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Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

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Yakima/Klickitat Fisheries Project Genetic Studies

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

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Executive Summary

Chapter 1: Chinook salmon of known gender were analyzed to determine the reliability of the two sets of genetic markers to identify gender. Samples of unknown gender collected at Roza Dam in 2005 were then analyzed to determine the sex of each fish. The analysis of known-sex samples resulted higher accuracy for the OTY2-WSU markers (Brunelli and Thorgaard, 2004) than the OT-24 markers (Clifton and Rodriquez 1997). Analysis of 140 samples of unknown gender in 2005 resulted in complete agreement between markers after seven samples were reanalyzed. The comparison of gender identifications based on morphology and genetics resulted in 22 of 140 (15.7%) individuals in both collections that had different gender determinations. Our investigations using these two different DNA markers for gender identification in Chinook suggested high, but not 100% accuracy.

Chapter 2: A maximum likelihood parentage assignment procedure was used to estimate the reproductive output of Chinook salmon spawners of hatchery- and natural-origin in the Cle Elum experimental spawning channel for the 2005 brood year. The assignments were based on offspring genotypes at 10 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999985. Two thousand eight hundred and ninety-two of 3,000 fry from the 2005 brood that were genotyped at six or more loci were assigned to a parental pair with 95% confidence. The number of progeny attributed to individual potential parents ranged from 0 to 465 for malesand from 0 to 257 for females. The average number of progeny attributed to hatchery-origin adult males was 1,688, while the average number attributed to hatchery-origin females was 1,483. In comparison, the average number of progeny attributed to natural-origin adult males was 1,000, while the average number attributed to natural-origin females was 1,409.

Chapter 3: A stock-of-origin assignment procedure based on 11 microsatellite loci was used to estimate the percentages of smolts from each of three spring and two fall Chinook salmon stocks outmigrating past Chandler Trap (Yakima River) from January – July 2005. A blind analysis using 100 known-origin samples resulted in approximately 97% (96/99) correct identification of stock-of-origin. Morphological assessment and genetic assignment of spring versus fall Chinook smolt were compared for the April, May, and June – July time strata; 1,003/1,044 (96.1%) smolts were identified the same using both procedures. Mixture analysis was conducted on a proportional subsample of 1,320 smolts. Assessment of five time strata (January – February, March, April, May, and June – July) during the outmigration revealed the largest percentage of the spring smolts to be from the upper Yakima River stock. During April, the percentage of smolts from American River and Naches River increased while the upper Yakima spring stocks declined. There was a large increase of the lower Yakima fall stock during the May stratum (77.5%) and over 73% of the total were by the two fall stocks in June/July.

Chapter 4: We continued our examination of the population genetic structure of steelhead (*Oncorhynchus mykiss*) in the Yakima River basin. Samples from Upper

Yakima River (at Roza Dam), Ahtanum Cr., Toppenish Cr., Satus Cr. and Naches River, and compared these wild collections to Skamania hatchery steelhead and several strains of hatchery rainbow trout planted into tributaries. Multi-locus genotypes were analyzed for 1211 individuals at ten microsatellite loci. Pairwise genotypic and F_{ST} tests indicated significant genetic differences among most tributaries and little genetic differentiation between collection years within tributaries, suggesting that most tributaries contain genetically differentiated populations. Collections from Roza Dam and Naches River were genetically similar, suggesting common ancestry and gene flow through straying. All collections were distinctly different from Skamania Hatchery steelhead and South Tacoma hatchery rainbow, although hatchery steelhead may have introgressed slightly into the Upper Yakima population and to a lesser degree into other collections.

Chapter 5: We reanalyzed data presented in our 2004 report on morphometric differences between hatchery-origin and natural-origin Upper Yakima spring chinook after one generation of hatchery culture, examining the possibility that some of the differences seen may have been due to inconsistent digitizing of landmark 7, the base of the tail. We redigitized landmark 7 for all fish, and redid the previous analysis with the corrected data. In this report we repeat key analyses with the redigitized data and compare the results with those of the previous report. We concluded that although the redigitization considerably reduced classification inaccuracy and perceived shape differences based on landmark 7, none of the previous conclusions were unjustified. Highly significant shape variation remains for all factors.

Chapter 6: Four-year old adult wild spring chinook at a supplementation hatchery were compared morphologically to their first-generation hatchery counterparts over three consecutive brood years using the method of thin-plate spline analysis on 12 digitized landmarks. Overall sex-specific canonical discriminant analysis (CDA) was able to classify females to origin with about 75% accuracy (up to 84% accuracy for one brood year) and was able to classify males to origin with about 65% accuracy (up to 89% accuracy for one brood year). Classification to brood year was about 61% accurate for females and males (and up to 73% for wild fish). Adjustment for size resulted in virtually no improvement in classification accuracy for females but improved accuracy in males about 5%. Although wild fish were on average larger than hatchery fish, allometry clearly had a minor impact on classification accuracy. Consensus shapes suggested that hatchery fish had larger heads, were more slender than wild fish, and differed in dorsal and anal fin base widths; analysis of variance of traditional body proportion measurements bore this out. Body proportion differences between hatchery and wild females ranged between 0.27 and 0.41 standard deviations; differences in males were somewhat less. After one generation of artificial rearing hatchery and wild fish statistically differ in morphology, and these small differences may be biologically important, they allow only moderate classification power.

Chapter 7: We present a major revision of the 2002 domestication monitoring plan, with much expanded detail on protocols and analyses for all traits and incorporation of results to date. More important, however, are revisions to include two major efforts to address

concerns raised in a recent issue paper on supplementation monitoring by the ISRP/ISAB (2005) and a comprehensive overview of supplementation by Goodman (2004). The first is a pedigree study called *Target Population Natural Replacement Rate*, in which the reproductive success in the wild of natural-origin and hatchery-origin fish can be compared. The second critical change is an expansion of trait A1, now called *Productivity: Female Recruits Produced per Naturally Spawning Female.*

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Chapter 1

DNA-Based Gender Determination of Hatchery-Origin Chinook Salmon Passing Roza Dam (Yakima River) in 2005

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Abstract

The objective of this task was to identify the gender of Chinook salmon using two sets of sex-linked molecular markers. Chinook salmon of known-gender were analyzed to determine the reliability of the two sets of markers to identify gender. Samples of unknown gender collected at Roza Dam in 2005 were then analyzed to determine the sex of each fish. The analysis of known-sex samples resulted higher accuracy for the OTY2-WSU markers (Brunelli and Thorgaard, 2004) than the OT-24 markers (Clifton and Rodriquez 1997). Analysis of the 2005 samples of unknown gender (total of 140 fish) resulted in complete agreement between markers after seven samples were re-analyzed to re-assess gender identification. The comparison of gender identifications based on morphology and genetics resulted in 22 of 140 (15.7%) individuals in both collections that had different gender determinations. Our investigations using these two different DNA markers for gender identification in Chinook suggested high, but not 100% accuracy.

Introduction

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

We screened approximately 140 hatchery-origin adult Chinook samples collected at Roza Dam for one or more DNA gender identification markers to estimate sex composition and age-specific sex composition for first generation hatchery returns (hatchery produced progeny of natural-origin recruits). The DNA samples are collected from fish passing Roza Dam because they give the best representative estimate of hatchery fish returns and they have a relatively large sample size.

Material and Methods

Collection of Known Gender
Three collections of Chinook salmon of known gender were analyzed by Kassler et al. (2004) to determine how well molecular techniques would assign gender. Results from Kassler et al. (2004) revealed accuracy to be between 74.5% and 100.0%. Based on these results, we analyzed three additional collections (04HR, 04GE, and 05MW) of Chinook salmon of known gender. One collection, 04HR (Cle Elum spawning channel, natural origin adults) was spring-run Chinook and two collections; 04GE and 05MW (Winfield Creek – Hoh River) were fall-run populations. Each collection was analyzed independently and then the accuracy of the DNA-based gender assignments were assessed by comparison with the assignments 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 2005 (N = 140 were analyzed; collections 05EA - adults and 05EB - jacks). 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 using the nucleospin tissue kits obtained from Machery-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 μL.

PCR and Gel Methods
Polymerase chain reaction (PCR) was used to amplify two sets of molecular markers (Clifton and Rodriquez 1997, Brunelli and Thorgaard 2004) that exist in distinct locations

on the Y chromosome or are linked to the Y chromosome to identify gender of Chinook salmon. Analysis of the markers described by Clifton and Rodriquez (1997) utilized primers p551 and p559, derived from a sex specific marker (OT-24), amplifying a 950 base pair fragment in males while females yielded a varied amount of a 950 base pair product but of lesser quantity than males. A second pair of primers (p709 and p710) derived from non-sex linked HSP30 (425 base pairs) is monomorphic in Chinook. The p709 and p710 primers were multiplexed with the OT-24 primers as a PCR control to determine that there had not been a false identification as a female due to PCR failure.

Brunelli and Thorgaard (2004) identified a primer sequence OTY2-WSU that allowed sex identification of male and female Chinook and other Pacific salmon species. A fragment of approximately 287 base pairs amplifies in males while females do not amplify any sex-specific products. A second set of primers amplifying the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH, approx. 750 base pairs) present in both males and females was multiplexed with the OTY2-WSU primers and used as a control to test for successful PCR amplification.

The polymerase chain reaction mixture contained the following for a 10 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.1 µM of each oligonucleotide primer, and 0.05 units *Taq* polymerase (Promega). Amplification was performed using an MJ Research PTC-200 thermocycler. The thermal profile for both gender markers was as follows: an initial denaturation step of 3 minutes at 94^{o}C; 35 cycles of 15 seconds at 94^{o}C, 30 seconds at 48^{o}C (Clifton-Rodriguez) or 63 ^{o}C (Brunelli-Thorgaard), and 1 minute at 72^{o}C; plus a final extension step at 70^{o}C for 30 minutes, followed by a final indefinite holding step at 10^{o}C.

Amplified products were separated electrophoretically using a 2.0% agarose gel (Agarose I (0710-100g) from AMRESCO), in 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 dH_20 was mixed, and 8µl of this mixture was loaded into the gel. Photographs of each gel were taken with a digital camera and used for scoring. A 100 base pair Kb ladder (New England Biolabs) was used to estimate size of fragments.

Scoring Methods

For each sample, gender was identified [by two different researchers] using both the OT-24/HSP30 and OTY2-WSU systems. A questionable gender identification (M? or F?) was given for each sample if there was some ambiguity to the banding pattern. A consensus identification was reached by evaluating the four scores (two researches using two methods). If all four scores were in agreement there was no question to the consensus identification. If the scores for one method were ambiguous or missing while the scores for the other method were good (i.e., not ambiguous) the consensus was based on the good scores. In cases where there were ambiguous scores for either technique, the

banding patterns for both methods were re-evaluated to determine if there had been an error in one set of scores or if the ambiguous score could be resolved. If differences occurred between the researchers for one of the methods, but there was agreement for the second method then the consensus reflected the good scores. In cases where there were good scores by both researchers for each method, but were different by method then the individual was excluded from any further assessment of accuracy because gender was not unambiguously determined. Samples were eliminated prior to assessment with the actual gender identification. Elimination of these samples does bias the overall percentages that were used in determining how well the techniques can correctly identify gender by decreasing the overall number of incorrect gender assignments.

Results

Analysis of Known Samples

A total of 70 samples were analyzed from the Cle Elum spawning channel (04HR). Five samples with different or ambiguous scores between the two techniques were dropped from further analysis or assessment (Table 1). The molecular gender identification for the remaining 65 samples was the same with the visual gender identification. The two collections from Winfield Creek (Hoh River; 04GE and 05MW) had four samples that were different or ambiguous and were not considered in this assessment of accuracy for known gender samples. The gender of the remaining samples was consistent between the genetic and visual gender methods.

Analysis of Unknown Samples

Analysis of the 2005 unknown samples (total of 140 fish) resulted in agreement between the two independent scorers and between both molecular methods, with the exception of four individuals (05EA – 7, 05EA – 79, 05EA – 99, 05EB – 2). Re-analysis of the four individuals resulted in agreement between scorers and techniques; therefore they were included with the final consensus scores. A list of the original scores for each technique and scorer along with the final consensus score is shown in Table 2.

Comparison of Unknown Samples Sexed by Morphology and Genetics

Sex identifications of unknown samples (05AE) based on morphology (sexed at the time the live fish were handled and passed at the dam) and genetics were different for 22 of 105 (20.95%) individuals (Appendix 1). Seventeen of the 22 (77.3%) differences were identified as females based on morphological characteristics and males by genetic analysis. The remaining five individuals were identified as males using morphological characteristics and females by genetic analysis. There were no differences between the morphological and genetic sex ID's for 35 jacks that were analyzed (WDFW collection code - 05EB).

Discussion

Genetic techniques can be used to identify the gender of live pre-spawned salmon when morphology-based identification is difficult. 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.

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. Nagler et al. (2001) suggest 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 2004).

Two independent genetic methods were tested and compared to determine if they would reveal the same gender identification. Nine samples were dropped due to inconsistencies in scores between methods or researchers and not given a consensus score. Of the remaining samples, there was consistent agreement of the consensus scores between the gender identification by both methods even when the identification was different than expected based on external morphology. Similar results by both methods support the possibility that samples 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 has the possibility that there has not been any sex reversal in these Chinook salmon, however if the sex identification markers are not linked to the sex determining region then sex reversal may still have occurred and not observed. If sequences of the Y chromosome had moved to the X chromosome then it seems possible that there would not be complete agreement between the gender identifications of the two methods unless the sex-linked markers are not linked with the sex determining region, therefore the same result is seen even when the sequences had moved.

The analysis of known gender samples was conducted on collections from two different geographic locations: Yakima River (Columbia River basin) and Winfield Creek (Hoh River - north Washington coast). A total of nine samples were dropped from the three collections because of disagreement among genetic techniques. Disagreement between the actual gender identification and genetic identification occurred mostly using the Clifton and Rodriquez method identifying females as males. This is not surprising given that the sex-determining band amplifies in females using this technique and therefore females could be mis-identified as males. All other samples were in 100% agreement with the actual gender identifications supporting the ability of the genetic analysis to correctly identify gender in Chinook.

Gender determinations of the unknown samples from 2005 agreed between scorers and methods used. Only four exceptions occurred, however when re-analyzed, consensus scores were determined.

Our comparison of the morphological and genetic methods for identifying gender resulted in a total (combined adult and jack collections) of 15.7% (22/140) of the samples that were identified differently. Most of these differences (over 77%) 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 sexually immature female and smaller male (individual that has not developed sexually dimorphic characteristics) are similar, and therefore difficult to distinguish.

Given that approximately 85% of the unknown samples and 100% of the known samples were identified the same gender by both the morphological assessment and genetic analysis, it would suggest that sex reversal had occurred in 15% or less of the samples that were analyzed. If sex reversal had occurred because of temperature or chemical fluctuations, then the expectation would be that some portion of the samples we analyzed would have been identified incorrectly as Nagler et al. (2001) reported.

Conclusions

Our investigations identifying gender in Chinook salmon using two different DNA markers suggested high, but not 100% accuracy. Using these DNA markers to determine the sex ratios of hatchery-origin Chinook passing Roza Dam we estimated 40 adult males: 65 adult females and 35 jacks in 2005.

Acknowledgements

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Nagler, J.J., J. Bouma, G.H. Thorgaard, and D.D. Dauble. 2001. High incidence of a male-specific genetic marker in phenotypic female Chinook salmon from the Columbia River. Environmental Health Perspectives 109:67-69.

Table 1. Gender determination for Chinook salmon using genetic analysis compared to the actual gender determined by gonads for three collections. Two independent gender identifications and a final consensus gender determination are shown. [methods: 'C - R' = Clifton &Rodriquez; 'B - T' = Brunelli & Thorgaard]. Cells highlighted grey in the consensus column identify individuals where a consensus was not determined. Consensus was reached by examining scores for each technique and scorer. Question marks (?) indicate unclear or ambiguous gender id for an individual score. An "X" identifies missing data for a given sample. If non-ambiguous gender id's were different for scorers (either technique) then no consensus score was given, however if both scores for one technique were ambiguous while scores for the other technique were non-ambiguous then the consensus score reflects the non-ambiguous score.

				C - R method		B - T method	
	Sample code	Actual	Consensus	Score #1	Score #2	Score #1	Score #2
1	04HR0001	F	**F**	F	F	F	F
2	04HR0002	F	**F**	F	F	F	F
3	04HR0003	M	**M**	M	M	M	M
4	04HR0004	M	**M**	M	M	M	M
5	04HR0005	F	**F**	M?	M?	F	F
6	04HR0006	F	**F**	F	F	F	F
7	04HR0007	M	**M**	M	M	M	M
8	04HR0008	M	**M**	M	M	M	M
9	04HR0009	F	**F**	F	F	F	F
10	04HR0010	F	**F**	F	F	F	F
11	04HR0011	F	**F**	F	F	F	F
12	04HR0012	M	**M**	M	M	M	M
13	04HR0013	M	**M**	M	M	M	M
14	04HR0014	F	**F**	F	F	F	F
15	04HR0015	F	**F**	F	F	X	X
16	04HR0016	M	**M**	M	M	M	M
17	04HR0017	M	**M**	M	M	M	M?
18	04HR0018	F	**F**	F	F	F	F
19	04HR0019	F	**F**	F	F	F	F
20	04HR0020	M	**M**	M	M	M	M
21	04HR0021	F	**F**	F	F	F	F
22	04HR0022	F	**F**	F	F	F	F
23	04HR0023	F	**F**	F	F	F	F
24	04HR0024	M	**M**	M	M	M	M
25	04HR0025	M	**M**	M	M	M	M
26	04HR0026	M	**M**	M	M	M	M
27	04HR0027	F	**F**	F	F	F	F
28	04HR0028	M	**M**	M	M	M	M
29	04HR0029	M	**M**	M	M	M	M
30	04HR0030	M	**M**	M	M	M	M

Table 1. continued

				C - R method		B - T method	
	Sample code	Actual	Consensus	Score #1	Score #2	Score #1	Score #2
31	04HR0031	M	**M**	M	M	M	M
32	04HR0032	M	**M**	M	M	M	M
33	04HR0033	M	**M**	M	M	M	M
34	04HR0034	M	**M**	M	M	M	M
35	04HR0035	F	**F**	F	F	F	F
36	04HR0036	M	**M**	M	M	M	M
37	04HR0037	F	**F**	F	F	F	F
38	04HR0038	F	**F**	F	F	F	F
39	04HR0039	M	**M**	M	M	M	M
40	04HR0040	F	**F**	F	F	F	F
41	04HR0041	F	**F**	F	F	F	F
42	04HR0042	F	**F**	F	F	F	F
43	04HR0043	M	**M**	M	M	M	M
44	04HR0044	M		M	M	F	F
45	04HR0045	M	**M**	M	M	M	M
46	04HR0046	M	**M**	M	M	M	M
47	04HR0047	M	**M**	M	M	M	M
48	04HR0048	F	**F**	F	F	F	F
49	04HR0049	M	**M**	M	M	M	M
50	04HR0050	F	**F**	F	F	F?	F
51	04HR0051	M	**M**	M	M	M	M
52	04HR0052	M	**M**	M	M	M	M
53	04HR0053	M	**M**	M	M	M	M
54	04HR0054	M	**M**	M	M	M	M
55	04HR0055	M	**M**	M	M	M	M
56	04HR0056	M	**M**	M	M	X	X
57	04HR0057	M		M?	F?	F	F?
58	04HR0058	M	**M**	M	M	M	M
59	04HR0059	M	**M**	M	M	M	M
60	04HR0060	M	**M**	M	M	M	M
61	04HR0061	M		M	M	M?	F
62	04HR0062	M		M	M	F?	F
63	04HR0063	M	**M**	M	M	M	M?
64	04HR0064	M	**M**	M	M	M	M
65	04HR0065	M	**M**	M	M	M	M
66	04HR0066	M	**M**	M	M	M	M
67	04HR0067	M	**M**	M	M	M	M
68	04HR0068	M	**M**	M	M	M	M
69	04HR0069	M		M?	F?	M?	M?
70	04HR0070	M	**M**	M	M	M	M

Table 1. continued

				C - R method		B - T method	
	Sample code	Actual	Consensus	Score #1	Score #2	Score #1	Score #2
1	04GE0235	F	**F**	F	F	F	F?
2	04GE0236	M	**M**	M	M?	M	M?
3	04GE0237	M	**M**	M	M	M	M
4	04GE0238	F	**F**	F	F	F	F
5	04GE0239	F	**F**	F	F	F	F
6	04GE0240	F	**F**	F	F	F	F
7	04GE0241	M	**M**	M	M	M	M
8	04GE0242	M	**M**	M	M	M	M?
9	04GE0243	M	**M**	M	M	M	M
10	04GE0244	M	**M**	M	M	M	M
11	04GE0245	M	**M**	M	M	M	M
12	04GE0246	F	**F**	F	F	F	F
13	04GE0247	M	**M**	M	M	M	M
14	04GE0248	F	**F**	F	F	F	F
15	04GE0249	F	**F**	F	F	F	F
16	04GE0250	F	**F**	F	F	F	F
17	04GE0251	M	**M**	M	M	M	M
18	04GE0252	M	**M**	M	M	M	M
19	04GE0253	M	**M**	M	M	M	M
20	04GE0254	M	**M**	M	M	M	M
21	04GE0255	F	**F**	F	F	F	F
22	04GE0256	M	**M**	M	M	M	M
23	04GE0257	M	**M**	M	M	M	M
24	04GE0258	M	**M**	M	M	M	M
25	04GE0259	M	**M**	M	M	M	M
26	04GE0260	M	**M**	M	M	M	M
27	04GE0261	F	**F**	F	F	F	F
28	04GE0262	M	**M**	M	M	M	M
29	04GE0263	M	**M**	M	M	M	M
30	04GE0264	F	**F**	F?	F	F	F
31	04GE0265	F	**F**	F	F	F	F
32	04GE0266	M	**M**	M	M	M	M
33	04GE0267	F	**F**	F	F	F	F
34	04GE0268	F	**F**	F	F	F	F?
35	04GE0269	F	**F**	F	F	F	F
36	04GE0270	M		M	M	F?	F
37	04GE0271	F	**F**	F	F	F	F
38	04GE0272	F	**F**	F	F	F	F
39	04GE0273	F	**F**	F	F	F	F
40	04GE0274	M	**M**	M	M	M	M

Table 1. continued

				C - R method		B - T method	
	Sample code	Actual	Consensus	Score #1	Score #2	Score #1	Score #2
1	05MW0001	F	**F**	F	F	F	F
2	05MW0002	F	**F**	F	F	F	F
3	05MW0003	M	**M**	M	M	M	M?
4	05MW0004	F	**F**	F	F	F	F
5	05MW0005	M	**M**	M	M	M	M
6	05MW0006	F	**F**	F	F	F	F
7	05MW0007	F	**F**	F	F	F	F
8	05MW0008	F	**F**	F	F	F	F
9	05MW0009	F	**F**	F	F	F	F
10	05MW0010	F	**F**	F	F	F	F
11	05MW0011	F	**F**	F	F	F	F
12	05MW0012	M	**M**	M	M	M?	M?
13	05MW0013	M		M	M	F	F
14	05MW0014	F	**F**	M?	M?	F	F
15	05MW0015	M	**M**	M	M	M	M
16	05MW0016	F	**F**	F	F	F	F
17	05MW0017	F	**F**	F	F	F	F
18	05MW0018	M	**M**	M	M	M	M
19	05MW0019	F	**F**	F	F	F	F
20	05MW0020	F	**F**	F	F	F	F
21	05MW0021	F	**F**	F	F	F	F
22	05MW0022	M	**M**	M	M	M	M
23	05MW0023	M	**M**	M	M	M	M?
24	05MW0024	M	**M**	M	M	M	M?
25	05MW0025	F	**F**	F	F	F	F
26	05MW0026	M	**M**	M	M	M	M
27	05MW0027	F	**F**	M	M?	F	F
28	05MW0028	M		M	M	F	F
29	05MW0029	F		M	M?	F	F
30	05MW0030	M	**M**	M	M	M	M
31	05MW0031	F	**F**	F	F	F	F
32	05MW0032	F	**F**	F	F	F	F
33	05MW0033	F	**F**	F	F	F	F
34	05MW0034	M	**M**	M	M	M	M
35	05MW0035	F	**F**	F	F	F	F
36	05MW0036	F	**F**	F	F	F	F
37	05MW0037	F	**F**	F	F	F	F
38	05MW0038	F	**F**	F	F	F	F
39	05MW0039	M	**M**	M	M	F?	F?

Table 2. Gender determination for Chinook salmon passed at Roza Dam in 2005. Two independent gender identifications and a final consensus gender determination are shown. [methods: 'C - R' = Clifton &Rodriquez; 'B - T' = Brunelli & Thorgaard] Consensus was reached by examining scores for each technique and scorer. Question marks (?) indicate unclear or ambiguous gender id for an individual score. Four individuals (05EA 7, 79, 99, and 05EB 2) were rerun (rr) because of unclear or ambiguous scores. The rerun (rr) score was used to determine the consensus score.

Unknown gender - Adults

			C - R method		B - T method	
		Consensus	Score #1	Score #2	Score #1	Score #2
1	05EA00001	**F**	F	F	F	F
2	05EA00002	**M**	M	M	M	M
3	05EA00003	**F**	F	F	F	F
4	05EA00004	**F**	F	F	F	F
5	05EA00005	**M**	M	M	M	M
6	05EA00006	**M**	M	M	M	M
7	05EA00007		M	M	F	M?
	05EA00007 rr	**M**	M	M	M	M
8	05EA00008	**F**	F	F	F	F
9	05EA00009	**F**	F	F	F	F
10	05EA00010	**F**	F	F	F	F
11	05EA00011	**F**	F	F	F	F
12	05EA00012	**F**	F	F	F	F
13	05EA00013	**F**	F	F	F	F
14	05EA00014	**M**	M	M	M	M
15	05EA00015	**F**	F	F	F	F
16	05EA00016	**F**	F	F	F	F
17	05EA00017	**F**	F	F	F	F
18	05EA00018	**M**	M	M	M	M
19	05EA00019	**M**	M	M	M	M
20	05EA00020	**M**	M	M	M	M
21	05EA00021	**F**	F	F	F	F
22	05EA00022	**F**	F	F	F	F
23	05EA00023	**F**	F	F	F	F
24	05EA00024	**M**	M	M	M	M
25	05EA00025	**F**	F	F	F	F
26	05EA00026	**F**	F	F	F	F
27	05EA00027	**M**	M	M	M	M
28	05EA00028	**M**	M	M	M	M
29	05EA00029	**F**	F	F	F	F
30	05EA00030	**M**	M	M	M	M
31	05EA00031	**F**	F	F	F	F

Table 2. continued

			C - R method		B - T method	
		Consensus	Score #1	Score #2	Score #1	Score #2
32	05EA00032	**M**	M	M	M	M
33	05EA00033	**M**	M	M	M	M
34	05EA00034	**F**	F	F	F	F
35	05EA00035	**M**	M	M	M	M
36	05EA00036	**F**	F	F	F	F
37	05EA00037	**F**	F	F	F	F
38	05EA00038	**M**	M	M	M	M
39	05EA00039	**M**	M	M	M	M
40	05EA00040	**F**	F	F	F	F
41	05EA00041	**F**	F	F	F	F
42	05EA00042	**F**	F	F	F	F
43	05EA00043	**M**	M	M	M	M
44	05EA00044	**F**	F	F	F	F
45	05EA00045	**F**	F	F	F	F
46	05EA00046	**M**	M	M	M	M
47	05EA00047	**F**	F	F	F	F
48	05EA00048	**F**	F	F	F	F
49	05EA00049	**F**	F	F	F	F
50	05EA00050	**F**	F	F	F	F
51	05EA00051	**M**	M	M	M	M
52	05EA00052	**F**	F	F	F	F
53	05EA00053	**F**	F	F	F	F
54	05EA00054	**F**	F	F	F	F
55	05EA00055	**F**	F	F	F	F
56	05EA00056	**M**	M	M	M	M
57	05EA00057	**F**	F	F	F	F
58	05EA00058	**M**	M	M	M	M
59	05EA00059	**M**	M	M	M	M
60	05EA00060	**M**	M	M	M	M
61	05EA00061	**F**	F	F	F	F
62	05EA00062	**F**	F	F	F	F
63	05EA00063	**M**	M	M	M	M
64	05EA00064	**F**	F	F	F	F
65	05EA00065	**F**	F	F	F	F
66	05EA00066	**F**	F	F	F	F
67	05EA00067	**M**	M	M	M	M
68	05EA00068	**F**	F	F	F	F?
69	05EA00069	**F**	F	F	F	F
70	05EA00070	**F**	F	F	F	F
71	05EA00071	**M**	M	M	M	M
72	05EA00072	**F**	F	F	F	F

Table 2. continued

			C - R method		B - T method	
		Consensus	Score #1	Score #2	Score #1	Score #2
73	05EA00073	**F**	F	F	F	F
74	05EA00074	**F**	F	F	F	F
75	05EA00075	**F**	F	F	F	F
76	05EA00076	**F**	F	F	F	F
77	05EA00077	**M**	M	M	M	M
78	05EA00078	**F**	F	F	F	F
79	05EA00079		M	M	F	M?
	05EA00079 rr	**M**	M	M	M	M
80	05EA00080	**M**	M	M	M	M
81	05EA00081	**F**	F	F	F	F
82	05EA00082	**M**	M	M	M	M
83	05EA00083	**F**	F	F	F	F
84	05EA00084	**F**	F	F	F	F
85	05EA00085	**M**	M	M	M	M
86	05EA00086	**F**	F	F	F	F
87	05EA00087	**M**	M	M	M	M
88	05EA00088	**F**	F	F	F	F
89	05EA00089	**F**	F	F	F	F
90	05EA00090	**M**	M	M	M	M
91	05EA00091	**F**	F	F	F	F
92	05EA00092	**F**	F	F	F	F
93	05EA00093	**M**	M	M	M	M
94	05EA00094	**F**	F	F	F	F
95	05EA00095	**F**	F	F	F	F
96	05EA00096	**M**	M	M	M	M
97	05EA00097	**M**	M	M	X	M
98	05EA00098	**F**	F	F	F	F
99	05EA00099		M	M	F	F
	05EA00099 rr	**M**	M	M	M	M
100	05EA00100	**F**	F	F	F	F
101	05EA00101	**F**	F	F	F	F
102	05EA00102	**M**	M	M	M	M
103	05EA00103	**M**	M	M	M	M
104	05EA00104	**F**	F	F	F	F
105	05EA00105	**F**	F	F	F	F

Table 2. continued

Unknown gender - Jacks

			C - R method		B - T method	
		Consensus	Score #1	Score #2	Score #1	Score #2
1	05EB00002		M	M	F	M
	05EB00002 rr	**M**	M	M	M	M
2	05EB00004	**M**	M	M	M	M
3	05EB00007	**M**	M	M	M	M
4	05EB00010	**M**	M	M	M	M
5	05EB00012	**M**	M	M	M	M
6	05EB00014	**M**	M	M	M	M
7	05EB00017	**M**	M	M	M	M
8	05EB00020	**M**	M	M	M	M
9	05EB00022	**M**	M	M	M	M
10	05EB00024	**M**	M	M	M	M
11	05EB00027	**M**	M	M	M	M
12	05EB00029	**M**	M	M	M	M
13	05EB00032	**M**	M	M	M	M
14	05EB00034	**M**	M	M	M	M
15	05EB00037	**M**	M	M	M	M
16	05EB00039	**M**	M	M	M	M
17	05EB00042	**M**	M	M	M	M
18	05EB00044	**M**	M	M	M	M
19	05EB00047	**M**	M	M	M	M
20	05EB00049	**M**	M	M	M	M
21	05EB00052	**M**	M	M	M	M
22	05EB00054	**M**	M	M	M	M
23	05EB00057	**M**	M	M	M	M
24	05EB00059	**M**	M	M	M	M
25	05EB00062	**M**	M	M	M	M
26	05EB00064	**M**	M	M	X	M
27	05EB00067	**M**	M	M	M	M
28	05EB00069	**M**	M	M	M	M
29	05EB00072	**M**	M	M	M	M
30	05EB00074	**M**	M	M	M	M
31	05EB00077	**M**	M	M	M	M
32	05EB00078	**M**	M	M	M	M
33	05EB00081	**M**	M	M	M	M
34	05EB00084	**M**	M	M	M	M
35	05EB00087	**M**	M	M	M	M

Appendix 1. Biological data and gender determination of Chinook salmon collected at Roza Dam in 2005 using morphological characteristics and genetic analysis.

