
6/1/2003	03BZ00056	M	J	47	38	1.4	3
6/2/2003	03BZ00059	M	J	44	37	1	
6/4/2003	03BZ00062	M	J	39	33	0.7	
6/5/2003	03BZ00065	M	J	50	43	1.5	3
6/5/2003	03BZ00068	M	J	54	44	1.9	3
6/5/2003	03BZ00071	M	J	45	38	1.1	3
6/6/2003	03BZ00074	M	J	47	39	1.2	3
6/7/2003	03BZ00077	M	J	43	36	0.9	
6/7/2003	03BZ00080	M	J	45	38	1.1	3
6/7/2003	03BZ00083	M	J	48	41	1.3	3
6/8/2003	03BZ00086	M	J	47	39	1.1	3
6/8/2003	03BZ00089	M	J	51	43	1.6	3
6/9/2003	03BZ00092	M	J	42.5	35.5	0.9	3
6/9/2003	03BZ00095	M	J	50	42	1.5	3
6/10/2003	03BZ00098	M	J	51	43	1.5	3
6/10/2003	03BZ00101	M	J	49.5	42	1.5	3
6/10/2003	03BZ00104	M	J	56	48	1.9	3
6/10/2003	03BZ00107	M	J	46	39	1.1	3
6/10/2003	03BZ00110	M	J	46	39	1.1	3
6/10/2003	03BZ00113	M	J	41	35	0.7	3
6/11/2003	03BZ00116	M	J	47.5	40.5	1.3	3
6/11/2003	03BZ00119	M	J	50	43	1.6	3
6/11/2003	03BZ00122	M	J	48	40	1.1	3
6/11/2003	03BZ00125	M	J	53	45	1.6	3
6/11/2003	03BZ00128	M	J	55	47	2	3
6/11/2003	03BZ00131	M	J	43	36	0.9	3
6/12/2003	03BZ00134	M	J	51.5	43.5	1.4	3
6/12/2003	03BZ00137	M	J	45	38	1	3
6/12/2003	03BZ00140	M	J	53	45	1.7	3
6/12/2003	03BZ00143	M	J	53	45	1.6	3
6/13/2003	03BZ00146	M	J	54	45	2.1	3
6/13/2003	03BZ00149	M	J	42	35	1	3
6/13/2003	03BZ00152	M	J	48	40	1.3	3
6/13/2003	03BZ00155	M	J	44	37	1	3

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
6/13/2003	03BZ00158	M	J	52	43	1.9	3
6/13/2003	03BZ00161	M	J	48	40	1.3	3
6/13/2003	03BZ00164	M	J	49	41	1.4	
6/14/2003	03BZ00167	M	J	56	46	2.3	3
6/14/2003	03BZ00170	M	J	51	42	1.7	3
6/14/2003	03BZ00173	M	J	48	39	1.4	3
6/14/2003	03BZ00176	M	J	49	40	1.6	3
6/14/2003	03BZ00179	M	J	51	42	1.5	3
6/14/2003	03BZ00182	M	J	49	40	1.4	3
6/15/2003	03BZ00185	M	J	46	38	1.3	3
6/15/2003	03BZ00188	M	J	53	43	1.8	3
6/15/2003	03BZ00191	M	J	52	43	1.7	3
6/15/2003	03BZ00194	M	J	47	38	1.3	3
6/16/2003	03BZ00197	M	J	50.5	42	1.6	3
6/17/2003	03BZ00200	M	J	52	43	1.7	3
6/17/2003	03BZ00203	M	J	49	39	1.5	3
6/17/2003	03BZ00206	M	J	50	41	1.6	3
6/18/2003	03BZ00209	M	J	54	46	1.9	3
6/18/2003	03BZ00212	M	J	40	33	0.6	3
6/18/2003	03BZ00215	M	J	57	48	2.2	3
6/19/2003	03BZ00218	M	J	53	45	1.8	3
6/19/2003	03BZ00221	M	J	51	44	1.7	3
6/19/2003	03BZ00224	M	J	51.5	44	1.7	
6/19/2003	03BZ00227	M	J	52	44	1.6	3
6/19/2003	03BZ00230	M	J	53	45	1.9	
6/19/2003	03BZ00233	M	J	42	34	0.8	3
6/19/2003	03BZ00236	M	J	56	47	2.3	3
6/20/2003	03BZ00239	M	J	47	38	1.2	3
6/20/2003	03BZ00242	M	J	45	36	1.1	3
6/20/2003	03BZ00245	M	J	53	44	1.9	3
6/20/2003	03BZ00248	M	J	49	40	1.5	3
6/20/2003	03BZ00251	M	J	45	36	1.1	3
6/21/2003	03BZ00254	M	J	46	38	1.1	3
6/21/2003	03BZ00257	M	J	47	39	1.1	3
6/22/2003	03BZ00260	M	J	59	48	2.5	3
6/22/2003	03BZ00263	M	J	45	37	1	3
6/23/2003	03BZ00266	M	J	51	43	1.5	3
6/24/2003	03BZ00269	M	J	52	44	1.7	3
6/24/2003	03BZ00272	M	J	49	42	1.4	3
6/24/2003	03BZ00275	M	J	56	48	2.7	3
6/25/2003	03BZ00278	M	J	41	34	0.6	3
6/25/2003	03BZ00281	M	J	53	45	1.7	3
6/25/2003	03BZ00284	M	J	46	38	1	3
6/26/2003	03BZ00287	M	J	56	47	2.2	3
6/26/2003	03BZ00290	M	J	54	46	1.9	3
6/27/2003	03BZ00293	M	J	59	49	2.7	3
6/27/2003	03BZ00296	M	J	52	42	1.7	3
6/27/2003	03BZ00299	M	J	45.5	36	1.1	3
6/28/2003	03BZ00302	F	J	57	48	2.3	3
6/28/2003	03BZ00306	M	J	52	43	1.7	3

Appendix 2. Biological data and gender determination of Chinook salmon collected at Roza Dam in 2003 using morphological characteristics and genetic analysis.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
4/29/2003	03BY00001	F	F	78	67	6.1	4
5/1/2003	03BY00003	M	M	86	74	7.9	5
5/7/2003	03BY00004	M	F	88	73	8.2	5
5/9/2003	03BY00007	M	F	74	60	4.9	4
5/12/2003	03BY00009	F	F	71	61	4.3	4
5/12/2003	03BY00011	M	F	85	72	7.3	5
5/12/2003	03BY00012	F	F	83	71	7.4	5
5/12/2003	03BY00015	F	F	80	67	6.3	
5/12/2003	03BY00018	F	F	80	69	6.5	5
5/12/2003	03BY00020	M	M	101	82	11.8	5
5/13/2003	03BY00021	F	F	82	70	6	5
5/13/2003	03BY00024	F	F	85	73	7.9	5
5/13/2003	03BY00027	F	F	85	74	7	
5/13/2003	03BY00029	F	F	70	60	4.3	4
5/13/2003	03BY00030	F	F	78	68	6.1	5
5/13/2003	03BY00033	F	F	85	74	7.1	5
5/13/2003	03BY00036	F	F	85	72	7.4	5
5/13/2003	03BY00038	M	M	93	77	9.4	
5/14/2003	03BY00039	F	F	86	74	7.2	5
5/14/2003	03BY00042	F	F	68	57	4.3	4
5/14/2003	03BY00045	F	F	85	72	6.8	5
5/14/2003	03BY00047	F	F	79	67	6	5
5/14/2003	03BY00048	M	F	66	56	3.9	4
5/14/2003	03BY00051	M	F	70	58	4	4
5/15/2003	03BY00054	F	F	83	72	6.6	5
5/15/2003	03BY00056	M	F	70	60	4.4	4
5/15/2003	03BY00057	F	F	84	73	7	5
5/16/2003	03BY00060	F	F	82	66	5.8	5
5/16/2003	03BY00063	F	F	70	59	3.8	4
5/16/2003	03BY00065	F	F	82	70	6.5	4
5/16/2003	03BY00066	F	F	70	58	4.3	4
5/16/2003	03BY00069	M	M	86	71	6.9	4
5/16/2003	03BY00072	F	F	73	61	4.6	4
5/16/2003	03BY00074	F	F	70	58	4.4	4
5/16/2003	03BY00075	F	F	86	72	7.1	5
5/16/2003	03BY00078	M	F	74	62	4.8	4
5/16/2003	03BY00081	F	F	73	61	4.4	4
5/16/2003	03BY00083	F	F	84	71	7	5
5/16/2003	03BY00084	F	F	83	69	7.4	5
5/18/2003	03BY00087	M	F	69	58	3.8	4
5/18/2003	03BY00090	M	F	70	59	4.2	4
5/19/2003	03BY00092	M	M	85	68	7	5
5/20/2003	03BY00093	F	F	86	75	7.4	5
5/20/2003	03BY00096	F	F	81	71	5.8	
5/20/2003	03BY00099	M	M	84	70	6.9	4
5/20/2003	03BY00101	F	F	78	68	5.9	5

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/20/2003	03BY00102	F	F	88	75	8.2	5
5/20/2003	03BY00105	F	F	70	59	4	4
5/20/2003	03BY00108	F	F	79	68	5.4	5
5/20/2003	03BY00110	F	F	89	77	7.8	5
5/21/2003	03BY00111	M	M	91	75	8.5	5
5/21/2003	03BY00114	M	F	70	59	4.3	4
5/21/2003	03BY00117	F	F	81	68	6.1	5
5/21/2003	03BY00119	M	F	71	58	4.3	4
5/22/2003	03BY00120	F	F	68	58	3.6	4
5/22/2003	03BY00123	F	F	73.5	63	4.2	4
5/22/2003	03BY00126	M	M	90	75	8.3	5
5/22/2003	03BY00128	F	F	83	71	6.4	5
5/22/2003	03BY00129	M	M	92	78	8.4	
5/22/2003	03BY00132	F	F	69	59	4.1	4
5/22/2003	03BY00135	F	F	76	64	5.2	5
5/22/2003	03BY00137	F	F	80.5	68	5.6	5
5/22/2003	03BY00138	M	F	77	66	5.5	4
5/22/2003	03BY00141	F	F	72	62	4.4	4
5/22/2003	03BY00144	F	F	72	62	4.8	4
5/22/2003	03BY00146	F	F	69	59	4.1	4
5/22/2003	03BY00147	M	F	66	56	3.4	
5/23/2003	03BY00150	F	F	86	72	6.9	5
5/23/2003	03BY00153	F	F	74	62	4.7	4
5/23/2003	03BY00155	M	F	71.5	60	4.8	4
5/23/2003	03BY00156	F	M	86	71	7.2	5
5/23/2003	03BY00159	F	F	67.5	58	3.9	4
5/23/2003	03BY00162	F	F	80.5	69	6	5
5/24/2003	03BY00164	F	F	68	56	3.6	4
5/24/2003	03BY00165	M	M	68	56	3.6	4
5/24/2003	03BY00168	M	M	78	66	6.2	4
5/24/2003	03BY00171	M	M	91	67	8	5
5/24/2003	03BY00173	M	F	70	59	4.4	4
5/24/2003	03BY00174	F	F	69.5	59	4.2	4
5/24/2003	03BY00177	F	F	89	75	8.1	5
5/25/2003	03BY00180	F	F	74.5	63	5.5	4
5/25/2003	03BY00182	F	F	82	69	6.2	5
5/25/2003	03BY00183	M	M	75	61.5	4.6	
5/25/2003	03BY00186	F	F	74.5	63	4.9	4
5/26/2003	03BY00189	F	F	67	57	3.2	4
5/26/2003	03BY00191	F	F	85	73	6.9	5
5/26/2003	03BY00192	F	F	86	73	7.1	5
5/26/2003	03BY00195	M	M	85	71	5.9	5
5/27/2003	03BY00198	M	F	70	60	3.5	5
5/28/2003	03BY00200	F	F	83	71	6.5	5
5/28/2003	03BY00201	F	F	68	58	3.4	4
5/28/2003	03BY00204	F	F	71	61	4	5
5/28/2003	03BY00207	F	F	69	59	4.2	4
5/28/2003	03BY00209	F	F	86	73	7.1	5
5/28/2003	03BY00210	F	F	73	63	4.3	4
5/28/2003	03BY00213	M	F	74	63	4.5	4

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/28/2003	03BY00216	M	F	72	61	4.4	4
5/30/2003	03BY00218	M	M	83	69	6.4	5
5/30/2003	03BY00219	F	F	83	69	7	5
5/30/2003	03BY00222	M	M	70.5	59	4.3	4
5/30/2003	03BY00225	M	M	71	59	4.5	4
5/30/2003	03BY00227	F	F	75	63.5	5.3	4
5/30/2003	03BY00228	F	F	78.5	66	5.7	4
5/31/2003	03BY00231	F	F	86	73	6.4	5
6/1/2003	03BY00234	F	F	79	67	6.7	5
6/6/2003	03BY00236	M	F	70	60	4.2	5
6/7/2003	03BY00237	M	M	85	72	6.5	5
6/8/2003	03BY00240	F	F	69	59	3.9	4
6/12/2003	03BY00243	F	F	75	65	5.4	4
6/13/2003	03BY00246	M	M	75	62	4.7	4
6/13/2003	03BY00247	F	F	74	64	5	4
6/13/2003	03BY00249	F	F	66	54	3.3	4
6/13/2003	03BY00252	F	F	84	70.5	6.1	
6/14/2003	03BY00255	F	F	69	58	3.8	4
6/14/2003	03BY00258	M	M	91	75	8.5	5
6/14/2003	03BY00261	F	F	80	66	5.6	5
6/14/2003	03BY00264	F	F	78	65	5.9	4
6/15/2003	03BY00267	F	F	66	55	3	4
6/16/2003	03BY00270	M	M	77.5	64.5	5.4	4
6/18/2003	03BY00273	M	M	71	59	3.7	4
6/18/2003	03BY00276	M	M	77	65	4.8	4
6/19/2003	03BY00279	F	F	75	64	4.8	4
6/19/2003	03BY00282	F	F	77	67	5.4	4
6/19/2003	03BY00285	M	F	69	59	3.6	4
6/20/2003	03BY00288	M	M	80	65	5.5	4
6/21/2003	03BY00291	M	F	75	63	4.6	4
6/21/2003	03BY00294	F	F	78	66	5.7	5
6/24/2003	03BY00297	F	F	76	65	4.7	4
6/26/2003	03BY00300	F	F	80	68	5.2	5
6/28/2003	03BY00303	F	F	81	70	5.2	5
6/29/2003	03BY00306	F	F	81	68	5.5	5
7/8/2003	03BY00309	M	F	76	65	3.8	5
7/17/2003	03BY00312	F	F	67	55	3.3	4
7/30/2003	03BY00315	M	M	71	61	4.2	4
8/4/2003	03BY00318	F	F	91	77	7.9	5
8/5/2003	03BY00321	F	F	77	67	4.9	4
8/15/2003	03BY00324	F	F	70	60	3.5	4
8/24/2003	03BY00327	F	F	84	70	5.6	5
9/1/2003	03BY00330	F	F	75	62	4.5	4
9/5/2003	03BY00333	F	F	55	46	1.7	3
9/5/2003	03BY00336	F	F	82	70	5.3	5
9/5/2003	03BY00339	F	F	76	64	4.2	4
9/5/2003	03BY00342	F	F	72	61	3.8	4
9/8/2003	03BY00345	F	F	88	74	6.2	5
5/22/2003	03BZ00002	M	J	43	36	0.6	3
5/24/2003	03BZ00005	M	J	54	45	1.9	3

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
5/25/2003	03BZ00008	M	J	50.5	43	1.6	3
5/26/2003	03BZ00011	M	J	50	42	1.6	3
5/26/2003	03BZ00014	M	J	48	40	1.2	3
5/26/2003	03BZ00017	M	J	50	42	1.4	3
5/27/2003	03BZ00020	M	J	47	39	1.2	3
5/27/2003	03BZ00023	M	J	47	40	1.2	3
5/27/2003	03BZ00026	M	J	48.5	40.5	1.3	3
5/28/2003	03BZ00029	M	J	45	38	1	3
5/28/2003	03BZ00032	M	J	44	37	0.9	
5/28/2003	03BZ00035	M	J	50	42	1.6	3
5/29/2003	03BZ00038	M	J	49	41	1.5	3
5/30/2003	03BZ00041	M	J	49	41	1.5	3
5/30/2003	03BZ00044	M	J	50	43	1.6	
5/30/2003	03BZ00047	M	J	46.5	39	1.2	3
5/31/2003	03BZ00050	M	J	47	39	1.3	3
6/1/2003	03BZ00053	M	J	51	43	1.7	3
6/1/2003	03BZ00056	M	J	47	38	1.4	3
6/2/2003	03BZ00059	M	J	44	37	1	
6/4/2003	03BZ00062	M	J	39	33	0.7	
6/5/2003	03BZ00065	M	J	50	43	1.5	3
6/5/2003	03BZ00068	M	J	54	44	1.9	3
6/5/2003	03BZ00071	M	J	45	38	1.1	3
6/6/2003	03BZ00074	M	J	47	39	1.2	3
6/7/2003	03BZ00077	M	J	43	36	0.9	
6/7/2003	03BZ00080	M	J	45	38	1.1	3
6/7/2003	03BZ00083	M	J	48	41	1.3	3
6/8/2003	03BZ00086	M	J	47	39	1.1	3
6/8/2003	03BZ00089	M	J	51	43	1.6	3
6/9/2003	03BZ00092	M	J	42.5	35.5	0.9	3
6/9/2003	03BZ00095	M	J	50	42	1.5	3
6/10/2003	03BZ00098	M	J	51	43	1.5	3
6/10/2003	03BZ00101	M	J	49.5	42	1.5	3
6/10/2003	03BZ00104	M	J	56	48	1.9	3
6/10/2003	03BZ00107	M	J	46	39	1.1	3
6/10/2003	03BZ00110	M	J	46	39	1.1	3
6/10/2003	03BZ00113	M	J	41	35	0.7	3
6/11/2003	03BZ00116	M	J	47.5	40.5	1.3	3
6/11/2003	03BZ00119	M	J	50	43	1.6	3
6/11/2003	03BZ00122	M	J	48	40	1.1	3
6/11/2003	03BZ00125	M	J	53	45	1.6	3
6/11/2003	03BZ00128	M	J	55	47	2	3
6/11/2003	03BZ00131	M	J	43	36	0.9	3
6/12/2003	03BZ00134	M	J	51.5	43.5	1.4	3
6/12/2003	03BZ00137	M	J	45	38	1	3
6/12/2003	03BZ00140	M	J	53	45	1.7	3
6/12/2003	03BZ00143	M	J	53	45	1.6	3
6/13/2003	03BZ00146	M	J	54	45	2.1	3
6/13/2003	03BZ00149	M	J	42	35	1	3
6/13/2003	03BZ00152	M	J	48	40	1.3	3
6/13/2003	03BZ00155	M	J	44	37	1	3

Appendix 2 continued.

Date	DNA Sample #	Genetic - ID	morph - ID	fork length	POH length	weight	age
6/13/2003	03BZ00158	M	J	52	43	1.9	3
6/13/2003	03BZ00161	M	J	48	40	1.3	3
6/13/2003	03BZ00164	M	J	49	41	1.4	
6/14/2003	03BZ00167	M	J	56	46	2.3	3
6/14/2003	03BZ00170	M	J	51	42	1.7	3
6/14/2003	03BZ00173	M	J	48	39	1.4	3
6/14/2003	03BZ00176	M	J	49	40	1.6	3
6/14/2003	03BZ00179	M	J	51	42	1.5	3
6/14/2003	03BZ00182	M	J	49	40	1.4	3
6/15/2003	03BZ00185	M	J	46	38	1.3	3
6/15/2003	03BZ00188	M	J	53	43	1.8	3
6/15/2003	03BZ00191	M	J	52	43	1.7	3
6/15/2003	03BZ00194	M	J	47	38	1.3	3
6/16/2003	03BZ00197	M	J	50.5	42	1.6	3
6/17/2003	03BZ00200	M	J	52	43	1.7	3
6/17/2003	03BZ00203	M	J	49	39	1.5	3
6/17/2003	03BZ00206	M	J	50	41	1.6	3
6/18/2003	03BZ00209	M	J	54	46	1.9	3
6/18/2003	03BZ00212	M	J	40	33	0.6	3
6/18/2003	03BZ00215	M	J	57	48	2.2	3
6/19/2003	03BZ00218	M	J	53	45	1.8	3
6/19/2003	03BZ00221	M	J	51	44	1.7	3
6/19/2003	03BZ00224	M	J	51.5	44	1.7	
6/19/2003	03BZ00227	M	J	52	44	1.6	3
6/19/2003	03BZ00230	M	J	53	45	1.9	
6/19/2003	03BZ00233	M	J	42	34	0.8	3
6/19/2003	03BZ00236	M	J	56	47	2.3	3
6/20/2003	03BZ00239	M	J	47	38	1.2	3
6/20/2003	03BZ00242	M	J	45	36	1.1	3
6/20/2003	03BZ00245	M	J	53	44	1.9	3
6/20/2003	03BZ00248	M	J	49	40	1.5	3
6/20/2003	03BZ00251	M	J	45	36	1.1	3
6/21/2003	03BZ00254	M	J	46	38	1.1	3
6/21/2003	03BZ00257	M	J	47	39	1.1	3
6/22/2003	03BZ00260	M	J	59	48	2.5	3
6/22/2003	03BZ00263	M	J	45	37	1	3
6/23/2003	03BZ00266	M	J	51	43	1.5	3
6/24/2003	03BZ00269	M	J	52	44	1.7	3
6/24/2003	03BZ00272	M	J	49	42	1.4	3
6/24/2003	03BZ00275	M	J	56	48	2.7	3
6/25/2003	03BZ00278	M	J	41	34	0.6	3
6/25/2003	03BZ00281	M	J	53	45	1.7	3
6/25/2003	03BZ00284	M	J	46	38	1	3
6/26/2003	03BZ00287	M	J	56	47	2.2	3
6/26/2003	03BZ00290	M	J	54	46	1.9	3
6/27/2003	03BZ00293	M	J	59	49	2.7	3
6/27/2003	03BZ00296	M	J	52	42	1.7	3
6/27/2003	03BZ00299	M	J	45.5	36	1.1	3
6/28/2003	03BZ00302	F	J	57	48	2.3	3
6/28/2003	03BZ00306	M	J	52	43	1.7	3

Chapter 2

Recent Developments in Genetic Risk Assessment of Integrated Hatchery Programs: Application to Upper Yakima Spring Chinook

Craig Busack, WDFW

Introduction

The purpose of the Yakima Fisheries Project, as stated in a letter from the Northwest Power Planning Council (now the Northwest Power and Conservation Council) was to test whether new methods of fish production can be used to increase harvest and natural production while conserving genetic resources.¹ Because of that charge, genetic risk has always been a serious topic of discussion in the project throughout its development. An early genetic risk assessment of the project (1990) may well have been the first genetic risk assessment in the region. The 1997 spring chinook monitoring plan (Busack et al. 1997) reviews potential monitoring measures aimed at genetic risk. Indeed, the entire recent reorientation of the project into an intensive study of domestication selection is an effort to address genetic risk.

Attempts to describe genetic risk in hatchery operations typically are discussions of risk concepts (e.g., Busack and Currens 1995) or generalized approaches to risk containment (e.g., Currens and Busack 1995). What has been missing is an actual quantitative assessment of risk. This is understandable, given the massive level of uncertainty about the actual genetic consequences of particular actions. Substantial progress has been made in the last few months toward quantitative assessment of domestication risk, however, and this has direct application to the Yakima spring chinook program. This is a very important development. For the first time, we can predict with some level of confidence the relative domestication impacts to be expected from integrated hatchery programs operated in various ways. We can for the first time say something quantitative about the benefits of wild-only broodstock collection. Also, we can predict how much more domestication the hatchery control line should experience than the supplementation line, and use this information in further development of the domestication monitoring plan.

The new development was made possible by the Hatchery Scientific Review Group (HSRG)², a group of agency and independent scientists convened to evaluate and make recommendations on hatchery operations in western Washington. Key to their evaluation is the concept that there are two basic ways to operate hatcheries: as *segregated* operations in which the intent is to not have interbreeding between hatchery-origin and natural-origin fish, and *integrated* operations, where this interbreeding is intentional

¹ Letter from NPPC to Joe Blum, director of Washington Department of Fish and Wildlife, 1990

² See www.hatcheryreform.com for description of group, process, and for work products.

(HSRG 2004). The YFP spring chinook program, and all other classical supplementation programs, are clearly integrated programs *sensu* HSRG.³ The most important effect of the HSRG review and recommendations is that it takes the basic approach used to do supplementation in the Yakima and elsewhere out of its narrow stock recovery role, and presents it as an option for both harvest augmentation and conservation operations. This new heightened emphasis on integrated programs comes at a time when there is still high uncertainty about the demographic and genetic outcomes such programs should serve to make the YFP spring chinook operation an even more important “experiment”.

Below is a diagram of an integrated program from Lynch and O’Hely (2001), that I previously presented in a report two years ago (Busack et al. 2002):

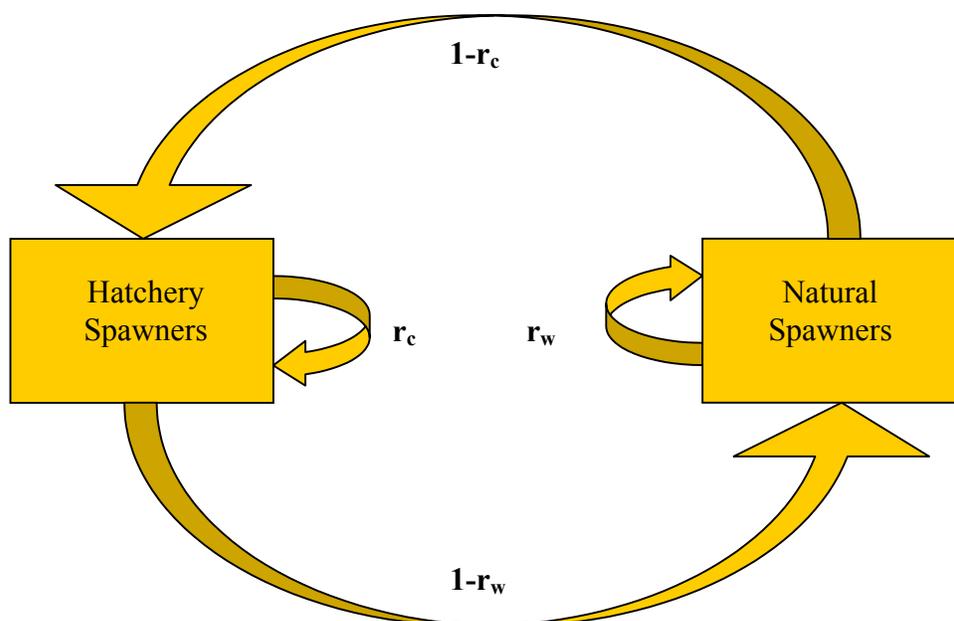


Fig. 1. Schematic of reproductive interactions between natural and hatchery subpopulations in an integrated production program (from Lynch and O’Hely, 2001)

The r coefficients on the diagram represent the proportions of spawners used in the two environments by origin. Thus r_c is the proportion of hatchery broodstock consisting of hatchery-origin fish, and $1-r_c$ is the proportion of hatchery broodstock consisting of natural-origin fish. Similarly, r_w is the proportion of natural spawners consisting of

³ The HSRG definition for integrated programs differs somewhat from the intuitive definition in that it is based on broodstock management. Technically, an integrated program can be one in which there is gene flow from the natural to the hatchery subcomponent, but no gene flow from hatchery to the natural subcomponent.

natural-origin fish, and $1-r_w$ is the proportion of natural spawners consisting of hatchery-origin fish. Although the diagram was designed with integrated programs in mind, it serves well to illustrate the interactions in any hatchery program. A typical hatchery program, in which most of the broodstock consists of returning hatchery fish, would have an r_c close to 1. In contrast, the YKFP spring chinook program has an r_c of 0.

An important, but perhaps not obvious feature of this diagram is that programs can be completely characterized by the gene flow rates. Because all the rates are proportions that add to 1 for both the natural and hatchery spawning components, only two rates are necessary for population characterization, either r_c and r_w or $(1-r_c)$ and $(1-r_w)$. The latter pair of coefficients will be very useful for our purposes.

The key finding of the HSRG, and the jumping off point for the original work reported on here is that if the proportion of natural-origin fish in the broodstock ($1-r_c$ in Fig. 1) exceeds the proportion of hatchery-origin fish on the spawning grounds ($1-r_w$ in Fig. 1), then natural forces would dominate artificial forces in the domestication process (HSRG 2003). This conclusion was derived from the domestication model of Ford (2002) by HSRG member D. Campton.

Analysis

Ford (2002) simulated domestication via a so-called infinite-alleles model. He assumed for a hypothetical trait a wild optimum (the trait value the population would evolve to without hatchery influence) and a hatchery optimum (the trait value the population would evolve to with only hatchery influence). Under the assumption of equal heritability of the trait in the two environments, the population would evolve to an equilibrium value dependent on the strength of selection, the two optima, and the two gene flow rates mentioned above. The result is summarized in his equation (7) (p.818):

$$\hat{z}_w = \frac{\sigma^2((1+p_c(h^2-1))\theta_w + (h^2-1)(p_w-1)\theta_c) + (\theta_c(\omega_w^2 - \omega_w^2 p_w) - \theta_w \omega_c^2 (p_c - 1))}{\sigma^2(2 - p_w - p_c + h^2(p_w + p_c - 1)) + \omega_w^2(1 - p_w) + \omega_c^2(1 - p_c)}$$

where \hat{z} is the equilibrium trait value, p_c and p_w are the proportions of hatchery-origin broodstock and natural-origin spawners (same as Lynch and O'Hely but using p rather than r), h^2 is the heritability of the trait, θ_c and θ_w are the hatchery and natural optima, σ^2 the variance of the trait, and ω_c and ω_w the width of the selection functions in the two environments. Translated to terms of this equation, what Campton found was that if $(1-p_c) > (1-p_w)$, then \hat{z} was guaranteed to be closer to the natural optimum than to the hatchery optimum. Thus, if the natural optimum was 20 and the hatchery was 10, the equilibrium value would be greater than 15. Thus the interpretation of this being a situation where natural forces dominate really stems from the result that the optimum is on the natural side of the intermediate trait value.

The work presented here began as a need to verify Campton's conclusion, but resulted in a considerable extension of it.

Before proceeding with the analysis, a standardization of terminology is useful. In parallel with the NOR/HOR convention that is now widespread in discussions of hatcheries, we will add the following additional terms: NOS and HOS, to represent natural-origin and hatchery-origin fish spawning in the natural environment; and NOB and HOB, to represent natural-origin and hatchery-origin fish in the broodstock. One final convention: let pNOB be the proportion of natural-origin fish in the broodstock, and pHOS be the proportion of hatchery-origin fish on the spawning grounds. Now

Campton's condition for natural dominance can be expressed as $\frac{pNOB}{pHOS} > 1$.

I wrote a simple FORTRAN program to solve Ford's equation 7 for varying pNOB and pHOS, assuming (as did Ford) a heritability of 0.5, and trait optima of 0 in the hatchery environment and 100 in the natural environment. I modeled both relatively strong and relatively weak selection, exactly as Ford did, by setting σ^2 to 10, and $\omega_c^2 = \omega_w^2 = 100$ for strong selection and $\omega_c^2 = \omega_w^2 = 1000$ for weak selection. I sorted the results for pNOB/pHOS proportion combinations yielding equilibrium trait values close to 50.

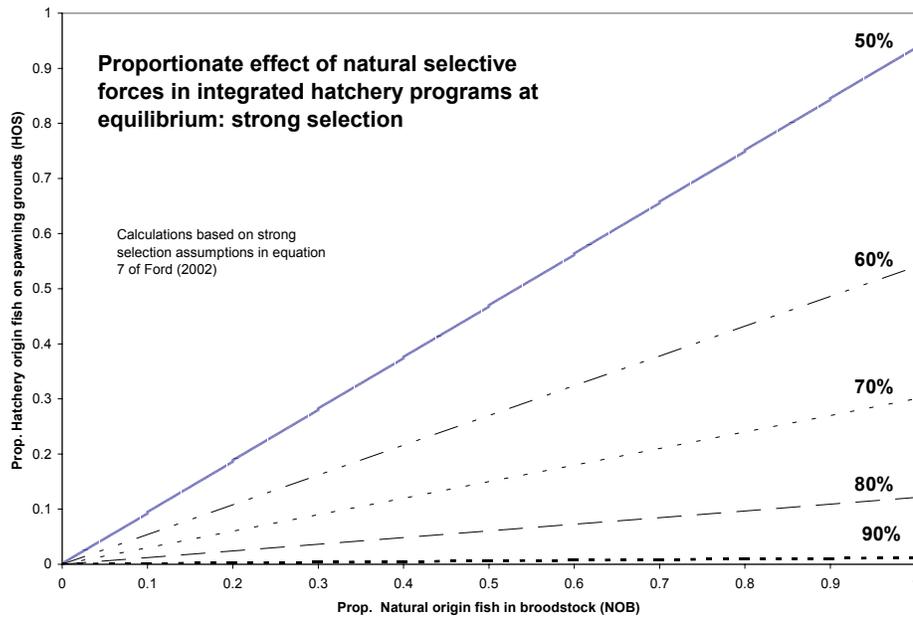
After verifying that values close to 50 were obtained when pNOB = pHOS, I then did a second set of runs, collecting combinations that yielded equilibrium trait values of 60, 70, 80, and 90, which represented higher levels of natural selection dominance. For example, if the equilibrium trait value is 60, it can be argued that the proportionate contribution of natural and artificial selection is 0.6 and 0.4, so natural selection is half again the magnitude of hatchery selection.

Results

In all cases, many combinations of NOB proportions and HOS proportions yielded a give equilibrium trait value, and the relationship differed only trivially from linear. This yields simple isopleths (Fig. 2).

The basic finding of the HSRG that having the pNOB be greater than pHOS will guarantee that natural forces dominate is borne out by the simulations. Equivalency of these two proportions defines a "50%" line for natural influence on the plane of all possible values for pNOB and pHOS. Conditions are somewhat more stringent for the case of strong selection, but this is probably not important at this stage of the discussion. The other isopleths are very interesting, because although the HSRG recommended condition is bounded by the 50% isopleth, it is obvious that the recommendations can be refined to set guidelines for other natural:artificial influence levels. In addition to 50% levels, we can set 60%, 70%, etc.

a)



b)

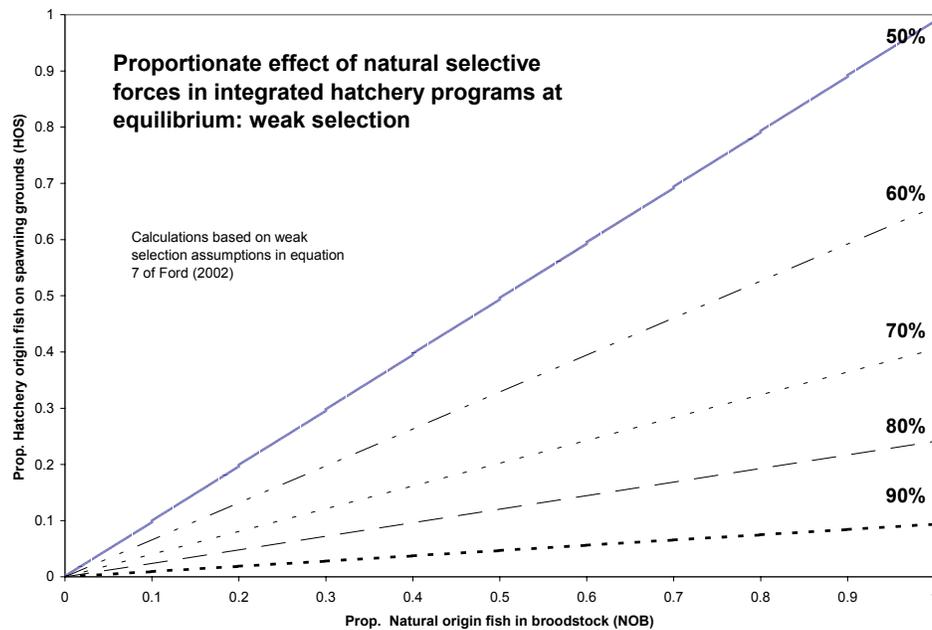


Fig. 2. Isopleths of equilibrium trait values, based on Ford (2002) under selection (expressed as proportion of distance between natural and hatchery trait optima) for varying proportions of natural-origin fish in the broodstock (pNOB) and hatchery-origin fish on the spawning grounds (pHOS). a) strong selection ($\omega^2 = 100, \sigma^2 = 10$); b) weak selection ($\omega^2 = 1000, \sigma^2 = 10$).

Interestingly, the isopleths are strongly related to Lynch and O'Hely's statistic for the percentage of time an allele spends in the natural environment in an integrated hatchery system (T) (Lynch and O'Hely 2001). This statistic is directly related to gene flow patterns among the hatchery and natural subcomponents of the population (p. 366):

$$\omega = \frac{1 - r_c}{2 - r_c - r_w}, \text{ or in our terminology } \omega = \frac{pNOB}{pNOB + PHOS},$$

where r_c is the proportion of the broodstock comprised by hatchery-origin fish, and r_w is the proportion of natural-origin fish on the spawning grounds. The relationship between the NOB proportion and HOS proportion required to generate a particular value for T is virtually the same as the relationship required for a corresponding level of natural influence using Ford's model. More simply stated, the NOB/HOS ratio required to insure a 60% level of natural dominance is almost exactly the same as the ratio required to guarantee an allele spends 60% of its time in the wild. This result makes complete sense, as strength of selection should depend on the proportion of time the population spends in the hatchery environment. This comparison is presented in Table 1 below. The data in the two Ford columns are the isopleth slopes.

Table 1. Ratio of proportion of natural-origin broodstock to proportion hatchery-origin spawners required to insure specified levels of natural influence dominance, compared to ratios required to achieve same level of Lynch and O'Hely's T statistic (proportion of time an allele spends in natural environment). Lynch and O'Hely values are constants; values from Ford are means (relationship is quasi-linear).			
Trait Equilibrium Value Expressed as Percentage of Natural Optimum	Ford Weak Selection	Ford Strong Selection	Corresponding Lynch and O'Hely Values
50%	1.01	1.06	1.0
60%	1.52	1.85	1.5
70%	2.47	3.33	2.33
80%	4.15	8.26	4
90%	10.67	83.3	9

Discussion

This analysis does not tell the entire story. It assumes a particular mode of selection, and considers only equilibrium conditions and not the rate of approach to them. Most importantly, it considers equilibrium trait value and not fitness. All these considerations are topics for future work. Taking these issues into account, however, the analysis still suggests that the pNOB/pHOS ratio and the balance of natural and artificial selective forces the ratio indicates in calculations based on Ford (2002) is a powerful tool for comparing the relative risk of various programs. Clearly, programs with high pNOB/pHOS ratios will be expected to have a lower domestication impact than programs with low ratios.

Hatchery-origin jacks began returning from the Yakima spring chinook program since 2000, and adults since 2001 (YKFP 2004). Table 2 below summarizes p HOS and pNOB/pHOS for the program for 2000-2003:

Table 2. Summary of pHOS and pNOB/pHOS for the Yakima spring chinook supplementation program for 2001-2003, based on Table 2 of YKFP(2004)				
Year	Adults Only		Adults and Jacks	
	pHOS	pNOB/pHOS	pHOS	pNOB/pHOS
2001	0.58	1.73	0.60	1.67
2002	0.77	1.30	0.76	1.31
2003	0.72	1.38	0.66	1.52

Comparing these numbers with the values in Table 1, or plotting on Fig. 2 (pNOB = 1.0), we see that so far the project is operating in the “properly integrated” region of the possible hatchery-natural influence state space, with a natural: hatchery influence ratio of about 55%:45%. Traditional hatchery programs typically have a high pHOS, but very low pNOB, so the natural:hatchery influence will be close to 0. What we can say about the Yakima supplementation is that at equilibrium traits will have changed somewhat less than half as much as they would have with a more traditional program. For purposes of the domestication study, the S line should be changing somewhat less than half as much as the HC line.

This analysis provides for the first time some insight into the importance of the 100% wild broodstock rule we use in the project. In theory any project can be run to a specified pNOB and pHOS within a state space determined by several demographic parameters (Busack, unpubl.). To actually run a project to a specified pNOB and pHOS requires perfect identification of fish as to origin and complete control over the hatchery-origin/natural-origin composition of broodstock and natural spawners, including the ability to remove as many hatchery-origin fish from the escapement as needed. In many places neither condition holds, but the first condition holds in several places, among them the Yakima spring chinook supplementation program. In this program we have chosen to use 100% natural-origin broodstock (pNOB=1.0), but have made no attempt to restrict spawning by hatchery-origin fish (pHOS). Fig. 2 shows quite clearly how powerful this approach is. With a pNOB of 1.0, we are guaranteed to be in the “properly integrated” region, where natural selective forces is equal to or greater than the hatchery selective forces.

Fig. 2 also shows, however, the advantage of restricting the proportion of hatchery-origin fish on the spawning grounds. By continuing with 100% natural-origin broodstock and limiting pHOS, we could achieve even higher levels of natural influence.