Date	DNA Sample #	Genetic ID	Morph ID	Fork Length	Poh Length	Weight	Age
05/09/2005	05EA00001	F	F	70.0	60.0	4.1	4
05/09/2005	05EA00002	M	M	75.0	63.0	5.2	4
05/10/2005	05EA00003	F	F	78.0	68.0	5.0	4
05/12/2005	05EA00004	F	F	70.0	60.0	4.2	4
05/13/2005	05EA00005	M	M	84.0	71.0	7.0	5
05/13/2005	05EA00006	M	F	67.5	56.5	3.5	4
05/13/2005	05EA00007	M	M	96.0	80.0	10.0	5
05/15/2005	05EA00008	F	F	82.0	70.0	6.4	
05/15/2005	05EA00009	F	F	69.0	59.0	3.9	4
05/15/2005	05EA00010	F	F	69.5	59.5	4.2	4
05/15/2005	05EA00011	F	F	71.0	61.0	4.2	4
05/15/2005	05EA00012	F	M	70.0	59.0	4.0	4
05/16/2005	05EA00013	F	F	69.0	58.0	3.6	4
05/16/2005	05EA00014	M	F	68.0	58.0	3.6	4
05/16/2005	05EA00015	F	F	67.5	58.0	3.8	4
05/17/2005	05EA00016	F	F	68.0	58.0	4.0	4
05/17/2005	05EA00017	F	F	73.0	63.0	4.9	4
05/17/2005	05EA00018	M	F	72.5	61.5	4.2	r
05/17/2005	05EA00019	M	M	71.0	60.0	4.6	4
05/17/2005	05EA00020	M	F	57.5	48.0	2.3	4
05/18/2005	05EA00021	F	F	65.5	55.5	3.6	4
05/18/2005	05EA00022	F	F	64.5	54.5	3.2	4
05/18/2005	05EA00023	F	F	72.0	62.0	4.2	4
05/18/2005	05EA00024	M	M	77.0	65.0	5.5	4
05/18/2005	05EA00025	F	F	69.0	59.0	3.7	4
05/18/2005	05EA00026	F	F	73.0	63.0	4.5	4
05/18/2005	05EA00027	M	M	77.0	65.0	5.7	4
05/18/2005	05EA00028	M	F	71.0	61.0	4.3	4
05/19/2005	05EA00029	F	F	64.0	54.0	3.2	r
05/19/2005	05EA00030	M	F	67.0	57.0	3.5	4
05/19/2005	05EA00031	F	F	68.5	58.5	3.9	4
05/19/2005	05EA00032	M	M	63.0	53.0	2.9	4
05/20/2005	05EA00033	M	F	73.0	61.0	4.2	4
05/20/2005	05EA00034	F	F	72.0	61.0	4.5	4
05/20/2005	05EA00035	M	F	65.0	55.0	3.1	4
05/20/2005	05EA00036	F	F	70.0	60.0	4.0	4
05/20/2005	05EA00037	F	F	75.0	63.0	4.7	5
05/21/2005	05EA00038	M	F	63.0	53.0	3.1	4
05/21/2005	05EA00039	M	M	75.0	63.0	5.0	4
05/22/2005	05EA00040	F	F	63.0	53.0	2.7	4
05/22/2005	05EA00041	F	F	69.0	59.0	3.6	4
05/22/2005	05EA00042	F	F	65.0	55.0	3.5	4
05/23/2005	05EA00043	M	M	72.0	61.0	4.0	4
05/23/2005	05EA00044	F	F	66.0	56.0	3.4	4
05/24/2005	05EA00045	F	F	74.0	64.0	5.0	4
05/24/2005	05EA00046	M	F	68.0	58.0	3.7	4
05/24/2005	05EA00047	F	F	73.0	63.0	4.8	4

Appendix 1. Continued

Date	DNA Sample #	Genetic ID	Morph ID	Fork Length	Poh Length	Weight	Age
05/25/2005	05EA00048	F	F	67.0	57.0	3.6	4
05/25/2005	05EA00049	F	F	70.0	60.0	4.0	4
05/26/2005	05EA00050	F	F	73.0	63.0	4.4	4
05/26/2005	05EA00051	M	M	68.0	57.0	3.5	4
05/26/2005	05EA00052	F	F	81.0	70.0	5.4	5
05/26/2005	05EA00053	F	F	63.0	53.0	2.9	r
05/26/2005	05EA00054	F	F	69.0	59.0	4.0	4
05/26/2005	05EA00055	F	F	70.0	60.0	3.9	4
05/26/2005	05EA00056	M	M	69.0	58.0	4.0	r
05/27/2005	05EA00057	F	F	71.0	60.0	4.1	4
05/27/2005	05EA00058	M	M	69.0	56.0	3.5	4
05/27/2005	05EA00059	M	M	75.0	62.0	4.9	r
05/27/2005	05EA00060	M	F	56.0	46.0	2.2	4
05/28/2005	05EA00061	F	F	86.0	74.0	9.9	5
05/28/2005	05EA00062	F	F	70.0	60.0	3.3	4
05/28/2005	05EA00063	M	F	67.0	56.0	3.8	r
05/29/2005	05EA00064	F	F	72.0	62.0	4.3	4
05/29/2005	05EA00065	F	F	75.0	64.0	4.7	4
05/29/2005	05EA00066	F	M	74.0	62.0	4.9	4
05/29/2005	05EA00067	M	F	72.0	60.0	5.3	4
05/29/2005	05EA00068	F	F	77.0	66.0	5.2	4
05/29/2005	05EA00069	F	M	74.0	62.0	4.6	4
05/29/2005	05EA00070	F	F	73.0	62.0	5.0	4
05/30/2005	05EA00071	M	F	66.0	56.0	3.2	4
05/30/2005	05EA00072	F	F	73.0	63.0	4.7	4
05/30/2005	05EA00073	F	F	81.0	69.0	6.0	5
05/30/2005	05EA00074	F	F	68.0	58.0	3.4	4
05/30/2005	05EA00075	F	F	84.0	72.0	6.1	4
05/31/2005	05EA00076	F	F	77.0	67.0	5.0	5
05/31/2005	05EA00077	M	F	65.5	55.0	3.7	r
05/31/2005	05EA00078	F	F	78.0	68.0	5.1	5
05/31/2005	05EA00079	M	F	72.0	62.0	4.2	4
06/01/2005	05EA00080	M	M	76.0	65.0	5.6	4
06/01/2005	05EA00081	F	F	70.0	60.0	3.8	4
06/01/2005	05EA00082	M	F	70.0	60.0	3.9	4
06/03/2005	05EA00083	F	F	64.0	53.0	2.9	4
06/05/2005	05EA00084	F	F	63.0	53.0	2.6	4
06/11/2005	05EA00085	M	M	62.0	52.0	2.6	4
06/11/2005	05EA00086	F	M	69.0	57.0	3.8	4
06/12/2005	05EA00087	M	M	64.0	52.0	2.6	r
06/13/2005	05EA00088	F	F	68.0	58.0	3.6	4
06/14/2005	05EA00089	F	F	70.0	59.0	3.9	4
06/15/2005	05EA00090	M	M	61.0	50.0	2.5	4
06/17/2005	05EA00091	F	F	71.0	60.0	4.1	4
06/18/2005	05EA00092	F	F	70.0	60.0	4.0	4
06/19/2005	05EA00093	M	M	75.0	62.0	4.3	4
06/19/2005	05EA00094	F	M	72.0	61.0	4.2	4
06/19/2005	05EA00095	F	F	66.0	55.0	3.9	4
06/20/2005	05EA00096	M	M	73.0	61.0	4.1	4

Appendix 1. Continued

Date	DNA Sample #	Genetic ID	Morph ID	Fork Length	Poh Length	Weight	Age
06/21/2005	05EA00097	**M**	**M**	76.0	65.0	5.0	4
06/21/2005	05EA00098	**F**	**F**	71.0	61.0	4.0	4
06/22/2005	05EA00099	**M**	**M**	72.0	60.0	3.8	4
06/24/2005	05EA00100	**F**	**F**	75.0	65.0	4.8	4
06/25/2005	05EA00101	**F**	**F**	77.0	67.0	5.4	4
06/27/2005	05EA00102	**M**	**M**	67.0	55.0	3.0	r
06/30/2005	05EA00103	**M**	**M**	68.0	58.0	3.9	4
07/08/2005	05EA00104	**F**	**F**	64.0	54.0	2.9	4
07/16/2005	05EA00105	**F**	**F**	65.0	55.0	2.8	4
05/26/2005	05EB00002	**M**	**J**	48.0	41.0	1.3	3
05/27/2005	05EB00004	**M**	**J**	45.0	37.0	1.2	3
05/29/2005	05EB00007	**M**	**J**	46.0	39.0	1.2	3
05/30/2005	05EB00010	**M**	**J**	43.0	37.0	0.9	3
05/30/2005	05EB00012	**M**	**J**	54.0	45.0	2.0	3
05/31/2005	05EB00014	**M**	**J**	48.0	44.0	1.4	3
05/31/2005	05EB00017	**M**	**J**	53.5	44.5	1.8	3
06/01/2005	05EB00020	**M**	**J**	49.0	41.0	1.5	3
06/01/2005	05EB00022	**M**	**J**	46.0	39.0	1.2	3
06/02/2005	05EB00024	**M**	**J**	48.0	41.0	1.5	3
06/03/2005	05EB00027	**M**	**J**	49.0	39.0	1.3	3
06/04/2005	05EB00029	**M**	**J**	47.0	40.0	1.2	3
06/05/2005	05EB00032	**M**	**J**	48.0	41.0	1.3	3
06/07/2005	05EB00034	**M**	**J**	42.0	35.0	0.8	3
06/10/2005	05EB00037	**M**	**J**	52.0	44.0	1.7	3
06/11/2005	05EB00039	**M**	**J**	47.0	39.0	1.3	3
06/12/2005	05EB00042	**M**	**J**	52.0	43.0	1.5	3
06/13/2005	05EB00044	**M**	**J**	51.0	43.0	1.5	3
06/15/2005	05EB00047	**M**	**J**	43.0	34.0	0.9	3
06/16/2005	05EB00049	**M**	**J**	44.0	38.0	1.1	3
06/17/2005	05EB00052	**M**	**J**	49.0	41.0	1.3	3
06/18/2005	05EB00054	**M**	**J**	54.0	45.0	1.9	3
06/19/2005	05EB00057	**M**	**J**	50.0	41.0	1.3	3
06/20/2005	05EB00059	**M**	**J**	42.0	36.0	0.9	3
06/21/2005	05EB00062	**M**	**J**	59.0	50.0	2.3	3
06/22/2005	05EB00064	**M**	**J**	49.0	42.0	1.4	3
06/23/2005	05EB00067	**M**	**J**	44.0	37.0	0.9	3
06/24/2005	05EB00069	**M**	**J**	42.0	35.0	0.8	3
06/26/2005	05EB00072	**M**	**J**	52.0	41.0	1.6	3
06/27/2005	05EB00074	**M**	**J**	50.0	42.0	1.6	3
06/29/2005	05EB00077	**M**	**J**	52.0	44.0	1.5	3
06/29/2005	05EB00078	**M**	**J**	44.0	37.0	0.9	3
07/03/2005	05EB00081	**M**	**J**	48.5	40.0	1.2	3
07/12/2005	05EB00084	**M**	**J**	59.0	50.0	2.1	3
08/01/2005	05EB00087	**M**	**J**	50.0	42.0	1.2	3

Chapter 2

DNA-Based Parentage Assignments of Chinook Salmon from the Cle Elum Spawning Channel in 2005

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Abstract

We used a maximum likelihood parentage assignment procedure to estimate the reproductive output of Chinook salmon spawners of hatchery- and natural-origin in the Cle Elum experimental spawning channel for the 2005 brood year. The assignments were based on offspring genotypes at 10 microsatellite loci. The probabilities of exclusion (inferring non-parentage by randomly picked adults) assuming neither parent was known were estimated to be 0.999985. Two thousand eight hundred and ninety-two of 3,000 fry from the 2005 brood that were genotyped at six or more loci were assigned to a parental pair with 95% confidence. We found no compelling evidence to suggest that un-genotyped parents spawned successfully in this year. The number of progeny attributed to individual potential parents was quite variable, ranging from 0 to 465 for all males (0 to 465 for adult males; 0 to 52 for jacks; 0 to 26 for precocious males) and from 0 to 257 for females. The average number of progeny attributed to hatchery-origin adult males was 1,688, while the average number attributed to hatchery-origin females was 1,483. In comparison, the average number of progeny attributed to natural-origin adult males was 1,000, while the average number attributed to natural-origin females was 1,409.

Introduction

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

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

Chinook salmon carrying visible external marks were released into the channel in September 2004. Hatchery-origin and natural-origin fish were released into shared spawning areas and allowed to select and compete for mates. Three age classes of males were released into the channel: adults, jacks, and yearling, non-migrant, or pre-migrant "precocious" males. Prior to the release of the potential spawners, researchers collected and preserved samples of fin tissue to enable genetic characterization of the potential spawners and to allow subsequent inference of parent/offspring relationships after juveniles were collected and genotyped. One group of researchers examined morphological characteristics of these potential parents and observed and recorded spawning area behaviors and interactions. The results of this work are described in a separate report. The potential parents' fin tissue samples and the collected progeny (fry) were delivered to the WDFW Genetics Laboratory in Olympia, Washington for genetic screening and parentage analysis. The genetic analyses provided direct, quantitative estimates of fry production by individual spawning Chinook salmon.

Materials and Methods

Collection of potential spawners – 2004
Fin tissue was collected from 23 adult females, 29 adult males, 4 jacks, and 14 precocious males (Table 1) prior to their release into the spawning channel during September 2004 and from an additional 27 yearling precocious males that were discovered alive in the spawning channel during spring 2005. The genetic analysis program Cervus 2.0 (Marshall et al. 1998) was used to check for identical multilocus genotypes among the potential parents. Data recorded for each released fish included gender, and whether it was of hatchery- or natural-origin (Table 1).

Collection of Fry
Fry collections occurred from December 02, 2004 to April 27, 2005. Fry samples were collected daily when fry were present. During that period 4,786 fry were collected. These collections were sub-sampled to select fry for genetic analysis based on the proportional temporal representation recorded during fry collections. A total of 3,000 fry were included in the genetic analysis.

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

PCR Methods
Potential spawners and offspring from 2004 were genotyped at 10 loci (Table 2). Potential spawners were screened twice and scored independently at all 10 loci by two biologists to minimize potential genotyping error of the parents. The number of alleles per locus among the candidate parents ranged from 10 at Ots-G474 to 35 at Omm-1080. Individual locus estimated exclusionary power (the average probability of excluding a single randomly-chosen unrelated individual as a candidate parent for a randomly drawn genotype from the population) with neither parent known ranged from 0.097 at Ots-G474 to 0.828 at Omm-1080. The estimated exclusionary power with neither parent known for the suite of 10 loci was 1.000000.

The polymerase chain reaction mixture contained the following for a 5 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units *Taq* polymerase (Promega). Amplification was performed using MJ Research PTC-200 thermocyclers. The thermal profile was as follows: an initial denaturation step of 3 minutes at 94^{o}C; 35 cycles of 15 seconds at 94^{o}C, 30 seconds at 49-58^{o}C, and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 30 minutes, followed by a final indefinite holding step at 4^{o}C.

Microsatellite DNA loci (Table 2) were amplified via the polymerase chain reaction (PCR) using fluorescent-labeled primers (obtained from Applied Biosystems or

Integrated DNA Technologies). Wherever feasible, loci were combined on the sequencer gels to increase efficiency and decrease costs.

Data were collected using an ABI-3730 Genetic Analyzer. Applied Biosystems Genemapper v.3.0 software was used to collect and analyze the raw data and to determine genotypes at each locus (based on estimated allele sizes in base pairs using an internal size standard). The output tables from Genemapper were imported into MS Excel where alleles were binned and named using MicrosatelliteBinner v.1.h (available from S.F. Young, WDFW). MicrosatelliteBinner identifies clusters of estimated PCR product sizes in the data and associates individual PCR fragments with those clusters. The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

Parentage Assignments

The dataset included 30,123 single-locus genotypes. A genotyping error rate in that dataset of 1.0% would result in 300 incorrect single-locus genotypes. Our error rate is unknown, but possibly greater than 1%. Since parentage analyses involve comparing genotypes of candidate parental pairs with offspring genotypes, genotyping errors can produce parent-offspring genotype mismatches and suggest exclusion of true parent-offspring pairings from consideration. Alternatively, genotyping errors can lead to failure to exclude parent-offspring pairings that are incorrect. We used a maximum likelihood procedure, implemented in Cervus 2.0 (Marshall et al. 1998) to infer parent-offspring relationships. The procedure uses allele frequency data to assign likelihoods to parent-offspring combinations, and allows mismatching genotypic data to be evaluated concurrently with matching genotype data.

Each fry was first assigned to all candidate female parents (dams) with positive LOD scores (log of odds). A total of 5,302 dam-fry assignments were possible when allowing all positive LOD scores. Fourteen fry were missing data for five or more loci and were removed. An additional four fry did not have a dam assigned to them and were also removed from further analysis. The fry and dam groups (N = 5,284) were then re-analyzed to assign the two most likely males (sires). Those assignments yielded a total of 10,576 possible dam-sire-fry combinations. Any fry-sire assignments with a negative LOD score were removed first (2,602 were removed) and then any fry-dam-sire combinations with more than two mismatching loci were excluded from further consideration (4,778 removed). The remaining assignments (N = 3,196) to a candidate dam-sire for each fry were then sorted to determine any fry that were only assigned one dam and one sire (N = 2,772). The remaining 424 duplicate dam-sire assignments to fry were then ranked by LOD scores and number of mismatches. Fry that were assigned with duplicate dam or sire were assigned parents if they had zero mismatches and the highest LOD score (N = 120). All remaining fry that assigned to a dam or sire with an equal number of mismatches or similar LOD scores were not assigned (N = 88). Cervus calculates delta, the difference in LOD scores between the most likely and next most likely parental assignments, for an offspring and then assesses the confidence in each

assignment by comparing delta values to values obtained in simulated assignments to correct parents.

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

Results

Parents

Genetic analysis revealed that all 97 fish released or found in the spawning channel had unique genotypes. These fish were divided into nine age class and origin categories (Table 1), as follows: 10 adult males of natural-origin; 19 adult males of hatchery origin; 12 females of natural-origin; 11 females of hatchery-origin; 2 hatchery jacks; 2 natural jacks; 7 precocious males of natural-origin; and 7 precocious males of hatchery-origin. There were 27 precocious males discovered that were not initially stocked in the channel, therefore their identity as a hatchery- or natural-origin is unknown.

Loci Screened

A total of 10 loci were screened and used in the analysis (Table 2). Number of alleles ranged from 11 - 35 (Ots-G474 and Omm-1080 respectively) and observed heterozygosity ranged from 0.351 – 0.959 (Ots-G474 and Omm-1080 respectively). Individual exclusionary power was over 42.0% for all loci when neither parent was known with the exception of Ots-G474 (9.7%). Exclusionary power was over 60.3% for all loci when one parent was known with the exception of Ots-G474 (24.8%). Cumulative exclusionary power was 1.000000 for analysis using all loci when neither parent was known or when one parent was known.

Parentage Assignments
Parentage assignments were made when genotype data was available for six or more loci. All 97 parents were genotyped at all ten loci while 2,986 of the 3,000 offspring were successfully genotyped at six or more loci (Table 3).

Of the 2,986 fry included in the analysis a total of 2,892 fry were assigned to a single male and female parent (2,892/2,986 = 96.9%; Table 4).

Evidence in the mismatched genotypes from the pilot study in 2000 -2001 suggested participation by two un-genotyped precocious male parents, but there was no compelling pattern suggesting similar activity in the 2004 – 2005 study. The possibility that un-genotyped males might have participated in spawning cannot be ruled-out, but if any did, they either contributed too few offspring to our sample or their genotypes were too similar to those of other males in the sample to present a compelling pattern.

Discussion

Parentage Assignments for Fry Collected in 2005
At least 96% success at inferring parent-offspring relationships with 95% confidence was achieved. Examination of Table 5 reveals a very uneven pattern of reproductive success among the candidate parents. Based on the subsample of 2,892 fry that were successfully assigned parents, the range of inferred reproductive output among males was 0 - 465 fry; the range for the same period in reproductive output among females was 0 - 257 fry. Some of the dam-sire matings we inferred are well supported (there were a lot of fry assigned to them) and some are weakly supported (not many if any fry were assigned to them). Caution should be used when interpreting dam-sire-fry combinations that were inferred rarely. Future integration of fecundity estimates for spawners will enrich the interpretation of these estimates of reproductive output.

Assessment of DNA Based Parentage Assignments from 2001 – 2005
The Cle Elum spawning channel was first utilized in 2001 to monitor spawning behavior and then reproductive success of the males (adult, jacks, and precocious) and females that were placed in the channel. Since then the channel has been used each year with varying numbers of males and females (Table 6). In 2001, the channel was stocked with wild adult Chinook only. The analysis from this year identified progeny that were not assigned to a known adult male (and female) and these progeny were subsequently thought to be the result of reproductive contributions by two un-genotyped precocious males that volitionally entered the spawning channel. The results from 2001 are therefore not directly comparable to the following years when both hatchery- and natural-origin males and females were placed in the channel. The channel was sub-divided into two replicated sections for the first three years. Results were tabulated separately for each section and then as a total for the entire channel. Adult male and female Chinook were placed into both sections of the channel and presumably could not move between sections. In contrast, precocious males could possibly move from one section to the next

because of small holes in the netting separating the sections so assignments of progeny were conducted with precocious males as a possible parent for either section.

The statistical method for assigning parentage has been consistent during the five years of analysis. A maximum likelihood based approach has been used to determine parentage instead of exclusion-based approach. The maximum likelihood approach allows for the assignment of a progeny to a male or female even when alleles from the candidate parents are not possible for a given progeny. An exclusionary approach will only allow exact matches among the progeny and both male and female parents; therefore the number of progeny to be assigned in most cases will be lower. In all five years of analysis, the same principals for maximum likelihood have been employed while the loci used (number and type) have varied. In 2001, a total of six loci were used for the analysis while in 2002 and 2003 eleven loci (nine loci were the same in those years) were used (Young and Kassler, 2005). In the 2004 and 2005 analyses, a different suite of 10 loci was used (Kassler, 2005). The cumulative exclusionary power of the loci that were selected each year was high providing confident parentage assignments even though different locus sets were used.

In 2002 and 2003, a similar number of males and females were used in the channel, however in 2004 and 2005 fewer adult males and females were used than in 2002 and 2003. After spawning was complete and the channel was being cleared a large number of precocious males were discovered and sampled in both 2004 and 2005. It was unknown if those precocious males were present during spawning and may have contributed to the overall reproductive success, therefore they were sampled and genotyped. Each year precocious males have been responsible for some reproductive success in the channel; therefore, these males were included in the analysis and account for the larger percentage of potential male parents in the 2004 and 2005 sample.

Interpretation of the inferred parental reproductive output based on parentage assignments by genetic analysis requires the consideration and analysis of individual fish attributes, including fecundity and body size, the closed nature of the experimental environment in which sub-dominant males had a more limited number of alternative females to court than they might have had in an open system, and relative stocking levels and synchronicity of spawning.

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electrofishing techniques. Jennifer Von Bargen (WDFW) processed the samples. Denise Hawkins and Ken Warheit for their editorial comments and review.

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Table 1. Potential Chinook salmon spawners in the Cle Elum experimental spawning channel.

Age class and origin	
	Stocked females
Hatchery	11
Natural	12
	Stocked males
Adult-Hatchery	19
Adult-Natural	10
Jack-Hatchery	2
Jack-Natural	2
Precocious-Hatchery	7
Precocious-Natural	7
	Volunteer males
Precocious-Unknown	27

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

					Individual locus		Cumulative		
Locus	N alleles	N parents genotyped	H_o(observed)	H_e (expected)	Excl(1)	Excl(2)	Excl(1)	Excl(2)	Estimated Null allele freq
Ogo-4[a]	11	97	0.732	0.787	0.423	0.603	0.423000	0.603000	+0.0361
Oki-100[b]	21	97	0.928	0.906	0.672	0.804	0.810744	0.922188	-0.0145
Omm-1080[c]	35	97	0.959	0.958	0.828	0.906	0.967448	0.992686	-0.0031
Ots-201b[b]	21	97	0.928	0.914	0.691	0.817	0.989941	0.998661	-0.0110
Ots-208b[d]	25	97	0.918	0.945	0.784	0.879	0.997827	0.999838	+0.0124
Ots-211[d]	23	97	0.897	0.929	0.735	0.847	0.999424	0.999975	+0.0146
Ots-212[d]	18	97	0.825	0.876	0.591	0.744	0.999765	0.999994	+0.0263
Ots-213[d]	23	97	0.918	0.937	0.759	0.863	0.999943	0.999999	+0.0078
Ots-G474[e]	10	97	0.351	0.425	0.097	0.248	0.999949	0.999999	+0.0892
Ssa-408[f]	18	97	0.763	0.923	0.714	0.833	0.999985	1.000000	+0.0925

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

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

Loci genotyped	Parents (04HR)	Offspring (05FD)
10	97	2,385
9	0	488
8	0	83
7	0	13
6	0	17
5	0	7
4	0	4
3	0	1
2	0	1
1	0	0
0	0	1
Totals	97	3,000

Table 4. Parentage distribution among spawning pairs inferred with 95% confidence for 2,892 Chinook salmon fry spawned in fall of 2004. The column and row headers describe adult females released and males released respectively. "n" for males indicates total number of progeny assigned to this male, "n" for females indicates a subtotal of the number of progeny assigned to this female (totals can be obtained by summing female "n" across pages [see Table 5 for totals]).

		Male	04HR0003	04HR0004	04HR0007	04HR0008	04HR0012	04HR0013	04HR0016	04HR0017	04HR0020	04HR0024	04HR0025	04HR0026	04HR0028	04HR0029	04HR0030	04HR0031	04HR0032	04HR0033	04HR0034	04HR0036	04HR0039
			A	A	A	A	A	A	A	A	A	A	A	A	J	A	A	J	J	A	A	A	A
	Origin		H	H	H	H	H	N	H	N	N	H	N	N	N	H	H	H	N	H	N	N	H
Female		n	90	81	240	367	165	165	25	465	2	35	7	6	29	8	0	5	52	202	180	10	0
04HR0001	H	142	-	-	-	-	-	132	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-
04HR0002	H	30	7	18	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-
04HR0005	H	91	-	-	-	-	20	-	-	23	-	-	2	-	-	-	-	-	-	2	44	-	-
04HR0006	N	171	-	-	13	127	-	25	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-
04HR0009	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0010	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0011	N	69	-	-	19	-	37	-	-	1	-	-	-	-	12	-	-	-	-	-	-	-	-
04HR0014	H	113	-	45	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	66	-	-	-
04HR0015	H	28	-	-	-	-	5	-	-	19	-	-	-	-	-	-	-	4	-	-	-	-	-
04HR0018	H	69	-	-	67	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
04HR0019	N	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	-
04HR0021	N	150	-	-	-	-	77	-	25	-	-	-	-	-	-	-	-	-	-	4	44	-	-
04HR0022	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0023	H	39	-	-	-	-	16	-	-	4	-	16	-	-	3	-	-	-	-	-	-	-	-
04HR0027	N	175	-	-	7	80	-	-	-	-	-	-	-	-	-	-	-	-	-	88	-	-	-
04HR0035	H	234	-	-	-	-	-	-	-	234	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0037	H	113	-	-	-	-	8	-	-	1	-	-	-	1	4	-	-	1	-	18	80	-	-
04HR0038	N	187	83	-	79	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-
04HR0040	N	97	-	-	26	44	-	-	-	-	-	-	-	-	-	-	-	-	27	-	-	-	-
04HR0041	N	86	-	-	-	-	-	-	-	86	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0042	H	111	-	-	-	-	-	-	-	97	-	-	-	-	-	2	-	-	-	-	12	-	-
04HR0048	N	115	-	-	29	73	-	8	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-
04HR0050	N	90	-	18	-	43	-	-	-	-	-	19	-	-	-	-	-	-	-	-	-	10	-

Table 4. continued.

Female	Origin	Male n	04HR0043 AH 63	04HR0044 AN 43	04HR0045 AH 66	04HR0046 AH 37	04HR0047 AH 99	04HR0049 AH 31	04HR0051 AH 154	04HR0052 AH 5	04HR0053 JH 12	04HR0054 AN 16	04HR0055 AN 106	04HR0056 AH 20	04HR0057 PN 0	04HR0058 PH 1	04HR0059 PN 13	04HR0060 PH 21	04HR0061 PN 1	04HR0062 PN 0	04HR0063 PH 7	04HR0064 PH 17	04HR0065 PH 0	04HR0066 PN 26
04HR0001	H	11	-	-	2	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	4	-	-	-
04HR0002	H	78	32	-	-	-	-	31	7	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-
04HR0005	H	29	-	-	-	-	-	-	6	-	-	-	22	-	-	-	1	-	-	-	-	-	-	-
04HR0006	N	21	-	-	-	-	-	-	19	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
04HR0009	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0010	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0011	N	22	4	-	-	-	-	-	17	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
04HR0014	H	144	-	-	37	-	-	-	79	-	-	-	28	-	-	-	-	-	-	-	-	-	-	-
04HR0015	H	61	-	-	23	-	-	-	19	-	-	3	-	12	-	-	4	-	-	-	-	-	-	-
04HR0018	H	15	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	14	-	-
04HR0019	N	12	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0021	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0022	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0023	H	28	14	-	-	-	1	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-
04HR0027	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0035	H	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
04HR0037	H	41	-	-	-	-	-	-	1	-	-	-	40	-	-	-	-	-	-	-	-	-	-	-
04HR0038	N	57	13	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
04HR0040	N	31	-	-	4	5	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	3	-	7
04HR0041	N	30	-	-	-	-	6	-	6	-	-	-	16	-	-	-	2	-	-	-	-	-	-	-
04HR0042	H	92	-	-	-	-	92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0048	N	58	-	-	-	32	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	19
04HR0050	N	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-

Table 4. continued.

		Male	04HR0067	04HR0068	04HR0069	04HR0070	04HR0071	04HR0072	04HR0073	04HR0074	04HR0075	04HR0076	04HR0077	04HR0078	04HR0079	04HR0080	04HR0081	04HR0082	04HR0083	04HR0084	04HR0085	04HR0086	04HR0087	04HR0088
			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Origin		N	H	N	H	??	??	??	??	??	??	??	??	??	??	??	??	??	??	??	??	??	??
Female		n	0	19	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
04HR0001	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0002	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0005	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0006	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0009	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0010	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0011	N	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0014	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0015	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0018	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0019	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0021	N	6	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0022	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0023	H	3	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0027	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0035	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0037	H	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0038	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0040	N	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0041	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0042	H	6	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0048	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04HR0050	N	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. continued.

		Male	04HR0089	04HR0090	04HR0091	04HR0092	04HR0093	04HR0094	04HR0095	04HR0096	04HR0097
			P	P	P	P	P	P	P	P	P
	Origin		??	??	??	??	??	??	??	??	??
Female		n	0	0	0	0	0	0	0	0	0
04HR0001	H	0	-	-	-	-	-	-	-	-	-
04HR0002	H	0	-	-	-	-	-	-	-	-	-
04HR0005	H	0	-	-	-	-	-	-	-	-	-
04HR0006	N	0	-	-	-	-	-	-	-	-	-
04HR0009	N	0	-	-	-	-	-	-	-	-	-
04HR0010	N	0	-	-	-	-	-	-	-	-	-
04HR0011	N	0	-	-	-	-	-	-	-	-	-
04HR0014	H	0	-	-	-	-	-	-	-	-	-
04HR0015	H	0	-	-	-	-	-	-	-	-	-
04HR0018	H	0	-	-	-	-	-	-	-	-	-
04HR0019	N	0	-	-	-	-	-	-	-	-	-
04HR0021	N	0	-	-	-	-	-	-	-	-	-
04HR0022	H	0	-	-	-	-	-	-	-	-	-
04HR0023	H	0	-	-	-	-	-	-	-	-	-
04HR0027	N	0	-	-	-	-	-	-	-	-	-
04HR0035	H	0	-	-	-	-	-	-	-	-	-
04HR0037	H	0	-	-	-	-	-	-	-	-	-
04HR0038	N	0	-	-	-	-	-	-	-	-	-
04HR0040	N	0	-	-	-	-	-	-	-	-	-
04HR0041	N	0	-	-	-	-	-	-	-	-	-
04HR0042	H	0	-	-	-	-	-	-	-	-	-
04HR0048	N	0	-	-	-	-	-	-	-	-	-
04HR0050	N	0	-	-	-	-	-	-	-	-	-

Table 5. Total number of offspring assigned to females and males in the spawning channel, life stage (A = adult, J = Jack, and P = precocious), and hatchery- (H) or natural-origin (N) designation. Unknown origin is identified by ??.

Females	Life Stage and H/W	Total Offspring	Males	Life Stage and H/W	Total Offspring	Males	Life Stage and H/W	Total Offspring
04HR0001	A - H	153	04HR0003	A - H	90	04HR0062	P - N	0
04HR0002	A - H	108	04HR0004	A - H	81	04HR0063	P - H	7
04HR0005	A - H	120	04HR0007	A - H	240	04HR0064	P - H	17
04HR0006	A - N	192	04HR0008	A - H	367	04HR0065	P - H	0
04HR0009	A - N	0	04HR0012	A - H	165	04HR0066	P - N	26
04HR0010	A - N	0	04HR0013	A - N	165	04HR0067	P - N	0
04HR0011	A - N	95	04HR0016	A - H	25	04HR0068	P - H	19
04HR0014	A - H	257	04HR0017	A - N	465	04HR0069	P - N	1
04HR0015	A - H	89	04HR0020	A - N	2	04HR0070	P - H	0
04HR0018	A - H	84	04HR0024	A - H	35	04HR0071	P - ??	0
04HR0019	A - N	36	04HR0025	A - N	7	04HR0072	P - ??	0
04HR0021	A - N	156	04HR0026	A - N	6	04HR0073	P - ??	0
04HR0022*	A - H	0	04HR0028	J - N	29	04HR0074	P - ??	0
04HR0023	A - H	70	04HR0029	A - H	8	04HR0075	P - ??	0
04HR0027	A - N	175	04HR0030	A - H	0	04HR0076	P - ??	0
04HR0035	A - H	239	04HR0031	J - H	5	04HR0077	P - ??	0
04HR0037	A - H	154	04HR0032	J - N	52	04HR0078	P - ??	0
04HR0038	A - N	244	04HR0033	A - H	202	04HR0079	P - ??	0
04HR0040	A - N	129	04HR0034	A - N	180	04HR0080	P - ??	0
04HR0041	A - N	116	04HR0036	A - N	10	04HR0081	P - ??	0
04HR0042	A - H	209	04HR0039	A - H	0	04HR0082	P - ??	0
04HR0048	A - N	173	04HR0043	A - H	63	04HR0083	P - ??	0
04HR0050	A - N	93	04HR0044	A - N	43	04HR0084	P - ??	0
			04HR0045	A - H	66	04HR0085	P - ??	0
		2,892	04HR0046	A - H	37	04HR0086	P - ??	0
			04HR0047	A - H	99	04HR0087	P - ??	0
			04HR0049	A - H	31	04HR0088	P - ??	0
			04HR0051	A - H	154	04HR0089	P - ??	0
			04HR0052	A - H	5	04HR0090	P - ??	0
			04HR0053	J - H	12	04HR0091	P - ??	0
			04HR0054	A - N	16	04HR0092	P - ??	0
			04HR0055	A - N	106	04HR0093	P - ??	0
			04HR0056	A - H	20	04HR0094	P - ??	0
			04HR0057	P - N	0	04HR0095	P - ??	0
			04HR0058	P - H	1	04HR0096	P - ??	0
			04HR0059	P - N	13	04HR0097	P - ??	0
			04HR0060	P - H	21			
			04HR0061	P - N	1			2,892

* = tissue sample was taken and female was genotyped, but was not used in the spawning channel

Table 6. Number of females and males in the Cle Elum spawning channel from 2001 - 2005. Data for the 2002 and 2003 brood years are shown as a total (above) and by channel section (below). Adults are assigned as Adult (A), Jack (J), or precocious (P) parents and from a hatchery (H) or natural (N) origin.

	2001		2002		2003		2004		2005	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
A - H			21	17	22	18	13	11	11	19
A - N	11	16	20	23	22	20	13	14	12	10
J - H				4		2		5		2
J - N		0		2		2		1		2
P - H				7		12		7		7
P - N		1		5		5		13		7
P - ?		1		7		2		22		27
Total	11	18	41	65	44	61	26	73	23	74

	2002				2003			
	Female		Male		Female		Male	
	Section1	Section2	Section1	Section2	Section1	Section2	Section1	Section2
A - H	11	10	10	7	11	11	11	7
A - N	10	10	12	11	11	11	11	9
J - H			2	2			1	1
J - N			2	0			1	1
P - H			7[a]				12[a]	
P - N			5[a]				5[a]	
P - ?			7[a]				2[a]	
Total	21	20	26	20[b]	22	22	24	18[b]

[a] = could have been present in either section

[b] = total does not include precocious males

Chapter 3

DNA-Based Stock-of-Origin Assignments of Chinook Salmon Smolts Outmigrating Past Chandler Trap (Yakima River) in 2005

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Abstract

A stock-of-origin assignment procedure based on 11 microsatellite loci was used to estimate the percentages of smolts from each of three spring and two fall Chinook salmon stocks outmigrating past Chandler Trap (Yakima River) from January – July 2005. Smolt outmigration in the Yakima River is comprised of a mixture of three spring stocks (upper Yakima River, American River, and Naches River) and two fall stocks (lower Yakima River and Marion Drain). A blind analysis using 100 known-origin samples resulted in approximately 97% (96/99) of the known-origin samples correctly identified to their stock-of-origin. Morphological assessment and genetic assignment of spring versus fall Chinook smolt were compared for the April, May, and June – July time strata; 1,003/1,044 (96.1%) smolts were identified the same using both procedures. Mixture analysis was conducted on a proportional subsample of 1,320 smolts. Assessment of five time strata (January – February, March, April, May, and June – July) during the outmigration revealed the largest percentage of the spring smolts to be from the upper Yakima River stock. During April, the percentage of smolts from American River and Naches River increased while the upper Yakima spring stocks declined. There was a large increase of the lower Yakima fall stock during the May stratum (77.5%) and over 73% of the total were by the two fall stocks in June/July.

Introduction

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

The methodology used in this study to estimate the stock-of-origin for individual fish in a mixture followed the general approach of Paetkau et al. (1995). The Paetkau et al. approach assumes that the individual genotype frequencies in each baseline stock are in Hardy-Weinberg equilibrium (H-W), that the loci are independent, and that the likelihood that an unknown individual comes from a baseline stock is equal to the H-W frequency of its multilocus genotype in that baseline stock. Inherent in the latter assumption is an expectation that the various potential source populations have an equal likelihood of contributing individuals to the unknown mixture sample. Using this basic approach, each unknown is assigned to the baseline population where its multilocus genotype has the highest probability of occurrence. However, when the multilocus genotype of an individual from the mixture has similar probabilities in two or more source populations, such a simple allocation is unreliable. Others have used the methodology developed by Paetkau et al. (1995) to provide robust stock-of-origin assignments of unknown individuals (Shriver et al. 1997 and Olsen et al. 2000).

Calculation of stock-of-origin for Chinook smolts trapped at Chandler trap throughout the entire outmigration (January through July) was hindered in the first few years of analysis for several reasons: non-representative temporal sampling of the downstream migration, past omission of the Marion Drain fall and lower Yakima River mainstem fall Chinook stocks from the DNA baseline, and by maintenance and other shutdowns of trap operations in December and January in many years. In the analyses of the 2004 and 2005 samples, attempts were made to eliminate the problems present in previous analyses. A new sampling design was initiated to provide a proportional sample of smolts outmigrating past Chandler trap and a larger number of smolts were analyzed. Repeated multi-year samples of all five-baseline stocks were used to characterize the potential sources of smolts in the Yakima basin.

This report presents the results of stock-of-origin assignments for 1,520 smolts collected at the Chandler trap during the 2005 outmigration and 100 known-origin test samples.

Materials and Methods

Collections
Sampling crews from the Yakama Nation and WDFW collected the adult spawning ground tissue samples included in the baseline used in this study. The tissue samples consisted of dry-mounted scales or fin tissue preserved in 100% ethanol from five baseline stocks collected across multiple years (American River spring, Naches River spring, upper Yakima River spring, Marion Drain fall, and lower Yakima River fall; Table 1 and Figure 1).

A total of 100 known-origin samples were collected by Yakama Nation biologists to serve as a test of the genetic analysis to correctly identify Chinook smolts to their stock-of-origin. The analysis of these samples was done as a "blind" test. The tissue samples were sent to Olympia for genetic analysis without identifying the stream location where each sample was collected. Upon completion of genetic analysis the assignments for the 100 samples were sent to Mark Johnston (Yakama Nation biologist) for comparison with the actual sampling location information. These known-origin samples were juveniles collected from a screw trap on the Naches River (a likely mixture of both the American River and Naches River spring stocks), Roza Dam (upper Yakima River spring stock), and Marion Drain Hatchery (Marion Drain and the lower Yakima River fall stocks).

An estimated total of 331,869 smolts passed the lower Yakima River at Chandler from January 24 – July 1, 2005. This estimate, derived by Doug Neeley and staff (Yakama Nation) was based on expansion of the total number of smolts counted at the Chandler trap (44,784) to account for trap efficiency, etc.

Smolts were categorized as spring or fall Chinook when they were intercepted at the Chandler trap based on anatomical characteristics. Three morphological features (length, size of the eye, and snout shape) were used to identify smolts as spring or fall (Mark Johnston, Yakama Nation; pers. comm.).

A sampling design was developed to provide smolt samples for genetic analysis that is proportional to the entire outmigration within each of the five targeted time strata (January – February, March, April, May, and June – July) to get reliable estimates of stock proportions. Each day, the total number of smolts at the trap was visually estimated before any processing occurred. If that number was below a predetermined threshold then a "standard" day's sample was taken (e.g. 10 fish). If the number of smolts was above the threshold then a "peak" day's sample was taken (e.g. 30 fish). The threshold and "standard" and "peak" numbers of samples to be taken varied for each of the time strata and were determined by analyzing the number of "peak" and "standard" days counted during the four years' of smolt outmigration. Upon completion of the

outmigration, the total estimated numbers of smolts passing the Chandler trap each day were plotted with the total number of genetic samples that had been collected. The available genetic samples were then randomly subsampled to obtain collections that were representative as possible of the smolts that were outmigrating on a given day for each stratum. Based on this sampling design, 3,930 Chinook smolt samples were collected (Figure 2). A total of 1,520 smolts were subsampled and used for the stock-of-origin analysis for smolts passing Chandler trap (Figure 3) and for pathology analysis to determine if pathology results revealed any stock-of-origin patterns. The number and distribution of smolts that were subsampled from each time stratum are shown in Figure 4.

DNA Extraction Methods

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

PCR Methods

The polymerase chain reaction mixture contained the following for a 5 µl reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, approx. 0.1 µM of each oligonucleotide primer, and 0.05 units *Taq* polymerase (Promega). Amplification was performed using MJ Research PTC-200 thermocyclers. The thermal profile was as follows: an initial denaturation step of 3 minutes at 94^{o}C; 35 cycles of 15 seconds at 94^{o}C, 30 seconds at 49-58^{o}C, and 1 minute at 72^{o}C; plus a final extension step at 72^{o}C for 30 minutes, followed by a final indefinite holding step at 10^{o}C.

Eleven microsatellite DNA loci (Appendix 1) were amplified via the polymerase chain reaction (PCR) using fluorescent-labeled primers (obtained from Applied Biosystems or Integrated DNA Technologies). Wherever feasible, loci were combined in poolplexes in the sequencer to increase efficiency and decrease costs.

Data were collected using an ABI-3730 Genetic Analyzer. Applied Biosystems GeneMapper v.3.0 software was used to collect and analyze the raw data and estimate fragment sizes. The output tables from GeneMapper were imported into MS Excel where final genotyping was accomplished – alleles were binned and named using MicrosatelliteBinner v.1.h (available from S.F. Young, WDFW). MicrosatelliteBinner identifies clusters of estimated PCR product sizes in the data and associates individual PCR fragments with those clusters. The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

Jackknife analysis of the baseline samples was conducted using WHICHRUN v 4.1 (Banks and Eichert 2000). The jackknife analysis provides a means for evaluating baseline stocks to determine if an unknown sample can be correctly assigned back to a

stock in the baseline. The jackknife analysis removes one individual and recalculates the allele frequencies for that baseline stock. The individual removed from the baseline is then treated as an unknown and assigned to its most likely stock-of-origin based on its multilocus genotype and the allele frequencies of the baseline populations. The process is repeated N times until each individual in the baseline has been removed and assigned to its most likely stock-of-origin. Then the percentage of individuals that assign to each baseline stock is calculated.

Stock-of-origin assignments in this study were accomplished using WHICHRUN (Banks and Eichert 2000) and a Visual Basic implementation of the Expectation-Maximization (EM) algorithm that simultaneously estimates admixture proportions and assigns individuals to candidate donor stocks using the program MIXASSIGN v1.1C (available from S.F. Young, WDFW) using a maximum likelihood-based adjustment to previously described tests (Paetkau et al. 1995). This 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 (e.g., Paetkau et al. 1995, Banks and Eichert 2000). A description and comparison of the procedure described by Paetkau et al. (1995) and Young is available in Young and Shaklee (2002).

Results

Collections

A total of 1,184 samples was analyzed from the five known Yakima River Chinook stocks across multiple collection years to create the baseline (Table 1). Baseline collections for each stock across years were combined for the stock-of-origin analysis. I analyzed a total of 1,520 unknown smolts from the Chandler trap.

PCR and Locus Assessment

The number of alleles per locus among all samples ranged from eight (*Ots-9*) to 48 (*Ots-208b*). PCR failure rates were highest in the baseline samples – missing data for individual loci ranged from 5.9% for upper Yakima R. spring to 15.6% for Naches River spring (Table 1). Missing data per locus ranged from 7.3% (*Ots-3M*) to 22.6% (*Ogo-4*; Appendix 1) in the baseline collections. Allele frequencies for baseline and smolt collections are in Appendix 2.

Jackknife of Baseline

Jackknife analysis of the baseline resulted in correct assignment of over 89.0% of individuals in both the upper Yakima River spring and the American River spring baseline stocks (Table 2). Incorrect assignment of the upper Yakima River spring and American River spring stocks occurred predominately to the Naches River spring stock. The Naches River spring stock had much lower assignment accuracy at 71.3% with incorrect assignments going equally to the upper Yakima River spring and American River spring. The two fall stocks (Marion Drain and lower Yakima River) assigned at

60.7% and 70.7% respectively with most incorrect assignments being to the other fall stock.

Analysis of Known-Origin Samples

A total of 100 known-origin samples were analyzed. One sample could not be assigned a stock-of-origin because of missing data for four or more loci. The remaining 99 samples were assigned a stock-of-origin using the genetic analysis (Table 3). Comparison of the genetic assignment with the collection information revealed two different results. Three spring-run smolts were assigned to a stock (or stock group) that was inconsistent with the collection notes regarding where they were obtained (smolts identified from the upper Yakima River stock were assigned to the Naches River stock by genetic analysis). Secondly, the assignments as spring stock versus fall stock were 100% accurate.

Comparison of Morphological ID and Genetic Assignment as SP vs F Chinook at Chandler

A comparison of the morphological assessment to genetic assignment was conducted for the April, May, and June-July time strata (no fall smolts were assigned genetically or morphologically during the January-February and March strata). A total of 419 of 431 smolts in April, 515 of 522 smolts in May, and 110 of 110 in the June/July time strata were analyzed (Appendix 3). Smolts that were not scored for four or more loci were not genetically assigned as a spring or fall and not included in this comparison. Results for the time strata were as follows. The April time stratum - 414 smolts were assigned identically using morphological and genetic methods (335 spring and 79 fall), 4 smolts identified as spring Chinook by morphology were assigned as fall Chinook by genetic analysis and 1 smolt identified as a fall Chinook by morphology was assigned as a spring Chinook by genetic analysis. The May time stratum - 509 smolts were assigned identically using morphological and genetic methods (51 spring and 458 fall), the remaining 6 smolts were identified as fall Chinook by morphology but were assigned as spring Chinook by genetic methods. The June/July time stratum – 80 smolts were assigned identically using morphological and genetic methods (80 fall), the remaining 30 smolts were identified as fall Chinook by morphology but were assigned as spring Chinook by genetic analysis.

Mixture Analysis

Stock composition estimates using WHICHRUN and MIXASSIGN were slightly different, however they showed the same patterns of abundance for the five stocks in each time strata (Table 4). The mixture composition estimates for the entire 2005 outmigration indicated that the largest overall percentage of spring smolts was from the upper Yakima River. The number and relative proportion of smolts from each stock varied substantially across the five time strata (January-February, March, April, May, and June-July). During the early and mid outmigration (January – April), the proportion of the three spring stocks was between 22 and 36% while the two fall stocks were almost non-existent (Table 4). The March stratum had more upper Yakima River smolts, but the sample size in that stratum was low. During the later portion of the outmigration (May and June-July time strata) the lower Yakima River fall smolts were most abundant

(70.0% - 77.5%), but a surprisingly high 24.5% upper Yakima spring smolts were in the June-July stratum.

Stock Identification for Pathology Samples
The mixture composition estimates for the pathology samples (N = 200) collected in 2005 revealed the largest overall percentage of smolts were from the American River spring stock (36.7%). The upper Yakima River spring stock accounted for 31.1% and Naches with 23.5% (Table 5). There were a few samples that were identified to the lower Yakima River and Marion Drain fall stocks (3.6 % - 5.1%).

Discussion

Collection of smolts at the Chandler Trap in 2005 utilized a sampling design intended to yield a sample that was proportional to the number of smolts passing the Chandler Trap. Sampling a proportional number of smolts was important to determine an accurate percentage of smolts from each stock that were outmigrating from the basin. Developing the sampling strategy for identifying a "standard" versus "peak" day of smolts that were in the trap and applying a sampling goal for those days allowed for a proportional sample. Subsampling the smolts collected for genetic analysis provided a best fit to the actual passage of smolts for a given day. Assessment of the plots in Figure 4 demonstrates how well the process worked during each of the five time strata.

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

A baseline of the five stocks in the Yakima River basin was created using 18 individual collections. The baseline collections as a whole had higher genotyping failure compared to the known-origin samples and the Chandler smolt samples. All five-baseline collections had approximately 15% missing genotypes, with the exception of the upper Yakima River (5.9%), while smolt, known-origin, and pathology samples had less than 5.7% missing genotypes. Scales were taken from carcasses on spawning grounds for most baseline collections, therefore, DNA quality was presumably poorer than the Chandler smolt collection and the known-origin collections where tissue was collected from live fish. The upper Yakima River tissue collections were also taken from live fish at the hatchery and, therefore, genotyping success was higher for this collection than the other baseline collections.

The jackknife analysis, using methods in WHICHRUN (Banks and Eichert 2000), of the baseline collections revealed good assignment power of the upper Yakima River spring and American River spring baseline stocks while the Naches River spring stock was lower. Incorrect assignment for the upper Yakima River and American River spring stocks went to the Naches River spring stock and incorrect assignment for the Naches River spring stock went equally to the upper Yakima River spring and American River spring stocks. Assignment of a fall smolt as a fall-run (not spring-run) was high, but assignment back to the actual fall stock of origin was only 60.7% - 70.7%. The results of this jackknife analysis suggest that this baseline is very good at distinguishing between spring and fall smolts. These results also suggest that smolts from the upper Yakima River spring and American River spring stocks will assign with high accuracy while a smolt from the Naches River spring may incorrectly assign to the upper Yakima River spring or American River spring stock about 30% of the time when this baseline is used.

The ability of the baseline to assign fish to the correct stock-of-origin was directly tested by analyzing 100 samples of known-origin in a blind test where the origins were unknown to those conducting the DNA analysis until the analysis was completed and reported. The known samples were collected from Roza Dam (the upper Yakima River spring stock), a screw trap on the Naches River (Naches and American River spring stocks were co-mingled), and Marion Drain hatchery (Marion Drain and the lower Yakima River fall stocks were co-mingled). One individual was not assigned because of missing data. Three spring-run individuals (3.0%) were not assigned to their known stock-of-origin using the genetic analysis. Error in assignment could be the result of an individual having a combination of alleles that is more likely in another baseline stock. This might result in an individual's probability of membership in two stocks being nearly equal. The probability is the log of probability raised to the 10^{th} between the log score of the most likely stock and the log score of the second most likely stock. The probability for one of the three samples was 3.0 indicating the smolt was less than three times more likely to belong in the first assigned stock than the second (Naches and American spring). Two of the assignments had a probability of one indicating the smolt was equally as likely to be from the first and second stock (Naches and upper Yakima River spring). All individuals from the fall stock were correctly identified as fall-run by the genetic analysis. The analysis of the known-origin samples suggests that with this baseline, we are able to assign Chinook smolts as spring or fall stock of origin 100% of the time and that approximately 97% of the time we correctly distinguish between upper Yakima River spring and Naches/American River spring. The blind test did not provide any information regarding the accuracy of assignments between the Naches and American Rivers stocks.

Assessment of spring or fall smolts by morphological and genetic analysis revealed good agreement between the two methods in the earlier time periods. Identification as a spring or fall smolt was the same for 923 out of 930 smolts in the April and May time strata. The comparison in the June – July strata revealed more differences (30 differences out of 110). In all 30 differences, the smolt was determined to be from a fall stock by morphology and spring stock by genetic analysis. Assessment of the 30 smolts that were different revealed a larger average size (91.2 mm) while the 80 fall smolts were smaller

(84.2 mm). A larger size would be expected if the smolts were from a spring stock instead of a fall stock, indicating these samples may be from a spring stock. There appears to be a systematic bias in the May – July time period because all discrepancies between the two methods showed "fall" for the morphological assessments but "spring" for the genetic analysis.

All of the assignments from the early time strata (January-February) were from each of the three spring stocks with the upper Yakima River spring stock accounting for the highest percentage (44.7%). The three spring stocks were almost equal in the January – February and April time strata while the March time stratum was dominated by the upper Yakima River spring stock. As expected, the May and June-July time strata were predominately composed of fall Chinook stocks. The relatively high percentage of the upper Yakima River spring stock in the June-July time stratum (24.6%) was surprising and unexpected. These genetic results should allow a reliable estimation of relative stock-specific smolt production in this system.

Analysis of the pathology samples revealed that each of the three spring stocks contributed over 20.0%, with the upper Yakima River and American River stocks accounting for the largest number of smolts (Table 5). Comparison of the results from 2004 and 2005 pathology samples reveal similar stock composition percentages for the three spring stocks. The 2005 samples included almost 9% fall smolts while there were no fall smolts in the 2004 collection. Assessment of the pathology results with the stock-of-origin assignments will provide a means to determine if diseases are more common to a stock within the Yakima River basin.

Additional analyses were conducted this year to improve assignment accuracy among the three spring stocks: re-analysis of existing loci and analysis of additional baseline collections from other collection years. Re-analysis of existing loci and samples provided more complete genotypes to calculate the allele frequencies of each baseline stock used in stock-of-origin assignments (approx. 10% of the genotypes for baseline collections were added). Analysis of additional baseline samples increased the overall sample size for each of the baseline stocks.

Assessment of DNA Mixture Assignments from 2000 – 2006

Mixed stock analysis has been conducted on Chandler smolts since 2000 (Young 2004, Kassler et al. 2005), however the sampling design for samples collected in 2000 – 2003 was not proportionalized during the run. The yearly assignments are therefore not comparable from those years. Beginning in 2004, staff at the Chandler trap utilized a sampling protocol to provide a number of smolts that was relative to the percentage of smolts passing that day. Samples were then subsampled at WDFW to provide a proportional number of samples that would represent the overall passage to be analyzed. Assignments for smolt samples collected in 2004 and 2005 are shown in Table 4 for comparison. The three stocks dominate the run from January to April while the fall stocks are the most abundant in May through July in both years.

Some notable differences exist in the percentage of each stock between 2004 and 2005: the American River spring Chinook is relatively more abundant in 2005 than in 2004; the percentage of all three spring stocks in the May stratum is higher in 2004, and the percentage of the upper Yakima River spring stock in the June – July time stratum in 2005 is higher than seen in 2004. These individual differences may represent yearly temporal variability in the outmigration of each individual stock while the overall abundance remains somewhat constant.