The current analysis is quite illuminating, but it is not the full story. For one thing, possible reproductive fitness deficits of hatchery-origin fish in the wild have not been included. The effect of this would be to make the real pHOS lower than the apparent pHOS. There are three larger issues as well, all currently under study:

1) the analysis does not directly say anything about fitness based on the trait. Fifty percent natural influence does not guarantee 50% fitness. The relationship between equilibrium trait values and fitness is non-linear and depends on strength of selection and the distance between natural and hatchery trait optima (see Ford’s equation 3). Under

strong selection and a large distance between optima, the relative fitness at a given level of natural influence may be lower than the proportion of natural influence, whereas with weak selection and small distance between optima, the equilibrium fitness may be higher. Also, the model is of a single trait. Total fitness loss will be multiplicative over all fitness-related traits that have undergone domestication.

2) the analysis is based on trait equilibria. Possibly equilibrium is achieved after such a long time that it is relevant, and what should be focused on is the speed at which change occurs.

3) the analysis is based on stabilizing selection. This is a very reasonable approach, as strong directional selection does not appear to common in nature (Kingsolver et al. 2001). Nevertheless directional selection should be explored.

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Chapter 3

Initial Morphometric Analysis of Upper Yakima and Naches Adult Spring Chinook: Progress on Trait A11 of Domestication Monitoring Plan

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Introduction

Hatchery and natural environments differ a great deal. Compared to their wild counterparts, hatchery juveniles experience much higher fish densities and a much less complex environment. Differences in spawning environment may also be quite important. Natural spawners must select redd sites, dig redds, attract and compete for mates, engage in courtship, deposit gametes, and guard them. Broodstock need do none of these things. Sexual selection, the genetic result of competition for mates, is a widespread phenomenon in animals and is considered to be an important evolutionary force. It can be inferred both from the obvious sexual dimorphisms observed in salmon and steelhead and from the research done on reproductive success (e.g., Fleming and Gross 1989) that this is also an important phenomenon in salmon, but obviously one which cannot take place in the hatchery environment. The multitude of ways in which hatchery environments may exert considerable selective pressures that differ from the natural environments suggests that domestication may involve large physiological changes, and it would not be unexpected that these changes would be manifested morphologically. In addition, the total relaxation in the hatchery environment from selective pressures ((Lynch and O'Hely 2001) related to spawning competence could easily cause changes in aspects of morphology that are critical to reproductive success in the wild.

There is a substantial literature on morphological differences between natural-origin and hatchery-origin fish in salmonids (see Swain et al. 1991 and Hard et al. 2000), particularly in coho salmon. In coho natural-origin and hatchery-origin fish differ at both the juvenile and adult life stage, and Swain et al. (1991) argue that the differences are predicted by obvious differences between the two types of environment. Their basic argument is that fish with deep bodies and large median fins are more capable of burst swimming and agonistic behaviors, characteristics that are important in stream life both as juveniles and during reproduction. Fish with more fusiform bodies are better suited to sustained swimming. In nature sustained swimming is important for migration, but the hatchery environment probably selects strongly for sustained swimming and selects against burst swimming (and possibly agonism). These conjectures are based on observations of natural populations. For example, in British Columbia, coho from interior, lake-rearing populations are more fusiform than coho from coastal, stream-

rearing populations ((Taylor and McPhail 1985a). These differences do correlate well with swimming performance (Taylor and McPhail 1985b). It was also established with lab experiments that these differences were at least in part genetic (Taylor and McPhail 1985b). Swain and Holtby (1989) found that in a single drainage, lake-rearing juvenile coho were more less colorful, more fusiform, and differed in pectoral fin placement from stream-rearing juveniles. They were also less aggressive.

The results from comparisons of natural-origin and hatchery-origin coho largely bears out the expectations. In a comparison of several wild and hatchery populations, Taylor (1986) found hatchery juveniles more streamlined and possessing smaller fins than wild fish, and also noted that among-population variability was lower in the hatchery populations than in the wild. In comparing adult wild and hatchery coho females at several traits Fleming and Gross (1989) found hatchery females to be smaller and more streamlined than wild females, and also to have smaller kypes. How much of these differences are attributable to true genetic differences rather than to phenotypic plasticity responding to the two environments is open to question, however. Swain et al. (1991) compared fish of wild and hatchery origin reared in the same environment and found that genetic differences were considerably smaller than those induced by the environment. Hard et al. (1999) found that morphology in juvenile chinook was under strong genetic control. The importance of environment was demonstrated dramatically in juvenile brown trout and Atlantic salmon by Pakkasmaa and Piironen(2000), who found strong responses in body depth and finnage in a month. Interestingly, they found the two species responded differently, with salmon becoming more robust in faster water and brown trout becoming more streamlined.

The morphological literature presents an intriguing picture. There are definite expectations based on the coho work of what the response due to domestication should be if morphology is under significant genetic control. Yet little work has been done apart from the coho work, and there is evidence that species may differ in their response (phenotypically at least) to hatchery environments. Another issue is the degree of genetic control of morphology. If morphology is closely related to survival, it is important to evaluate both the genetic and environmental effects of hatchery rearing on it.

Here we report on the first attempt to characterize morphological variation in Yakima basin spring chinook, a comparison of 2003 natural-origin spawners from the Upper Yakima population, 2003 hatchery-origin spawners from the Upper Yakima population, and 2003 Naches spawners. In the terminology of the domestication monitoring plan, this is the beginning of work on trait A11, a comparison of the S, HC, and WC lines.

Materials and Methods

In 2003, all spawners at CESRF, and some surplus jacks and males, were photographed with a digital camera immediately after they were killed and before they were spawned. Photographs were done on a light stand with camera in a fixed position, with fins pinned out to make landmarks more visible. In addition, a pin was placed at the base of the

skull, another landmark. A meter stick or measuring tape was included in each picture to provide a scale for the digitized measurements that were to be made later. The same process was conducted throughout the spawning season on the Naches spawning ground using a portable platform and a unipod to standardize the distance from camera to specimen. The Naches operation differed significantly from the CESRF operation in specimen quality. Fresh, unspawned fish were photographed at CESRF, but on the Naches all fish were spawned out, and many had been dead for many days. Any carcass encountered that was likely to yield a clear view of the landmarks, regardless of age, was photographed. In many cases, at both CESRF and on the Naches, multiple photographs were made of a single specimen.

The resulting .jpeg photo files were scanned for quality and then compiled into files for analysis, one per specimen, using the TPSUTIL program.¹ The files created by TPSUTIL are .tps format, which is required for data acquisition and analysis by other programs in F.J. Rohlf's TPS (thin-plate spline) series. Combined and sex-specific files were created for hatchery-origin (HO) spawners at CESRF that were being used to found the hatchery-control line (HC), natural-origin (NO) spawners from the supplementation line (S), and Naches spawners. The resulting number of specimens available for analysis is shown below in Table 1.

Images were digitized on a desktop computer using program TPSDIG². Digitizing basically involves placing the clicking cursor on a series of body-shape landmarks to create a series of coordinates representing fish's shape in the XY-plane. We used virtually the same 13 landmarks as Hard et al. (2000): 1) tip of snout, 2) base of skull, 3) anterior insertion of dorsal, 4) posterior insertion of dorsal, 5) anterior insertion of adipose, 6) dorsal insertion of caudal, 7) posterior tip of body (junction of posterior-most point of body and caudal), 8) ventral insertion of caudal, 9) posterior insertion of anal, 10) anterior insertion of anal, 11) anterior insertion of pelvic, 12) anterior insertion of pectoral, and 13) posterior tip of maxillary. Our landmarks 12 and 13 differed from those of Hard et al. (2000) in that where they used the body edge directly below the fin insertion, we used the actual point of fin insertion. All digitizing was done by Germaine Hart.

	U. Yakima Natural Origin	U. Yakima Hatchery Origin	Naches
Females	179	48	27
Males	77	32	11
Jacks	26	17	4

¹ Rohlf, F.J. 2004. TPSUTIL, version 1.28. Program for data file creation and editing. Available from Department of Ecology and Evolution, State University of New York at Stony Brook through <http://life.bio.sunysb.edu/morph>.

² Rohlf, F.J. 2004. TPSDIG, version 1.40. Program for digitizing images for thin-plate spline analysis. Available from Department of Ecology and Evolution, State University of New York at Stony Brook through <http://life.bio.sunysb.edu/morph>.

The basic approach we have taken for morphometric analysis is thin-plate spline analysis ((Bookstein 1991). This technique is mathematically complex, and abounds in confusing terminology, but a fairly understandable description of the application to salmon shapes is found in Hard et al. ((2000). Thin-plate spline analysis has become the predominant method of morphometric analysis, as it deals much better with shape allometry due to size than the previous widespread method of sheared principal components (Parsons et al. 2003). The thin-plate spline approach that considers decomposes over deviations of the individual shapes from a group consensus shape into a series of arrays. These arrays represent two-dimensional planes, one a flat XY plane, but the others distorted by bending into a third dimension. These arrays are called partial warps. N landmarks will generate $2N-4$ partial warps ($N-2$ x,y pairs) (Bookstein 1991); thus the present analysis resulted in 22 partial warps. The partial warps can then be used as vectors for multivariate analysis such as principal components, canonical variates, and discriminant analysis (see Hard et al. (2000) for examples). We used program TPSRELW³ to generate consensus shapes, partial warps, and relative warps. Because males and females differ obviously in shape at spawning, we analyzed males and females separately. We also examined variation among fish from the three source populations (U. Yakima NO. U. Yakima HO, and Naches) and between jacks and 4-year old males. This examination was done by exploration of consensus shapes and by canonical variate analysis (CVA).

Results

Landmarks and Relative Warps

Obviously, depending on the form of variation, and the way the fish is positioned, the landmarks will vary in how they contribute to overall morphological variation. Fig. 1 shows the relative contribution of the 13 landmarks for both females and males. The pattern was similarly for both sexes, with the caudal peduncle being most important (landmarks 5,6,8,and 9), and the dorsal peduncle (5 and 6) being less important than the ventral (8 and 9). The posterior end of the body (landmark 7) was not a great contributor. There was little variation importance between the hatchery-origin and natural-origin upper Yakima fish. The Naches fish differed somewhat from the U. Yakima fish in that in both sexes landmark 7 was more important. The meaning of this is unclear at this point, as any consideration of differences in shape between the U. Yakima specimens and the Naches specimens could to some extent be an artifact caused by the Naches fish being days-old carcasses whereas the U. Yakima fish were very fresh. There was no obvious difference between the relative significance of landmarks in jacks and all males, but stronger comparison of adult males vs. jacks has not been done.

The relative warps analysis showed that about half the variation in the partial warps could be represented by the first two relative warps (Fig. 2); this was true of both sexes. Naches males appear to be an exception, but this may just be due to the fact that only 14

³ Rohlf, F.J. 2004. TPSRELW, version 1.39. Program for thin-plate spline analysis of digitized landmark data to produce relative warps. Available from Department of Ecology and Evolution, State University of New York at Stony Brook through <http://life.bio.sunysb.edu/morph>.

relative warps could be derived for them. Apparently the number of relative warps cannot exceed $N-1$, where N is the sample size. The approximately 50% contribution by the first two warps is line with the value Hard et al. (2000) found for adult coho. Plotting the specimens by scores at relative warps 1 and 2 on scattergrams (Fig. 3) shows quite well that these two composite measures do not do a good job of discriminating the hatchery-origin and natural-origin U. Yakima fish. Naches fish, especially females, tend to cluster somewhat separately from the U. Yakima fish. There was no clear pattern of variation between jacks and 4-year old males, but this was not examined in detail due to the small sample sizes (e.g., 4 for Naches).

Consensus Shapes

The RELW program allows exploration of how deviation at relative warps deforms the consensus fish shape by keeping the consensus landmark values in place but distorting the grid on which they are placed, a method introduced by Thompson (1917). Figs. 4a and 4b show the impact of deviations in the four quadrants of the cartesian plane : positive(+) relwarp1, + relwarp2; + relwarp1, negative (-) relwarp 2; - relwarp1, + relwarp2; and - relwarp1, -relwarp2. The diagonal deformations (e.g. upper left [-,+], lower right[+,-]) show the same type of shape change. There are undoubtedly subtle differences here, but the only visually obvious ones in females (Fig. 4a) are body depth ([-,+],[+,-]) and mid-body twisting ([+,+],[,-,-]). The male patterns are more difficult. Males show the same mid-body depth deformation as the two warps increase or decrease together, but deformations in the other axis appear to be related to depth over the entire body.

Consensus shapes derived from separate warp analysis are shown for the three groups and two sexes are shown in Fig. 5. Natural-origin and hatchery-origin U. Yakima females differ subtly in several areas, and appear differ only slightly in body depth, with hatchery-origin fish possibly being thinner. The Naches consensus shape is definitely shallower in the mid-body but also appears to be narrower over the entire body. In males the consensus shape for the natural-origin fish appears deeper than the hatchery-origin fish. Comparisons between the U. Yakima shape and the Naches shape are difficult because of the differing orientation of the Naches shape, but it does not appear to have a shallower body. The head seems somewhat narrower, however. Obviously a more complete analysis could be done by measuring the distances between landmarks.

Canonical Variate Analysis

Subtle though the differences in shape appear to be between the three groups of fish, canonical variate analysis of the partial warps shows high discriminatory power. Figs. 6a and 6b are scattergram plots of the specimens at the first two canonical variates. There is remarkable consistency between presentations for the two sexes. In both cases, the Naches fish are intermediate between the natural-origin and hatchery-origin U. Yakima fish at CV 2 and considerably lower in CV 1 score. There is no overlap between the Naches and U. Yakima specimens for either sex. Using canonical variates as a discriminant function to classify specimens as to group was very effective. Female specimens classified with overall 93% accuracy, with only one incorrect classification of

an U. Yakima fish as a Naches fish, and no incorrect classification of a Naches fish as an U. Yakima fish. The largest misclassification category was natural-origin fish being classified as hatchery-origin. The jackknifed results, simulating sampling error, had an overall classification accuracy of 91%.

Table 2a. Classification of spring chinook females by canonical variates				
Group	Classification			Percent Correct
	U. Yakima NO	U. Yakima HO	Naches	
U. Yakima NO	163	15	1	91
U. Yakima HO	2	46	0	96
Naches	0	0	27	100
Total	165	61	28	93

Table 2b. Jackknifed classification of spring chinook females by canonical variates				
Group	Classification			Percent Correct
	U. Yakima NO	U. Yakima HO	Naches	
U. Yakima NO	161	17	1	90
U. Yakima HO	5	43	0	90
Naches	0	0	27	100
Total	166	60	28	91

Classification of males (Tables 3a,b) was nearly as successful as that of females, with an overall classification success rate of 90%. The same pattern in misclassification held: discrimination was very good between U. Yakima and Naches, and natural-origin U. Yakima fish were more often misclassified as hatchery-origin than the reverse.

We finally investigated classification via canonical variates for 4-year old males only (Tables 4a,b). The pattern of misclassification did not change, but the classification success rate improved to 94% (88% jackknifed). This suggests that jacks vary less across groups than 4-year old males.

Table 3a. Classification of spring chinook males (4-yr olds and jacks) by canonical variates				
	Classification			
Group	U. Yakima NO	U. Yakima HO	Naches	Percent Correct
U. Yakima NO	99	14	0	88
U. Yakima HO	2	47	0	96
Naches	1	1	13	87
Total	102	62	13	90

Table 3b. Jackknifed classification of spring chinook males (4-yr olds and jacks) by canonical variates				
	Classification			
Group	U. Yakima NO	U. Yakima HO	Naches	Percent Correct
U. Yakima NO	95	18	0	84
U. Yakima HO	7	42	0	86
Naches	1	1	13	87
Total	103	61	13	85

Table 4a. Classification of spring chinook males (4-yr olds only) by canonical variates				
	Classification			
Group	U. Yakima NO	U. Yakima HO	Naches	Percent Correct
U. Yakima NO	80	7	0	92
U. Yakima HO	1	31	0	97
Naches	0	0	11	100
Total	81	38	11	94

Table 4b. Jackknifed classification of spring chinook males (4-yr olds only) by canonical variates				
Group	Classification			Percent Correct
	U. Yakima NO	U. Yakima HO	Naches	
U. Yakima NO	77	10	0	89
U. Yakima HO	3	29	0	91
Naches	1	1	9	82
Total	103	61	13	88

Discussion

The work reported here is obviously preliminary. We have just begun doing this type of work, and there is much more to be done in terms of analysis. The differences we have seen between hatchery-origin and natural-origin fish need to be characterized in a less mathematical and more biologically understandable way, a constant challenge with this type of analysis. Comparisons of between-landmark distances need to be explored and used to supplement the inferences based on the thin-plate spline analysis.

The differences observed between the Naches and U. Yakima populations are interesting, but need to be regarded as quite preliminary. The Naches carcasses were usually several days old when photographed, and if body shape changed following death, the comparison of Naches and U. Yakima could be biased. To test this, we need to photograph some U. Yakima carcasses of comparable condition on the spawning grounds. Another possible concern is the small sample size for Naches. However, given the environmental differences between the Naches and Upper Yakima systems, and all the other differences we have noted between the two populations, and the body of literature on among-population morphological variation in coho, it would certainly not be unexpected that the two populations would differ morphologically.

The observation that the U. Yakima hatchery-origin and natural-origin fish differ morphologically is also not surprising, given the other studies of hatchery-wild differences cited earlier. It is exciting, however, that we were able to see a difference in a single year's sampling, with the limited sampling opportunities we have for hatchery-origin fish. The challenges now, aside from being able to better describe the differences we have found, are to evaluate among-year variation and to determine how much of the difference is genetic. Measurements on the next generation of HC line returnees will be critical for this last step.

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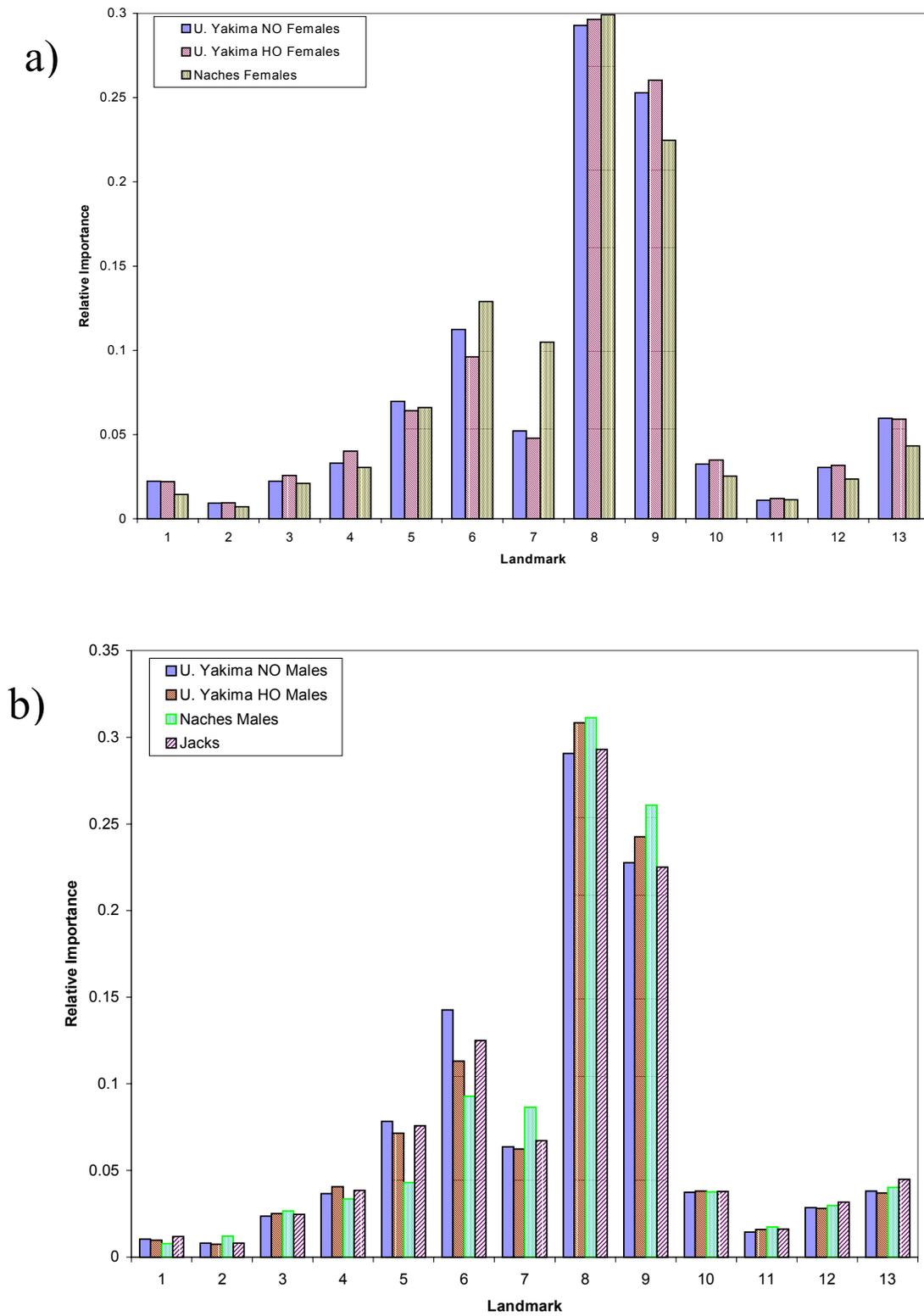


Fig. 1. Relative contribution of the 13 body-shape landmarks to morphometric variation in a) female, and b) male spring chinook.