Acknowledgements

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

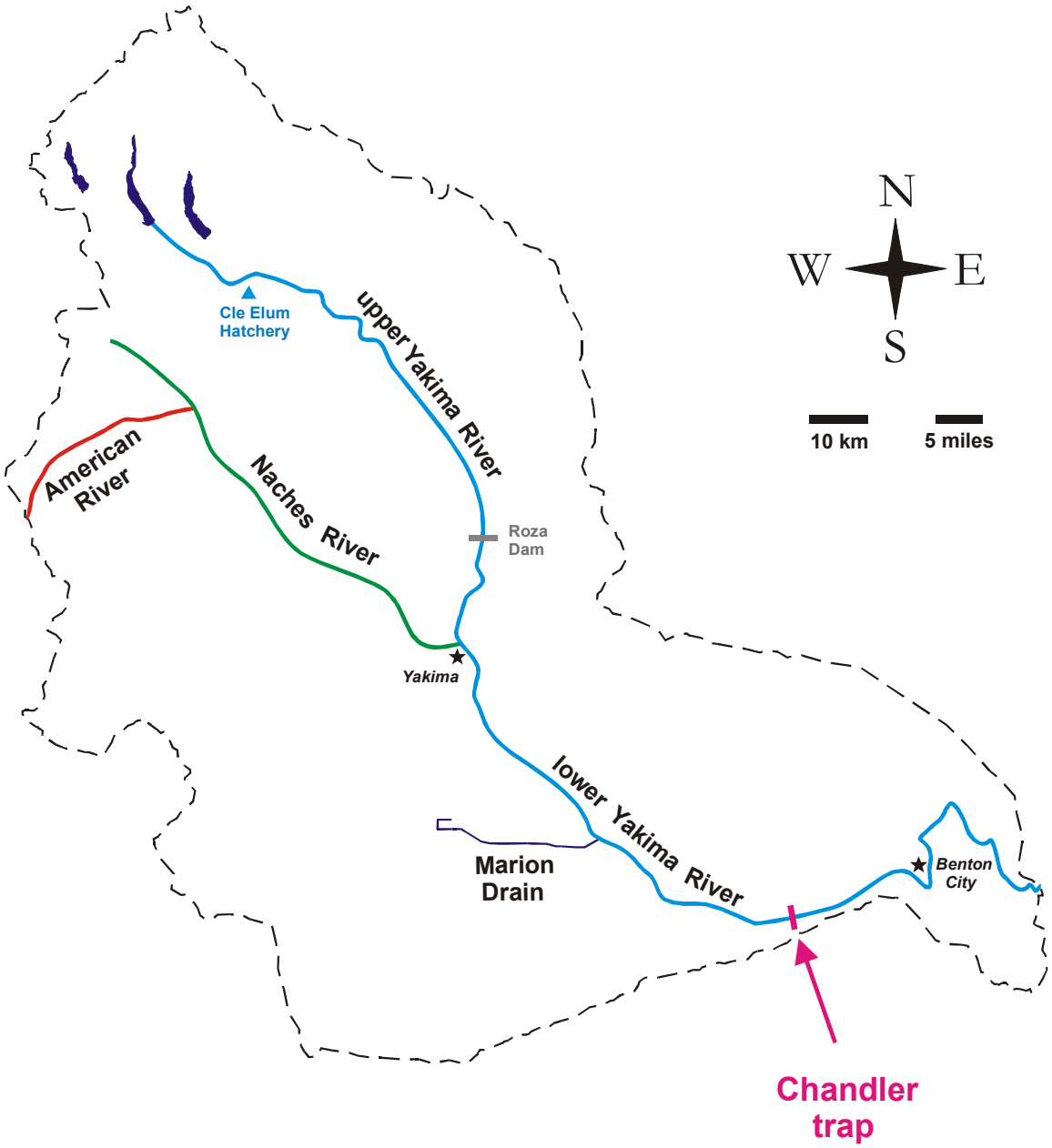


Figure 2. Temporal distribution of outmigrant Chinook passage at Chandler trap and of genetic sample collection.

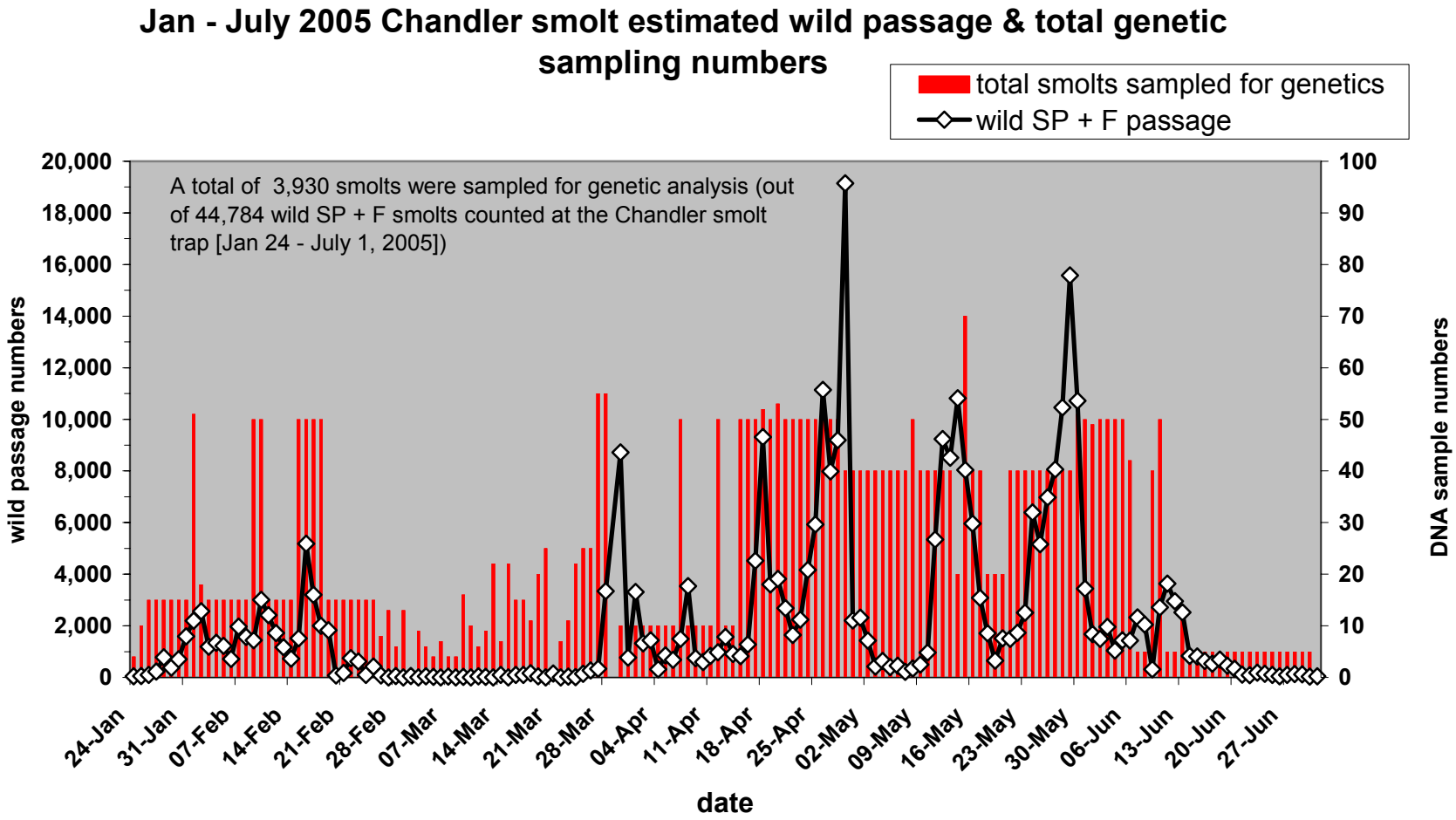


Figure 3. Temporal distribution of outmigrant Chinook passage at Chandler trap and of genetic sample collection including both smolt and pathology smolt collections.

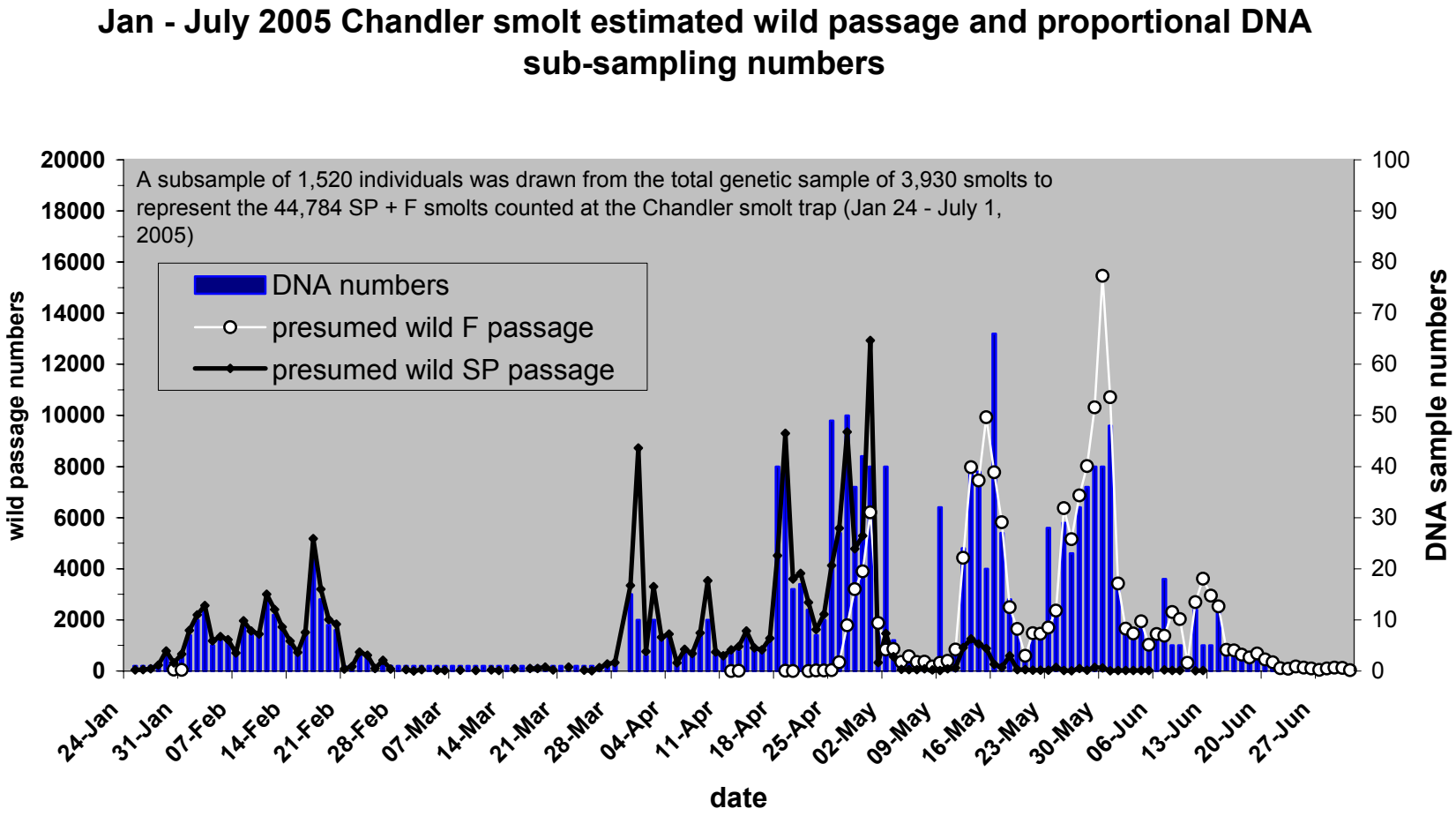
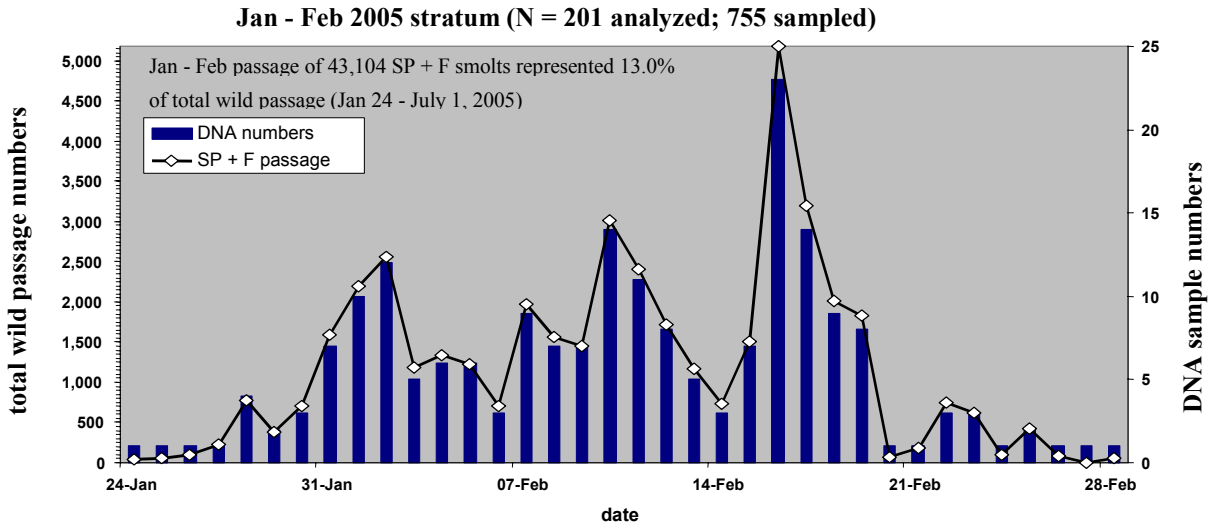


Figure 4. Proportional sampling of smolts for genetic analysis during each of five time strata



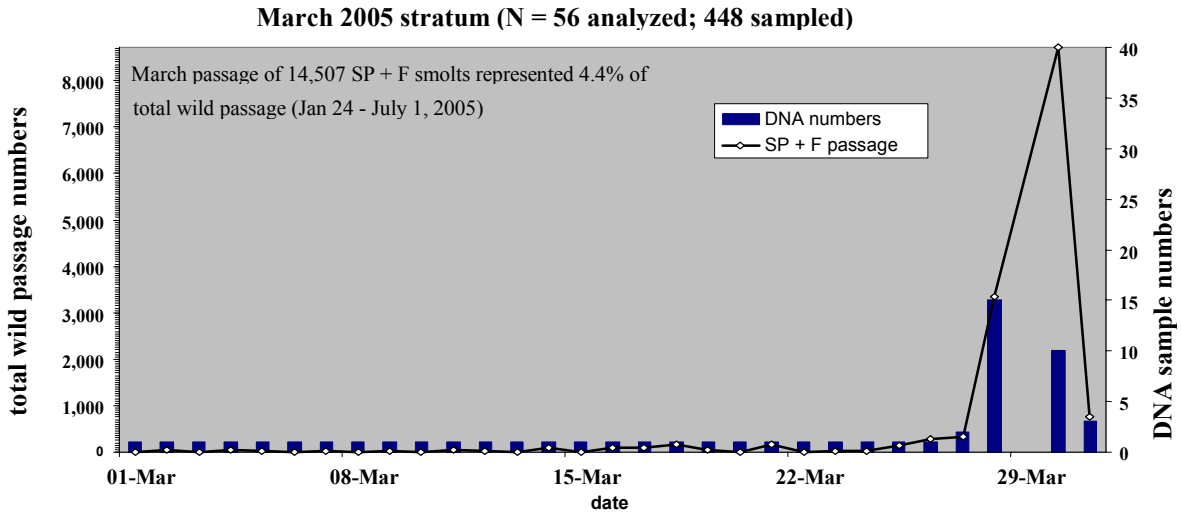


Figure 4. Continued.

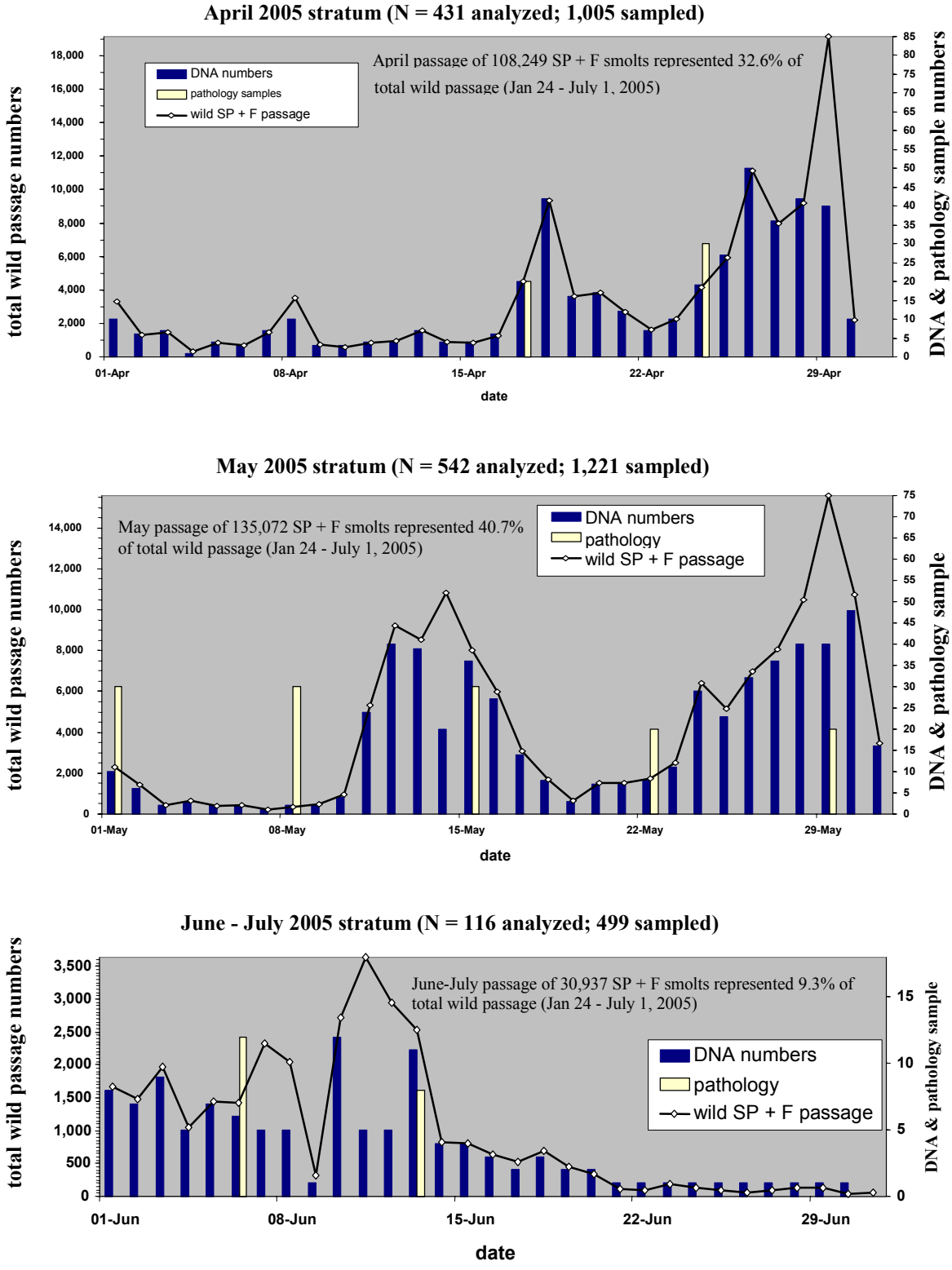


Table 1. Chinook salmon collections used for the baseline, smolt analysis, analysis of known-origin samples, and pathology samples analyzed in this study with the percentage of single locus genotypes missing. Collection codes that are highlighted identify collections added to the baseline in 2005.

Collection Source	Collection code	# processed	% Single locus genotypes missing
American River spring	89AG	80	12.7
	91DQ	102	18.6
	93DO	18	9.1
	total	*200*	**15.4%**
Naches River spring	89AC	76	13.2
	89AI	26	18.9
	93DQ	50	14.2
	93DR	32	24.4
Little Naches River spring	04BI	42	3.5
	04EM	56	22.7
	total	282	**15.6%**
upper Yakima River spring	92DN	24	9.5
	97DA	123	8.9
	03GO	99	1.4
	total	246	**5.9%**
Marion Drain fall	89BX	100	13.0
	92FQ	92	5.4
	93DY	8	8.0
	05LU	65	33.1
	total	265	**15.2%**
lower Yakima River fall	90DF	109	15.3
	93DW	82	11.5
	total	191	**13.7%**
Chandler Trap smolts 2005	05AB	1,320	**3.5%**
Chandler Trap pathology 2005	05AC	200	**4.0%**
Known-origin fish 2005	05AE	100	**5.7%**

Table 2. Jackknife of baseline collections. Rows identify the percentage of individuals that assign back to each baseline stock from the total number in that baseline stock. Columns identify the total percentage of individuals that assign to a baseline stock across all stocks.

	upper Yakima R. SP	Naches R. SP	American R. SP	Marion Drain F	lower Yakima R. F	Unassigned[1]
upper Yakima R. SP	89.0%	9.3%	0.8%	0.0%	0.0%	0.8%
Naches R. SP	13.1%	71.3%	13.5%	0.0%	0.0%	2.1%
American R. SP	0.0%	5.5%	92.5%	0.0%	0.0%	2.0%
Marion Drain F	0.0%	1.2%	1.2%	60.7%	34.3%	2.6%
lower Yakima R. F	0.0%	1.0%	0.0%	28.3%	70.7%	0.0%

[1] - fish were unassigned because of missing data for four or more loci

Table 3. Analysis of known-origin samples. The known-origin and the genetic stock assignment for each fish is tabulated. The probability is the assessment of the log of probabilities to determine that the stock assignment by genetic analysis from the assigned stock is "X" times more likely than the second most likely stock. Individuals highlighted in grey identify those where known-origin and genetic assignment disagree.

Fish ID	Known-Origin	Stock Assignment by Genetic Analysis	Probability
05AE0001	Naches R. SP	American R. SP	100
05AE0002	upper Yakima R. SP	*Naches R. SP/upper Yakima R. SP	1
05AE0003	Naches R. SP	American R. SP	681
05AE0004	Marion Drain F	lower Yakima R. F	2197
05AE0005	upper Yakima R. SP	upper Yakima R. SP	235
05AE0006	upper Yakima R. SP	upper Yakima R. SP	112
05AE0007	Marion Drain F	lower Yakima R. F	52
05AE0008	upper Yakima R. SP	upper Yakima R. SP	1133
05AE0009	Marion Drain F	lower Yakima R. F	1528
05AE0010	Naches R. SP	Naches R. SP	59
05AE0011	Marion Drain F	lower Yakima R. F	520
05AE0012	upper Yakima R. SP	upper Yakima R. SP	29
05AE0013	Naches R. SP	American R. SP	13
05AE0014	Marion Drain F	lower Yakima R. F	92
05AE0015	Marion Drain F	lower Yakima R. F	402
05AE0016	Naches R. SP	American R. SP	8
05AE0017	Marion Drain F	lower Yakima R. F	1214
05AE0018	Naches R. SP	American R. SP	380
05AE0019	Marion Drain F	lower Yakima R. F	90
05AE0020	upper Yakima R. SP	upper Yakima R. SP	28
05AE0021	Naches R. SP	Naches R. SP	4
05AE0022	Marion Drain F	lower Yakima R. F	4699
05AE0023	Marion Drain F	lower Yakima R. F	834
05AE0024	Marion Drain F	lower Yakima R. F	7253
05AE0025	Marion Drain F	lower Yakima R. F	6018
05AE0026	Naches R. SP	American R. SP	19
05AE0027	Naches R. SP	American R. SP	8124
05AE0028	Naches R. SP	American R. SP	90
05AE0029	Marion Drain F	lower Yakima R. F	25
05AE0030	Naches R. SP	American R. SP	1564
05AE0031	upper Yakima R. SP	upper Yakima R. SP	216
05AE0032	upper Yakima R. SP	Naches R. SP	3
05AE0033	upper Yakima R. SP	upper Yakima R. SP	3561
05AE0034	Naches R. SP	Naches R. SP	115
05AE0035	Marion Drain F	Marion Drain F	6
05AE0036	Naches R. SP	Naches R. SP	657
05AE0037	Marion Drain F	lower Yakima R. F	689
05AE0038	upper Yakima R. SP	upper Yakima R. SP	54
05AE0039	Naches R. SP	American R. SP	4044
05AE0040	upper Yakima R. SP	upper Yakima R. SP	1087
05AE0041	Naches R. SP	American R. SP	6195
05AE0042	Marion Drain F	lower Yakima R. F	37
05AE0043	Marion Drain F	lower Yakima R. F	4783
05AE0044	Marion Drain F	lower Yakima R. F	46

Table 3. continued.

Fish ID	Known-Origin	Stock Assignment by Genetic Analysis	Likelihood ratio
05AE0045	upper Yakima R. SP	upper Yakima R. SP	327
05AE0046	Marion Drain F	lower Yakima R. F	9912
05AE0047	Naches R. SP	Naches R. SP	21
05AE0048	Marion Drain F	lower Yakima R. F	16871
05AE0049	Marion Drain F	lower Yakima R. F	30
05AE0050	Naches R. SP	American R. SP	104
05AE0051	upper Yakima R. SP	upper Yakima R. SP	70
05AE0052	upper Yakima R. SP	upper Yakima R. SP	350
05AE0053	Marion Drain F	lower Yakima R. F	24
05AE0054	Marion Drain F	lower Yakima R. F	2872
05AE0055	Naches R. SP	American R. SP	825
05AE0056	upper Yakima R. SP	upper Yakima R. SP	96
05AE0057	Naches R. SP	Naches R. SP	25
05AE0058	Naches R. SP	American R. SP	17
05AE0059	Marion Drain F	lower Yakima R. F	714
05AE0060	Marion Drain F	lower Yakima R. F	332
05AE0061	upper Yakima R. SP	upper Yakima R. SP	22
05AE0062	Naches R. SP	American R. SP	435
05AE0063	upper Yakima R. SP	upper Yakima R. SP	760
05AE0064	upper Yakima R. SP	upper Yakima R. SP	26
05AE0065	Naches R. SP	American R. SP	12
05AE0066	Naches R. SP	American R. SP	1
05AE0067	Marion Drain F	lower Yakima R. F	32
05AE0068	Naches R. SP	Naches R. SP	1158
05AE0069	Naches R. SP	Naches R. SP	77
05AE0070	Naches R. SP	Naches R. SP	10
05AE0071	Naches R. SP	American R. SP	17
05AE0072	upper Yakima R. SP	upper Yakima R. SP	2
05AE0073	Marion Drain F	lower Yakima R. F	23
05AE0074	Naches R. SP	Naches R. SP	10
05AE0075	upper Yakima R. SP	upper Yakima R. SP	4220
05AE0076	upper Yakima R. SP	upper Yakima R. SP	377
05AE0077	upper Yakima R. SP	upper Yakima R. SP	425
05AE0078	upper Yakima R. SP	upper Yakima R. SP	68808
05AE0079	upper Yakima R. SP	upper Yakima R. SP	252
05AE0080	upper Yakima R. SP	upper Yakima R. SP	2
05AE0081	Marion Drain F	lower Yakima R. F	2899
05AE0082	upper Yakima R. SP	upper Yakima R. SP	29
05AE0083	Marion Drain F	lower Yakima R. F	39
05AE0084	upper Yakima R. SP	upper Yakima R. SP	31
05AE0085	Naches R. SP	American R. SP	1331
05AE0086	upper Yakima R. SP	upper Yakima R. SP	2102
05AE0087	upper Yakima R. SP	upper Yakima R. SP	16
05AE0088	upper Yakima R. SP	upper Yakima R. SP	1039
05AE0089	Marion Drain F	lower Yakima R. F	12921
05AE0090	Naches R. SP	Naches R. SP	254
05AE0091	upper Yakima R. SP	upper Yakima R. SP	5
05AE0092	upper Yakima R. SP	upper Yakima R. SP	469
05AE0093	Naches R. SP	Naches R. SP	72

Table 3. continued.

Fish ID	Known-Origin	Stock Assignment by Genetic Analysis	Likelihood ratio
05AE0094	upper Yakima R. SP	*Naches R. SP/upper Yakima R. SP	1
05AE0095	Marion Drain F	lower Yakima R. F	488
05AE0096	Naches R. SP	American R. SP	19
05AE0097	Marion Drain F	lower Yakima R. F	280320
05AE0098	Naches R. SP	-	-
05AE0099	Marion Drain F	lower Yakima R. F	102
05AE0100	upper Yakima R. SP	upper Yakima R. SP	16994

* - two stocks are shown because there was an equal probabilty of either stock being the correct stock-of-origin

Collection location of the American R. and Naches R. stocks was in the Naches R., therefore the two stocks were co-mingled and could not be discrimated.

Table 4. Stock composition estimates for each of five time strata for passage smolt samples collected at Chandler trap in 2004 (using 10 loci) and 2005 (using 11 loci).

2004	January – February	March	April	May	June – July
N - total	264	166	415	515[1]	140
N – unassigned[2]	0	2	1	2	8
American R. SP	4.9%	3.1%	20.8%	21.0%	2.9%
Naches R. SP	31.1%	24.4%	35.5%	19.2%	2.9%
upper Yakima R. SP	63.6%	72.5%	43.7%	15.5%	5.9%
Marion Drain F	0.0%	0.0%	0.0%	1.9%	15.5%
lower Yakima R. F	0.4%	0.0%	0.0%	42.4%	72.8%

[1] two samples were dropped from the May time strata because they were identified as non-Chinook

[2] fish were unassigned because of missing data for five or more loci

2005	January – February	March	April	May	June – July
N - total	201	56	431	522	110
N – unassigned[1]	4	3	12	7	0
American R. SP	20.3%	18.9%	22.9%	3.5%	0.0%
Naches R. SP	35.0%	3.8%	30.1%	2.7%	2.7%
upper Yakima R. SP	44.7%	77.4%	27.2%	4.7%	24.6%
Marion Drain F	0.0%	0.0%	6.0%	11.7%	2.7%
lower Yakima R. F	0.0%	0.0%	13.8%	77.5%	70.0%

[1] fish were unassigned because of missing data for four or more loci

Table 5. Stock composition estimates for each of five time strata for pathology smolt samples collected at Chandler trap in 2004 (using 10 loci) and 2005 (using 11 loci).

2004	Pathology samples
N - total	200
N – unassigned[1]	3
American R. SP	36.0%
Naches R. SP	35.5%
upper Yakima R. SP	27.5%
Marion Drain F	0.5%
lower Yakima R. F	0.5%

[1] fish were unassigned because of missing data for five or more loci

2005	Pathology samples
N - total	200
N – unassigned[2]	4
American R. SP	36.7%
Naches R. SP	23.5%
upper Yakima R. SP	31.1%
Marion Drain F	5.1%
lower Yakima R. F	3.6%

[2] fish were unassigned because of missing data for four or more loci

Appendix 1. The 11 microsatellite loci screened and the percentage of missing data for the 2005 baseline, smolt, known-origin, and pathology samples.

Locus	Repeat (bp)	Allelic range (bp)	Observed # of alleles	% missing data baseline N = 1,184	% missing data smolts N = 1,320	% missing data knowns N = 100	% missing data pathology N = 200
Oki-100	4	221-374	37	17.3	5.8	1.0	1.5
Ots-201b	4	168-338	43	12.2	2.1	0.0	1.5
Ots-208b	4	184-369	48	15.9	6.9	0.0	3.5
Ssa-408	4	211-338	33	10.0	2.7	0.0	2.5
Ogo-2	2	231-267	19	9.5	2.2	21.0	4.5
Ssa-197	4	181-318	36	16.9	3.7	3.0	3.5
Ogo-4	2	165-202	16	22.6	1.8	8.0	3.5
Ots-G474	4	183-247	16	9.9	1.3	3.0	4.5
Ots-213	4	228-385	40	14.9	5.9	24.0	5.0
Ots-3M	2	159-189	14	7.3	4.2	2.0	5.5
Ots-9	2	127-143	8	9.0	2.2	1.0	8.5

Appendix 2. Allele Frequencies.

Oki-100	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	161	211	234	223	151	1244
221	---	---	---	0.004	0.007	0.004
233	---	0.005	---	---	0.003	0.001
237	---	---	---	---	0.010	0.004
240	---	---	---	0.004	0.010	0.005
244	---	---	---	0.002	0.003	0.005
248	---	0.005	0.015	0.004	0.010	0.004
252	---	0.002	0.015	0.011	0.010	0.016
256	0.031	0.055	0.045	0.022	0.023	0.029
260	0.006	0.019	0.030	0.025	0.013	0.030
264	0.003	0.036	0.096	0.034	0.053	0.040
268	0.115	0.102	0.128	0.025	0.030	0.067
272	0.245	0.130	0.013	0.036	0.010	0.066
276	0.124	0.197	0.233	0.029	0.040	0.119
279	0.068	0.043	0.079	0.031	0.033	0.064
283	0.056	0.062	0.060	0.047	0.076	0.063
287	0.053	0.017	0.056	0.074	0.070	0.055
291	0.006	0.066	0.047	0.047	0.050	0.051
295	0.081	0.064	0.047	0.054	0.086	0.066
299	0.003	0.024	0.002	0.038	0.063	0.038
303	0.034	0.014	0.041	0.067	0.050	0.039
307	---	0.050	0.043	0.045	0.066	0.046
311	0.124	0.059	0.015	0.083	0.073	0.055
315	0.019	0.007	0.015	0.063	0.050	0.027
319	0.028	0.017	0.011	0.027	0.056	0.034
323	---	0.017	0.006	0.056	0.023	0.021
327	---	0.009	---	0.054	0.017	0.009
331	---	---	---	0.036	0.013	0.020
335	---	0.002	0.004	0.063	0.013	0.008
339	---	---	---	0.002	0.003	0.003
343	---	---	---	---	0.017	0.002
347	0.003	---	---	0.002	0.007	0.001
351	---	---	---	0.004	0.007	0.003
355	---	---	---	0.004	0.007	0.003
366	---	---	---	---	---	0.001
370	---	---	---	0.002	---	0.004
374	---	---	---	0.002	---	---

Appendix 2. Continued

Ots-201b	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	182	242	228	227	161	1292
168	---	---	---	0.024	---	---
172	---	---	---	0.002	0.022	0.003
176	---	---	---	0.002	0.006	---
180	---	---	---	---	0.006	---
184	---	0.002	---	0.002	0.006	0.003
188	---	---	---	---	---	0.001
192	---	0.002	---	0.004	0.009	0.007
196	0.003	0.014	---	0.035	0.068	0.027
200	0.060	0.062	0.004	0.022	0.034	0.031
204	0.124	0.043	0.009	0.013	0.019	0.030
207	---	0.045	0.132	0.024	0.028	0.052
211	0.025	0.043	0.029	0.009	0.006	0.025
215	0.151	0.130	0.136	0.048	0.047	0.097
219	0.239	0.159	0.096	0.115	0.050	0.106
223	0.148	0.056	0.044	0.009	0.043	0.054
227	0.005	---	0.022	0.088	0.056	0.031
231	0.005	0.008	0.015	0.035	0.031	0.027
235	0.066	0.062	0.050	0.046	0.028	0.041
239	0.080	0.074	0.020	0.013	0.028	0.050
242	---	0.021	0.011	0.048	0.059	0.032
246	0.027	0.072	0.114	0.079	0.096	0.066
250	0.008	0.054	0.114	0.145	0.090	0.094
254	0.008	0.021	0.009	0.090	0.106	0.049
258	---	0.029	0.094	0.048	0.040	0.046
262	---	---	0.009	0.022	0.019	0.012
266	---	---	---	0.004	0.022	0.012
270	0.003	0.002	---	0.002	0.003	0.005
274	0.005	0.004	---	0.011	0.009	0.003
278	0.003	0.010	0.004	0.022	0.006	0.008
282	0.022	0.021	0.007	0.002	0.009	0.012
286	0.014	0.033	0.035	---	---	0.026
290	---	0.023	0.039	---	0.012	0.014
294	0.003	---	---	---	0.003	0.004
298	---	---	0.004	0.009	0.003	0.013
301	---	0.002	---	0.007	0.009	0.002
306	---	---	---	0.002	---	0.001
310	---	0.006	0.002	0.004	0.006	0.006
314	---	---	---	0.007	0.003	0.002
318	---	---	---	0.002	---	0.001
322	---	---	---	---	0.003	0.005
326	---	---	---	---	---	0.001
334	---	---	---	---	0.009	---
338	---	---	---	---	0.003	0.001

Appendix 2. Continued

Ots-208b	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	155	224	232	222	163	1229
184	---	0.007	---	0.002	---	0.002
188	---	---	---	---	0.006	---
192	---	0.002	0.002	0.005	0.006	0.011
196	---	0.011	0.052	0.011	0.015	0.022
200	---	---	0.009	0.020	0.021	0.019
204	0.013	---	0.009	0.014	0.021	0.015
208	0.006	0.011	0.011	0.011	0.034	0.019
212	---	0.020	0.013	0.020	0.040	0.026
216	---	0.007	---	0.032	0.043	0.015
219	---	0.022	0.024	0.011	0.025	0.017
223	0.013	0.020	0.011	0.016	0.018	0.012
227	0.013	0.060	0.058	0.023	0.025	0.041
231	0.087	0.063	0.019	0.029	0.046	0.044
235	0.084	0.047	0.063	0.050	0.046	0.056
239	0.023	0.087	0.129	0.059	0.046	0.058
243	0.084	0.060	0.063	0.027	0.028	0.055
247	0.061	0.083	0.119	0.050	0.052	0.081
251	0.016	0.080	0.063	0.092	0.061	0.044
255	0.139	0.071	0.026	0.025	0.037	0.044
259	0.181	0.076	0.034	0.068	0.037	0.076
263	0.129	0.042	0.050	0.034	0.021	0.046
267	0.110	0.054	0.043	0.032	0.028	0.038
271	0.035	0.020	0.047	0.032	0.037	0.037
275	0.003	0.042	0.019	0.027	0.037	0.027
279	---	0.013	0.047	0.050	0.034	0.036
283	---	0.045	0.045	0.018	0.052	0.038
287	0.003	0.020	0.009	0.047	0.006	0.012
291	---	0.016	0.002	0.016	0.025	0.011
295	---	0.004	0.009	0.005	0.025	0.017
298	---	---	0.019	0.025	0.012	0.012
303	---	---	---	0.027	0.015	0.011
307	---	---	---	0.014	0.018	0.008
311	---	---	---	0.014	0.012	0.012
315	---	---	---	0.007	0.018	0.007
319	---	---	---	0.032	0.015	0.008
323	---	---	---	0.016	0.018	0.006
327	---	---	---	0.009	---	0.006
331	---	---	---	0.020	0.009	0.003
336	---	---	---	0.002	---	0.002
340	---	---	---	0.002	---	0.001
344	---	0.016	0.002	0.002	---	---
348	---	---	0.002	---	---	0.001
352	---	---	0.002	---	---	0.001
356	---	---	---	---	0.003	0.002
360	---	---	---	---	0.003	---
364	---	---	---	---	0.003	---
365	---	---	---	0.007	---	---
369	---	---	---	0.002	---	---

Appendix 2. Continued

Ssa-408	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	183	248	230	231	174	1284
211	0.254	0.071	0.078	0.006	0.003	0.072
214	0.164	0.198	0.070	0.013	0.003	0.066
218	0.142	0.058	0.020	0.019	0.023	0.060
222	0.008	0.109	0.159	0.061	0.078	0.094
226	0.036	0.089	0.139	0.113	0.078	0.084
230	---	0.014	0.013	0.024	0.046	0.018
234	---	0.012	0.002	0.136	0.063	0.022
238	---	0.010	0.074	0.175	0.147	0.090
242	0.016	0.046	0.020	0.082	0.095	0.059
245	0.112	0.077	0.087	0.065	0.066	0.084
249	0.025	0.129	0.070	0.013	0.046	0.065
253	0.158	0.101	0.041	0.074	0.063	0.086
257	0.003	0.022	0.061	0.054	0.092	0.056
261	0.049	0.018	0.059	0.039	0.029	0.039
265	---	0.006	0.033	0.030	0.057	0.028
269	0.027	0.024	0.017	0.006	0.014	0.020
273	---	0.006	---	0.009	0.003	0.003
276	---	---	---	0.004	0.020	0.008
280	---	---	0.004	0.006	0.006	0.002
284	---	---	---	0.011	0.009	0.007
288	---	---	---	---	0.009	0.001
292	---	---	---	0.002	0.006	---
296	---	---	---	0.004	0.003	0.001
300	---	---	0.007	0.002	0.006	0.004
304	---	0.002	0.039	0.002	0.003	0.009
308	---	---	---	0.002	---	0.003
312	---	---	0.007	0.032	0.020	0.014
316	---	0.002	---	---	0.009	---
320	---	0.004	---	0.013	0.003	0.003
324	---	0.002	0.002	---	---	0.001
328	0.003	---	---	---	0.003	---
332	---	---	---	---	---	0.001
338	0.003	---	---	---	---	---

Appendix 2. Continued

Ogo-2	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	189	248	233	217	184	1291
231	---	---	0.002	---	---	0.002
234	---	---	---	0.021	0.014	0.009
236	---	---	---	---	0.003	0.001
238	---	0.008	---	0.012	0.016	0.005
240	---	---	---	---	0.003	0.002
242	0.241	0.151	0.225	0.055	0.019	0.138
244	0.476	0.474	0.313	0.032	0.024	0.225
246	0.190	0.107	0.097	0.009	0.022	0.064
248	0.024	0.115	0.082	0.161	0.185	0.120
250	---	0.065	0.082	0.200	0.190	0.140
252	0.005	0.046	0.139	0.115	0.128	0.100
254	---	---	0.004	0.037	0.041	0.015
256	0.048	0.022	0.054	0.251	0.166	0.100
258	---	---	0.002	0.025	0.038	0.026
260	---	0.006	---	0.044	0.106	0.034
262	0.016	0.002	---	0.025	0.019	0.014
264	---	0.002	---	0.007	0.016	0.006
266	---	0.002	---	0.005	0.011	---

Ogo-4	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	134	202	232	205	144	1296
165	0.041	0.027	0.047	0.244	0.309	0.179
167	---	---	---	0.002	---	0.001
169	0.067	0.037	0.075	0.346	0.389	0.194
171	---	0.015	0.004	0.11	0.087	0.052
173	---	0.005	---	0.007	0.024	0.008
175	---	---	---	0.039	0.038	0.016
182	0.377	0.272	0.194	0.002	---	0.135
184	---	0.002	---	0.022	0.038	0.019
186	---	---	---	0.002	---	0.002
188	0.127	0.158	0.125	0.005	---	0.071
190	0.007	0.134	0.345	0.071	0.007	0.118
192	0.015	0.069	0.097	0.134	0.063	0.060
194	0.127	0.101	0.037	---	0.01	0.047
196	0.09	0.111	0.063	0.015	0.035	0.058
198	0.149	0.067	0.013	---	---	0.038
202	---	---	---	---	---	0.003

Appendix 2. Continued

Ssa-197	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	153	250	229	225	127	1271
185	---	---	---	---	0.012	0.004
189	---	0.002	---	0.004	---	0.003
193	---	---	0.013	0.002	0.008	0.007
197	---	0.012	0.013	0.060	0.059	0.021
201	0.010	0.002	0.002	0.040	0.043	0.033
205	---	0.008	0.011	0.078	0.055	0.044
209	0.007	0.018	0.052	0.038	0.035	0.043
213	0.056	0.058	0.026	0.036	0.059	0.044
217	---	0.002	---	0.087	0.047	0.021
221	---	0.002	0.013	0.027	0.031	0.019
225	---	---	---	0.047	0.020	0.012
229	---	0.002	---	0.029	0.028	0.017
233	---	---	---	0.016	0.004	0.007
237	---	---	0.007	0.004	0.004	0.006
241	---	---	---	0.007	0.016	0.004
244	0.003	---	---	0.004	0.008	0.008
248	0.108	0.040	0.007	0.009	0.004	0.031
253	0.029	0.024	0.022	0.027	0.008	0.020
257	0.042	0.018	0.028	0.027	0.047	0.026
261	0.082	0.030	0.087	0.029	0.071	0.048
265	0.078	0.068	0.020	0.064	0.031	0.047
269	0.114	0.160	0.175	0.069	0.063	0.109
273	0.147	0.116	0.138	0.067	0.071	0.086
277	0.101	0.080	0.094	0.069	0.091	0.093
281	0.078	0.100	0.074	0.040	0.051	0.068
285	0.026	0.062	0.070	0.022	0.051	0.049
289	0.010	0.056	0.124	0.051	0.028	0.060
293	0.016	0.048	0.020	0.018	0.012	0.022
297	0.039	0.016	0.004	0.011	0.024	0.018
301	0.049	0.026	---	0.007	0.008	0.015
305	---	0.004	---	0.007	0.004	0.001
309	---	0.012	---	0.002	0.004	0.003
312	---	0.002	---	---	---	---
314	---	---	---	0.004	0.004	0.004
318	0.003	0.032	---	---	---	0.006

Appendix 2. Continued

Ots-213	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	152	232	227	219	177	1242
228	0.003	---	---	---	---	---
232	---	---	---	---	---	0.001
240	---	0.002	---	0.005	---	0.006
244	---	---	---	0.005	0.011	0.002
248	---	---	---	0.011	0.006	0.006
251	0.010	0.004	0.011	0.018	---	0.006
255	---	0.030	0.018	0.021	0.020	0.032
259	0.003	0.013	0.055	0.002	0.014	0.014
263	---	0.032	0.018	0.002	0.008	0.012
267	---	0.004	0.037	---	0.008	0.017
271	---	0.004	---	0.005	0.011	0.011
275	---	---	---	0.016	0.008	0.016
279	---	0.004	0.022	0.007	0.014	0.012
283	---	0.006	0.020	0.014	0.014	0.015
287	---	0.026	0.015	0.030	0.045	0.030
291	0.059	0.103	0.130	0.055	0.071	0.073
295	0.036	0.103	0.055	0.068	0.071	0.091
299	0.095	0.078	0.084	0.071	0.065	0.079
303	0.164	0.110	0.081	0.046	0.116	0.101
307	---	0.041	0.026	0.087	0.065	0.033
311	0.089	0.065	0.009	0.059	0.042	0.039
315	0.181	0.095	0.073	0.110	0.068	0.084
319	0.063	0.063	0.037	0.055	0.068	0.049
323	0.155	0.073	0.075	0.053	0.042	0.072
327	0.066	0.065	0.081	0.034	0.034	0.056
331	0.003	0.006	0.042	0.037	0.017	0.037
335	0.020	0.043	0.084	0.025	0.037	0.031
339	0.053	0.022	0.002	0.018	0.028	0.014
343	---	0.006	0.011	0.007	0.017	0.008
347	---	---	0.002	0.021	0.008	0.003
351	---	---	0.004	0.037	0.011	0.009
355	---	---	0.007	0.018	0.014	0.006
359	---	---	---	0.005	0.006	0.007
362	---	---	---	0.002	0.014	0.006
366	---	---	---	0.030	0.023	0.010
370	---	---	---	0.011	0.014	0.006
374	---	---	---	0.005	0.006	0.002
377	---	---	---	0.002	0.003	0.004
381	---	---	---	0.005	---	---
385	---	---	---	0.007	---	---

Appendix 2. Continued

Ots-G474	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	183	242	238	231	181	1303
183	---	---	---	0.002	0.008	0.006
187	0.667	0.800	0.830	0.032	0.008	0.440
191	---	---	---	0.002	0.003	0.003
199	0.082	0.085	0.065	0.489	0.517	0.287
203	---	---	---	0.104	0.083	0.048
207	---	---	---	0.013	0.011	0.011
211	---	0.006	0.002	0.143	0.141	0.049
215	---	0.006	0.048	0.071	0.105	0.056
219	---	---	---	0.110	0.075	0.020
223	---	---	---	0.009	0.008	0.007
227	0.014	0.017	0.015	0.015	0.028	0.018
231	0.008	0.014	0.008	---	0.011	0.005
235	0.202	0.068	0.027	0.002	0.003	0.038
239	0.027	0.004	0.002	0.006	---	0.006
243	---	---	0.002	---	---	0.003
247	---	---	---	---	---	0.002

Ots-3M	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	192	254	235	234	183	1265
159	---	0.002	---	---	---	0.002
161	---	---	---	---	0.003	0.002
167	---	---	---	0.013	0.003	0.001
169	---	0.024	0.017	0.021	0.014	0.021
171	---	0.004	0.002	---	---	---
173	---	0.024	0.051	0.024	0.049	0.046
175	0.008	0.047	0.160	0.094	0.098	0.076
177	0.060	0.183	0.177	0.303	0.314	0.245
179	0.906	0.650	0.513	0.288	0.216	0.446
181	0.026	0.059	0.055	0.162	0.120	0.094
183	---	0.008	0.017	0.071	0.142	0.052
185	---	---	---	0.024	0.036	0.012
187	---	---	---	---	0.005	0.004
189	---	---	0.009	---	---	---

Ots-9	American R. SP	Naches R. SP	upper Yakima R. SP	Marion Drain F	lower Yakima R. F	smolts at Chandler trap
(N)	177	265	228	239	169	1291
127	---	---	---	0.002	---	---
130	0.014	0.011	---	---	---	0.004
132	0.014	0.100	0.125	0.169	0.077	0.110
134	0.387	0.374	0.480	0.385	0.518	0.438
136	0.412	0.317	0.294	0.092	0.089	0.198
138	0.172	0.142	0.092	0.347	0.314	0.236
141	---	0.040	0.007	---	0.003	0.012
143	---	0.017	0.002	0.004	---	0.002

Appendix 3. Assignment as a spring or fall Chinook from morphological assignment and genetic analysis in 2005.

April time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
02-Apr	05AB1206	SP	SP
02-Apr	05AB1207	SP	SP
02-Apr	05AB1208	SP	SP
02-Apr	05AB1209	SP	SP
02-Apr	05AB1210	SP	SP
02-Apr	05AB1211	SP	SP
02-Apr	05AB1212	SP	SP
02-Apr	05AB1213	SP	SP
02-Apr	05AB1214	SP	SP
02-Apr	05AB1215	SP	SP
03-Apr	05AB1216	SP	SP
03-Apr	05AB1217	SP	SP
03-Apr	05AB1219	SP	SP
03-Apr	05AB1220	SP	SP
03-Apr	05AB1222	SP	SP
03-Apr	05AB1224	SP	SP
04-Apr	05AB1226	SP	SP
04-Apr	05AB1227	SP	SP
04-Apr	05AB1229	SP	SP
04-Apr	05AB1230	SP	SP
04-Apr	05AB1231	SP	SP
04-Apr	05AB1234	SP	SP
04-Apr	05AB1235	SP	SP
05-Apr	05AB1238	SP	SP
06-Apr	05AB1246	SP	SP
06-Apr	05AB1248	SP	SP
06-Apr	05AB1251	SP	SP
06-Apr	05AB1254	SP	SP
07-Apr	05AB1256	-	SP
07-Apr	05AB1259	-	SP
07-Apr	05AB1263	SP	SP
08-Apr	05AB1266	SP	SP
08-Apr	05AB1267	SP	SP
08-Apr	05AB1274	SP	SP
08-Apr	05AB1282	SP	SP
08-Apr	05AB1291	SP	SP
08-Apr	05AB1300	SP	SP
08-Apr	05AB1308	SP	SP
09-Apr	05AB1316	SP	SP
09-Apr	05AB1317	SP	SP
09-Apr	05AB1318	SP	SP
09-Apr	05AB1319	SP	SP
09-Apr	05AB1320	SP	SP
09-Apr	05AB1321	SP	SP
09-Apr	05AB1322	SP	SP
09-Apr	05AB1323	SP	SP
09-Apr	05AB1324	SP	SP
09-Apr	05AB1325	SP	SP
10-Apr	05AB1326	SP	SP
10-Apr	05AB1329	SP	SP
10-Apr	05AB1333	SP	SP
11-Apr	05AB1336	SP	SP
11-Apr	05AB1339	SP	SP
11-Apr	05AB1343	SP	SP
12-Apr	05AB1346	SP	SP
12-Apr	05AB1348	SP	SP
12-Apr	05AB1351	SP	SP
12-Apr	05AB1353	SP	SP
13-Apr	05AB1356	SP	SP
13-Apr	05AB1367	-	SP
13-Apr	05AB1380	-	SP
13-Apr	05AB1394	SP	SP
14-Apr	05AB1406	SP	SP
14-Apr	05AB1407	SP	SP
14-Apr	05AB1408	SP	SP
14-Apr	05AB1410	SP	SP
14-Apr	05AB1411	SP	SP
14-Apr	05AB1413	SP	SP
14-Apr	05AB1414	SP	SP
15-Apr	05AB1416	SP	SP
15-Apr	05AB1418	SP	SP
15-Apr	05AB1420	SP	SP
15-Apr	05AB1423	SP	SP
16-Apr	05AB1426	SP	SP
16-Apr	05AB1439	SP	SP
16-Apr	05AB1452	SP	SP
16-Apr	05AB1465	SP	SP
17-Apr	05AB1476	-	SP
17-Apr	05AB1487	SP	SP
17-Apr	05AB1496	SP	SP
17-Apr	05AB1507	SP	SP
17-Apr	05AB1517	SP	SP
17-Apr	05AB1522	SP	SP
18-Apr	05AB1546	SP	SP
18-Apr	05AB1548	SP	SP
18-Apr	05AB1550	SP	SP
18-Apr	05AB1552	SP	SP
18-Apr	05AB1553	-	SP
18-Apr	05AB1554	SP	SP
18-Apr	05AB1556	SP	SP
18-Apr	05AB1557	SP	SP
18-Apr	05AB1559	SP	SP
18-Apr	05AB1560	SP	SP
18-Apr	05AB1562	SP	SP
18-Apr	05AB1563	SP	SP
18-Apr	05AB1564	SP	SP

Appendix 3 continued.

April time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
18-Apr	05AB1565	SP	SP
18-Apr	05AB1566	SP	SP
18-Apr	05AB1568	SP	SP
18-Apr	05AB1569	SP	SP
18-Apr	05AB1570	SP	SP
18-Apr	05AB1571	SP	SP
18-Apr	05AB1572	SP	SP
19-Apr	05AB1576	SP	SP
19-Apr	05AB1577	SP	SP
19-Apr	05AB1578	SP	SP
19-Apr	05AB1579	SP	SP
19-Apr	05AB1580	SP	SP
19-Apr	05AB1581	SP	SP
19-Apr	05AB1583	SP	SP
19-Apr	05AB1584	SP	SP
19-Apr	05AB1585	SP	SP
19-Apr	05AB1586	SP	SP
19-Apr	05AB1587	SP	SP
19-Apr	05AB1588	SP	SP
19-Apr	05AB1590	SP	SP
19-Apr	05AB1591	SP	SP
19-Apr	05AB1592	SP	SP
19-Apr	05AB1593	SP	SP
19-Apr	05AB1594	SP	SP
19-Apr	05AB1596	SP	SP
19-Apr	05AB1597	SP	SP
19-Apr	05AB1598	SP	SP
19-Apr	05AB1599	SP	SP
19-Apr	05AB1600	SP	SP
19-Apr	05AB1602	SP	SP
19-Apr	05AB1603	SP	SP
19-Apr	05AB1604	SP	SP
19-Apr	05AB1605	SP	SP
19-Apr	05AB1606	SP	SP
19-Apr	05AB1608	SP	SP
19-Apr	05AB1609	SP	SP
19-Apr	05AB1610	SP	SP
19-Apr	05AB1611	SP	SP
19-Apr	05AB1612	SP	SP
19-Apr	05AB1614	SP	SP
19-Apr	05AB1615	SP	SP
19-Apr	05AB1616	SP	SP
19-Apr	05AB1617	SP	SP
19-Apr	05AB1618	SP	SP
19-Apr	05AB1620	SP	SP
19-Apr	05AB1621	SP	SP
19-Apr	05AB1622	-	SP
19-Apr	05AB1623	SP	SP
19-Apr	05AB1624	SP	SP
20-Apr	05AB1628	SP	SP
20-Apr	05AB1631	SP	SP
20-Apr	05AB1634	SP	SP
20-Apr	05AB1637	SP	SP
20-Apr	05AB1640	SP	SP
20-Apr	05AB1643	SP	SP
20-Apr	05AB1646	SP	SP
20-Apr	05AB1649	SP	SP
20-Apr	05AB1652	SP	SP
20-Apr	05AB1655	SP	SP
20-Apr	05AB1658	SP	SP
20-Apr	05AB1661	SP	SP
20-Apr	05AB1664	SP	SP
20-Apr	05AB1667	SP	SP
20-Apr	05AB1670	SP	SP
20-Apr	05AB1673	-	SP
21-Apr	05AB1678	SP	SP
21-Apr	05AB1681	SP	SP
21-Apr	05AB1684	SP	SP
21-Apr	05AB1687	SP	SP
21-Apr	05AB1690	SP	SP
21-Apr	05AB1693	SP	SP
21-Apr	05AB1696	SP	SP
21-Apr	05AB1699	SP	SP
21-Apr	05AB1702	SP	SP
21-Apr	05AB1705	SP	SP
21-Apr	05AB1708	SP	SP
21-Apr	05AB1711	SP	SP
21-Apr	05AB1714	SP	SP
21-Apr	05AB1717	SP	SP
21-Apr	05AB1720	SP	SP
21-Apr	05AB1723	SP	SP
21-Apr	05AB1728	SP	SP
22-Apr	05AB1731	SP	SP
22-Apr	05AB1735	SP	SP
22-Apr	05AB1739	F	SP
22-Apr	05AB1743	SP	SP
22-Apr	05AB1747	-	SP
22-Apr	05AB1751	SP	SP
22-Apr	05AB1755	SP	SP
22-Apr	05AB1759	SP	SP
22-Apr	05AB1763	SP	SP
22-Apr	05AB1766	SP	SP
22-Apr	05AB1769	SP	SP
22-Apr	05AB1773	SP	SP
23-Apr	05AB1781	SP	SP
23-Apr	05AB1788	SP	SP
23-Apr	05AB1794	SP	SP
23-Apr	05AB1801	SP	SP

Appendix 3 continued.

April time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
23-Apr	05AB1808	-	SP
23-Apr	05AB1815	SP	SP
23-Apr	05AB1824	SP	SP
24-Apr	05AB1831	SP	SP
24-Apr	05AB1836	SP	SP
24-Apr	05AB1841	SP	SP
24-Apr	05AB1846	SP	SP
24-Apr	05AB1851	SP	SP
24-Apr	05AB1856	SP	SP
24-Apr	05AB1861	SP	SP
24-Apr	05AB1866	SP	SP
24-Apr	05AB1871	SP	SP
24-Apr	05AB1876	SP	SP
25-Apr	05AB1881	SP	SP
25-Apr	05AB1882	SP	SP
25-Apr	05AB1883	SP	SP
25-Apr	05AB1884	SP	SP
25-Apr	05AB1885	SP	SP
25-Apr	05AB1886	SP	SP
25-Apr	05AB1887	SP	SP
25-Apr	05AB1888	SP	SP
25-Apr	05AB1889	SP	SP
25-Apr	05AB1890	SP	SP
25-Apr	05AB1891	SP	SP
25-Apr	05AB1892	SP	SP
25-Apr	05AB1893	-	SP
25-Apr	05AB1894	-	SP
25-Apr	05AB1895	SP	SP
25-Apr	05AB1896	SP	SP
25-Apr	05AB1897	SP	SP
25-Apr	05AB1898	SP	SP
25-Apr	05AB1899	SP	SP
26-Apr	05AB1901	SP	SP
26-Apr	05AB1902	SP	SP
26-Apr	05AB1904	F	SP
26-Apr	05AB1906	SP	SP
26-Apr	05AB1908	SP	SP
26-Apr	05AB1910	SP	SP
26-Apr	05AB1912	SP	SP
26-Apr	05AB1914	SP	SP
26-Apr	05AB1916	SP	SP
26-Apr	05AB1918	SP	SP
26-Apr	05AB1920	SP	SP
26-Apr	05AB1922	SP	SP
26-Apr	05AB1924	SP	SP
26-Apr	05AB1926	SP	SP
26-Apr	05AB1928	SP	SP
26-Apr	05AB1930	SP	SP
26-Apr	05AB1932	SP	SP
26-Apr	05AB1934	SP	SP
26-Apr	05AB1936	SP	SP
26-Apr	05AB1938	SP	SP
26-Apr	05AB1940	SP	SP
26-Apr	05AB1942	SP	SP
26-Apr	05AB1944	SP	SP
26-Apr	05AB1945	SP	SP
26-Apr	05AB1947	SP	SP
26-Apr	05AB1949	SP	SP
26-Apr	05AB1950	SP	SP
27-Apr	05AB1951	F	F
27-Apr	05AB1952	SP	SP
27-Apr	05AB1953	SP	SP
27-Apr	05AB1954	F	F
27-Apr	05AB1955	SP	SP
27-Apr	05AB1956	SP	SP
27-Apr	05AB1957	F	F
27-Apr	05AB1958	SP	SP
27-Apr	05AB1959	SP	SP
27-Apr	05AB1960	SP	SP
27-Apr	05AB1961	F	F
27-Apr	05AB1962	SP	SP
27-Apr	05AB1963	F	F
27-Apr	05AB1964	SP	SP
27-Apr	05AB1965	F	F
27-Apr	05AB1966	SP	SP
27-Apr	05AB1967	SP	SP
27-Apr	05AB1968	SP	SP
27-Apr	05AB1969	SP	SP
27-Apr	05AB1970	SP	SP
27-Apr	05AB1971	SP	SP
27-Apr	05AB1972	F	F
27-Apr	05AB1973	F	F
27-Apr	05AB1974	SP	SP
27-Apr	05AB1975	F	F
27-Apr	05AB1976	SP	SP
27-Apr	05AB1977	SP	SP
27-Apr	05AB1978	SP	SP
27-Apr	05AB1979	F	F
27-Apr	05AB1980	SP	SP
27-Apr	05AB1981	SP	SP
27-Apr	05AB1982	F	F
27-Apr	05AB1983	F	F
27-Apr	05AB1984	F	F
27-Apr	05AB1985	SP	SP
27-Apr	05AB1986	SP	F
27-Apr	05AB1987	F	F
27-Apr	05AB1988	F	F
27-Apr	05AB1989	SP	SP

Appendix 3 continued.