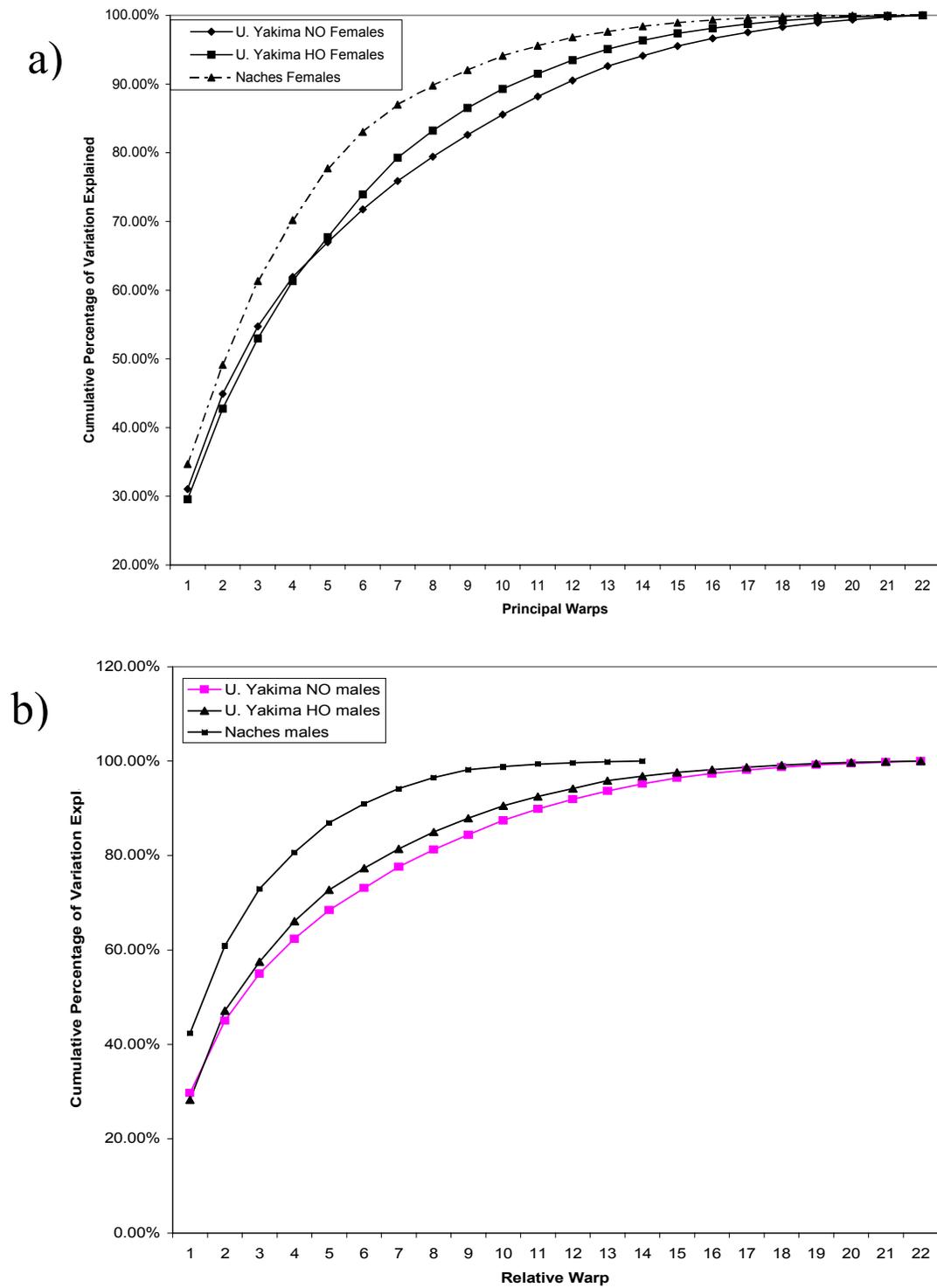


Fig. 2 Cumulative proportion of morphometric variation in a) female and b) male spring chinook captured by principal warps. The first two principal warps account for between 45 and 49% of the shape variation in females and 45 and 60% of the shape variation in males (45-47 if Naches males, which were only allowed 14 warps, are excluded).

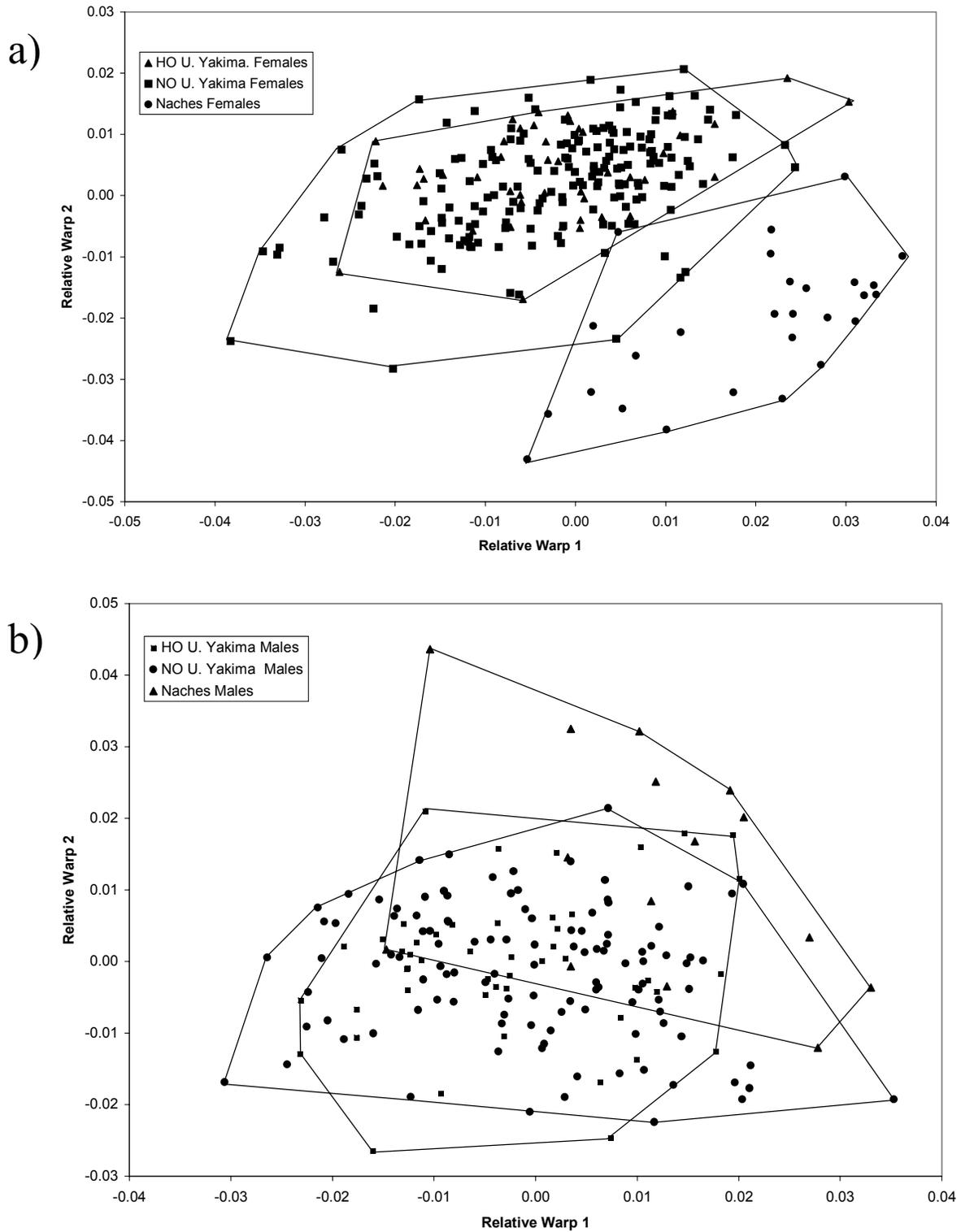


Fig. 3. Individual ordination of a) male and b) female spring chinook in the study by scores at relative warps 1 and 2. Minimum spanning convex polygons have been drawn around each group to illustrate group differences.

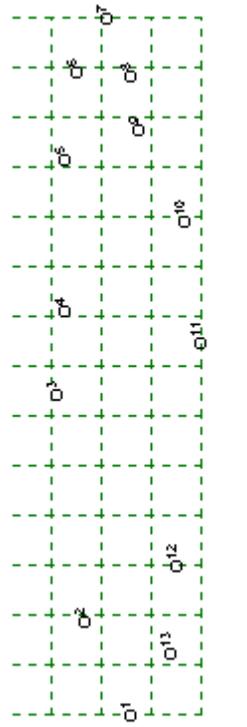


Fig. 4a. Female consensus shape (center) and deformations associated with relative warps 1 and 2. For each figure, deformed shape was based on specimen farthest from origin in each rel warp 1, rel warp 2 quadrant).

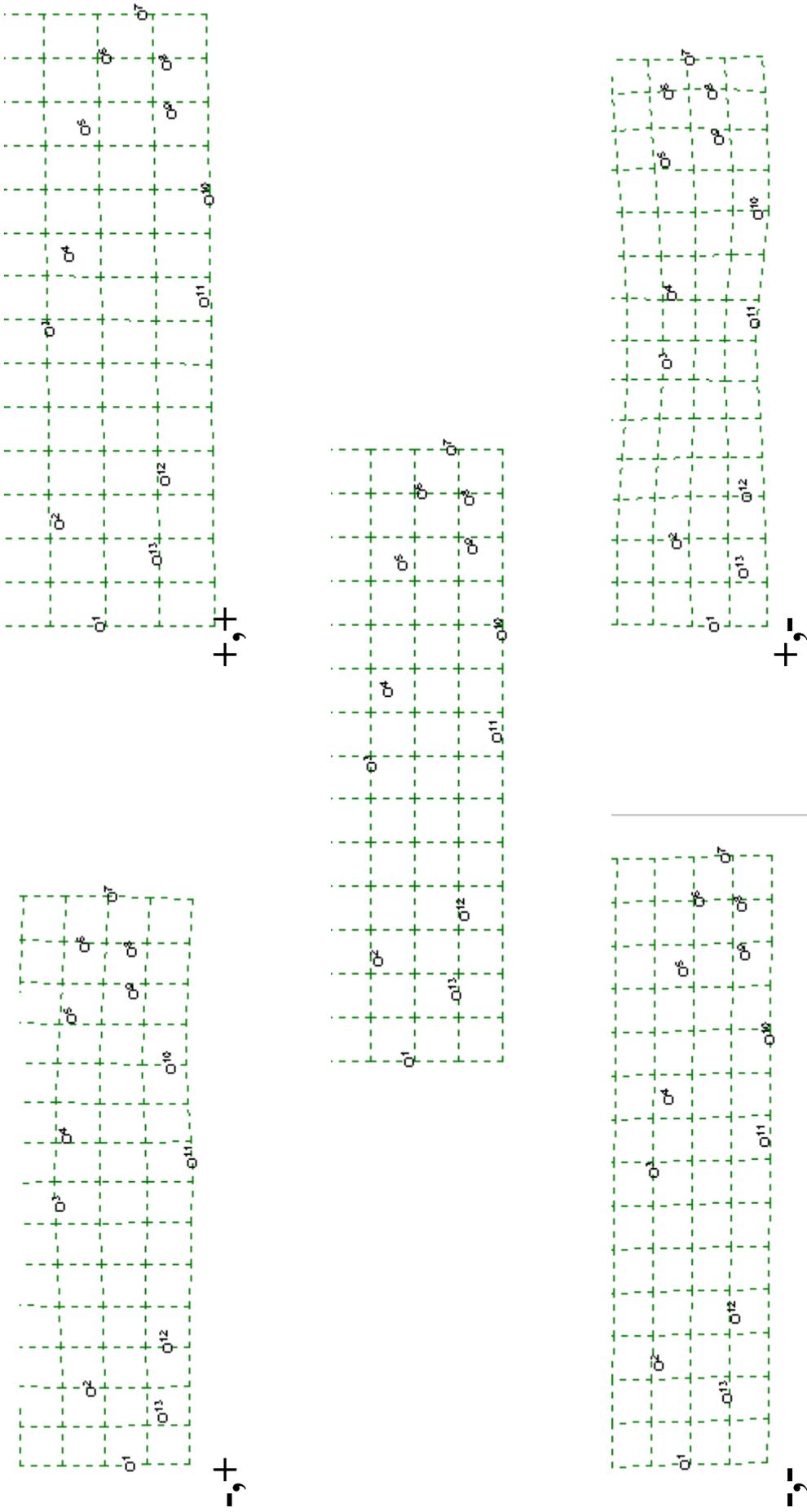
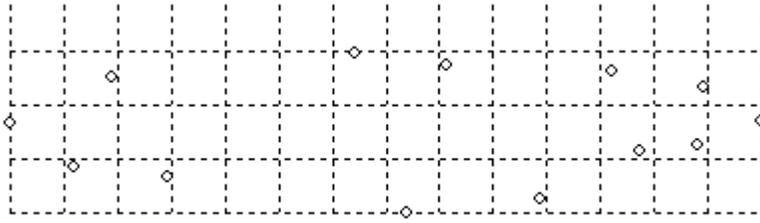
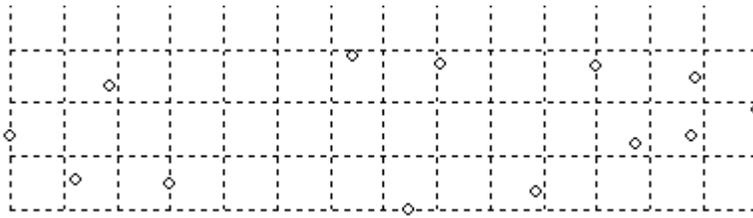


Fig. 4b. Male consensus shape (center) and deformations associated with relative warps 1 and 2. For each figure, deformed shape was based on specimen farthest from origin in each rel warp 1, rel warp 2 quadrant).

U. Yakima NO Females



U. Yakima HO Females



Naches Females

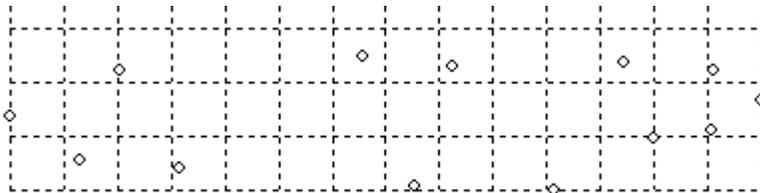
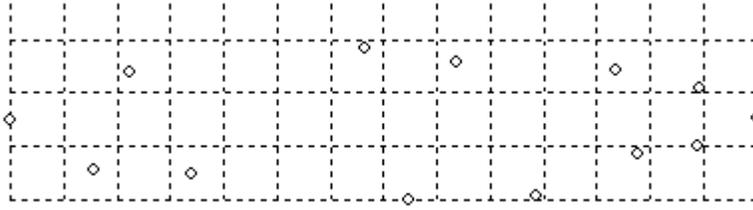
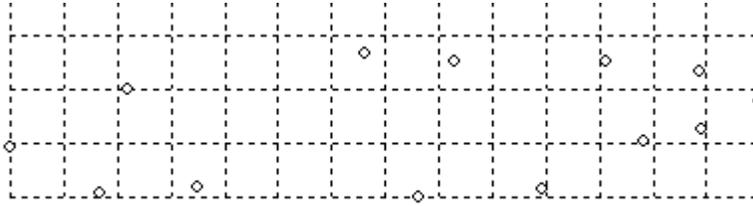


Fig. 5a. Consensus shapes of female Upper Yakima natural-origin (NO), Upper Yakima hatchery-origin (HO), and Naches spring chinook.

U. Yakima NO males



U. Yakima HO males



Naches males

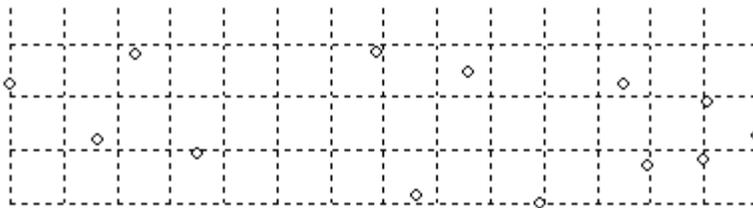


Figure 5b. Consensus shapes of male Upper Yakima natural-origin (NO), Upper Yakima hatchery-origin (HO), and Naches spring chinook.

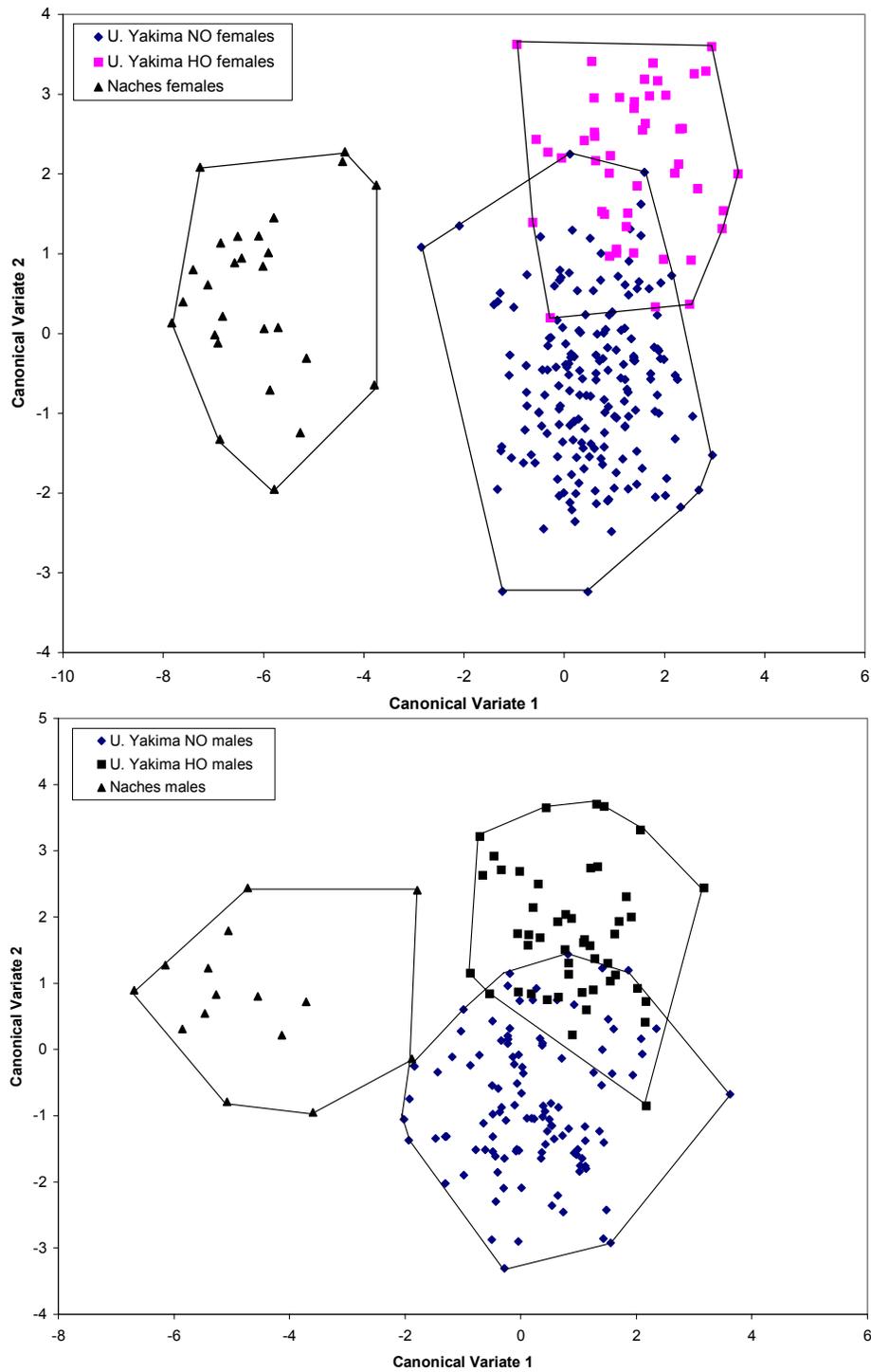


Fig. 6. Scattergram based on canonical variate analysis of partial warps 1 and 2 for a) female and b) male spring chinook .

Chapter 4

Further Development of Spring Chinook Domestication Monitoring Plan

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Introduction

In 2001 radical changes were proposed to domestication monitoring in the YKFP spring chinook program in response to comments from the Independent Scientific Review Panel (ISRP) (Busack et al. 2002). These changes culminated in a plan to use the Naches population as a wild control (WC) line, and to create a hatchery control (HC) line from the Upper Yakima population. The HC line was to be handled exactly the same as the normal supplementation production (now called the S line), except that it was to be completely closed, and not allowed to spawn in the wild (Busack et al. 2003). In attempting to implement the new domestication monitoring plan, problems arose in 2003 with implementing both the WC and the HC line.

Implementing Monitoring with the WC Line

The basic plan for using the Naches population was to use adults in the basin whenever possible, but for reproductive traits and for several juvenile traits, to spawn small numbers of Naches adults and take their gametes to CESRF for early development and rearing of fry. To minimize the impact on the population, we planned to use partially spawned adult females captured on the spawning grounds. This plan conflicted with fish health concerns, as the project pathologist was unwilling to allow Naches gametes at CESRF unless they were certified disease free. This requirement necessitated two important changes to the plan for using Naches fish. First, the disease screening requires lethal sampling, so the plan to use partially spawned fish that would then be returned to the river had to be scrapped. We decided to sacrifice and spawn the fish and then return excess fertilized eggs to the river for incubation using egg boxes.

The second and more disruptive change the fish health concerns caused was a need for rearing Naches eggs at a location other than CESRF until the adults could be certified disease free. The site decided upon was the YKFP Nelson Springs facility. This was good site for incubation in that the incubation system could be easily monitored, but had the disadvantage of high temperatures and low flow. The solution, developed by Steve Schroder, Curt Knudsen and Gene Sanborn, was a system of chillers to reduce water temperatures and misting boxes to keep eggs moist with minimal water expenditure. The

water supply was also fitted with a 1: filter to reduce the risk of *Saprolegnia* infection and to keep particulates out of the misting system. The system was tested using chum eggs in October 2003 at WDFW's George Adams Hatchery in Puget Sound. The fertilized eggs of four female chum were mixed and split into two groups, one incubated in Heath trays and one incubated in the misting system. Survival was very high (~99%) in both incubation environments, with no significant difference between them. The system was also tested on site at Nelson Springs to make sure that temperatures could be adequately regulated.

Because of the unforeseen need for the fish health precautions and resultant engineering, eggs were not taken from Naches fish in 2003, but at present it appears that Naches sampling can be done in 2004.

Issues with HC Line Precocious Males

Fundamental to the domestication monitoring design is isolation between the WC, HC, and S lines. WC and S isolation is achieved by the two populations spawning in different rivers. The HC and S situation is a bit more complicated, as they inhabit the same river. Here isolation is achieved by allowing the HC line to spawn only in the hatchery. No HC fish are allowed to pass above Roza. All HC returnees are either used for HC spawning at CESRF, or are surplus (Busack et al. 2003). There is a potential problem with HC precocious males spawning in the wild with S-line fish, however. Gene flow in this manner between the two lines could make the lines less distinct, handicapping comparisons between them of how much less domesticating the supplementation environment is than the "pure" hatchery environment. Perhaps more importantly, it could also make the supplementation environment look more domesticating than it really is, a very undesirable outcome.

We attempted to assess the potential impact of precocious HC-line males spawning in the wild in last year's report (Busack and Knudsen 2003b). The approach we used was power analysis. Predictably, as the proportionate contribution of precocious HC males increased, the power to detect WC-S differences increased and the power to detect HC-S differences decreased because the S line was becoming more domesticated than it otherwise would have. This analysis was somewhat unsatisfactory, however, in that it only addressed power to detect differences, not the magnitude of the actual differences. A new approach was needed, and that work was undertaken in 2003.

Understanding how precocious HC-line males spawning in the wild with S-line females may affect the domestication research is complex. There are two key steps: 1) what is the potential genetic contribution to be expected from a given spawning event; and 2) for a given genetic contribution, how much will it affect HC-S and S-WC comparisons.

1. What is the potential genetic contribution of precocious HC-line males?

The genetic contribution depends on how many of them are on the spawning grounds, how successful they are relative to other males, and how many adult males there are on the spawning grounds. Our best information (Pearsons et al. 2003) is that the HC line can be expected to produce 0.233 precocious males on the spawning grounds per HC-line female spawned in the hatchery. Because we expect to spawn ~36 HC females, we would expect ~8 precocious males on the spawning grounds. Our best estimate of relative reproductive success (Schroder et al. 2003) is 0.28; i.e., a precocious male is 28% as effective as an adult male. Table 1 shows the expected genetic contribution to the S line from precocious males for varying numbers of natural spawners. In addition to the 0.233 and 0.28 values, higher values for both rate of precocious male production and precocious success rate were used to illustrate worse case scenarios. For expected and larger than expected natural spawning events, the impact was generally a fraction of a percent. Only for very low population sizes and much higher than observed precocious

Table 1. Expected genetic contribution (%) to S line from precocious HC line males spawning in wild for different rates of precocial production and levels of reproductive success relative to full-sized males. Observed precocious production rate is 0.233, observed precocious reproductive success is 0.28.					
Naturally Spawning Pairs	Precocious production per female spawned/precocious relative reproductive success				
	0.233/0.28	0.233/0.5	0.5/0.28	0.5/0.5	1.0/1.0
150	0.54%	0.96%	0.85%	1.51%	4.04%
250	0.37%	0.66%	0.63%	1.13%	3.30%
500	0.21%	0.37%	0.39%	0.69%	2.26%
750	0.14%	0.26%	0.28%	0.50%	1.72%
1000	0.11%	0.20%	0.22%	0.39%	1.39%
2000	0.06%	0.10%	0.12%	0.21%	0.78%
3000	0.04%	0.07%	0.08%	0.14%	0.55%
4000	0.03%	0.05%	0.06%	0.11%	0.42%
5000	0.02%	0.04%	0.05%	0.09%	0.35%

production rates and reproductive success rates was the genetic contribution above 2%.

This analysis does not tell the entire story. Because of the unique nature of the Yakima spring chinook supplementation effort, the contribution will be alternatively magnified as wild fish are used as broodstock, and then “demagnified” as they spawn in the wild. We modeled accumulation of genetic impacts under this regime and found that the long-term accumulation of genetic impacts is somewhat less than the annual accumulation. Thus, if precocious males contribute 0.2% per year, the long-term accumulation will be less than 0.2% per generation.

2. How much will a given level of gene flow from the HC line to the S line from precocious males affect S-HC and S-WC comparisons?

We considered this only for WC-S comparisons, assuming that the general result would hold for HC-S comparisons.

We modeled three levels of domestication in the S line- 1%, 2%, and 5% per generation, three levels of HC line domestication relative to the S line- 1.5, 3, and 5- and gene flow rates from the HC to S line of 2% per generation. Table 2 below presents the percentage of the difference between the WC and S lines relative to the starting state due to gene flow from precocials. This table is easy to misinterpret. If the difference between the WC and S lines, for example, is 10 cm, and the table entry is 2.5, this means that 2.5% of the 10 cm difference- 0.25 cm- was caused by the gene flow. This table represents a near-worst case scenario, as a gene flow rate of 2% is much larger than expected (Table

Table 2. Percentage of expected difference over generations between S and WC lines due to gene flow from HC precocious males for a precocious contribution rate of 2% for differing levels of S-line domestication (g) and domestication of HC line relative to S line (1.5, 3, and 5).

Gens	g=1%			g=2%			g=5%		
	Relative Domestication of HC			Relative Domestication of HC			Relative Domestication of HC		
	1.5	3	5	1.5	3	5	1.5	3	5
1	1.5	2.9	4.8	1.5	2.9	4.8	1.5	2.9	4.8
2	1.7	3.9	6.6	1.7	3.9	6.7	1.8	4.0	7.0
3	2.0	4.8	8.5	2.0	4.9	8.7	2.1	5.2	9.5
4	2.2	5.8	10.3	2.3	5.9	10.8	2.4	6.5	12.2
5	2.5	6.7	12.1	2.5	7.0	12.8	2.7	7.8	15.2

1). The effects are quite small except for very high levels of HC line domestication. The effects do build up over generations, but after several generations they are often still quite small. The tabulated results do show, however, that if domestication is very high in the HC line, the accumulation rate of genetic impact in the S line with a 2% gene flow rate can be significant.

We conclude from these analyses that 1) gene flow from HC precocious males is likely to be quite low, and 2) even at gene flow rates substantially larger than expected, the accumulated genetic impact over the first few generations poses little threat to the integrity of the S-HC and S-WC comparisons. There seems to be little risk to the monitoring effort from precocious HC males. However, we should monitor the situation with precocious males carefully, and be prepared to reconsider the HC line should precocious gene flow rates be much higher than expected.

Based on this analysis, MIPT recommended in May 2003 to proceed with the HC line, but to do so with the above mentioned cautions.

Other Aspects of Domestication Research/Monitoring Plan Development

We began last year in a systematic attempt to do power analyses for all traits in the domestication monitoring plan (Busack and Knudsen 2003a). The concerns with the Naches fish and precocious HC fish, and actual implementation of several domestication

monitoring measures precluded doing further power analysis work this year. Implementation of several monitoring measures allowed considerable refinement of some of the trait-specific aspects of the domestication research/monitoring plan. We also revised the plan to incorporate a better designation system for the various types of fish involved in the individual trait comparisons. The new system is fully explained in the revised version of the plan, which is attached as an appendix.

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Appendix
Draft Design for Domestication Monitoring in the Yakima Spring Chinook
Supplementation Program

Design for Domestication Monitoring of the Yakima Spring Chinook Supplementation Program

Yakima/Klickitat Fisheries Project Monitoring Implementation Planning Team

Revised May 4, 2004

Introduction

We propose 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 HC line an effective size of at least 100 per generation. A larger line of HC fish was deemed to be politically untenable because of the large number of fish that would potentially have to be removed at Roza Dam. 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.

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.

This is a living document. Our goal in this document is to provide a short but reasonably comprehensive write-up on each trait so that a reader can quickly grasp what we are doing, how and why we are doing it, and what our experimental power is. Write-ups for the individual traits vary widely in how closely they have achieved this. Although some have been extensively developed, at this point the write-up for no trait is as complete as intended. Some write-ups date back to the last comprehensive evaluation of the plan by MIPT, on 11/07/2002; others have been very recently revised. Dates on which substantive changes are made in protocol, power, or analytical approach have been made are noted in the individual write-ups.

Nomenclature for Experimental Groups

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

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

SN_p- hatchery-origin juveniles from the supplementation line that are subjected to experimental rearing conditions that differ from the normal hatchery environment. These fish are from eggs taken from S-line production, so are identical to juvenile SH fish, except for rearing conditions.

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

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

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

HC_p- juveniles from the HC line that are reared under experimental conditions that differ from the normal hatchery environment.

WC-natural-origin fish from the wild control line. This designation is used for both juveniles and adults. Any natural-origin fish in the Naches qualifies as an WC fish.

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

<i>Trait</i>	<i>Revised</i>	11/07/02
A1. Adult Recruits/Adult-Adult Survival		
<i>Justification</i>		
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.		
<i>Location(s)</i>		
Roza and Prosser Dams, Upper Yakima, Naches, American spawning ground		
<i>Start Date</i>		
2002		
<i>Frequency</i>		
Annually		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
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).		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
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.		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	11/07/02
A2. Age composition by sex		
Justification		
Location(s)		
RAMF, CESRF, Naches spawning grounds		
Start Date		
2002		
Frequency		
Annually		
Groups Compared:		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
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 $\alpha=0.05$ (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.		

<i>Trait</i>	<i>Revised</i>	11/07/02
A3. Size-at-age by sex		
<i>Justification</i>		
<i>Location(s)</i>		
RAMF, CESRF, and Naches spawning grounds		
<i>Start Date</i>		
<i>Frequency</i>		
Annually		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
Protocol same as for trait A2 (same fish) but with post-orbital hypural (POH) lengths measured		
<i>Expectations/Hypotheses</i>		
<p>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.</p>		
<i>Analytical/Statistical Methods and Issues</i>		
<p>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.</p>		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	11/08/02
A4. Sex ratio at age		
<i>Justification</i>		
<i>Location(s)</i>		
RAMF, CESRF, and Naches spawning grounds		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
Protocol same as for trait A2 (same fish).		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
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.		
<i>Power Analysis Completed?</i>		
No.		

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

<i>Trait</i>	<i>Revised</i>	11/08/02
A6. Spawning timing		
<i>Justification</i>		
<i>Location(s)</i>		
CESRF, Upper Yakima and Naches spawning grounds		
<i>Start Date</i>		
2002		
<i>Frequency</i>		
Annual		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
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.		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
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 groups 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.		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	11/08/02
A7. Fecundity		
Justification		
Location(s)		
CESRF		
Start Date		
2002		
Frequency		
Annual		
Groups Compared:		
HC,SN, SH (Hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
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 broodstock in the two lines. WC is not included because we intend to collect partially spawned females and thus will not be able to get total egg 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 the S and HC lines, and the decrease should be greater in HC.		
Analytical/Statistical Methods and Issues		
Within years, analysis of covariance will be used to compare body traits vs. fecundity within age classes. Analysis of variance will be used within years to compare absolute fecundities within age classes. Over years analysis of covariance will be used on mean fecundity by age to detect trend differences among groups. Naches females, because there will be so few of them, should represent a variety of sizes.		
Power Analysis Completed?		
Partially.		

<i>Trait</i>	<i>Revised</i>	11/08/02
A8. Egg size		
<i>Justification</i>		
<i>Location(s)</i>		
CESRF, Naches spawning grounds		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
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 spawning grounds.		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
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.		
<i>Power Analysis Completed?</i>		
Partially.		

<i>Trait</i>	<i>Revised</i>	11/08/02
A9. Reproductive effort		
Justification		
Location(s)		
CESRF		
Start Date		
Frequency		
Groups Compared:		
HC,SN, SH (Hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
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?		
No.		

<i>Trait</i>	<i>Revised</i>	11/08/02
A10. Male and female fertility		
<i>Justification</i>		
<i>Location(s)</i>		
CESRF		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
<i>Protocol</i>		
<p>Estimate fertility of WC, HC, SH, and SN fish by doing <i>inter se</i> (within group) 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.</p>		
<i>Expectations/Hypotheses</i>		
<p>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.</p>		
<i>Analytical/Statistical Methods and Issues</i>		
<p>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.</p>		
<i>Power Analysis Completed?</i>		
No.		

Trait	Revised	1/06/04
A11. Adult morphology at spawning		
Justification		
Based on earlier work (see expectations/hypotheses), domestication can be expected to cause changes in body shape, especially those aspects of shape that are secondary sexual characteristics		
Location(s)		
CESRF and possibly some effort on Naches spawning grounds		
Start Date		
2003		
Frequency		
Annually		
Groups Compared:		
WC,HC,SN, SH (Naches wild, hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
Protocol		
Collect digitized measurement data from lateral image landmarks on photos of adults. Develop orthogonal variables with which to compare WC, HC, SH, and SN fish. Same fish used for traits A7- A10. Requires holding some SH origin males and females (about 30 pairs) to maturity at hatchery in addition to the SN fish that will be held for S broodstock and the HC fish that will be used for HC broodstock. Data on Naches fish will be collected from carcasses on spawning grounds. Program TPSDig (http://life.bio.sunysb.edu/morph/index.html) will be used to mark the coordinates of 13 landmarks. These are the same 13 used by Hard (2000): 1) tip of snout, 2) base of skull, 3) anterior dorsal insertion, 4) posterior dorsal insertion, 5) anterior adipose insertion, 6) dorsal caudal insertion, 7) posterior end of body, 8) ventral caudal insertion, 9) posterior anal fin insertion, 10) anterior anal fin insertion, 11) anterior insertion of pelvic, 12) anterior insertion of pectoral, 13) distal tip of maxillary.		
Expectations/Hypotheses		
If domestication does not occur no changes in morphology will occur. If domestication does occur, we expect secondary sexual characteristics in both sexes to become less pronounced; e.g., reduced kype length, reduced body depth, less fusiform body shape, smaller adipose fins (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). We expect to use the TPS (http://life.bio.sunysb.edu/morph/index.html) and IMP (http://www.canisius.edu/~sheets/morphsoft.html) software packages.		
Power Analysis Completed?		
No. Power analysis will not be possible until analysis of 2003 spawners is complete. At that time we will know what the variances of landmark measurements are, and can then do a power analysis.		

<i>Trait</i>	<i>Revised</i>	11/07/02
A12. Adult spawning behavior		
Justification		
Location(s)		
Cle Elum experimental spawning channel		
Start Date		
Frequency		
Groups Compared:		
HC,SN,SH (Hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
Protocol		
<p>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.</p>		
Expectations/Hypotheses		
<p>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.</p> <p>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.</p>		
Analytical/Statistical Methods and Issues		
<p>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.</p>		
Power Analysis Completed?		
No.		

<i>Trait</i>	<i>Revised</i>	11/07/02
A13. Adult spawning success		
Justification		
Location(s)		
Cle Elum experimental spawning channel		
Start Date		
Frequency		
Groups Compared:		
HC,SN,SH (Hatchery control, natural-origin Upper Yakima, hatchery-origin fish from CESRF)		
Protocol		
<p>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.</p>		
Expectations/Hypotheses		
<p>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.</p>		
Analytical/Statistical Methods and Issues		
<p>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.</p>		
Power Analysis Completed?		
No.		

<i>Trait</i>	<i>Revised</i>	5/04/04
J1. Emergence timing		
<i>Justification</i>		
<i>Location(s)</i>		
Cle Elum Supplementation and Research Facility incubation room		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
WC _p , SN _p , SH _p , HC _p (Hatchery-origin progeny of Naches fish, ordinary CESRF S-line juveniles, hatchery-origin progeny of supplementation fish produced by CESRF, hatchery-control juveniles)		
<i>Protocol</i>		
Compare emergence timing of fish from different groups produced by <i>inter se</i> matings (same matings in trait A10). Eggs will be housed in 100-egg upwelling incubation chambers that allow fish to voluntarily exit. Number of fish exiting will be noted daily. Eggs used will be those from the studies of adult reproductive traits.		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
Two within-year analyses will be performed: 1) a nonparametric or parametric analysis of variance will be used to compare duration of emergence. If egg size and duration are correlated, then analysis of covariance will be used to correct for this factor; 2) analysis of covariance will be used to compare median date of emergence among groups. Over years, analysis of covariance will be used to examine differences in trends in these two variables.		
<i>Power Analysis</i>		
No.		

<i>Trait</i>	<i>Revised</i>	5/04/04
J2. K_D at emergence		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility incubation room		
Start Date		
Frequency		
Groups Compared:		
WC _p , SN _p , SH _p , HC _p (Hatchery-origin progeny of Naches fish, ordinary CESRF S-line juveniles, hatchery-origin progeny of supplementation fish produced by CESRF, hatchery-control juveniles)		
Protocol		
Compare developmental condition at emergence (K_D , Bams 1970) of fish from different groups produced by <i>inter se</i> 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		
No.		

<i>Trait</i>	<i>Revised</i>	5/04/04
J3. Egg-fry survival		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility incubation room		
Start Date		
Frequency		
Groups Compared:		
WC _p , SN _p , SH _p , HC _p (Hatchery-origin progeny of Naches fish, ordinary CESRF S-line juveniles, hatchery-origin progeny of supplementation fish produced by CESRF, hatchery-control juveniles)		
Protocol		
Compare egg-to-fry survival of fish from different groups produced by <i>inter se</i> 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?		
No.		

<i>Trait</i>	<i>Revised</i>	5/04/04
J4. Occurrence of developmental abnormalities		
Justification		
Location(s)		
Cle Elum Supplementation and Research Facility incubation room		
Start Date		
Frequency		
Groups Compared:		
WC _p , SN _p , SH _p , HC _p (Hatchery-origin progeny of Naches fish, ordinary CESRF S-line juveniles, hatchery-origin progeny of supplementation fish produced by CESRF, hatchery-control juveniles)		
Protocol		
Compare the percentage of abnormally appearing alevins originating from each group using the progeny produced from the <i>inter se</i> 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 groups is also expected to increase but at a lower rate than that expressed by the HC line. 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?		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J5. Fry-smolt survival in a hatchery environment		
<i>Justification</i>		
<i>Location(s)</i>		
Cle Elum Supplementation and Research Facility		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
SH,HC (Juveniles from normal supplementation production at CESRF vs hatchery control line juveniles)		
<i>Protocol</i>		
<p>Compare the fry-to-smolt survival of supplementation and hatchery control line fish 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 SH and HC fish would have an unacceptably high impact on the Naches population.</p>		
<i>Expectations/Hypotheses</i>		
<p>If domestication does not occur, we would expect mortality rates to be comparable in the HC and SH groups. If domestication does occur, we would expect HC fish to have lower mortality rates during the rearing period (Reisenbichler and McIntyre 1977).</p>		
<i>Analytical/Statistical Methods and Issues</i>		
<p>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.</p>		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J6. Juvenile morphology at release		
Justification		
Location(s)		
HC Acclimation site		
Start Date		
Frequency		
Groups Compared:		
SH,HC (Juveniles from normal supplementation production at CESRF vs hatchery control line juveniles)		
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?		
No.		