April time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
27-Apr	05AB1990	F	F
27-Apr	05AB1991	SP	SP
27-Apr	05AB1992	F	F
27-Apr	05AB1993	F	F
27-Apr	05AB1994	SP	SP
27-Apr	05AB1995	SP	SP
27-Apr	05AB1996	SP	SP
27-Apr	05AB1997	SP	SP
27-Apr	05AB1998	F	F
27-Apr	05AB1999	SP	SP
27-Apr	05AB2000	SP	SP
28-Apr	05AB2001	SP	SP
28-Apr	05AB2002	SP	SP
28-Apr	05AB2003	F	F
28-Apr	05AB2005	SP	SP
28-Apr	05AB2006	SP	SP
28-Apr	05AB2007	F	F
28-Apr	05AB2009	SP	SP
28-Apr	05AB2010	SP	SP
28-Apr	05AB2011	SP	SP
28-Apr	05AB2012	SP	SP
28-Apr	05AB2014	F	F
28-Apr	05AB2015	SP	SP
28-Apr	05AB2016	SP	SP
28-Apr	05AB2017	F	F
28-Apr	05AB2019	F	F
28-Apr	05AB2020	F	F
28-Apr	05AB2021	F	F
28-Apr	05AB2022	SP	SP
28-Apr	05AB2024	SP	SP
28-Apr	05AB2025	SP	SP
28-Apr	05AB2026	F	SP
28-Apr	05AB2028	SP	SP
28-Apr	05AB2029	F	F
28-Apr	05AB2030	F	F
28-Apr	05AB2032	F	F
28-Apr	05AB2033	F	F
28-Apr	05AB2034	F	F
28-Apr	05AB2036	SP	SP
28-Apr	05AB2037	F	F
28-Apr	05AB2038	F	F
28-Apr	05AB2040	F	F
28-Apr	05AB2041	SP	SP
28-Apr	05AB2042	F	F
28-Apr	05AB2044	SP	SP
28-Apr	05AB2048	F	F
28-Apr	05AB2049	SP	SP
29-Apr	05AB2051	SP	SP
29-Apr	05AB2052	F	F
29-Apr	05AB2053	SP	SP
29-Apr	05AB2054	SP	SP
29-Apr	05AB2055	SP	SP
29-Apr	05AB2057	SP	SP
29-Apr	05AB2058	SP	SP
29-Apr	05AB2059	SP	SP
29-Apr	05AB2060	F	F
29-Apr	05AB2061	SP	SP
29-Apr	05AB2063	F	F
29-Apr	05AB2064	F	F
29-Apr	05AB2065	SP	SP
29-Apr	05AB2066	F	F
29-Apr	05AB2067	SP	SP
29-Apr	05AB2069	SP	SP
29-Apr	05AB2070	F	F
29-Apr	05AB2071	F	F
29-Apr	05AB2072	SP	SP
29-Apr	05AB2073	SP	SP
29-Apr	05AB2075	F	F
29-Apr	05AB2076	SP	SP
29-Apr	05AB2077	F	F
29-Apr	05AB2078	F	F
29-Apr	05AB2079	F	F
29-Apr	05AB2081	F	SP
29-Apr	05AB2082	SP	SP
29-Apr	05AB2083	SP	SP
29-Apr	05AB2084	SP	SP
29-Apr	05AB2085	F	F
29-Apr	05AB2087	SP	SP
29-Apr	05AB2088	F	F
29-Apr	05AB2089	F	F
29-Apr	05AB2090	SP	SP
29-Apr	05AB2091	F	F
29-Apr	05AB2093	F	F
29-Apr	05AB2094	SP	SP
29-Apr	05AB2095	SP	SP
29-Apr	05AB2096	SP	SP
29-Apr	05AB2098	F	F
29-Apr	05AB2099	F	F
29-Apr	05AB2100	F	F
30-Apr	05AB2101	F	F
30-Apr	05AB2102	F	F
30-Apr	05AB2103	F	F
30-Apr	05AB2104	F	F
30-Apr	05AB2105	F	F
30-Apr	05AB2106	F	F
30-Apr	05AB2107	F	F
30-Apr	05AB2108	F	F
30-Apr	05AB2109	F	F

Appendix 3 continued.

April time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
30-Apr	05AB2110	F	F
30-Apr	05AB2111	F	F
30-Apr	05AB2112	F	F
30-Apr	05AB2113	F	F
30-Apr	05AB2114	F	F
30-Apr	05AB2115	F	F
30-Apr	05AB2116	F	F
30-Apr	05AB2117	F	F
30-Apr	05AB2118	F	F
30-Apr	05AB2119	F	F
30-Apr	05AB2120	F	F
30-Apr	05AB2121	SP	SP
30-Apr	05AB2122	SP	SP
30-Apr	05AB2123	SP	SP
30-Apr	05AB2124	SP	SP
30-Apr	05AB2125	SP	SP
30-Apr	05AB2126	SP	SP
30-Apr	05AB2127	SP	SP
30-Apr	05AB2128	SP	SP
30-Apr	05AB2129	SP	SP
30-Apr	05AB2130	SP	SP
30-Apr	05AB2131	SP	SP
30-Apr	05AB2132	SP	SP
30-Apr	05AB2133	SP	SP
30-Apr	05AB2134	SP	SP
30-Apr	05AB2135	SP	SP
30-Apr	05AB2136	SP	SP
30-Apr	05AB2137	SP	SP
30-Apr	05AB2138	SP	SP
30-Apr	05AB2139	SP	SP
30-Apr	05AB2140	SP	SP
01-May	05AB2141	F	F
01-May	05AB2145	SP	SP
01-May	05AB2149	SP	SP
01-May	05AB2153	F	F
01-May	05AB2157	F	F
01-May	05AB2161	F	F
01-May	05AB2165	SP	SP
01-May	05AB2169	SP	SP
01-May	05AB2173	SP	SP
01-May	05AB2177	SP	SP

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
02-May	05AB2181	SP	SP
02-May	05AB2182	SP	SP
02-May	05AB2183	SP	SP
02-May	05AB2184	SP	SP
02-May	05AB2185	SP	SP
02-May	05AB2186	SP	SP
02-May	05AB2187	SP	SP
02-May	05AB2188	SP	SP
02-May	05AB2189	SP	SP
02-May	05AB2190	SP	SP
03-May	05AB2191	SP	SP
03-May	05AB2197	F	F
03-May	05AB2204	F	F
03-May	05AB2210	SP	SP
03-May	05AB2218	SP	SP
03-May	05AB2226	F	F
04-May	05AB2231	F	F
04-May	05AB2250	F	F
05-May	05AB2271	SP	SP
05-May	05AB2284	F	F
05-May	05AB2298	F	F
06-May	05AB2311	SP	SP
06-May	05AB2333	SP	SP
07-May	05AB2351	F	F
07-May	05AB2371	SP	SP
08-May	05AB2391	F	F
09-May	05AB2431	F	F
09-May	05AB2447	F	F
10-May	05AB2451	F	F
10-May	05AB2471	F	F
11-May	05AB2491	F	F
11-May	05AB2501	F	F
11-May	05AB2512	F	F
11-May	05AB2522	F	F
12-May	05AB2531	SP	SP
12-May	05AB2532	SP	SP
12-May	05AB2534	SP	SP
12-May	05AB2535	SP	SP
12-May	05AB2537	SP	SP
12-May	05AB2538	SP	SP
12-May	05AB2540	SP	SP
12-May	05AB2541	SP	SP
12-May	05AB2543	F	F
12-May	05AB2544	F	F
12-May	05AB2546	F	F
12-May	05AB2547	F	F
12-May	05AB2549	F	F
12-May	05AB2550	F	F
12-May	05AB2552	F	F
12-May	05AB2553	F	F
12-May	05AB2555	F	F
12-May	05AB2556	F	F
12-May	05AB2558	F	F
12-May	05AB2559	F	F
12-May	05AB2561	F	F

Appendix 3 continued.

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
12-May	05AB2562	F	F
12-May	05AB2564	F	F
12-May	05AB2565	F	F
13-May	05AB2571	SP	SP
13-May	05AB2572	SP	SP
13-May	05AB2573	SP	SP
13-May	05AB2574	SP	SP
13-May	05AB2575	SP	SP
13-May	05AB2576	SP	SP
13-May	05AB2577	SP	SP
13-May	05AB2578	SP	SP
13-May	05AB2579	SP	SP
13-May	05AB2580	SP	SP
13-May	05AB2581	SP	SP
13-May	05AB2582	SP	SP
13-May	05AB2583	SP	SP
13-May	05AB2584	SP	SP
13-May	05AB2585	SP	SP
13-May	05AB2586	SP	SP
13-May	05AB2587	SP	SP
13-May	05AB2588	SP	SP
13-May	05AB2589	SP	SP
13-May	05AB2590	SP	SP
13-May	05AB2591	F	F
13-May	05AB2592	F	F
13-May	05AB2593	F	F
13-May	05AB2594	F	F
13-May	05AB2595	F	F
13-May	05AB2596	F	F
13-May	05AB2597	F	F
13-May	05AB2598	F	F
13-May	05AB2599	F	F
13-May	05AB2600	F	F
13-May	05AB2601	F	F
13-May	05AB2602	F	F
13-May	05AB2603	F	F
13-May	05AB2604	F	F
13-May	05AB2605	F	F
13-May	05AB2606	F	F
13-May	05AB2607	F	F
13-May	05AB2608	F	F
13-May	05AB2609	F	F
13-May	05AB2610	F	F
14-May	05AB2611	F	F
14-May	05AB2612	F	F
14-May	05AB2613	F	F
14-May	05AB2614	F	F
14-May	05AB2615	F	F
14-May	05AB2616	F	F
14-May	05AB2617	F	F
14-May	05AB2618	F	F
14-May	05AB2619	F	F
14-May	05AB2620	F	F
14-May	05AB2621	F	F
14-May	05AB2622	F	F
14-May	05AB2623	F	F
14-May	05AB2624	-	F
14-May	05AB2625	F	F
14-May	05AB2626	F	F
14-May	05AB2627	F	F
14-May	05AB2628	F	F
14-May	05AB2629	F	F
14-May	05AB2630	F	F
14-May	05AB2631	F	F
14-May	05AB2632	-	F
14-May	05AB2633	F	F
14-May	05AB2634	F	F
14-May	05AB2635	F	F
14-May	05AB2636	F	F
14-May	05AB2637	F	F
14-May	05AB2638	F	F
14-May	05AB2639	F	F
14-May	05AB2640	F	F
14-May	05AB2641	F	F
14-May	05AB2642	F	F
14-May	05AB2643	F	F
14-May	05AB2644	F	F
14-May	05AB2645	F	F
14-May	05AB2646	F	F
14-May	05AB2647	F	F
14-May	05AB2648	F	F
14-May	05AB2649	F	F
15-May	05AB2651	F	F
15-May	05AB2652	F	F
15-May	05AB2653	F	F
15-May	05AB2654	F	F
15-May	05AB2655	F	F
15-May	05AB2656	F	F
15-May	05AB2657	F	F
15-May	05AB2658	F	F
15-May	05AB2659	F	F
15-May	05AB2660	F	F
15-May	05AB2661	F	F
15-May	05AB2662	F	F
15-May	05AB2663	F	F
15-May	05AB2664	F	F
15-May	05AB2665	F	F
15-May	05AB2666	F	F

Appendix 3 continued.

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
15-May	05AB2667	F	F
15-May	05AB2668	F	F
15-May	05AB2669	F	F
15-May	05AB2670	F	F
16-May	05AB2671	F	F
16-May	05AB2672	F	F
16-May	05AB2673	F	F
16-May	05AB2674	F	F
16-May	05AB2675	F	F
16-May	05AB2676	F	F
16-May	05AB2677	F	F
16-May	05AB2678	F	F
16-May	05AB2679	F	F
16-May	05AB2680	F	F
16-May	05AB2681	F	F
16-May	05AB2682	F	F
16-May	05AB2683	F	F
16-May	05AB2684	F	F
16-May	05AB2685	F	F
16-May	05AB2686	F	F
16-May	05AB2687	F	F
16-May	05AB2688	F	F
16-May	05AB2689	F	F
16-May	05AB2690	F	F
16-May	05AB2691	F	F
16-May	05AB2692	F	F
16-May	05AB2693	F	F
16-May	05AB2694	F	F
16-May	05AB2695	F	F
16-May	05AB2696	F	F
16-May	05AB2697	F	F
16-May	05AB2698	F	F
16-May	05AB2699	F	F
16-May	05AB2700	F	F
16-May	05AB2701	F	F
16-May	05AB2702	F	F
16-May	05AB2703	F	F
16-May	05AB2704	F	F
16-May	05AB2705	F	F
16-May	05AB2706	F	F
17-May	05AB2711	F	F
17-May	05AB2712	SP	SP
17-May	05AB2714	F	F
17-May	05AB2715	F	F
17-May	05AB2717	SP	SP
17-May	05AB2718	F	F
17-May	05AB2720	F	F
17-May	05AB2721	F	F
17-May	05AB2723	F	F
17-May	05AB2724	F	F
17-May	05AB2726	F	F
17-May	05AB2727	F	F
17-May	05AB2729	F	F
17-May	05AB2730	SP	SP
17-May	05AB2732	F	F
17-May	05AB2733	F	F
17-May	05AB2735	F	F
17-May	05AB2736	F	F
17-May	05AB2738	F	F
17-May	05AB2739	F	F
17-May	05AB2741	F	F
17-May	05AB2742	F	F
17-May	05AB2744	F	F
17-May	05AB2745	-	F
17-May	05AB2747	F	F
17-May	05AB2748	F	F
17-May	05AB2750	F	F
18-May	05AB2751	F	F
18-May	05AB2754	F	F
18-May	05AB2757	F	F
18-May	05AB2760	F	F
18-May	05AB2763	F	F
18-May	05AB2766	F	F
18-May	05AB2769	F	F
18-May	05AB2772	F	F
18-May	05AB2775	F	F
18-May	05AB2778	F	F
18-May	05AB2781	F	F
18-May	05AB2784	F	F
18-May	05AB2787	SP	SP
18-May	05AB2790	SP	SP
19-May	05AB2791	F	F
19-May	05AB2794	F	F
19-May	05AB2796	F	F
19-May	05AB2799	F	F
19-May	05AB2801	F	F
19-May	05AB2804	F	F
19-May	05AB2806	F	F
19-May	05AB2809	F	F
20-May	05AB2811	SP	SP
20-May	05AB2818	F	F
20-May	05AB2826	F	F
21-May	05AB2831	F	F
21-May	05AB2834	F	F
21-May	05AB2837	F	F
21-May	05AB2840	F	F
21-May	05AB2843	F	F
21-May	05AB2846	F	F

Appendix 3 continued.

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
21-May	05AB2849	F	F
22-May	05AB2851	F	F
22-May	05AB2857	F	F
22-May	05AB2862	F	F
22-May	05AB2867	F	F
22-May	05AB2872	F	F
22-May	05AB2878	F	F
22-May	05AB2884	F	F
23-May	05AB2893	F	F
23-May	05AB2895	F	F
23-May	05AB2898	F	F
23-May	05AB2901	F	F
23-May	05AB2904	F	F
23-May	05AB2907	F	F
23-May	05AB2908	F	F
23-May	05AB2910	F	F
24-May	05AB2911	F	F
24-May	05AB2915	F	F
24-May	05AB2919	F	F
24-May	05AB2923	F	F
24-May	05AB2927	F	F
24-May	05AB2931	F	F
24-May	05AB2935	F	F
24-May	05AB2939	F	F
24-May	05AB2943	F	F
24-May	05AB2947	F	F
24-May	05AB2949	F	F
25-May	05AB2951	F	F
25-May	05AB2952	F	F
25-May	05AB2953	F	F
25-May	05AB2955	F	F
25-May	05AB2956	F	F
25-May	05AB2957	F	F
25-May	05AB2959	F	F
25-May	05AB2960	F	F
25-May	05AB2961	F	F
25-May	05AB2963	F	F
25-May	05AB2964	F	F
25-May	05AB2965	F	F
25-May	05AB2967	F	F
25-May	05AB2968	F	F
25-May	05AB2969	F	F
25-May	05AB2971	F	F
25-May	05AB2972	F	F
25-May	05AB2973	SP	F
25-May	05AB2975	F	F
25-May	05AB2976	F	F
25-May	05AB2977	F	F
25-May	05AB2979	F	F
25-May	05AB2980	F	F
25-May	05AB2981	-	F
25-May	05AB2983	F	F
25-May	05AB2984	F	F
25-May	05AB2986	F	F
25-May	05AB2988	F	F
25-May	05AB2989	F	F
26-May	05AB2991	F	F
26-May	05AB2992	F	F
26-May	05AB2993	F	F
26-May	05AB2995	F	F
26-May	05AB2996	F	F
26-May	05AB2997	F	F
26-May	05AB2999	F	F
26-May	05AB3000	F	F
26-May	05AB3001	-	F
26-May	05AB3003	F	F
26-May	05AB3004	F	F
26-May	05AB3005	F	F
26-May	05AB3007	F	F
26-May	05AB3008	F	F
26-May	05AB3009	F	F
26-May	05AB3011	F	F
26-May	05AB3012	F	F
26-May	05AB3013	F	F
26-May	05AB3015	F	F
26-May	05AB3019	F	F
26-May	05AB3020	F	F
26-May	05AB3023	F	F
26-May	05AB3024	F	F
27-May	05AB3031	F	F
27-May	05AB3032	F	F
27-May	05AB3033	F	F
27-May	05AB3034	F	F
27-May	05AB3035	F	F
27-May	05AB3037	F	F
27-May	05AB3038	F	F
27-May	05AB3039	SP	F
27-May	05AB3040	F	F
27-May	05AB3041	F	F
27-May	05AB3043	F	F
27-May	05AB3044	F	F
27-May	05AB3045	F	F
27-May	05AB3046	F	F
27-May	05AB3047	F	F
27-May	05AB3049	F	F
27-May	05AB3050	F	F
27-May	05AB3051	F	F
27-May	05AB3052	F	F

Appendix 3 continued.

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
27-May	05AB3053	F	F
27-May	05AB3055	F	F
27-May	05AB3056	F	F
27-May	05AB3057	F	F
27-May	05AB3058	F	F
27-May	05AB3059	F	F
27-May	05AB3061	F	F
27-May	05AB3062	F	F
27-May	05AB3063	F	F
27-May	05AB3064	F	F
27-May	05AB3065	F	F
27-May	05AB3067	F	F
27-May	05AB3068	F	F
28-May	05AB3071	F	F
28-May	05AB3072	F	F
28-May	05AB3073	F	F
28-May	05AB3074	SP	F
28-May	05AB3075	F	F
28-May	05AB3076	F	F
28-May	05AB3077	F	F
28-May	05AB3078	F	F
28-May	05AB3079	F	F
28-May	05AB3080	F	F
28-May	05AB3082	F	F
28-May	05AB3083	F	F
28-May	05AB3084	F	F
28-May	05AB3085	F	F
28-May	05AB3086	F	F
28-May	05AB3087	F	F
28-May	05AB3088	F	F
28-May	05AB3089	F	F
28-May	05AB3090	F	F
28-May	05AB3091	F	F
28-May	05AB3093	SP	F
28-May	05AB3094	-	F
28-May	05AB3095	F	F
28-May	05AB3096	F	F
28-May	05AB3097	F	F
28-May	05AB3098	F	F
28-May	05AB3099	F	F
28-May	05AB3100	F	F
28-May	05AB3101	F	F
28-May	05AB3102	F	F
28-May	05AB3104	F	F
28-May	05AB3105	F	F
28-May	05AB3106	F	F
28-May	05AB3107	F	F
28-May	05AB3108	F	F
28-May	05AB3109	F	F
29-May	05AB3111	F	F
29-May	05AB3112	F	F
29-May	05AB3113	F	F
29-May	05AB3114	F	F
29-May	05AB3115	F	F
29-May	05AB3116	F	F
29-May	05AB3117	F	F
29-May	05AB3118	F	F
29-May	05AB3119	-	F
29-May	05AB3120	F	F
29-May	05AB3121	F	F
29-May	05AB3122	F	F
29-May	05AB3123	F	F
29-May	05AB3124	F	F
29-May	05AB3125	F	F
29-May	05AB3126	F	F
29-May	05AB3127	F	F
29-May	05AB3128	F	F
29-May	05AB3129	F	F
29-May	05AB3130	F	F
29-May	05AB3131	F	F
29-May	05AB3132	F	F
29-May	05AB3133	F	F
29-May	05AB3134	F	F
29-May	05AB3135	F	F
29-May	05AB3136	F	F
29-May	05AB3137	F	F
29-May	05AB3138	F	F
29-May	05AB3139	F	F
29-May	05AB3140	F	F
29-May	05AB3141	F	F
29-May	05AB3142	F	F
29-May	05AB3143	F	F
29-May	05AB3144	F	F
29-May	05AB3145	F	F
29-May	05AB3146	F	F
29-May	05AB3147	F	F
29-May	05AB3148	F	F
29-May	05AB3149	F	F
29-May	05AB3150	F	F
30-May	05AB3151	F	F
30-May	05AB3152	F	F
30-May	05AB3153	F	F
30-May	05AB3154	F	F
30-May	05AB3155	F	F
30-May	05AB3156	SP	F
30-May	05AB3157	F	F
30-May	05AB3158	F	F
30-May	05AB3159	F	F

Appendix 3 continued.

May time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
30-May	05AB3160	F	F
30-May	05AB3161	F	F
30-May	05AB3162	F	F
30-May	05AB3163	F	F
30-May	05AB3164	F	F
30-May	05AB3165	F	F
30-May	05AB3166	F	F
30-May	05AB3167	F	F
30-May	05AB3168	F	F
30-May	05AB3169	F	F
30-May	05AB3170	F	F
31-May	05AB3171	F	F
31-May	05AB3172	F	F
31-May	05AB3173	F	F
31-May	05AB3174	F	F
31-May	05AB3175	F	F
31-May	05AB3176	F	F
31-May	05AB3177	F	F
31-May	05AB3178	F	F
31-May	05AB3179	F	F
31-May	05AB3180	F	F
31-May	05AB3181	F	F
31-May	05AB3182	F	F
31-May	05AB3183	F	F
31-May	05AB3184	F	F
31-May	05AB3185	F	F
31-May	05AB3186	F	F
31-May	05AB3187	F	F
31-May	05AB3188	F	F
31-May	05AB3189	F	F
31-May	05AB3190	F	F
31-May	05AB3191	F	F
31-May	05AB3192	F	F
31-May	05AB3193	F	F
31-May	05AB3194	F	F
31-May	05AB3195	F	F
31-May	05AB3196	F	F
31-May	05AB3197	F	F
31-May	05AB3198	F	F
31-May	05AB3199	F	F
31-May	05AB3200	F	F
31-May	05AB3201	F	F
31-May	05AB3202	F	F
31-May	05AB3203	F	F
31-May	05AB3204	F	F
31-May	05AB3205	F	F
31-May	05AB3206	F	F
31-May	05AB3207	F	F
31-May	05AB3208	F	F
31-May	05AB3209	F	F
31-May	05AB3210	F	F
31-May	05AB3211	F	F
31-May	05AB3212	F	F
31-May	05AB3213	F	F
31-May	05AB3214	F	F
31-May	05AB3215	F	F
31-May	05AB3216	F	F
31-May	05AB3217	F	F
31-May	05AB3218	F	F
01-Jun	05AB3222	F	F
01-Jun	05AB3225	F	F
01-Jun	05AB3228	F	F
01-Jun	05AB3231	F	F
01-Jun	05AB3234	F	F
01-Jun	05AB3237	F	F
01-Jun	05AB3240	SP	F
01-Jun	05AB3243	F	F
01-Jun	05AB3246	F	F
01-Jun	05AB3249	F	F
01-Jun	05AB3252	F	F
01-Jun	05AB3255	F	F
01-Jun	05AB3258	F	F
01-Jun	05AB3261	F	F
01-Jun	05AB3264	F	F
01-Jun	05AB3267	F	F

June - July time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
02-Jun	05AB3272	F	F
02-Jun	05AB3277	F	F
02-Jun	05AB3283	F	F
02-Jun	05AB3289	F	F
02-Jun	05AB3295	F	F
02-Jun	05AB3301	F	F
02-Jun	05AB3307	F	F
02-Jun	05AB3314	SP	F
03-Jun	05AB3321	F	F
03-Jun	05AB3328	F	F
03-Jun	05AB3335	F	F
03-Jun	05AB3342	F	F
03-Jun	05AB3349	F	F
03-Jun	05AB3356	F	F
03-Jun	05AB3364	F	F
04-Jun	05AB3371	F	F
04-Jun	05AB3376	F	F
04-Jun	05AB3381	SP	F
04-Jun	05AB3386	SP	F
04-Jun	05AB3391	F	F
04-Jun	05AB3396	F	F

Appendix 3 continued.

June - July time stratum

Date	Animal ID	Genetic Assignment	Morphological Assignment
04-Jun	05AB3402	F	F
04-Jun	05AB3408	F	F
04-Jun	05AB3415	F	F
05-Jun	05AB3421	F	F
05-Jun	05AB3431	F	F
05-Jun	05AB3441	F	F
05-Jun	05AB3453	F	F
05-Jun	05AB3467	SP	F
06-Jun	05AB3471	SP	F
06-Jun	05AB3479	SP	F
06-Jun	05AB3486	F	F
06-Jun	05AB3493	F	F
06-Jun	05AB3500	F	F
06-Jun	05AB3506	SP	F
06-Jun	05AB3513	SP	F
07-Jun	05AB3521	F	F
07-Jun	05AB3524	F	F
07-Jun	05AB3529	SP	F
07-Jun	05AB3534	F	F
07-Jun	05AB3539	F	F
07-Jun	05AB3544	SP	F
08-Jun	05AB3551	F	F
08-Jun	05AB3552	F	F
08-Jun	05AB3553	F	F
08-Jun	05AB3554	F	F
08-Jun	05AB3555	F	F
09-Jun	05AB3556	F	F
09-Jun	05AB3557	SP	F
09-Jun	05AB3558	SP	F
09-Jun	05AB3559	F	F
09-Jun	05AB3560	SP	F
10-Jun	05AB3566	SP	F
11-Jun	05AB3601	SP	F
11-Jun	05AB3605	SP	F
11-Jun	05AB3609	F	F
11-Jun	05AB3613	F	F
11-Jun	05AB3617	SP	F
11-Jun	05AB3621	F	F
11-Jun	05AB3625	F	F
11-Jun	05AB3629	F	F
11-Jun	05AB3633	F	F
11-Jun	05AB3637	SP	F
11-Jun	05AB3641	F	F
11-Jun	05AB3646	F	F
12-Jun	05AB3651	F	F
12-Jun	05AB3652	SP	F
12-Jun	05AB3653	SP	F
12-Jun	05AB3654	SP	F
12-Jun	05AB3655	F	F
13-Jun	05AB3656	F	F
13-Jun	05AB3657	F	F
13-Jun	05AB3658	SP	F
13-Jun	05AB3659	F	F
13-Jun	05AB3660	SP	F
14-Jun	05AB3661	F	F
14-Jun	05AB3662	F	F
14-Jun	05AB3663	F	F
14-Jun	05AB3664	F	F
14-Jun	05AB3665	F	F
15-Jun	05AB3666	F	F
15-Jun	05AB3667	F	F
15-Jun	05AB3668	SP	F
15-Jun	05AB3669	F	F
16-Jun	05AB3671	F	F
16-Jun	05AB3672	SP	F
16-Jun	05AB3673	SP	F
16-Jun	05AB3674	F	F
17-Jun	05AB3676	F	F
17-Jun	05AB3678	F	F
17-Jun	05AB3680	SP	F
18-Jun	05AB3681	SP	F
18-Jun	05AB3684	F	F
19-Jun	05AB3686	F	F
19-Jun	05AB3688	SP	F
19-Jun	05AB3690	F	F
20-Jun	05AB3691	F	F
20-Jun	05AB3695	F	F
21-Jun	05AB3696	F	F
21-Jun	05AB3697	F	F
22-Jun	05AB3702	F	F
23-Jun	05AB3710	F	F
24-Jun	05AB3712	F	F
25-Jun	05AB3717	SP	F
26-Jun	05AB3721	F	F
27-Jun	05AB3728	F	F
28-Jun	05AB3734	F	F
29-Jun	05AB3738	F	F
30-Jun	05AB3741	F	F
01-Jul	05AB3750	F	F

Chapter 4

Microsatellite DNA Study of Population Genetic Structure among Yakima Basin Steelhead

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Abstract

We examined the population genetic structure of steelhead (*Oncorhynchus mykiss*) collected from tributaries in the Yakima River basin including: Upper Yakima River (at Roza Dam), Ahtanum Cr., Toppenish Cr., Satus Cr. and Naches River, and compared these wild collections to Skamania hatchery steelhead and several strains of hatchery rainbow trout planted into tributaries. We assessed spatial and temporal patterns of genetic diversity within and among collections and explored the possibility hatchery steelhead and rainbow trout had introgressed into native steelhead populations. Multi-locus genotypes were analyzed for 1211 individuals at ten microsatellite loci. Pairwise genotypic and F_{ST} tests indicated significant genetic differences among most tributaries and little genetic differentiation between collection years within tributaries, suggesting that most tributaries contain genetically differentiated populations. Collections from Roza Dam and Naches River were genetically similar, suggesting common ancestry and gene flow through straying. All collections were distinctly different from Skamania Hatchery steelhead and South Tacoma hatchery rainbow, although hatchery steelhead may have introgressed slightly into the Upper Yakima population and to a lesser degree into other collections. Slight relationships to Skamania hatchery could also be artifacts of shared polymorphisms or shared ancestry rather than introgression.