<i>Trait</i>	<i>Revised</i>	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		
Groups Compared:		
a)SN,SH,HC (Natural-origin Upper Yakima smolts, smolts from CESRF supplementation production, HC smolts)		
b)SN,SH, HC, WC (The same as (a) but including Naches smolts)		
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 at this point 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 groups to be comparable. SN fish are expected to survive at higher rates. This phenomenon has been observed in many other salmonid populations. If domestication does occur, we would expect SH smolts to survive at higher rates than HC individuals but not as well as SN fish. The comparisons involving SN need to be interpreted carefully, because they include only SN fish that 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?		
No.		

<i>Trait</i>	<i>Revised</i>	11/08/03
J9. Natural Smolt Production		
<i>Justification</i>		
<i>Location(s)</i>		
Chandler Smolt Facility.		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
WC, SN, SH, HC (Naches smolts, natural-origin upper Yakima smolts, smolts from CESRF supplementation production, hatchery-control line smolts)		
<i>Protocol</i>		
Outmigrating smolts made up of a mixture of WC, SN, SH and HC 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 River or upper Yakima. Marked fish will be assigned to HC and SH groups 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 group 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 groups.		
<i>Expectations/Hypotheses</i>		
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 fish to have higher productivity than HC individuals. The WC smolt productivity is unknown relative to the other groups. Its primary use is as a wild control benchmark against which the trends observed over time in the upper Yakima groups will be compared.		
<i>Analytical/Statistical Methods and Issues</i>		
Within year analysis will consist of the total number of smolts produced each year by group with confidence intervals.		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J10. Smolt-to-adult survival of hatchery-origin fish		
<i>Justification</i>		
<i>Location(s)</i>		
From one acclimation site to RAMF		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
SH,HC		
<i>Protocol</i>		
<p>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.</p>		
<i>Expectations/Hypotheses</i>		
<p>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.</p>		
<i>Analytical/Statistical Methods and Issues</i>		
<p>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.</p>		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J11. Smolt out-migration timing		
Justification		
Location(s)		
From one acclimation site to downstream monitoring sites		
Start Date		
Frequency		
Groups Compared:		
SN,SH,HC (Natural-origin upper Yakima smolts, smolts from CESRF supplementation production, hatchery-control line smolts)		
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 Kolmogorov-Smirnov tests. Analysis of covariance will be used to ascertain if genetically based trends in median out-migration timing occur in HC and SH fish. SN data will not be included in this analysis.		
Power Analysis Completed?		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J12. Food conversion efficiency		
<i>Justification</i>		
<i>Location(s)</i>		
Cle Elum Supplementation and Research Facility and smolt acclimation sites		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
SH,HC (Supplementation and hatchery-control line juveniles at CESRF)		
<i>Protocol</i>		
<p>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.</p>		
<i>Expectations/Hypotheses</i>		
<p>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.).</p>		
<i>Analytical/Statistical Methods and Issues</i>		
<p>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</p>		
<i>Power Analysis Completed?</i>		
No.		

<i>Trait</i>	<i>Revised</i>	4/28/03
J13. Juvenile Length-Weight Relationships		
<i>Justification</i>		
<i>Location(s)</i>		
CESRF and smolt acclimation sites		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
SH,HC (Supplementation and hatchery-control line juveniles at CESRF)		
<i>Protocol</i>		
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.		
<i>Expectations/Hypotheses</i>		
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.		
<i>Analytical/Statistical Methods and Issues</i>		
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		
<i>Power Analysis Completed?</i>		
No.		

Trait	Revised	5/04/04
J14. Agonistic-competitive behavior a) Contest competition b) Scramble competition c) Aggression)		
Justification		
<i>Competition and aggression has been demonstrated to be influenced by domestication</i>		
Location(s)		
Cle Elum Supplementation and Research Facility		
Start Date		
<i>June, July 2003-2004 (HC_p, SN_p), June 2005 (HC_p, SN_p, WC_p)</i>		
Frequency		
<i>Annual (Trials to be conducted daily from June through October)</i>		
Groups Compared:		
WC _p , SN _p , HC _p (Hatchery progeny of Naches adults, juveniles from CESRF supplementation production, hatchery control line juveniles)		
Protocol		
<p>Juvenile fish produced from the crosses used in J3 will be test subjects. Dominance and aggressiveness will be compared to the WC_p. Two types of dominance trials will be performed. The first will test for contest competition (14a) and the second scramble competition (14b). In this behavioral assay, three group comparisons will be made: WC_p vs. HC, WC_p vs. SH, and SH vs. HC. Size-matched pairs of fish (each fish represents a different group) will be simultaneously introduced into tanks. In the test of contest competition, fish will be placed into tanks that have one optimal location (possessing one piece of cover and a single tube used to introduce food and velocity in the water column). Dominance will be assigned to the fish that obtains the most food, dominates the majority of the agonistic contests, and spends the most time adjacent to the food tube and cover. In the test of scramble competition, no cover will be provided, water will be introduced through a tube as before, and food will be introduced in different locations on the surface of the water. Dominance will be assigned to the fish that eats the most food items and dominates the most agonistic contests. Trials will be conducted for 7 days.</p> <p>Aggression (14c) will be tested in the same way as the trials to determine dominance under contest competition (14a), however fish will be of the same origin (WC_p vs. WC_p, SH vs. SH, HC vs. HC). Number of trials will be determined by power analysis (see below)</p>		
Expectations/Hypotheses		
<p>If domestication does not occur, we would expect HC, WC_p, and SH fish to have equivalent levels of aggression and dominance. If domestication does occur, we would expect the following results ordered from most to least: contest competition dominance WC_p >SH>HC; scramble competition dominance HC>SH>WC; and aggressiveness WC_p >SH>HC or HC>SH> WC_p. In addition, we would expect that these differences would be accentuated with time. How aggressive and dominant WC_p fish may be is unknown, but their behavior is not expected to change over time and therefore they will act as a valuable reference.</p>		
Analytical/Statistical Methods and Issues		
<p>Within a year three to four separate Chi-square analyses will be performed, comparing fish with individuals from each of the three groups. Analysis of covariance will be used to ascertain if trends in dominance among the comparisons diverge over time.</p>		
Power Analysis Completed?		
Some Analysis Completed (see next page)		

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	5/04/04
<p>J15. Predator avoidance a) Line comparison b) Survivor vs. Naïve comparison</p>		
<p>Justification</p>		
<p><i>Predation has been demonstrated to be influenced by domestication</i></p>		
<p>Location(s)</p>		
<p>Cle Elum Supplementation and Research Facility</p>		
<p>Start Date</p>		
<p><i>April 2003, 2004 (HC_p, SN_p), April 2005 (HC_p, SN_p, WC_p)</i></p>		
<p>Frequency</p>		
<p><i>Annual (Trials to be conducted weekly from April through June)</i></p>		
<p>Groups Compared:</p>		
<p>WC_p, SN_p, HC_p (Hatchery progeny of Naches adults, juveniles from CESRF supplementation production, hatchery control line juveniles)</p>		
<p>Protocol</p>		
<p>Two types of predator trials will be conducted on alternate weeks. The first set of trials will be used to determine if domestication affects the survivability of fry (15a). The second set of trials will be used to determine if fish that survive a predator challenge survive better than naïve fish (15b). Enhanced survival of “surviving” fish may be due to learned predator avoidance (experience) or innate predator avoidance abilities (genetic). To avoid pseudo-replication, multiple arenas possessing different individual fish predators will be established. There will be 8 arenas which will consist of 8 x 10 foot net pens. A total of 8 net pens will be placed in a single hatchery raceway. For trait 15a, between 67 (3 line comparison) and 100 (2 line comparison) size-matched fish from each line will be simultaneously introduced 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, that corresponds to approximately 50% of the introduced fish have been eaten (e.g., 4 days), survivors will be removed from each arena and enumerated. The fish that survive the predator challenges will be held in tanks until the start of trials intended to test survival differences between surviving fish and naïve fish (15b). In these trials, 100 fish that survived in trials from 15a and 100 naïve fish from the same line will be introduced simultaneously into arenas. All other methods and analyses will be the same as those for 15a. Fish predators will be changed after each trial to avoid pseudo-replication. This assay is being performed to determine if innate anti-predator behaviors differ among the lines.</p>		
<p>Expectations/Hypotheses</p>		
<p>If domestication does not occur, we would expect fish from all lines to survive at equal rates. In addition, the expression and use of innate anti-predator behaviors should remain constant within a line over time. If domestication does occur, we would expect WC fish to have the highest survival rates followed by SH, and HC individuals in that order. We would also expect that “survivor” fish would survive better than naïve fish regardless of line. However, we would expect that the amount of difference in survival would be in the following order WC_p>SH>HC. This might be expected if domestication decreases the ability of fish to learn.</p>		
<p>Analytical/Statistical Methods and Issues</p>		
<p>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.</p>		
<p>Power Analysis Completed?</p>		
<p>No</p>		

<i>Trait</i>	<i>Revised</i>	4/28/03
J16. Incidence of precocialism in production raceways		
<i>Justification</i>		
<i>Location(s)</i>		
One smolt acclimation site		
<i>Start Date</i>		
<i>Frequency</i>		
<i>Groups Compared:</i>		
SH,HC (Juveniles from CESRF supplementation production and the hatchery control line)		
<i>Protocol</i>		
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.		
<i>Expectations/Hypotheses</i>		
If domestication does not occur, we would expect HC and SH fish to have equivalent rates of precocial development. If domestication does occur, we would expect HC fish to have a lower incidence of precocialism.		
<i>Analytical/Statistical Methods and Issues</i>		
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		
<i>Power Analysis Completed?</i>		
No.		

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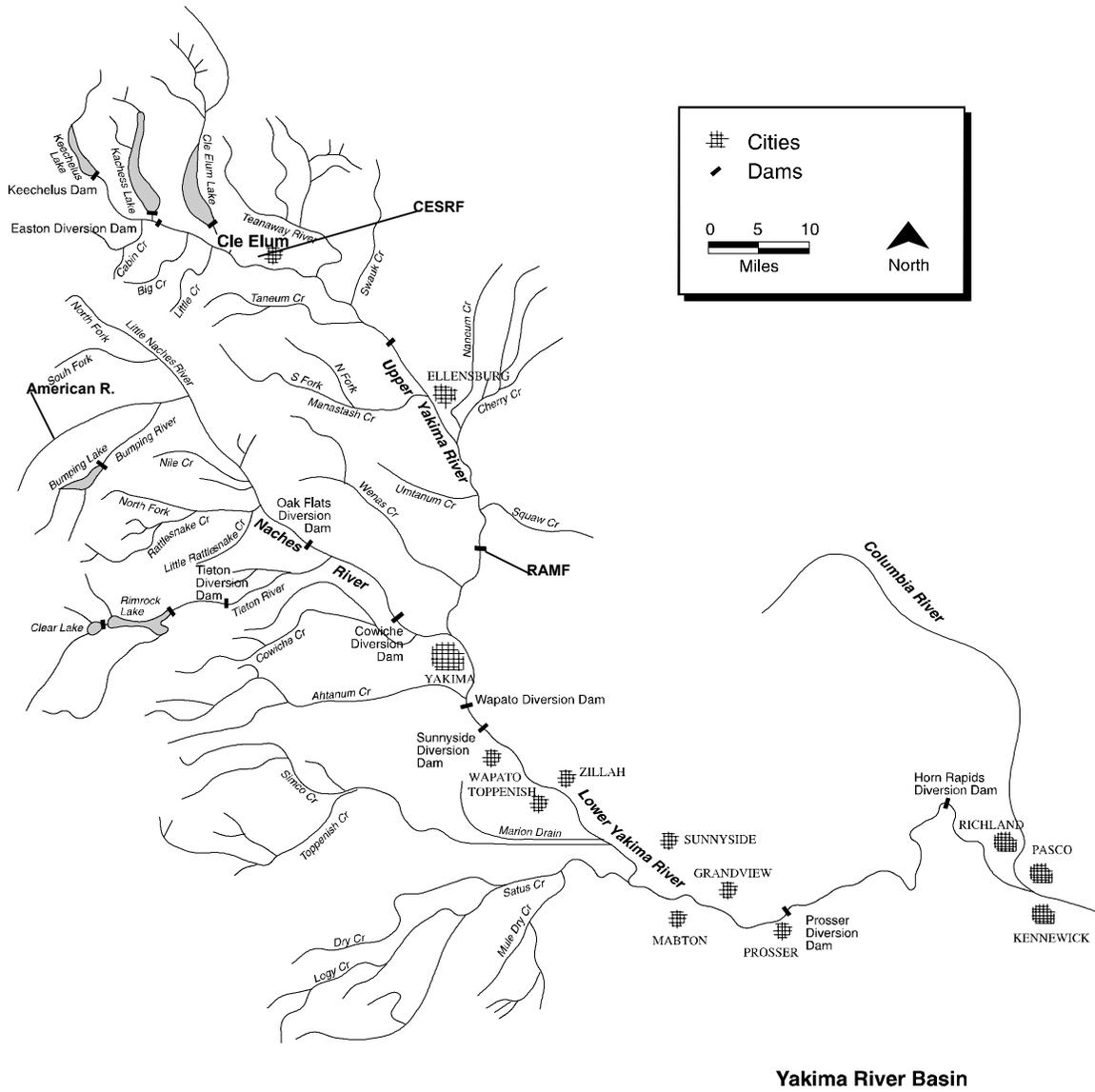


Figure 1. Map of Yakima basin.

Chapter 5

Modeling the Effective Size Advantage of Factorial Mating in Hatcheries

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Introduction

For generations hatchery personnel have been mating fish by fertilizing buckets of eggs from many females with buckets of sperm from many males. This has been shown to have the potential of reducing effective size because under these circumstances males can vary greatly in their fertilization success (Gharrett and Shirley 1985; Withler 1988; Withler and Beacham 1994). The result has been a move to single-pair matings. Often over the last few years we have heard recommendations made that as an alternative to single-pair mating, that fish be mated to more than one other fish, an approach called matrix or factorial mating (Campton in press; Currens et al. 1998; Fiumera et al. 2004; Kapuscinski and Miller 1993). For example, if four males and four females are available for spawning, the milt from each male and eggs from each female can be divided into two or more aliquots. In the full factorial design, every male could be mated to every female and vice versa, but a partial factorial scheme in which each fish is mated to two other fish is also possible.

Fig. 1 depicts the four regular factorial designs possible with 10 males and 10 females. Note that the single-pair design can also be thought of as a 1x1 partial factorial. Irregular designs are also possible when equal numbers of the two sexes are not available, of course, such as 3 x 2.

Two lines of reasoning have been presented for the superiority of the factorial over the single-pair design. The first is increased genetic diversity. It seems reasonable that mating every available fish with every other will increase the diversity of genotypes produced over what would be produced by single-pair mating. In small populations this added diversity may be valuable in that some high-fitness genotypes may occur that would not occur under single-pair mating. The second argument, and the one we explore in this paper, is that factorial mating may actually increase the genetic effective size over that achievable with single pair mating.

The effective size, calculated (Hedrick 1983) for each sex¹, is

¹ This is technically the formula for variance effective size, but the principle holds true as well for inbreeding effective size.

$$N_e = \frac{N\bar{k} - 1}{\bar{k} - 1 + \frac{V_k}{k}} \quad (1)$$

where N is the number of breeders, \bar{k} is the mean family size, and V_k is the variance in family size. In single-pair mating, the reproductive success of every fish is tied directly to the reproductive success of its single mate. The variance of family size for males will thus always be equal to the variance of family size for females. If pairs differ considerably in reproductive potential (in terms of number of returning adult progeny), due to gamete quality or genetic composition the variance of family size will be large, decreasing the effective size for both sexes.

A factorial mating scheme differs significantly from the single pair design. The complete linkage between the reproductive potentials of mates is broken. In a factorial system, the reproductive potential of any fish has a greater probability of being expressed because the fish is mated to more than one, perhaps several mates. For example, under a single-pair regime, the entire reproductive potential of a male could be lost by mating him to a female with underripe eggs. In a factorial scheme, he would be mated to at least one other female, which may very well not have green eggs, providing a greater certainty of realization of his reproductive potential. Factorial mating schemes offer many more combinations of mates than a single pair design. In the situation depicted in Fig. 1, where 10 fish of each sex are available, 10 matings will be made in the single-pair design, 20 in the 2x2 design, 50 in the 5x5, and 100 in the full factorial (10x10). It seems reasonable to expect that the bet-hedging strategy afforded by factorial mating systems could decrease the variance of family size relative to what it would be under single-pair mating.

Our interest in this mating system began with the implementation of the YKFP spring chinook program, for which broodstock were first collected in 1997. With the emphasis on genetic conservation that the project had as its foundation it seemed reasonable to investigate this type of mating system. We began a series of computer simulations, and found out early on, that if fish vary in their potential to produce offspring, then factorial mating had the potential to increase effective size over what could be achieved by single-pair mating. Moreover, a significant proportion of the benefit of full factorial mating could be achieved going from single-pair mating to 2x2 partial factorial mating. Based on this work, and because it also facilitates fertility and egg quality studies, we have used partial factorial mating regularly in CESRF spawning operations.

This modeling has been presented in fragmentary form for several years at Project Annual Reviews. Here we present the completed series of computer simulations done to evaluate the effective size benefit over single-pair mating, and to quantify the relative benefits offered by various partial factorial designs.

Methods

We modeled a situation where mating k males and k females as single-pairs (1x1, 2x2, etc.) was compared to mating the same fish in regular partial or full factorial for population sizes of 10, 20, 40, and 120 pairs. Partial factorial designs of 2x2, 5x5, 10x10, 20x20, and 40x40 were modeled.

The runs for a given population size included all the possible partial factorial designs that were possible. For example, for 10 pairs, 2x2 and 5x5 were the only partial designs possible. Population sizes were held constant: the k pairs of spawners produced k pairs of spawners for the next generation.

The key to the modeling was varying the reproductive value of the spawners. For purposes of this model we define reproductive value as the ability to produce progeny which will return as adults. There are any number of ways to simulate reproductive values. The method we used was random generation of identically distributed normal deviates, with the exception that when negative values occurred, they were set to zero. Overall levels of reproductive value variation were set by varying the coefficient of variation (CV). CV values of 0, 0.5, 1.0, 2.0, 5.0, and 10.0 were simulated. The reproductive value of a mating was then computed as either: a) the arithmetic mean of the male and female reproductive values, called the additive method; b) the product of the two reproductive values, called the multiplicative method; and c) the harmonic mean of the reproductive values. If either fish in the pair had a reproductive value of zero, the reproductive value for the pair was zero.

The raw reproductive values for all matings were then converted to relative values, and finally normalized to add to one. At this point the reproductive values for each mating became the mating's relative probability of producing an adult offspring. This is the step that is most likely to cause confusion, so it will be explained in greater detail using a simple example for the additive case. Consider a mating design with only two fish of each sex, and assume a reproductive value CV of 2 is being simulated, with reproductive values of the two sexes being weighted equally. Reproductive values for each animal are randomly generated from a normal distribution with mean μ and specified coefficient of variation. The value of μ is irrelevant, since the variance is always scaled to it, but a value of 500 was used. The initial reproductive values: 1=426.3, 2=643.0, 1=145.4, 2=704.9. Weighting the reproductive values of the two sexes equally, we get the following reproductive values for the four possible matings(shaded):

		426.3	643.0
		145.4	704.9
	145.4	285.85	394.20
	704.9	565.60	673.95

Normalizing by dividing each mating reproductive value by the total of all mating reproductive values, we get:

		426.3	643.0
		145.4	704.9
	145.4	0.1489	0.2054
	704.9	0.2946	0.3511

The table entries can now be considered the relative probability of the four matings in the factorial design producing progeny. Looked at another way, which is critical to the approach we used for generating progeny, if a fish produced by this mating design returns, the probability is 35.1% that it came from the 2x2 mating, 14.9% that it came from the returns that came from the 1x1 mating, etc. For the single-pair situation, the raw reproductive values are normalized by dividing by the sum of just the matings in the single pair design (1x1 and 2x2):

		426.3	643.0
		145.4	0.2978
		704.9	0.7022

Thus, a fish produced by the single-pair design has a 70.2% probability of coming from one mating and a 29.8% probability of coming from the other.