Introduction

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

The reduced abundance of steelhead led to hatchery propagation within the basin and introduction of non-local stocks of steelhead and rainbow trout from hatcheries outside the basin. Phelps *et al.* (2000) summarized hatchery steelhead releases into the Yakima Basin as follows: 1) an average of over 65,000 smolts were released per year from 1961-1986; 2) smolts of Priest Rapids, Klickitat, and other unspecified Columbia River hatchery strains were released from 1961-1971; 3) only Skamania smolts were released from 1972-1986; 4) only 'Yakima' smolts derived from adults intercepted at Prosser Dam have been released since 1987. Because Prosser is below all steelhead spawning areas, the adults used for this program would have been a mixture of any distinct populations in the basin. Up to 1975, most releases were into the mainstem Yakima River above Roza Dam with modest releases into Naches River and Ahtanum Creek (Phelps *et al.* 2000). From 1975 to 1989, fish were mainly released into portions of the Naches drainage. Records indicate no hatchery plants into Satus Creek. Over three million hatchery rainbow trout (South Tacoma and Goldendale hatchery strains) have also been released into the Yakima River basin since 1950 (Campton and Johnston 1985) and interbreeding between rainbow and steelhead is possible.

Earlier genetic work by WDFW using allozymes indicated the existence of at least three genetically distinct groups of steelhead in the Yakima basin: Satus Cr., Toppenish Cr., and UpperYakima-Naches (Phelps 2000). Satus and Toppenish were quite distinct from each other. Fish in the Upper Yakima and the Naches rivers were not well characterized, and it appeared that they may have been significantly impacted by hatchery plants. More recently, we have employed DNA microsatellites to elucidate the genetic structure of steelhead in the Yakima basin. Loxterman and Young (2003) examined fish collected from Roza Dam, Ahtanum Cr., Satus Cr, and Toppenish Cr. in 2000 and 2001. The four groups were found to differ significantly. Samples from Naches River, Skamania Hatchery, and another collection year from Roza Dam were included (Small *et al.* 2005) to explore the relationship between fish collected at Roza Dam and in the Naches River and estimate hatchery introgression in fish populations exposed to hatchery steelhead.

The current study extends the earlier microsatellite work with further examination of among-year variability by adding additional samples from Roza Dam and Naches River.

Methods

Samples and Microsatellites

We extracted DNA from 238 tissue samples from Roza Dam and Naches River in 2005 using Nucleospin columns from Macherey-Nagel and manufacturers standard protocols. These collections were compared to samples representing four collections sampled in both 2000 and 2001, (Roza Dam sampled also in 2003), Naches River sampled in 2004 and Skamania steelhead from Reiter Ponds from 2001 (Table 1). In one analysis we included adult hatchery rainbow samples from Spokane, South Tacoma, Goldendale and Eells Springs hatcheries to examine the relationship between hatchery rainbow and steelhead. Fish from the first three hatcheries represent the three major trout strains used in Washington (Crawford 1979); the Eells Springs collection represents the Mt. Whitney strain, a California import that is also used in the state.

Yakama Nation personnel collected fin clips from live juvenile fish, presumably smolts in Satus Creek, Toppenish Creek, Ahtanum Creek, and Naches River (Figure 1). All collections were made over two months during the steelhead outmigration period using screw traps, and fish were actively migrating fish of smolt size (25-30 cm) and coloration (Mark Johnston, YN, pers. comm.). Roza Dam samples were collected from returning adults intercepted at the Roza Dam trap.

Ten microsatellite loci were assayed using fluorescently labeled primers following multiplex protocols developed in the WDFW Genetics Lab (Table 2). We conducted PCRs in 10µl volumes with 1.5mM $MgCl_2$ in 1X PCR buffer with 2mM of each dNTP and 0.05 units Taq polymerase. Genotypes were previously generated from PCR products using an Applied Biosystems (ABI) 3100 automated sequencer for 2000 and 2001 collections (except Skamania Hatchery) and an ABI 3730 automated sequencer for 2003 Roza Dam, 2004 Naches River and Skamania Hatchery. Data from the ABI 3100 was standardized to 3730 microsatellite allele sizes (alleles run differently on the 3100 and 3730 platforms) by running a subset of samples on both platforms and standardizing allele mobilities. Systematic differences may remain, however, since the 3730 is more sensitive and thus may detect more of the larger sized alleles that can be difficult to detect on the 3100. We used GENESCAN (Version 3.1) and GENOTYPER (Version 2.1) software to collect and analyze 3100 microsatellite data and GENEMAPPER software (Version 3.0) to analyze and bin 3730 microsatellite data. Data from the 2005 collections were generated using the ABI 3730 and GENEMAPPER 3.0.

Statistical Analyses

General measures of within-population genetic diversity including average heterozygosity and allelic richness were computed for each collection using FSTAT (Version 2.9.3.2, Goudet 1995). Tests for Hardy-Weinberg (HW) proportions for each locus and genotypic linkage disequilibrium between all pairs of loci within each collection were conducted using GENEPOP (Version 3.3, Raymond and Rousset 1995) and statistical significance was evaluated using a Bonferroni correction of *P*-values (Rice

1989). Departures from expected HW equilibrium and linkage among loci can indicate the following: non-random mating, mixing of distinct breeding groups (populations or families) in a collection, population bottlenecks or recent interbreeding between distinct populations.

To assess population structure among steelhead collections, we computed pairwise estimates of genetic differentiation between collections. We generated estimates of genotypic population differentiation using GENEPOP 3.3 and we used ARLEQUIN ver. 3.0 (Schneider *et al.* 2000) to compute measures of population subdivision (Θ, an estimate of F_{ST}) between all pairs of collections. In addition to providing a statistic for differentiation between populations, pairwise F_{ST} estimates give a measure of the magnitude of differences. Statistical significance of F_{ST} estimates was tested using 10,000 permutations and was evaluated using a Bonferroni correction of *P*-values.

Genetic distance between pairs of collections was estimated using the Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza and Edwards 1967) as calculated in PHYLIP (Version 3.572, Felsenstein 1993). The distance matrices (based on 1000 bootstrap resamplings) were used to construct neighbor-joining trees using the NEIGHBOR algorithm as implemented in PHYLIP. A bootstrap consensus tree was constructed using the CONSENSE option in PHYLIP. Trees were drawn using TREEVIEW (Version 1.6.5, Page 1996). The four hatchery rainbow trout collections, (Spokane, Goldendale, South Tacoma and Eells Spring hatcheries) all derived from McCloud River broodstock and other broodstock in varying proportions (Crawford 1979, Busack and Gall 1980), were included in a cluster analysis to explore the possibility hatchery rainbow trout had hybridized with native steelhead.

STRUCTURE 2.1 (Pritchard *et al.* 2000) was used to estimate introgression by hatchery fish and estimate relationships between Naches River fish and fish collected at Roza Dam. For hatchery introgression, individual collections (all collection years from a single location) were examined in a series of pairwise tests with Skamania Hatchery fish. Naches River and Roza Dam were examined in a Naches-Roza Dam relationship test. STRUCTURE sorts individuals in order to achieve Hardy-Weinberg equilibrium and linkage equilibrium in the hypothetical clusters or populations. To test for hatchery introgression runs were carried out comparing each collection to Skamania Hatchery fish and two clusters were hypothesized. The percentage of membership in both clusters was calculated for an individual (and averaged for the population), giving an estimate of the individual's (and population's) ancestry. Collections with high hatchery introgression might display mixed ancestry or mixed membership in individuals, with ancestry shared with Skamania Hatchery fish. Introgressed fish are suggested by membership in the cluster occupied by Skamania Hatchery fish. For each test, the program was run 10 times with 20,000 burn-in runs and 180,000 iterations.

We used an assignment test in GeneClass2 (Piry *et al.* 2004) to examine the likelihood that, based upon the genotype of the fish and allele frequencies in reference collections, an individual fish originated in the collection where it was sampled. High assignments back to river or creek of origin indicates that genetic structure occurs at the level of

individual rivers. We used the partial Bayesian method with prior assumption of equal probability for allele frequencies at each locus in each population with temporal collections grouped by tributary.

Results and Discussion

A total of 1211 individuals were compared at ten microsatellite loci. All loci were polymorphic with number of alleles per locus ranging from nine to 41. Genetic diversity, as estimated by average heterozygosity, was similar among all populations and ranged from 0.712 to 0.802 (Table 1). Toppenish collections were significantly lower in estimated heterozygosity (combined heterozygosity 0.698, $P = 0.009$) than collections from other tributaries. The lower heterozygosity of Toppenish Cr. fish had also been noted in earlier allozyme work (Phelps 2000). Heterozygosity estimates in this study are slightly higher than the range of average heterozygosity estimates reported for other collections of steelhead (0.41 – 0.72, Wenburg *et al.* 1996; 0.66 – 0.72, Nielsen 1999).

A similar pattern occurred with estimates of allelic richness, with richness ranging from 10.39 alleles (01Toppenish) to 14.48 alleles (01Roza Dam, Table 1). Allelic richness was significantly higher in Roza Dam collections (combined richness = 14.3, $P = 0.015$ from test with 1000 permutations). Higher richness in Roza Dam may reflect a larger effective population, sampling from multiple spawning groups above the Roza Dam, or that they have received allelic infusions via strays from other populations or hatchery fish.

Tests for Hardy-Weinberg (HWE) proportions were examined to assess the validity of the underlying assumptions of the models used to interpret genetic variation and explore collections for signals indicating inbreeding from small population size or admixture (rainbow and steelhead mixed in collections, hatchery steelhead and wild steelhead mixed in collections, or different wild spawner groups represented in collections). Most collections showed deviations from HWE in tests of loci within populations and three deviations remained significant after Bonferroni corrections (corrected P value = 0.00038, Table 3). Nine collections deviated from HWE over all loci with four deviations remaining significant after corrections, including both collection years from Naches River (Table 3). All deviations were for deficits of heterozygotes, suggesting small effective population sizes or mixing of reproductively isolated groups within the collections, possibly due to including some rainbow trout or due to subdivision among steelhead breeding groups within tributaries. We further suspect mixtures since most loci in these collections had consistent, although not significant, patterns of deficits of heterozygotes, rather than a mix of heterozygote and homozygote deficiencies (positive and negative F_{IS} values) characteristic of random mating.

In addition to tests for HWE, we tested for linkage disequilibrium between pairs of loci in each collection to look for evidence of non-random mating. Most collections exhibited little or no linkage disequilibrium; however, 17 of 45 possible pairs of loci deviated

significantly from equilibrium in the 2000 Ahtanum Creek collection (Table 1). While significant linkage disequilibrium likely does not indicate physical linkage (since different locus pairs were linked in different collections), this pattern suggests that the 2000 Ahtanum Creek collection could have experienced a recent bottleneck or have a smaller effective population size relative to the other collections, or that the sample could be a mixture of two or more populations of steelhead and/or a mixture of steelhead and rainbow trout, or that there was a recent infusion of non-native fish into the population. When a differentiated group of fish mates with the local population, HWE is re-established within one or two generations, but linkage disequilibrium decays more slowly. Small effective population size is less likely since the 2001 Ahtanum Creek collection does not exhibit the same pattern and allelic richness is similar to other collections. The more likely explanation is that the sample is a mixture of steelhead and rainbow trout or had recently received migrants. In their review of steelhead stock structure, Phelps *et al.* (2000) reported that both Satus and Toppenish creeks exhibited no gene flow between hatchery-origin rainbow trout and steelhead, and the native steelhead collections. However, Ahtanum Creek has more resident rainbow trout relative to Satus and Toppenish creeks (J. Hubble, pers. comm.), making a mixture of steelhead and rainbow trout possible in a collection of juvenile samples.

Pairwise tests of genotypic differentiation indicated differences in genotype distributions among most collections (Table 4). Significant genotypic differentiation occurred between all collections from different tributaries, but, with the exception of Ahtanum Creek, temporal samples from the same tributary were not differentiated. The two Ahtanum Creek collections may have differed if the 2000 Ahtanum Creek sample was a mixture of rainbow and steelhead or was composed of juveniles originating from a few families. The overall pattern suggests strong, temporally stable spatial genetic structure among most steelhead populations in the Yakima drainage.

Genetic structure was further examined by testing for population subdivision using *F*-statistics (Table 4). While the pairwise genotypic test uses genotypic frequency differences between collections to determine structure, *F*-statistics assess population genetic structure using the differences in heterozygous genotypes between collections. Specifically, the test assesses differences between heterozygosity observed with the collections grouped versus heterozygosity expected based upon mean allele frequencies with the collections grouped. If collections are from the same randomly mating population, allele frequencies will be similar, observed and expected heterozygosity will be similar and F_{ST} will not be significant. If collections are reproductively isolated, allele frequencies are different, collections will have different types of heterozygotes based upon their allele frequencies and there will be a deficit of expected heterozygotes when collections are grouped into a single large collection; F_{ST} will be significant and the deficit of expected heterozygotes gives a measure of the magnitude of genetic differences. Pairwise F_{ST} tests showed the same temporal patterns as genotypic tests but further illustrated a close relationship between Naches River and Roza Dam collections (Table 4), with several low and non-significant values. Pairwise F_{ST} values suggested a slightly closer genetic relationship between Skamania Hatchery and Roza Dam and Naches River collections than between Skamania Hatchery and other collections. Pairwise genotypic and F_{ST} tests can differ for several reasons. Genotypic tests are

extremely sensitive and a significant value may result if there are differences in genotypic distributions at a single locus, possibly the result of a null allele or scoring errors, and be biologically meaningless. Furthermore, pairwise F_{ST} tests are more conservative since they examine the proportion of genetic variation between the two collections without regard for the identity of specific alleles. Thus, if populations were homozygous for different alleles at the same locus, genotypic tests might indicate a significant difference and F_{ST} tests might indicate no difference.

Results from this study indicate that most collections of steelhead included in this report represent different genetic stocks with different gene pools and that the Naches River and Roza Dam collections are genetically closely related. To further illustrate relationships, we constructed a neighbor-joining (NJ) tree based on genetic distance among collections. In the first tree (Figure 2a), hatchery rainbow collections were included; hatchery rainbow were clearly different from steelhead collections (100% bootstrap support for hatchery rainbow branch) and were thus not considered further in other analyses. Collections from different years within tributaries grouped together with bootstrap support, further supporting temporal stability. Toppenish samples had the longest branch length, indicating higher differentiation. Roza Dam samples form a single group with short branch lengths and low bootstrap support, suggesting low genetic distinction. This lack of distinction could arise from sampling adults destined for other spawning areas, such as the Naches River. The Naches samples have similarly short branch lengths but higher bootstrap support, indicating that the two samples are genetically similar to each other and different from the other collections. Ahtanum samples grouped together but branch lengths were long, indicating some differentiation between collections, and bootstrap support was low. In the consensus NJ tree without hatchery rainbow (Figure 2b), most tributary groups were on single, supported branches except samples from Roza Dam, which divided into two branches. If family groups were represented in smolt samples, distinction and strong bootstrap support could reflect family distinction. In the absence of the hatchery rainbow samples, the Ahtanum samples were on a supported branch with Satus and Toppenish creeks collections. However, Satus and Toppenish collections are still separated from Ahtanum and each other with strong bootstrap support. The shifting of Ahtanum creek collections away from the Satus and Toppenish creeks collections towards the hatchery collections when the hatchery rainbow were included in the cluster analysis suggests that Ahtanum creek collections may have included some rainbow trout. Skamania Hatchery joined the Roza-Naches group with no bootstrap support and a long branch length, indicating that the hatchery collection is genetically distinct. The consensus trees support strong geographic structure and little temporal genetic structure in these collections.

The STRUCTURE analysis also supports temporal stability within tributaries and indicated low hatchery introgression (Table 5, Figure 3, Figure 4). Table 5 shows the ancestry of each population averaged over all individuals. Each individual's ancestry is illustrated in Figures 3 and 4 where each individual is represented by a single bar divided into two shades of grey which represent the portion of ancestry of the individual in the two possible populations, wild (dark grey) or Skamania hatchery (light grey). In an analysis with all samples and K = 6 (not shown), collections shared highest ancestry in

the group containing the other year collections from within the tributary. When averaged over all individuals in the sample, Naches River and Roza Dam collections had slightly higher ancestry in the Skamania Hatchery cluster than Ahtanum, Satus and Toppenish creeks collections in pairwise tests (Table 5), and, particularly in the Roza Dam collections, several individual fish shared moderate to high ancestry with Skamania Hatchery fish (Figure 3). When comparing results between collections, Roza Dam fish had the most individuals with Skamania-type ancestry, and the highest Skamania-type ancestry when averaged over all individuals (Table 5). Further STRUCTURE analysis compared Naches River and Roza Dam collections in a pairwise test and to Skamania Hatchery fish in a three-way test (Table 5, Figure 4). Naches River and Roza Dam collections shared more ancestry with each other than with Skamania Hatchery fish. Naches River fish also appeared more distinct than Roza Dam fish since they had a higher proportion of ancestry in a single cluster (Table 5), and more individual fish were assigned to a predominantly "Naches" cluster (Figure 4). This pattern reiterates the cluster analysis where the Naches River collections grouped with much higher bootstrap support than the Roza Dam collections (Figure 2a). However, Naches River samples were smolts and Roza Dam samples were adults, thus the distinction of the Naches River samples could represent partially a family-type distinction.

GeneClass2 assignments further supported genetic patterns in the study (Table 6). Although correct assignment (assignment to the collection the fish originated in) to individual collections (not shown) was low in most samples except 00Ahtanum and Skamania Hatchery, misassignments were mostly to the other year class from the tributary. When collection years were grouped, individuals assigned well (> 69%) to tributaries (Table 6). A relationship between Naches River and Roza Dam fish was also suggested by misassignments: the highest portion of misassigned Naches River fish (18%) were misassigned to Roza Dam and the highest portion of misassigned Roza Dam fish (10%) were misassigned to Naches River. Few individuals were assigned as Skamania Hatchery fish, supporting the genetic division between wild fish and hatchery fish.

Samples from the screw trap on the Naches River and the trap at Roza Dam potentially include fish from multiple spawning groups or subpopulations. Both samples were obtained from locations downstream of multiple tributaries that may support subpopulations of steelhead. All samples from each year had positive F_{IS} values when summed over all loci (Table 3) and most individual loci within collections had positive, although not significant, F_{IS} values, indicating a trend toward homozygote excess, often indicative of samples containing a mixture of breeding groups. We explored the possibility of substructure within samples by calculating identity values between individuals within collections and calculating the mean and variance of the identity value over all individuals using the program IDENTIX Version1.1 (Belkhir *et al.* 2002) with 1000 permutations (Table 7). Even if the mean value is not significantly higher than a random value, high variance may indicate several independent groups of related individuals in the sample. Collection mean identities were near the mean for the permutation, but variance was high for 00Roza, 01Roza, and 05Roza (top 3% above the mean variance), suggesting that they may have contained some subpopulations. Mean

identity values were higher in most juvenile samples (Toppenish, Ahtanum, Satus creeks) than in adult samples (Roza Dam, Skamania Hatchery) and variances were in the top 0.001% above the mean, suggesting that juvenile samples contained family groups.

In a second analysis, Roza Dam samples were analyzed using STRUCTURE to see if the program detected population structure within the data set indicating subpopulations. As the number of hypothetical groups was increased, most individuals were subdivided among the hypothesized groups. But in several individuals ancestry remained assigned primarily to a single group even as the number of groups increased. This could indicate some subpopulation structure or could possibly be individuals belonging to family groups. However, the program was unable to resolve the data set into discrete subpopulations or breeding groups.

Conclusion

Earlier analyses (e.g. Phelps *et al.* 2000) using allozymes concluded that there were at least three distinct groups of steelhead in the Yakima basin: Toppenish Cr., Satus Cr., and Upper Yakima/Naches. The earlier sampling effort was unable to clearly differentiate Upper Yakima and Naches steelhead, and our ability to differentiate these two groups may have been complicated by gene flow from releases of hatchery steelhead and rainbow trout. Our more recent results from DNA analysis (Loxterman and Young 2003) supports the earlier conclusion that Toppenish and Satus are distinct and additionally showed that Ahtanum Creek steelhead are also distinct, although with less bootstrap support than the other two groups.

The Roza-Naches relationship remains unresolved after the second year of Naches River sampling and the fourth year of Roza Dam sampling. Although the cluster analysis showed that Naches River samples formed a group with high bootstrap support and Roza Dam samples formed their own group with less support, internodal distances (branch lengths between bifurcating branching points) were small indicating low genetic distinction between the groups. Furthermore, although genotypic tests indicated significant differences in genotypic distributions, pairwise F_{ST} values were universally low and most were non-significant, suggesting that collections from the Naches River and Roza Dam were from the same or closely related gene pools. It is highly likely that the groups share common ancestry as members of the same gene pool recolonized the region following glacial retreat and that contemporary gene flow through reciprocal straying has prevented divergence. Alternatively, contemporary gene flow may be low or absent but the habitat above collection points may support populations that are large enough that differentiation through genetic drift is slow. Genetic homogenization from infusions into the gene pools by Skamania Hatchery fish seems unlikely: only Roza Dam collections appeared to share much ancestry with Skamania Hatchery in the Bayesian analysis. Sampling may have contributed to the lack of resolution. Since collection points were within 15 miles of each other (Figure 1), if fish were not imminently ready to spawn, some fish collected at Roza Dam may have been exploring rather than en route to

spawning grounds above Roza Dam. Thus, Roza Dam samples may have contained steelhead ultimately destined for Naches River. If this were the case, collections of smolts from the upper Yakima drainage would be more genetically distinct than the adult collection, and collections of spawning adults from both the upper Yakima and Naches drainages would indicate more genetic distinction.

The Upper Yakima and Naches subbasins are both large enough to contain more than a single steelhead population, but sampling has not been directed toward detecting multiple populations. Using the tools available to us we were unable to detect multiple populations in the possibly mixed samples from these areas, but this analysis was not diagnostic. The possibility of multiple populations in these areas should be addressed through sampling of subregions. This would also minimize the possibility of collecting out of tributary fish that happen to be sampled at the collection points.

In the current work we also explored the possibility of gene flow from hatchery steelhead and rainbow trout. Hatchery steelhead may have introgressed slightly into the Upper Yakima and possibly Naches populations, but the Yakima basin steelhead were still genetically quite distinct from the Skamania stock. Although hatchery rainbow trout have been planted into some tributaries, candidate hatchery trout collections were far more different from wild steelhead than were hatchery steelhead. The true gene flow picture may be quite complicated, however. Campton and Johnston (1985) found allozymic evidence of introgression from hatchery rainbow trout into Upper Yakima resident rainbow trout populations. Pearsons *et al.* (in press) reported that reproductive interactions between steelhead and rainbow trout appeared to vary considerably, both temporally and spatially. Adequate understanding of the population structure of Yakima basin *O. mykiss* will require sampling of resident as well as anadromous fish.

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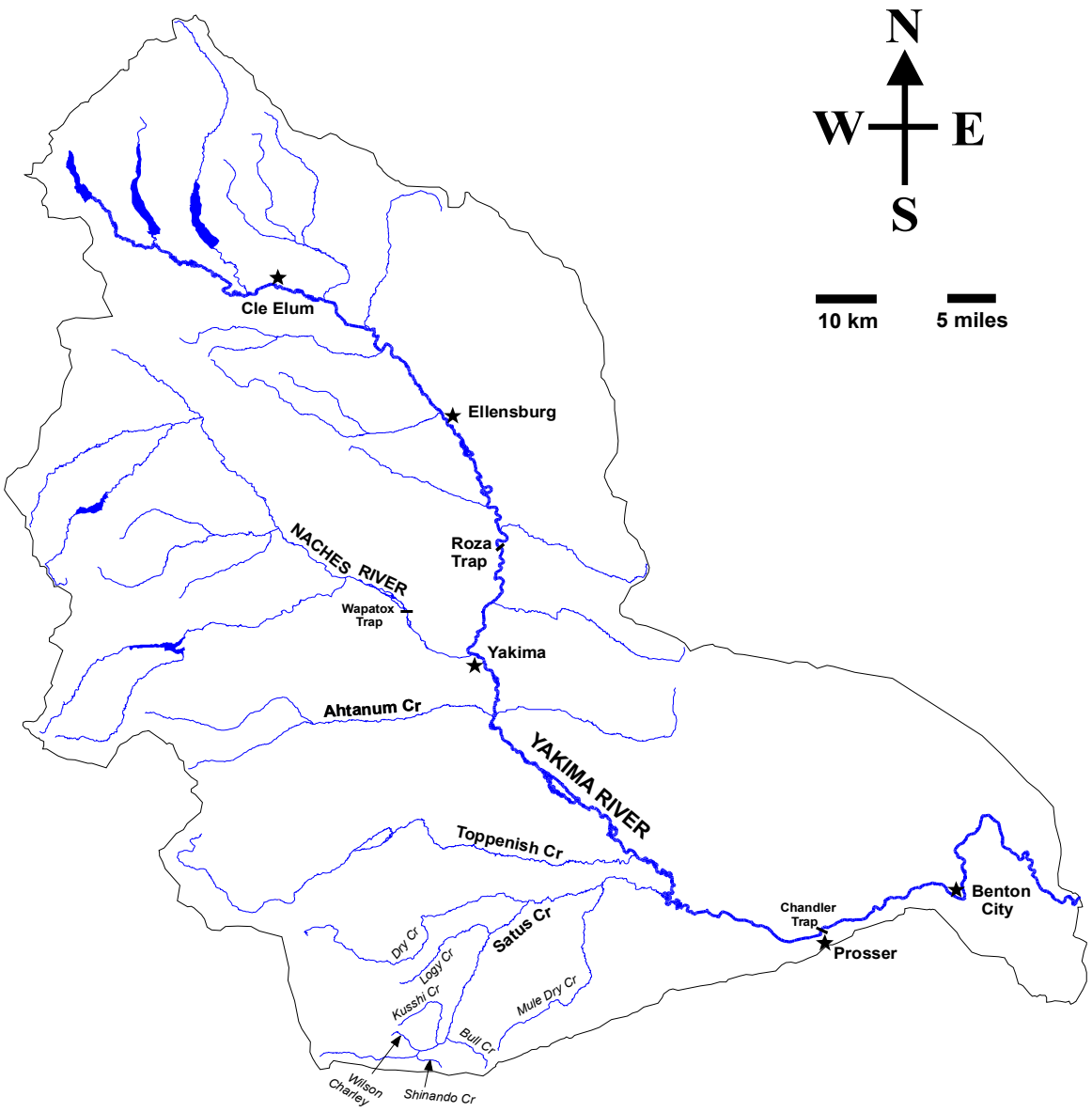
Figure 1. Map of the Yakima River basin showing tributary locations for collections. Map was generated by Jim Shaklee, WDFW.



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

Figure 2a. Consensus neighbor joining tree of Cavalli-Sforza and Edwards genetic chord distances (1967) among steelhead collections from the Yakima River basin, hatchery steelhead from Skamania Hatchery and hatchery rainbow from Spokane, Eells Springs, South Tacoma, and Goldendale hatcheries. Numbers at the nodes indicate the percentage of 1000 trees in which collections beyond the nodes grouped together. Abbreviations follow Table 1.