For each of the $2k$ progeny generated for each mating design, the originating mating was determined by a multinomial sampling process, using these relative reproductive values, and the male and female involved in the cross were noted. After all the $2k$ progeny were generated, the variance in family size of males and females was calculated, and the effective size computed according to equation 1.

An early reviewer of this work was concerned that the factorial mating system, by creating large numbers of half-sib families, would serve to increase inbreeding over time. To answer this concern, we included a single-locus genetic system and ran each simulation over twenty generations, starting each run with the population of $2n$ fish having $4n$ unique alleles and tracking the accumulation of homozygosity over time.

Each population size-CV-reproductive value combining method-partial factorial combination was run 5000 times and the results averaged.

Programming Details

The models were written and compiled in Lahey Fortran90, version 3.0. Routines RAN1 and GASDEV from Press et al. (1986) were used for random number generation and for generation of normal deviates, respectively. The model runs were done on a desktop personal computer.

Results

Running the simulations over 20 generations showed clearly that inbreeding buildup was not a side effect of factorial mating. Population trajectories indicated a constant among-family

variance over time. The mean among-family variance over time and replicates was used for tabulation of results.

The results for populations consisting of 10, 20, 40, and 120 pairs are presented in Tables 1-4, respectively. If not already obvious, it is clear from the simulations that factorial mating has no impact if there is no reproductive value difference among the fish ($CV=0$). Effective size declines rapidly as reproductive value CV increases, no matter what the reproductive value combining mode is. The effective size depression is least within additive combination, and greatest with multiplicative combination. Harmonic combination is much closer to multiplicative than additive in its effect. This is all to be expected, because multiplying and or taking harmonic means of reproductive values yields a wider range of values, and thus among family variances, than taking arithmetic means.

Table 5 summarizes the effective size advantage of full factorial mating over single-pair mating. With additive reproductive-value combination, full factorial mating resulted in effective sizes between 1.14 and 1.33 that achieved under single-pair mating. The advantage increased as the reproductive value CV increased, but size of population had no discernable effect. Under multiplicative reproductive-value combination, full factorial mating resulted in effective sizes 1.55 to 2.81 that achieved under single-pair mating. Again, the advantage increased as CV increased, but also increased with population size. The reason for this is not clear.

Under harmonic reproductive-value combination, full factorial mating resulted in an effective size advantage of 1.14 to 2.39, with a pronounced increase in advantage as CV increased. The pattern across different population sizes was in sharp contrast to the additive and multiplicative schemes, however. Not only was there no trend toward increase as population size increased, but the advantage varied considerably and apparently randomly over the different population sizes. The lack of systematic pattern makes it very unlikely that this fluctuation has any biological basis. It is probably just a reflection of the fact that the number of replicates was not large enough to reflect the true pattern. It is also likely, however, that because there is absolutely no indication of the advantage increasing as population size increases, that the real situation may be one of no increase as population size increases.

As mentioned above, early on in the study we noticed that an appreciable portion of the effective size advantage of full factorial mating could be achieved by switching from single-pair mating to 2×2 partial factorials. This is summarized over all model runs in Table 6. Under additive reproductive-value combination, about half (.44-.56) the full effective size benefit can be achieved with 2×2 mating. This is constant over CV and population size. Under multiplicative combination the benefit is somewhat lower and considerably more variable (.27-.45) and decreases with increasing CV and population size. The same phenomenon seems to hold true for harmonic reproductive value combination (.31-.49).

Upon examination of all the results, a fascinating and extremely useful result became obvious. Under the same conditions of reproductive value CV and reproductive value combination, a $k \times k$ factorial mating block, be it a full factorial or a partial factorial in a larger design will have the same effective size advantage for the fish involved in the block. For example, the advantage

realized from a 10x10 mating block in a population of 40 pairs is the same as that realized from a 10x10 full factorial in a population of 10 pairs. This conclusion is summarized in Table 7, but the reader can easily check by comparing Tables 1-4. Knowing this, the effective size advantage for an irregular factorial mating design can easily be computed. For example, assume additive reproductive value combination and a CV of 3, if 20 pairs are mated in a 10x10 partial factorial design. According to Table 2, this should result in an effective size of 28.84. Using Table 7, we see the result should be $14.45 + 14.45$ (because there will be 2 10x10 blocks) = 28.90. No suppose the fish were instead mated as a 10x10, a 5x5, two 2x2's and a 1x1. The result is $14.45 + 7.04 + 2(2.62) + 1.18 = 27.91$, almost as good. Table 7 is also useful in that it allows the effective size of mating designs not modeled (3x3, 4x4, etc) to be estimated by regression.

Discussion

Reproductive Value Simulation and Weighting

Our primary concern in generating reproductive value variation was coming up with a simple method that would result in substantial, but realistic differences in reproductive values of matings. We feel the methods we used, all based a truncated normal distribution achieved this. One possible benchmark is the maximum family size variance possible with extreme reproductive value variation. The maximum value a male or female family size variance can take on, v_{\max} , in a mating design involving k adults of each sex that produces $2k$ progeny is $4(k-1)$, achieved when one sire or one dam is responsible for all the progeny. v_{\max} values for k of 5, 10, 20, and 50, are then 16, 36, 76, and 196. Simulated values achieved v_{\max} in the case of $k=5$ (Figs. 1a and 3a), but did not increase much for larger designs, so fell considerably short of v_{\max} : the $k=10$ scenario achieved about 60% of v_{\max} , the $k=20$ scenario achieved about 30%, and the $k=50$ scenario achieved about 10%. Variances closer to v_{\max} probably could be achieved by modeling higher CV's, but it is unclear how useful this would be, because as k increases, v_{\max} becomes increasingly unrealistic biologically. Having only one male out of five produce all of ten returning offspring seems reasonable; having only one out of 50 produce all 100 returning offspring seems unlikely.

Data on variance of family size, measured as returning adults, is very sparse in salmonids. Simon et al.(1986) released 21-45 full-sib families of coho salmon (*Oncorhynchus kisutch*) smolts annually for five years and identified returnees by family. Because the population they studied was fluctuating in size, the ratio of family size variance to mean family size is more relevant to the present discussion than absolute family size variance. This ratio varied from 1.57 to 3.82. Scaling this to a stable population situation (mean family size of 2) such as we modeled here, the corrected ratio would be roughly 3 to 8. Our simulations achieved variances in that range at CV's of 0.5 to 2, so although our simulations included values that are realistic, based on this single paper, we modeled situations with much higher variances. Another interesting observation from this study that bears on the extreme variance case is that 0%, 0%, 20%, 16%, and 5% of families released returned no adults in the five years of the study. Once again, there seems to be no need, based on empirical data, to achieve v_{\max} in the simulations.

There is probably an endless number of ways to model the reproductive value variation, and we make no claims that ours is a particularly good one. A realistic one would consider all the genetic and phenotypic and stochastic factors that determine the distribution of family survivorship, but the data just aren't there to do that. Our scheme, we feel, is adequate. It generates reproductive variability that covers a reasonable range of situations. We doubt that any other more biologically correct scheme will change the overall results of the modeling.

A paper similar to this report (Fiumera et al. 2004) recently appeared in the literature. We have not studied the paper in detail, but the authors appear to have approached the problem similar to the way we did. They randomly generated three levels of sterility, used a uniform distribution to simulate reproductive value of non-sterile fish, and used both additive and multiplicative methods for combining breeding values. Their focus was a supplementation program for redhorse (*Moxostoma robustum*), a depressed catostomid native to the southeastern U.S. This is a small program (maximum spawners 1993-2002 was 39), and probably because of the size of the program, they did not do as large a series of simulations as we did in terms of population size. They did explore the possibility of differing numbers of males and females, which we did not do. Overall their results agreed well with ours. They found that full factorial mating could increase effective size in their supplementation program by 19%.

Although there are countless ways to do these simulations, the result seems clear. Factorial mating can be expected to improve effective size considerably over single-pair mating, so it should be considered in any program where effective size is a concern. Factorial designs are logistically demanding, but methods have been developed to make them more workable in a hatchery setting (Currens et al. 1998). Large designs may not be practical, but 2x2 matings may be possible, and 2x2 matings alone will achieve an appreciable proportion of the benefit that would have been achieved by full factorial mating. Proof of this is the fact that we have been using partial factorial matings, primarily 2x2 and 3x3, at CESRF since 1997. Finally, the relative benefits of any partial mating scheme, regular or irregular, can be determined easily to inform decisions about mating protocols.

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Table 1. Realized effective size for a population of 10 pairs, with different levels of breeding value variation, under different mating regimes, for three modes of breeding value combination.

a) Additive

Breeding Value CV	Mating Design			
	1x1	2x2	5x5	10x10
0	19.50	19.49	19.51	19.48
1	14.77	15.81	16.55	16.81
2	12.37	13.77	14.78	15.15
3	11.43	12.91	14.00	14.39
5	10.58	12.12	13.27	13.70
10	9.92	11.49	12.68	13.16

b) Multiplicative

Breeding Value CV	Mating Design			
	1x1	2x2	5x5	10x10
0	19.50	19.49	19.51	19.48
1	7.50	9.36	10.98	11.65
2	4.67	6.28	7.98	8.77
3	3.91	5.31	6.94	7.74
5	3.39	4.59	6.13	6.94
10	3.05	4.11	5.56	6.35

c) Harmonic

Breeding Value CV	Mating Design			
	1x1	2x2	5x5	10x10
0	19.51	19.51	19.50	19.50
1	9.78	11.75	13.25	13.82
2	5.88	7.90	9.79	10.59
3	4.73	6.57	8.50	9.40
5	3.96	5.57	7.47	8.39
10	3.48	4.89	6.72	7.66

Table 2. Realized effective size for a population of 20 pairs, with different levels of breeding value variation, under different mating regimes, for three modes of breeding value combination.

a) Additive						
Breeding Value CV	Mating Design					
	1x1	2x2	5x5	10x10	20x20	
0	39.54	39.49	39.52	39.53	39.46	
1	29.95	32.00	33.32	33.80	34.04	
2	25.13	27.78	29.67	30.37	30.73	
3	23.16	26.01	28.09	28.84	29.18	
5	21.56	24.43	26.62	27.44	27.84	
10	20.24	23.24	25.50	26.31	26.78	

b) Multiplicative						
Breeding Value CV	Mating Design					
	1x1	2x2	5x5	10x10	20x20	
0	39.51	39.48	39.53	39.51	39.52	
1	14.94	18.62	21.81	23.12	23.85	
2	9.02	12.26	15.62	17.20	18.12	
3	7.35	10.24	13.52	15.15	16.08	
5	6.15	8.73	11.87	13.47	14.49	
10	5.34	7.71	10.66	12.27	13.28	

c) Harmonic						
Breeding Value CV	Mating Design					
	1x1	2x2	5x5	10x10	20x20	
0	39.51	39.48	39.53	39.51	39.52	
1	20.44	24.03	26.83	27.95	28.50	
2	12.51	16.42	19.99	21.51	22.36	
3	10.01	13.79	17.41	19.09	20.01	
5	8.19	11.67	15.37	17.09	18.08	
10	6.94	10.21	13.84	15.58	16.62	

Table 3. Realized effective size for a population of 40 pairs, with different levels of breeding value variation, under different mating regimes, for three modes of breeding value combination.

a) Additive							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	
0	79.44	79.50	79.52	79.58	79.44	79.48	
1	60.24	64.23	66.85	67.79	68.24	68.48	
2	50.64	55.83	59.49	60.84	61.48	61.89	
3	46.79	52.29	56.19	57.74	58.52	58.91	
5	43.45	49.20	53.33	55.02	55.81	56.20	
10	40.87	46.65	51.08	52.66	53.57	54.00	
b) Multiplicative							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	
0	79.50	79.52	79.52	79.48	79.52	79.50	
1	29.78	37.12	43.45	46.08	47.51	48.21	
2	17.83	24.23	30.87	34.05	35.84	36.84	
3	14.31	20.17	26.64	29.83	31.72	32.77	
5	11.79	17.02	23.27	26.46	28.43	29.56	
10	10.16	14.93	20.82	24.06	26.04	27.16	
c) Harmonic							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	
0	79.56	79.54	79.48	79.48	79.52	79.50	
1	60.24	64.23	66.85	67.79	68.24	68.48	
2	25.88	33.48	40.31	43.26	44.83	45.68	
3	20.93	28.15	35.22	38.41	40.18	41.18	
5	17.25	24.09	31.18	34.42	36.36	37.38	
10	14.69	21.11	28.06	31.45	33.42	34.59	

Table 4. Realized effective size for a population of 120 pairs, with different levels of breeding value variation, under different mating regimes, for three modes of breeding value combination.

a) Additive							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	239.38	239.50	239.44	239.44	239.56	239.38	239.32
1	181.47	193.30	201.01	203.83	205.18	205.84	206.38
2	152.72	167.95	178.83	182.68	184.76	185.69	186.49
3	141.17	157.31	169.05	173.43	175.65	176.72	177.70
5	131.16	148.09	160.39	165.09	167.42	168.72	169.68
10	123.47	140.55	153.48	158.24	160.52	161.12	153.97
b) Multiplicative							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	239.50	239.50	239.50	239.50	239.50	239.50	239.50
1	89.33	110.88	129.88	137.70	142.05	144.28	145.77
2	52.79	71.99	91.98	101.34	106.75	109.55	111.67
3	42.25	59.50	78.88	88.57	94.24	97.42	99.58
5	34.65	50.11	68.71	78.38	84.24	87.57	89.91
10	29.48	43.66	61.29	70.91	76.97	80.32	82.76
c) Harmonic							
Breeding Value CV	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	239.50	239.56	239.50	239.44	239.50	239.56	239.50
1	126.30	147.00	162.98	169.05	172.27	173.96	175.07
2	79.46	101.54	121.60	130.27	135.01	137.51	139.22
3	64.59	85.77	106.39	115.64	120.96	123.76	125.66
5	53.51	73.34	94.12	103.72	109.41	112.39	114.57
10	45.89	64.48	84.89	94.77	100.59	103.81	106.04

Table 5. Realized effective size for partial mating blocks, for different levels of breeding value variation, for three modes of breeding value combination.

a) Additive							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	1.99	3.99	9.98	19.95	39.93	79.79	239.32
1	1.51	3.22	8.38	16.99	34.20	68.61	206.38
2	1.27	2.80	7.45	15.22	30.79	61.90	186.49
3	1.18	2.62	7.04	14.45	29.28	58.91	177.70
5	1.09	2.47	6.68	13.76	27.90	56.24	169.68
10	1.03	2.34	6.39	13.19	26.75	53.71	153.97
b) Multiplicative							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	2.00	3.99	9.98	19.96	39.92	79.83	239.50
1	0.74	1.85	5.41	11.48	23.68	48.09	145.77
2	0.44	1.20	3.83	8.45	17.79	36.52	111.67
3	0.35	0.99	3.29	7.38	15.71	32.47	99.58
5	0.29	0.84	2.86	6.53	14.04	29.19	89.91
10	0.25	0.73	2.55	5.91	12.83	26.77	82.76
c) Harmonic							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	2.00	3.99	9.98	19.95	39.92	79.85	239.50
1	1.05	2.45	6.79	14.09	28.71	57.99	175.07
2	0.66	1.69	5.07	10.86	22.50	45.84	139.22
3	0.54	1.43	4.43	9.64	20.16	41.25	125.66
5	0.45	1.22	3.92	8.64	18.24	37.46	114.57
10	0.38	1.07	3.54	7.90	16.76	34.60	106.04

Table 6. Proportion of effective size gain to be realized under full factorial mating that is achieved by using 2x2 partial factorial for populations of 20, 40, 80, and 240 pairs, with different levels of breeding value variation, under different mating regimes, for three modes of breeding value combination.

a) Additive

Breeding Value CV	Population Size			
	20	40	80	240
1	0.51	0.50	0.48	0.47
2	0.50	0.47	0.46	0.45
3	0.50	0.47	0.45	0.44
5	0.49	0.46	0.45	0.44
10	0.48	0.46	0.44	0.56

b) Multiplicative

Breeding Value CV	Population Size			
	20	40	80	240
1	0.45	0.41	0.40	0.38
2	0.39	0.36	0.34	0.33
3	0.36	0.33	0.32	0.30
5	0.34	0.31	0.29	0.28
10	0.32	0.30	0.28	0.27

c) Harmonic

Breeding Value CV	Population Size			
	20	40	80	240
1	0.49	0.45	0.48	0.42
2	0.43	0.40	0.38	0.37
3	0.39	0.38	0.36	0.35
5	0.36	0.35	0.34	0.32
10	0.34	0.34	0.32	0.31

Table 7. Realized effective size for partial mating blocks, for different levels of breeding value variation, for three modes of breeding value combination.

a) Additive							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	1.99	3.99	9.98	19.95	39.93	79.79	239.32
1	1.51	3.22	8.38	16.99	34.20	68.61	206.38
2	1.27	2.80	7.45	15.22	30.79	61.90	186.49
3	1.18	2.62	7.04	14.45	29.28	58.91	177.70
5	1.09	2.47	6.68	13.76	27.90	56.24	169.68
10	1.03	2.34	6.39	13.19	26.75	53.71	153.97

b) Multiplicative							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	2.00	3.99	9.98	19.96	39.92	79.83	239.50
1	0.74	1.85	5.41	11.48	23.68	48.09	145.77
2	0.44	1.20	3.83	8.45	17.79	36.52	111.67
3	0.35	0.99	3.29	7.38	15.71	32.47	99.58
5	0.29	0.84	2.86	6.53	14.04	29.19	89.91
10	0.25	0.73	2.55	5.91	12.83	26.77	82.76

c) Harmonic							
Breeding Value CV	Mating Design						
	1x1	2x2	5x5	10x10	20x20	40x40	120x120
0	2.00	3.99	9.98	19.95	39.92	79.85	239.50
1	1.05	2.45	6.79	14.09	28.71	57.99	175.07
2	0.66	1.69	5.07	10.86	22.50	45.84	139.22
3	0.54	1.43	4.43	9.64	20.16	41.25	125.66
5	0.45	1.22	3.92	8.64	18.24	37.46	114.57
10	0.38	1.07	3.54	7.90	16.76	34.60	106.04

Chapter 6

Year 2003 Chandler Chinook Smolt Stock-of-Origin Assignments

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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. Domestication monitoring plans require discrimination of the three spring Chinook salmon stocks in the basin: in addition to the ongoing upper 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. Accurate assignments to stock of origin of Chinook smolts captured at the Chandler fish passage facility will allow researchers and managers to estimate production by the three spring Chinook stocks, assess smolt-to-smolt survival and stock-specific fish health parameters, and evaluate stock-specific condition factors.

WDFW has developed the methodology to estimate the stock-of-origin of individual fish in a mixture (reported elsewhere). Implementing that methodology with Chinook trapped at Chandler has been hindered by marginally adequate genetic characterizations of the American River and Naches River stocks, by non-representative temporal sampling of the downstream migration passing Chandler, and by past omission of the Marion Drain and Yakima River mainstem spawning fall Chinook stocks from the DNA baseline that provides the standard for individual assignments.

Methods

Sampling crews from WDFW and the Yakama Indian Nation (YIN) provided fin clips preserved in 100% ethanol from 415 spring Chinook spawners from American River, Naches River and upper Yakima River, 236 fall Chinook spawners from Marion Drain and the Yakima River mainstem (Figure 1), and a collection of juvenile Chinook salmon downstream migrants intercepted at the Chandler fish passage facility on the Yakima River (Table 1) The spawning area samples (baseline) represent the presumed donor populations that generated the migrant sample (mixture).

A total of 14 loci were screened in the baseline populations and in the Chandler trap mixture sample but one locus (*One-114*) was omitted from all analyses because of unreliable scoring. The baseline and mixture samples were characterized using 11

microsatellite DNA loci; three additional loci were dropped because of scoring problems (Table 2). The microsatellite loci were amplified using the polymerase chain reaction (PCR) and fractionated by capillary electrophoresis in an Applied Biosystems 3730 DNA Analyzer. Oligonucleotide PCR product lengths were estimated in base pairs (bp) using Applied Biosystems Genemapper version 3.0 software. The estimated oligonucleotide fragment lengths were aggregated into non-contiguous allele classes (bins) by identifying discontinuities in the distributions of estimated fragment lengths. PCR, electrophoresis, and allele binning protocols are available from the WDFW Genetics Laboratory in Olympia, WA.

Stock-of-origin assignments in this study were accomplished with a Visual Basic implementation of the Expectation Maximization (EM) algorithm, that simultaneously estimates admixture proportions and assigns 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).

Mixture composition estimates were generated for 10 April to 30 April, 1 May to 14 May, and after 15 May to see if we could discern temporal trends in the relative abundances of the contributing populations.

Results

The downstream migration extended from at least the middle of December, 2002 through the middle of July, 2003, the Chinook smolt sample was collected during April and May 2003 (Figure 2). The sampling dates for the later smolt samples are unavailable.

PCR failure rates were high in the baseline samples – missing data over all collections ranged from 22% to 60% (Table 2). We were unable to observe 60%, 55%, and 49% of the alleles for the loci *Omm-1135*, *Ots-2M*, and *Ots-1*, respectively, so we eliminated those loci from the analysis. Allele frequencies and missing data rates are in Table 3.

The mixture composition estimates for the 2003 outmigration indicated that American River spring Chinook contributed 12-16% of the mixture during the April – May period (Table 4.) The estimated contribution of upper Yakima spring Chinook declined from 12% in April to 6% after 15 May. The estimated Naches spring Chinook contribution ranged from 75% to 79% (Table 4). Contributions of the two fall stocks combined (lower Yakima and Marion Drain) were estimated to be less than 1% in every stratum.

Discussion

The poor concordance of the temporal pattern of the Chandler smolt sampling and the temporal pattern of downstream Chinook migration past the Chandler trap suggest that

estimates of relative abundance of the three spring Chinook stocks should be used with caution and probably should not be extrapolated to estimates of relative production in the three stocks. WDFW and YIN have devised a sampling plan to provide a better representation of the downstream migration in 2004.

WDFW will strive to enhance the Yakima basin Chinook salmon baseline for the analysis of the 2004 outmigrants. The poor PCR and genotyping success for the American and Naches River collections likely was due to poor sample quality. WDFW lab staff noted during sample preparation that several American River samples smelled rotten and at least one had a maggot in the sample vial. Locus selection might also have played a part. We will re-extract DNA template from archived material from these populations and we will use a newly assembled suite of microsatellite loci that has been adopted by our lab and other collaborating labs as a standardized coast-wide suite for Chinook salmon. After we have reconstructed the baseline, we will reanalyze the previous smolt samples from Chandler.

Acknowledgements

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Literature cited

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Table 1. Chinook salmon collections analyzed in this study.

Collection Source	Lab code	# processed	# analyzed*
American River spring Chinook	01FG	96	71
	02IJ	192	45
	92DJ	96	89
	<i>total</i>	<i>384</i>	<i>205</i>
Naches River spring Chinook	01FH	96	78
	02IK	192	40
	92DM	96	92
	<i>total</i>	<i>384</i>	<i>210</i>
upper Yakima River spring Chinook	02BK	105	105
Marion Drain fall Chinook	92FQ	92	90
Yakima River fall Chinook	98FB	79	41
Chandler trap Chinook smolts	03BX	708	702

* All animals genotyped at one or more loci were analyzed

Table 2. The microsatellite loci used in this study and some of their characteristics.

Locus	Predominant allele increment (bp)	Allelic range (bp)	# of alleles	% missing data
Ogo-2	2	201-254	24	22%
Ogo-4	2	128-172	16	24%
One-8	2	154-192	23	27%
Ots-1	2	174-237	15	49%
Ots-101	4	146-278	29	30%
Ots-3M	2	127-188	12	25%
Ots-2M	2	134-172	26	55%
Ssa-197	4	160-306	32	42%
Omm-1135	2	199-224	10	60%
Omm-1142	2	143-252	26	39%
Omy-1001	2	206-358	58	29%

Table 3. Allele frequencies

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Ogo-2						
Alleles scored:	352	236	210	160	60	1018
% missing data:	14%	44%	0%	11%	26%	22%
Allele			Relative frequencies			
201	-	0.004	-	-	-	
202	-	-	0.019	-	-	
206	-	-	-	0.013	0.033	
207	-	0.004	-	-	0.033	
208	0.003	-	-	-	-	
209	-	0.008	-	0.019	0.017	
210	0.003	-	-	-	-	
211	0.003	0.004	-	-	-	
214	0.631	0.589	-	0.069	0.050	
215	-	-	0.529	-	-	
217	0.241	0.097	-	0.025	0.017	
219	0.057	0.085	-	0.156	0.217	
220	-	-	0.210	-	-	
221	0.003	0.110	-	0.206	0.217	
223	-	0.030	0.171	0.106	0.100	
225	-	-	0.005	0.019	0.050	
227	0.048	0.051	0.067	0.294	0.100	
229	-	0.008	-	0.006	0.050	
231	0.003	0.008	-	0.044	0.033	
233	0.009	-	-	0.019	-	
235	-	-	-	-	0.050	
237	-	-	-	0.019	-	
239	-	-	-	-	0.033	
254	-	-	-	0.006	-	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Ogo-4						
Alleles scored:	328	228	210	166	62	994
% missing data:	20%	46%	0%	8%	23%	24%
Allele			Relative frequencies			
128	-	-	-	-	0.032	
134	0.030	0.026	0.024	0.217	0.339	
138	0.061	0.035	0.062	0.325	0.323	
141	-	-	0.048	0.145	0.113	
142	-	0.013	-	-	-	
145	-	-	-	0.066	0.032	
148	-	-	-	-	0.016	
152	0.357	0.298	0.162	-	0.032	
155	-	0.009	-	0.024	0.032	
157	-	0.004	-	-	0.016	
159	0.137	0.114	0.157	0.006	-	
161	0.009	0.162	0.319	0.072	-	
165	0.305	0.294	0.229	0.145	0.065	
169	0.098	0.035	-	-	-	
171	-	0.009	-	-	-	
172	0.003	-	-	-	-	
Locus: Ots-1						
Alleles scored:	130	156	208	132	40	666
% missing data:	68%	63%	1%	27%	51%	49%
Allele			Relative frequencies			
174	-	0.006	-	-	-	
177	-	0.013	-	-	-	
178	0.008	0.019	0.010	-	-	
180	0.023	0.006	0.024	-	-	
182	-	-	-	-	0.050	
183	-	0.051	0.005	-	-	
184	0.762	0.468	0.365	0.235	0.175	
185	-	-	0.005	-	-	
186	-	0.026	0.067	0.144	0.050	
188	0.023	0.026	0.029	0.038	0.025	
190	-	-	-	-	0.025	
194	0.185	0.378	0.495	0.538	0.625	
196	-	-	-	0.038	0.025	
198	-	-	-	0.008	0.025	
237	-	0.006	-	-	-	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: One-8						
Alleles scored:	290	228	206	172	60	956
% missing data:	29%	46%	2%	4%	26%	27%
Allele			Relative frequencies			
154	0.010	0.004	-	-	-	
155	0.034	0.110	0.141	0.047	0.050	
157	-	0.013	-	-	-	
159	0.076	0.039	0.029	0.180	0.217	
161	-	0.009	0.073	0.180	0.167	
162	-	0.004	-	-	-	
164	0.003	0.013	-	-	-	
165	0.034	0.079	0.209	0.006	0.033	
168	0.393	0.382	0.286	0.209	0.217	
170	0.121	0.057	0.078	0.012	-	
172	0.252	0.154	0.107	0.058	0.083	
175	-	-	0.005	-	-	
176	-	0.022	0.029	0.128	0.100	
178	-	-	-	0.023	-	
180	-	-	-	0.012	-	
181	-	-	-	0.006	-	
182	-	0.018	-	0.052	0.100	
183	0.003	-	-	-	-	
184	0.052	0.018	0.005	-	-	
186	0.003	0.022	-	-	-	
188	0.017	0.057	0.039	0.076	0.033	
191	-	-	-	0.006	-	
192	-	-	-	0.006	-	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Ots-101						
Alleles scored:	246	254	208	132	66	906
% missing data:	40%	40%	1%	27%	19%	30%
Allele			Relative frequencies			
146	-	0.004	0.014	-	-	
152	-	0.012	0.005	-	-	
156	-	0.008	-	0.008	0.030	
160	0.004	0.016	0.072	0.008	0.015	
164	0.008	0.035	0.005	0.023	0.045	
168	0.008	0.020	0.087	0.038	0.015	
172	0.118	0.142	0.029	0.098	0.045	
176	0.041	0.106	0.135	0.076	0.091	
180	0.089	0.102	0.063	0.038	0.061	
184	0.024	0.043	0.029	0.083	0.030	
188	-	-	0.005	0.106	0.061	
192	0.114	0.071	0.005	0.098	0.091	
196	0.102	0.059	0.005	0.015	0.061	
200	0.199	0.118	0.091	0.053	0.076	
204	0.008	0.031	0.058	0.068	0.121	
208	0.049	0.039	0.077	0.076	-	
212	0.020	0.028	0.063	0.045	0.045	
216	0.012	0.031	0.029	0.053	0.045	
220	0.049	0.024	0.034	0.053	0.030	
224	0.033	0.004	0.077	0.015	0.030	
228	0.004	0.035	0.053	-	0.030	
232	0.012	0.008	0.010	0.008	0.015	
238	0.089	0.024	0.005	0.008	-	
240	0.012	0.004	0.048	0.030	-	
244	-	0.020	-	-	0.015	
250	0.004	0.008	-	-	0.030	
254	-	0.004	-	-	-	
270	-	0.004	0.005	-	-	
278	-	-	-	-	0.015	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Ots-3M						
Alleles scored:	282	268	208	152	72	982
% missing data:	31%	36%	1%	16%	11%	25%
Allele			Relative frequencies			
127	0.004	-	-	-	-	
136	-	-	-	0.026	-	
137	0.004	0.004	0.010	0.020	-	
140	-	-	-	-	0.014	
142	-	0.034	0.038	0.013	0.083	
144	0.018	0.041	0.106	0.132	0.083	
147	0.092	0.209	0.178	0.309	0.278	
149	0.851	0.679	0.567	0.289	0.208	
151	0.032	0.030	0.048	0.132	0.194	
153	-	0.004	0.048	0.059	0.125	
155	-	-	-	0.020	0.014	
188	-	-	0.005	-	-	
Locus: Omm-1135						
Alleles possible (=2N):	30	78	208	160	42	518
% missing data:	93%	81%	1%	11%	48%	60%
Allele			Relative frequencies			
199	0.800	0.744	0.726	0.313	0.286	
201	0.100	0.013	0.048	0.031	0.024	
203	-	-	-	-	0.024	
208	-	0.013	-	-	-	
209	0.067	0.038	0.111	0.150	0.071	
216	0.033	0.103	0.082	0.394	0.405	
218	-	-	-	0.025	0.071	
220	-	0.090	0.034	0.075	0.095	
222	-	-	-	0.006	-	
224	-	-	-	0.006	0.024	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Ssa-197						
Alleles scored:	132	218	204	148	50	752
% missing data:	68%	48%	3%	18%	38%	42%
Allele			Relative frequencies			
160	-	-	-	0.007	-	
164	-	-	0.010	-	0.020	
168	-	0.014	0.015	0.061	0.120	
172	0.008	0.005	0.005	0.014	0.060	
176	-	-	0.010	0.074	0.040	
180	-	0.005	0.034	0.027	0.040	
184	0.015	0.023	0.049	0.041	0.020	
188	-	-	-	0.095	0.060	
192	-	-	0.005	0.041	-	
196	-	-	-	0.034	0.020	
200	-	-	-	0.027	0.020	
204	-	-	-	0.041	0.020	
208	-	-	-	0.014	0.020	
212	-	-	-	0.007	0.020	
216	-	-	-	-	0.020	
220	0.152	0.055	0.020	0.007	-	
224	0.008	0.014	0.015	0.034	-	
228	0.091	0.037	0.005	0.020	0.020	
232	0.038	0.018	0.127	0.020	0.040	
240	0.152	0.188	0.167	0.135	0.140	
244	0.136	0.133	0.108	0.074	0.060	
248	0.098	0.165	0.108	0.054	0.080	
252	0.114	0.151	0.118	0.020	0.040	
256	0.008	0.046	0.054	0.027	0.080	
260	0.030	0.046	0.093	0.061	0.020	
264	-	0.018	0.049	0.027	-	
268	0.106	0.009	0.010	0.020	0.040	
272	0.030	0.014	-	0.007	-	
276	-	0.005	-	0.014	-	
280	-	0.005	-	-	-	
288	-	0.041	-	-	-	
306	0.015	0.009	-	-	-	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Omm-1142						
Alleles scored:	142	246	208	156	40	792
% missing data:	65%	41%	1%	13%	51%	39%
Allele	Relative frequencies					
143	-	-	-	0.019	-	
145	-	-	-	0.006	-	
147	0.592	0.484	0.279	0.218	0.125	
149	0.021	0.020	0.024	-	-	
151	0.035	0.069	-	0.058	0.100	
156	-	0.004	0.010	-	-	
158	0.042	0.016	0.029	0.141	0.075	
160	-	0.008	-	0.103	0.075	
162	-	-	-	0.006	0.025	
170	-	-	-	0.019	-	
172	0.007	0.004	0.077	0.006	-	
174	-	-	-	0.006	-	
178	-	-	-	-	0.075	
180	-	-	0.038	0.045	0.150	
184	0.183	0.187	0.293	0.122	0.125	
186	0.021	-	0.111	0.090	0.200	
188	-	0.004	-	0.051	-	
190	-	0.004	0.019	-	-	
192	-	-	-	0.006	-	
198	-	0.004	-	-	-	
200	0.007	0.008	0.053	0.090	0.050	
202	0.007	0.008	0.010	-	-	
204	-	-	0.005	-	-	
206	0.085	0.171	0.053	0.013	-	
207	-	0.004	-	-	-	
252	-	0.004	-	-	-	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Omy-1001						
Alleles scored:	230	314	204	138	38	924
% missing data:	44%	25%	3%	23%	53%	29%
Allele			Relative frequencies			
206	0.009	0.006	0.015	-	-	
210	0.039	0.016	-	-	-	
214	0.061	0.048	0.059	-	-	
216	-	0.003	-	-	-	
218	0.070	0.064	0.078	0.007	-	
222	0.039	0.045	0.108	-	-	
224	0.022	-	-	-	-	
226	0.039	0.013	0.059	0.029	-	
228	0.009	0.006	-	-	-	
230	0.004	0.006	0.025	-	-	
232	-	-	-	0.007	-	
234	0.009	0.006	-	0.029	-	
238	0.004	-	-	0.007	0.053	
242	-	-	-	-	0.026	
246	-	-	0.020	0.007	-	
248	-	-	-	0.022	0.026	
252	-	-	-	-	0.053	
254	-	-	0.015	0.080	-	
256	-	-	-	0.029	-	
258	0.009	0.010	-	0.014	0.053	
260	0.004	0.003	0.029	0.007	0.053	
262	-	-	0.010	-	-	
264	-	-	0.010	0.029	0.132	
266	0.009	0.006	0.054	0.072	-	
268	-	0.010	-	0.072	-	
270	0.022	0.032	0.054	-	-	
272	0.030	0.070	0.010	0.043	0.053	
274	0.009	0.029	0.034	0.014	0.026	
276	0.013	0.006	0.078	0.022	-	
278	0.048	0.076	0.039	0.014	0.026	
280	0.004	0.006	0.005	0.065	0.053	
282	0.026	0.045	0.015	0.022	-	
284	-	-	-	0.101	0.026	
286	0.004	0.013	-	-	-	
288	0.035	0.035	-	0.029	0.026	
290	0.030	0.038	0.010	0.022	0.053	
292	0.035	0.035	0.005	0.051	-	
294	0.022	0.013	0.020	0.007	0.053	

Table 3. Allele frequencies (continued).

Population:	American sp	Naches sp	upper Yakima sp	Marion fall	Yakima fall	All
Alleles possible (=2N):	410	420	210	180	81	1301
Locus: Omy-1001 (continued)						
296	0.122	0.121	0.054	0.014	-	
298	-	0.006	-	0.029	-	
300	0.043	0.003	0.029	0.058	0.105	
302	-	0.010	-	0.007	-	
304	0.026	0.035	0.039	0.014	0.026	
306	0.009	0.006	-	0.022	-	
308	0.048	0.048	0.025	-	-	
310	0.013	0.019	-	-	-	
312	0.026	0.029	0.044	0.007	-	
316	0.017	-	0.034	0.007	0.026	
320	0.057	0.054	0.010	-	0.026	
324	0.017	0.019	-	-	-	
328	-	0.003	0.010	-	-	
330	0.009	-	-	0.007	-	
332	-	-	0.005	-	-	
338	0.004	0.006	-	0.014	0.026	
342	-	-	-	-	0.026	
346	-	-	-	-	0.026	
350	-	-	-	0.014	0.026	
358	0.004	-	-	-	-	

Table 4. Mixture composition estimates.

2003	10-30 April	1-14 May	After 15 May*	Entire sample**
N	156	241	305	702
American SP	12%	13%	16%	13%
Naches SP	75%	79%	78%	80%
upper Yakima SP	12%	8%	6%	7%

* Collection dates not available for the final 68 samples taken after 15 May 2003

** stock-of-origin assignment proportions for the entire outmigration period represent a single EM estimate for all samples, not an average of the individual time strata estimates

Figure 1. Chinook salmon spawning streams and the location of the Chandler trap in the Yakima basin, Washington.

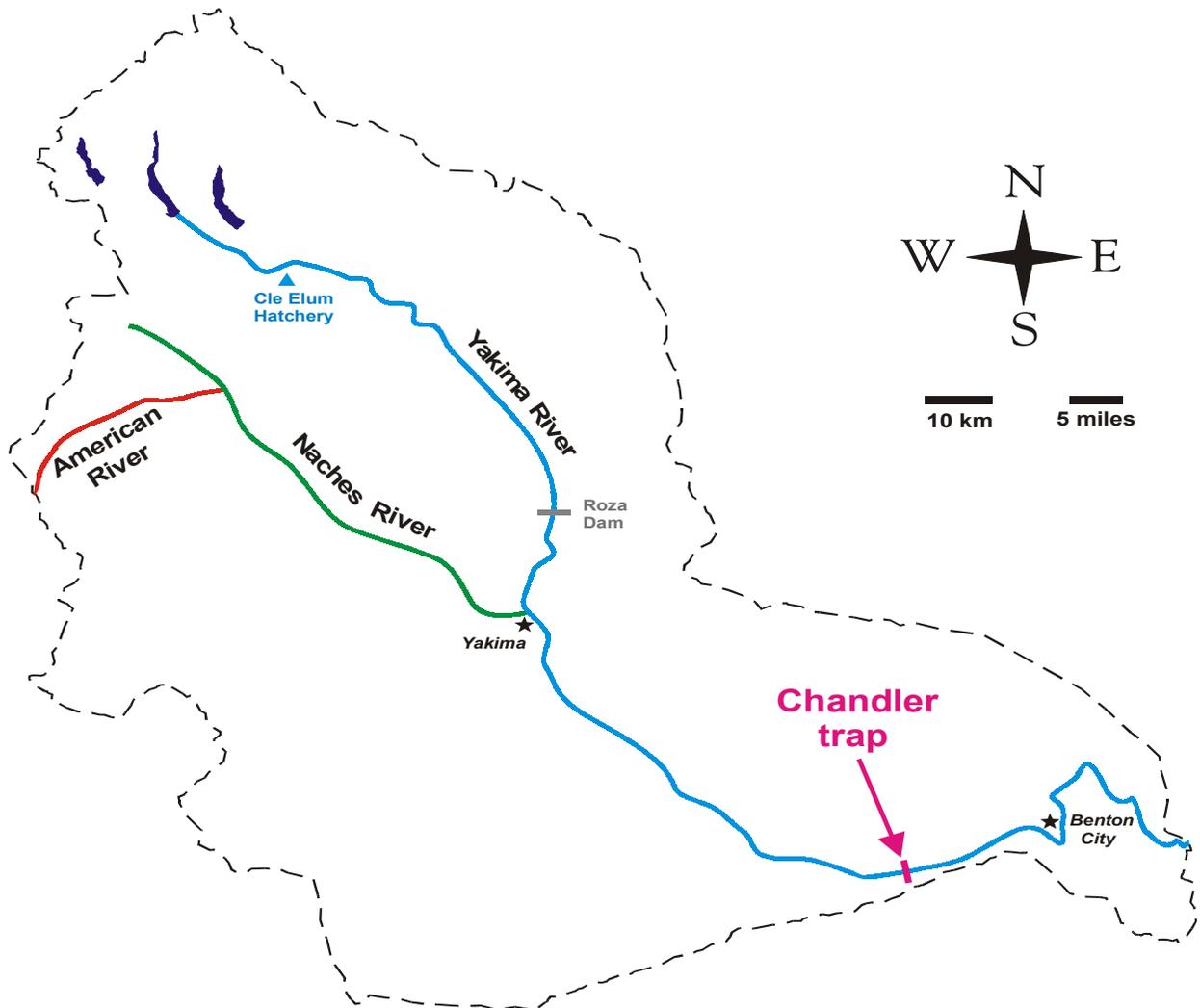


Figure 2. Temporal distribution during 2002 of outmigrant Chinook passage at Chandler trap and of genetic sample collection. There are no passage numbers prior to 1 March.