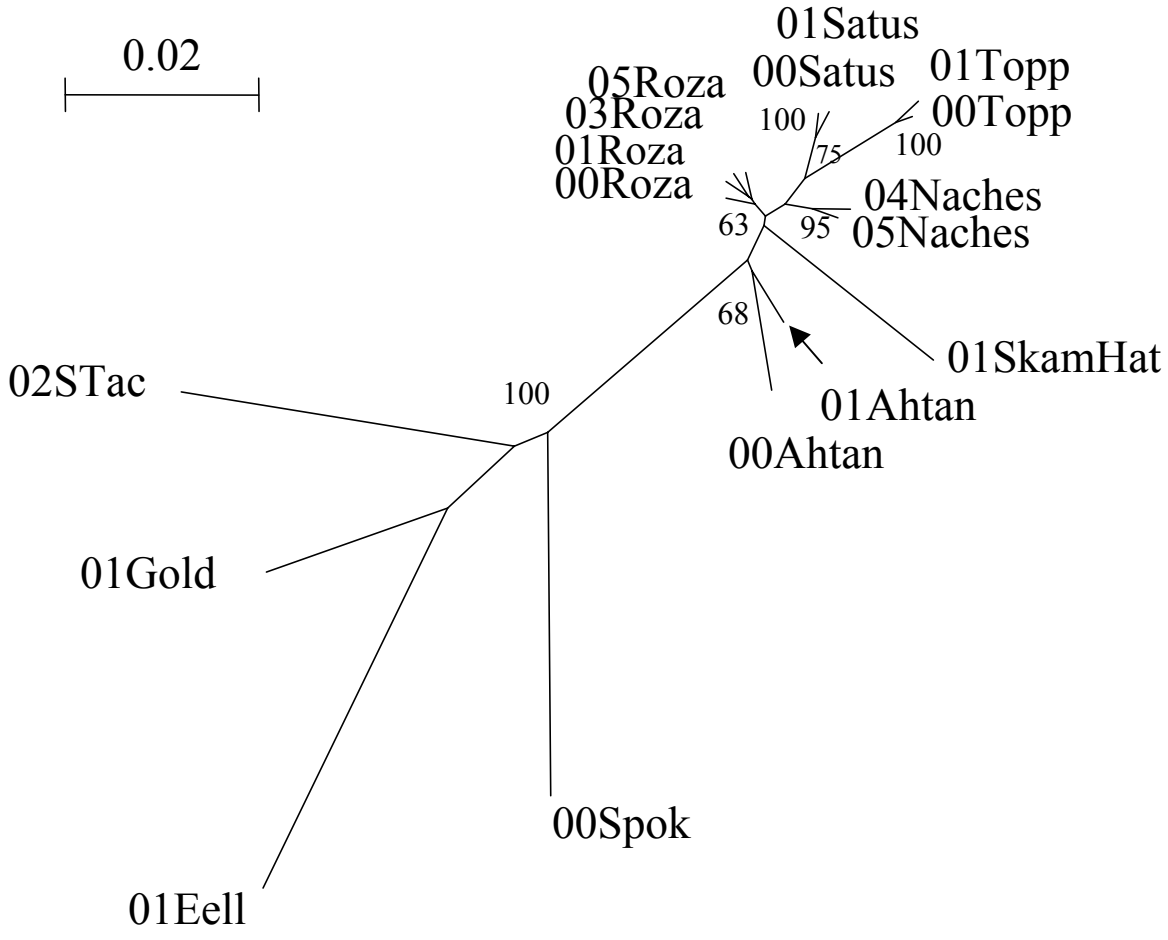


Figure 2b. Consensus neighbor joining tree of Cavalli-Sforza and Edwards genetic chord distances (1967) among steelhead collections from the Yakima River basin. Numbers at the nodes indicate the percentage of 1000 trees in which collections beyond the nodes grouped together.

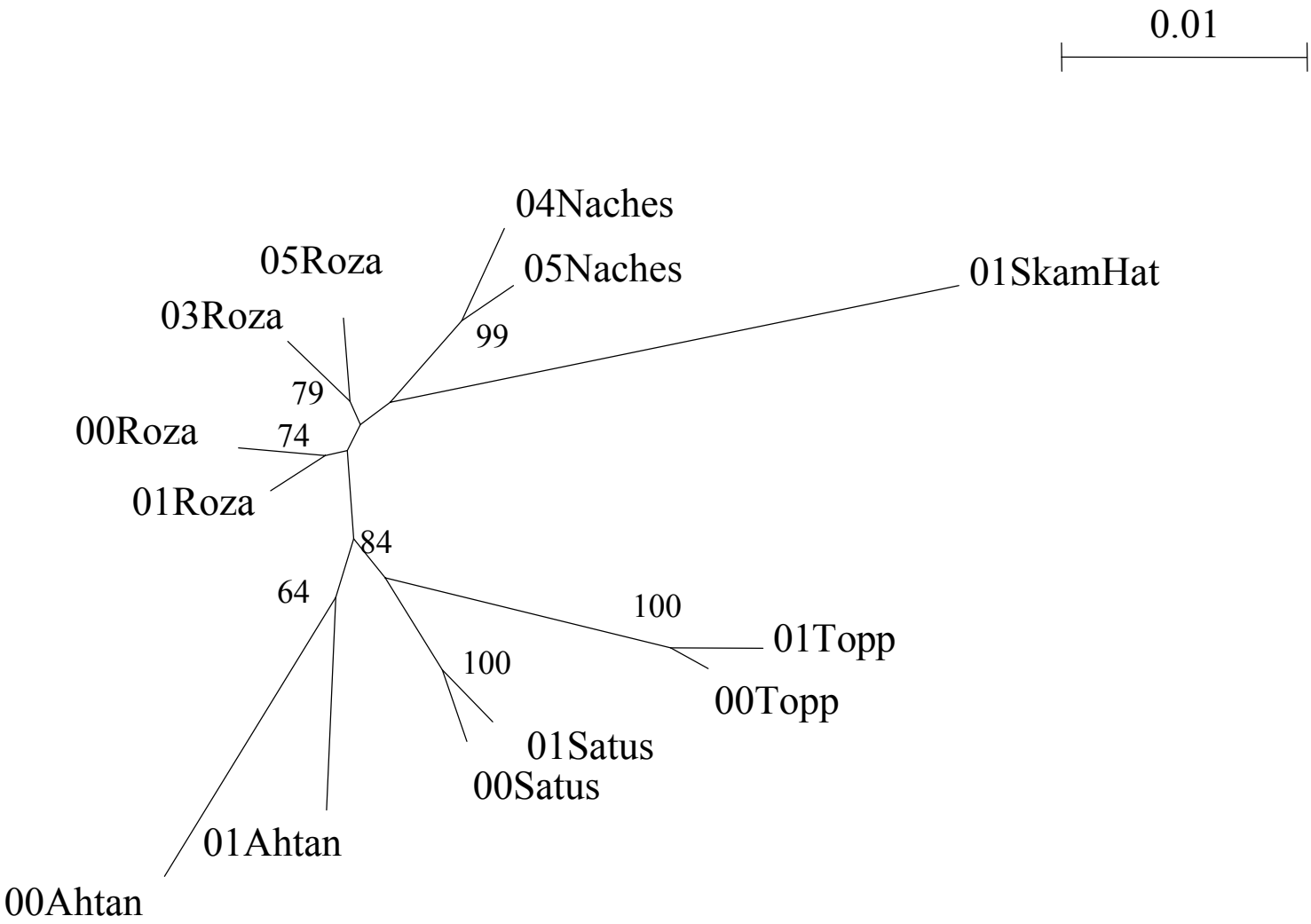


Figure 3. Estimated population structure from pairwise Bayesian analyses. Each individual is represented by a single bar divided into two shades of grey which represent the portion of ancestry of the individual in the two possible populations, wild (dark) or Skamania hatchery (light). See Table 5 for ancestry proportions averaged over all individuals.

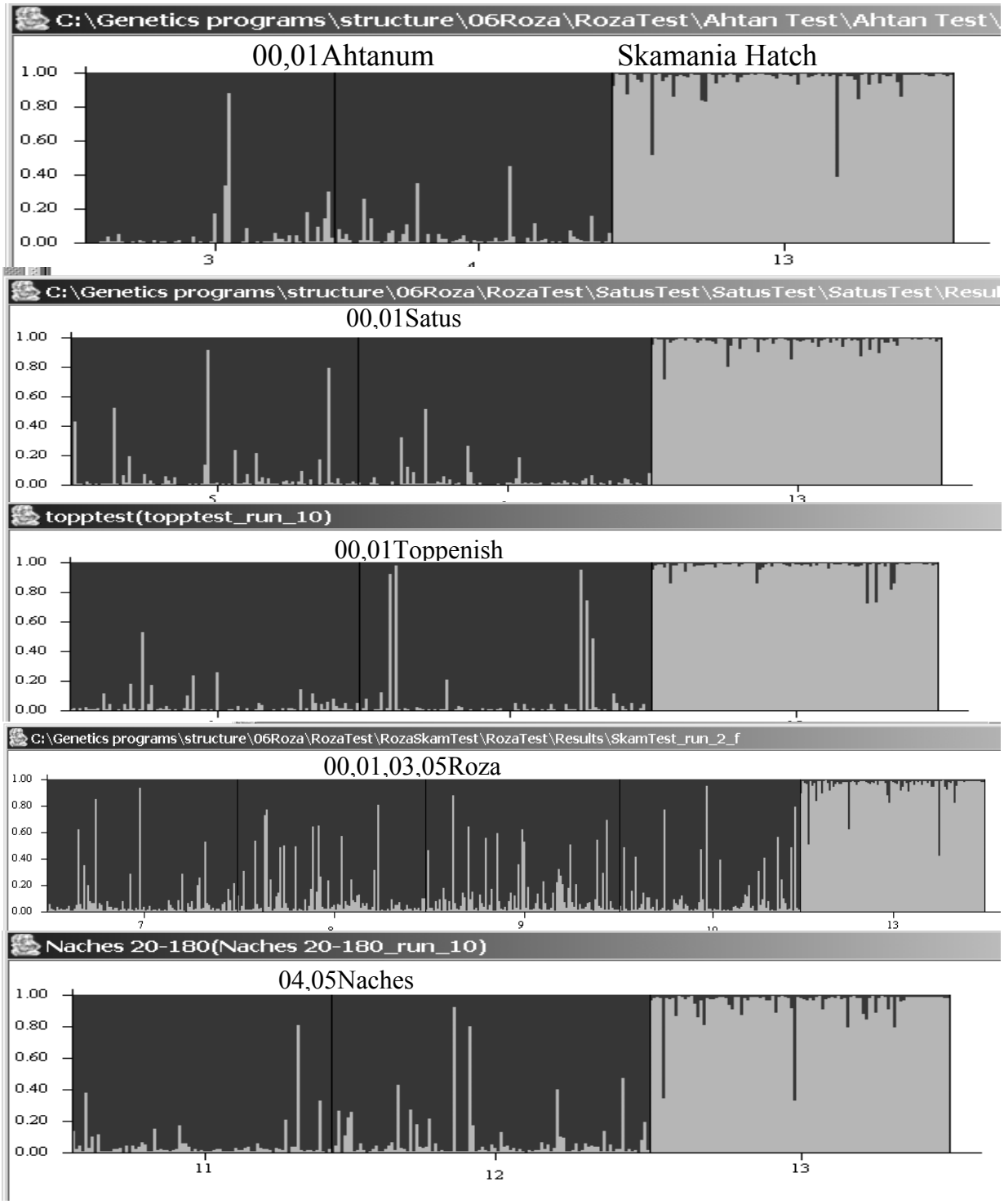


Figure 4. Estimated population structure from Bayesian analyses. Each individual is represented by a single bar divided into colors, which represent the portion of ancestry of the individual in the two possible populations (Roza (light grey) or Naches (medium grey)) in the top bar, or three possible populations (Roza (light grey), Naches (dark grey) or Skamania Hatchery (medium grey)) in the lower bar. See Table 5 for ancestry proportions averaged over all individuals.

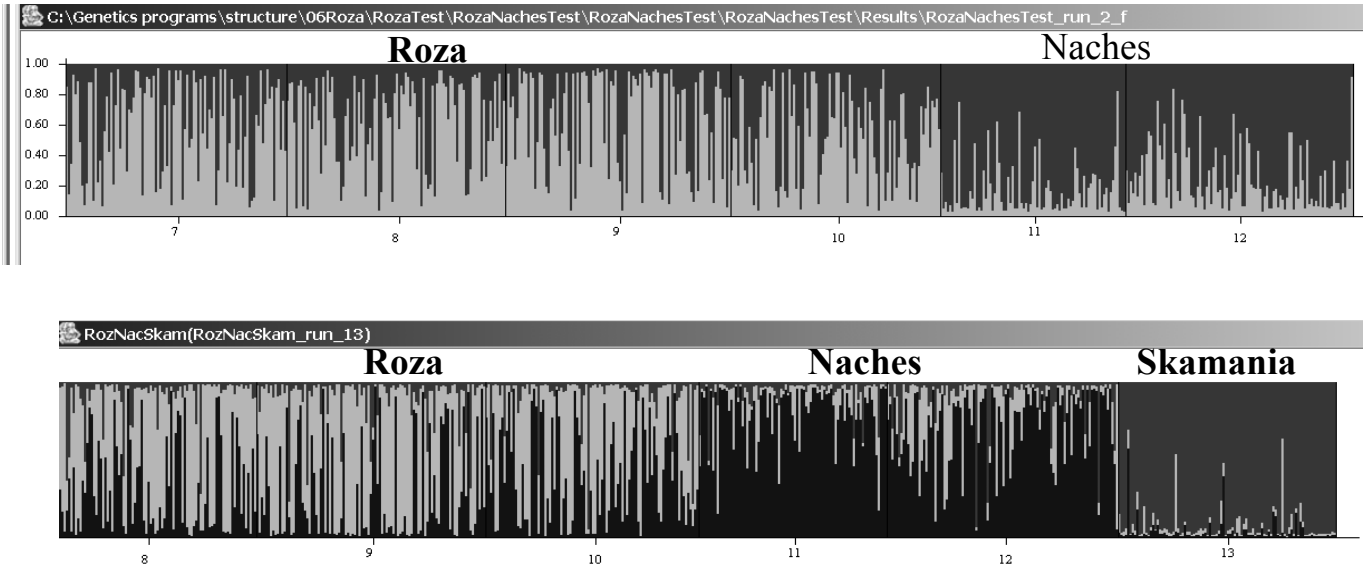


Table 1. Statistics for study samples including collection code, abbreviations used in tables and figures, number of fish used in the study (amplified at 5 or more loci), expected heterozygosity (H_e), allelic richness (rich) and number of locus pairs in linkage disequilibrium (link).

	Code	Abbreviation	N > 5 loci	H_e	rich	link
Toppenish Creek	00AB	00Topp	97	0.712	11.02	1
Toppenish Creek	01AU	01Topp	98	0.77	10.39	1
Ahtanum Creek	00AI	00Ahtan	71	0.769	11.87	21
Ahtanum Creek	01AX	01Ahtan	78	0.732	13.34	3
Satus Creek	00CS	00Satus	95	0.796	12.27	0
Satus Creek	01AW	01Satus	97	0.752	11.38	1
Roza Dam	00AC	00Roza	100	0.794	13.99	0
Roza Dam	01AV	01Roza	98	0.768	14.48	4
Roza Dam	03LA	03Roza	99	0.748	14.46	0
Roza Dam	05AD	05Roza	94	0.776	13.84	1
Naches River	04BH	04Naches	84	0.802	13.08	0
Naches River	05AZ	05Naches	102	0.749	13.76	1
Skamania	01GG	01SkamHat	96	0.763	12.02	2
Hatchery rainbow						
Goldendale Hatchery	01JB	01Gold	48	0.660	6.32	0
South Tacoma Hatchery	02BK	02STac	50	0.580	4.60	0
Eells Springs Hatchery	01OA	01Eell	89	0.580	4.24	0
Spokane Hatchery	00DF	00Spok	96	0.700	6.35	1

Table 2. Information for multiplexes and loci. PCR details include annealing temperature (^{0}C), number of cycles, primer concentration (in μM), number of alleles observed in this study, size range (in basepairs), observed heterozygosity (H_o), repeat unit size (in basepairs), and *P* value for deviation from Hardy-Weinberg equilibrium (HWE). G_{ST} (equivalent to F_{ST}) was calculated using FSTAT2.9.3. Reference for primer sequences are under source.

Multiplex	Anneal T	cycles	Locus	conc [μM]	#alleles	range	H_o	repeat	HWE P	Gst	Source
Omy-B2	55	26	One-102	0.05	23	188-290	0.851	4	0.0535	0.056	Olsen *et al.* 2000
			One-114	0.05	24	181-276	0.878	4	0.0523	0.024	Olsen *et al.* 2000
			Ots-100	0.04	26	168-224	0.856	2	0.0223	0.021	Olsen *et al.* 2000
Omy-C2	55	28	One-101	0.02	19	119-275	0.466	4	0.0002	0.065	Olsen *et al.* 2000
			One-108	0.02	35	161-337	0.862	4	< 0.0001	0.025	Olsen *et al.* 2000
			Ots-103	0.015	9	56-90	0.200	4	0.1092	0.018	Small *et al.* 1998
Omy-F2	52	25	Oki-10	0.02	17	92-172	0.794	2	0.2534	0.043	Smith *et al.* 1998
			Omm-1128	0.08	41	211-404	0.900	4	< 0.0001	0.023	Rexroad III *et al.* 2001
			One-18	0.07	10	166-186	0.748	2	0.0742	0.037	Scribner *et al.* 1996
			Omy-1001	0.03	30	163-249	0.895	2	0.0868	0.036	Paul Bentzen, pers. comm.

Table 3. Loci information for each collection. Under each collection is the F_{IS} value at each locus, underlined values were significant before Bonferroni corrections and bold type values were significant after corrections. Population name abbreviations follow Table 1.

	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
One-102	-0.065	-0.042	0.041	0.024	0.015	0.016	0	0.036	0.009	-0.001	0.07	0.004	-0.007
One-114	0.088	0.015	0.029	0.048	0.011	-0.028	0.026	0.043	-0.006	0.029	0.017	0.029	0.008
Ots-100	0.062	0.07	0.018	-0.014	0.065	0.02	-0.053	0.032	-0.009	0.039	0.031	0.006	-0.072
One-101	-0.046	0.27	-0.053	-0.109	0.238	0.051	0.098	0.179	0.128	-0.012	0.418	0.132	0.25
One-108	-0.051	-0.004	-0.022	-0.046	0.017	0.008	0.064	0.087	**0.142**	0.047	0.008	0.066	0.002
Ots-103	0.151	-0.014	-0.057	-0.021	-0.03	0.01	-0.008	0.136	-0.036	0.205	0.093	0.266	0.029
Oki-10	0.126	0.064	0.024	-0.044	0.057	-0.095	0.028	0.026	0.042	0.064	0.012	0.033	-0.034
Omm-1128	0.015	0.068	-0.075	0.045	**0.144**	0.008	-0.026	0.035	-0.015	-0.008	0.093	-0.015	**0.135**
Omy-1001	0.026	-0.014	-0.031	0.011	0.022	0.007	-0.011	-0.002	-0.037	-0.039	0.023	0.062	0.007
One-18	0.035	0.004	0.031	-0.074	0.105	0.034	0.089	-0.016	0.003	0.06	-0.032	0.094	0.036
All	0.026	0.04	-0.005	-0.011	0.061	0.001	0.019	0.045	0.022	0.025	0.055	0.049	0.025
P-value	0.0479	0.0068	0.6277	0.7891	**0.0001**	0.4806	0.0643	**0.0002**	0.0308	0.0285	**0.0003**	**0.0003**	0.0404

Table 4. Results from pairwise genotypic differentiation tests from FSTAT2.9.3 and pairwise F_{ST} tests from ARLEQUIN. Upper triangular matrix has the *P* value for pairwise genotypic tests summed over all loci. Lower triangular matrix has pairwise F_{ST} values. All significant values are in bold type.

	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
00Topp	0	0.00291	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
01Topp	0.00434	0	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Ahtan	**0.05276**	**0.05772**	0	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
01Ahtan	**0.04329**	**0.05078**	**0.02488**	0	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Satus	**0.04557**	**0.04263**	**0.03524**	**0.02724**	0	0.51913	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
01Satus	**0.03230**	**0.03119**	**0.03466**	**0.03116**	0.00011	0	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Roza	**0.03494**	**0.04433**	**0.03146**	**0.02105**	**0.01661**	**0.01521**	0	0.10163	**0.00001**	**0.00003**	**0.00001**	**0.00001**	**0.00001**
01Roza	**0.03493**	**0.04174**	**0.02926**	**0.01847**	**0.01497**	**0.01504**	0.00026	0	**0.00001**	**0.00008**	**0.00001**	**0.00001**	**0.00001**
03Roza	**0.03807**	**0.04623**	**0.02898**	**0.01682**	**0.02233**	**0.02232**	0.00373	0.0036	0	0.01286	**0.00001**	**0.00001**	**0.00001**
05Roza	**0.03059**	**0.03918**	**0.02878**	**0.01782**	**0.01467**	**0.01325**	0.00119	0.0006	0.00352	0	**0.00001**	**0.00001**	**0.00001**
04Naches	**0.03448**	**0.04122**	**0.02511**	**0.01373**	**0.00603**	**0.00733**	**0.00618**	**0.00705**	**0.00544**	0.00278	0	0.16249	**0.00001**
05Naches	**0.02768**	**0.03537**	**0.02818**	**0.01124**	**0.01187**	**0.00943**	0.00038	0.00015	-0.00348	-0.00324	-0.00392	0	**0.00001**
01SkamHat	**0.06613**	**0.07178**	**0.06272**	**0.04650**	**0.06290**	**0.06005**	**0.03213**	**0.03241**	**0.02156**	**0.03196**	**0.04549**	**0.02997**	0

Table 5. Estimation of hatchery introgression and population differentiation using Bayesian analysis. Values are ancestry proportions assigned to two (or three) possible groups, averaged over all individuals. Highest ancestry values are in bold type. Abbreviations follow Table 1.

	1	2	N
00Ahtan	**0.957**	0.043	70
01Ahtan	**0.961**	0.039	78
01SkamHat	0.036	**0.964**	96
00Satus	**0.946**	0.054	95
01Satus	**0.970**	0.030	97
01SkamHat	0.025	**0.975**	96
00Topp	**0.966**	0.034	97
01Topp	**0.941**	0.059	98
01SkamHat	0.024	**0.976**	96
00Roza	**0.920**	0.080	99
01Roza	**0.881**	0.119	98
03Roza	**0.872**	0.128	101
05Roza	**0.894**	0.106	94
01SkamHat	0.045	**0.955**	96
04Naches	**0.951**	0.049	83
05Naches	**0.927**	0.073	102
01SkamHat	0.047	**0.953**	96
Roza-Naches			
00Roza	**0.609**	0.391	99
01Roza	**0.626**	0.374	98
03Roza	**0.651**	0.349	101
05Roza	**0.534**	0.466	94
04Naches	0.179	**0.821**	83
05Naches	0.247	**0.753**	102
Roza-Naches-Skamania			
	1	2	3
00Roza	**0.584**	0.357	0.059
01Roza	**0.587**	0.328	0.085
03Roza	**0.601**	0.304	0.094
05Roza	**0.490**	0.427	0.082
04Naches	0.129	**0.812**	0.059
05Naches	0.192	**0.714**	0.093
01SkamHat	0.042	0.040	**0.919**

Table 6. Bayesian assignment test results from GeneClass2. Fish were given the most likely assignment to a collection (multiple collection years combined) based upon the genotype of the fish and the allele frequencies of the collections in the baseline. Columns show the number of fish from a single collection assigned to various collections. Numbers of fish assigned to collection of origin are in bold type and also in "Total correct" row. Correct, unambiguous assignments were at least 100 times more likely than the second possibility in the baseline and correct, ambiguous assignments had the highest assignment probability but were less than 100 times more likely than the second possibility (number in parenthesis). Total correct includes both unambiguous and ambiguous assignments. Abbreviations follow Table 1.

	Topp	Ahtan	Satus	Roza	Naches	SkamHat
Topp	**116 (53)**	9*	6*	1 (3)	5*	
Ahtan	1 (6)	**65 (45)**	4*	1 (21)	6*	2*
Satus	8*	6*	**53 (100)**	2 (22)	12*	
Roza	1 (8)	16*	20*	**94 (202)**	33*	1 (3)
Naches	3*	8*	8*	4 (37)	**41 (87)**	1*
SkamHat			1*	5*	1 (1)	**74 (15)**
corr	169	110	153	296	128	89
total	196	149	192	392	186	96
%corr	86.22	73.83	79.69	75.51	68.82	92.71

Table 7. Mean and variance of identity values (Belhkir *et al.* 2002) for Upper Yakima and Naches River steelhead. Variance in top 0.001% indicated with ***, variance in top 3% indicated by *.

	Mean	Variance
00Topp	0.291	0.014***
01Topp	0.299	0.015***
00Ahtan	0.238	0.018***
01Ahtan	0.216	0.013***
00Satus	0.250	0.013***
01Satus	0.259	0.014***
00Roza	0.198	0.0102*
01Roza	0.192	0.0115*
03Roza	0.190	0.0081
05Roza	0.193	0.0090*
04Naches	0.209	0.0109
05Naches	0.189	0.0110
01SkamHat	0.240	0.0130

Appendix I. Allele frequencies at ten microsatellite loci sampled from Yakima River and Skamania Hatchery steelhead. Abbreviations follow Table 1.

One-102	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
188	0.0109	0	0.0547	0.0705	0.0225	0.0103	0.0365	0.0319	0.0446	0.0435	0.05	0.0385	0.1062
192	0.038	0.0781	0.0938	0.1154	0.0506	0.0464	0.1146	0.133	0.2475	0.087	0.1	0.1264	0.1062
196	0.087	0.0521	0.1562	0.1987	0.2921	0.232	0.0938	0.0957	0.099	0.1033	0.0714	0.0934	0.0063
200	0	0	0.0234	0.0705	0.0225	0.0206	0.0417	0.0426	0.0545	0.0598	0.0714	0.0714	0.0125
204	0.1359	0.0677	0.0078	0.0577	0.1124	0.1392	0.1406	0.0798	0.0891	0.0924	0.1714	0.1319	0
208	0.2446	0.2604	0.1328	0.1795	0.0618	0.0876	0.1458	0.1649	0.1287	0.1413	0.1571	0.1209	0.1313
212	0.0163	0.026	0.3828	0.0385	0.0169	0.0258	0.0469	0.0691	0.0594	0.0489	0.0429	0.022	0.0625
216	0.3261	0.4115	0.0703	0.0577	0.1798	0.2784	0.0833	0.0851	0.0644	0.1141	0.0786	0.0879	0
221	0.0163	0.0208	0.0391	0.0769	0.0337	0.0309	0.0573	0.016	0.0347	0.0326	0.0143	0.0165	0.0187
225	0.038	0.0052	0.0078	0.0321	0.0843	0.0206	0.0573	0.0851	0.0545	0.0652	0.1143	0.1154	0.0813
229	0	0	0	0.0128	0.0169	0.0412	0.0365	0.0532	0.0396	0.0707	0	0.0165	0.0125
233	0	0	0.0156	0.0192	0.0225	0.0052	0.0156	0.016	0	0.0109	0.0214	0.0165	0
237	0.038	0.0365	0	0	0.0112	0.0052	0.0208	0.0213	0.0297	0.0217	0.0071	0.022	0.25
241	0	0	0	0.0128	0	0	0	0	0	0	0.0071	0.011	0.0813
245	0.0217	0.0312	0.0156	0.0064	0.0056	0.0052	0.0104	0.0213	0	0.0272	0.0143	0.022	0.0312
249	0.0054	0.0052	0	0	0.0112	0.0052	0.0104	0.0053	0.0099	0.0217	0.0071	0.011	0.0125
253	0.0054	0.0052	0	0.0385	0	0.0206	0.0573	0.0426	0.0198	0.0435	0.0214	0.022	0.0437
257	0.0163	0	0	0	0.0449	0.0258	0	0	0.005	0.0054	0.0214	0.0385	0.0187
261	0	0	0	0.0064	0.0056	0	0	0.0053	0.005	0	0	0.0055	0.0187
265	0	0	0	0.0064	0.0056	0	0.0156	0	0.005	0.0054	0.0071	0.0055	0
269	0	0	0	0	0	0	0	0.0106	0	0.0054	0	0	0
273	0	0	0	0	0	0	0	0	0	0	0.0214	0	0
290	0	0	0	0	0	0	0.0156	0.0213	0.0099	0	0	0.0055	0.0063
samples:	92	96	64	78	89	97	96	94	101	92	70	91	80

One-114	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
181	0	0	0.0469	0.0128	0.0057	0	0.0052	0	0.005	0	0	0.011	0
185	0	0	0	0	0	0	0.0052	0	0.005	0.0054	0.007	0.011	0.0705
189	0.038	0.0263	0.0391	0.1154	0.0517	0.0737	0.0464	0.0521	0.04	0.0326	0.0211	0.033	0.0385
193	0.0163	0.0053	0.0625	0.0256	0.046	0.0263	0.0773	0.0938	0.1	0.1196	0.169	0.1813	0.109
197	0.0109	0.0105	0	0.0577	0.1437	0.1105	0.0515	0.0885	0.055	0.0924	0.0704	0.0714	0.0385
201	0.1522	0.1211	0.0781	0.1154	0.1379	0.1	0.0825	0.0625	0.06	0.0761	0.0563	0.0769	0.0705
205	0.2446	0.2947	0.125	0.0769	0.1724	0.1789	0.1237	0.1875	0.125	0.1576	0.1901	0.1703	0.1923
209	0.0109	0.0158	0.0391	0.1026	0.023	0.0421	0.0361	0.0677	0.12	0.0598	0.0282	0.0275	0.0577
213	0.0109	0.0053	0.0391	0.0577	0.0632	0.0474	0.0412	0.0312	0.02	0.0435	0.0282	0.033	0.0641
217	0.0272	0.0421	0.0078	0.0128	0.0977	0.0737	0.0722	0.0781	0.055	0.0924	0.0915	0.1099	0.1987
221	0.038	0.0105	0.2031	0.109	0.069	0.1158	0.1031	0.0938	0.115	0.0598	0.0986	0.022	0.0128
225	0.2391	0.2368	0.0859	0.0705	0.0287	0.0684	0.067	0.0521	0.05	0.0924	0.0634	0.0659	0.0321
229	0.1033	0.1368	0.1484	0.0641	0.0172	0.0211	0.0825	0.0417	0.055	0.0326	0.0563	0.0495	0.0449
233	0.0435	0.0368	0.0234	0.0064	0	0.0053	0.0619	0.0729	0.065	0.0489	0.0211	0.033	0.0064
236	0.0326	0.0316	0	0.0064	0.0517	0.0579	0.0567	0.026	0.065	0.0543	0.0211	0.0165	0
240	0.0109	0.0105	0.0312	0.0321	0.0402	0.0211	0.0515	0.0365	0.03	0.0217	0	0.0055	0
244	0.0163	0.0105	0.0078	0.0256	0.0057	0.0105	0.0103	0.0052	0.005	0.0109	0.007	0.011	0.0064
248	0	0.0053	0	0.0128	0.0172	0	0	0.0052	0.01	0	0.0493	0.0549	0

One-114cont	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
252	0.0054	0	0.0078	0	0.0115	0.0368	0.0052	0	0.005	0	0	0	0.0064
256	0	0	0.0312	0.0449	0.0057	0.0053	0	0	0.005	0	0.007	0	0.0064
260	0	0	0	0.0128	0.0115	0	0.0103	0.0052	0.01	0	0	0	0.0385
264	0	0	0.0234	0.0385	0	0	0	0	0	0	0	0	0
268	0	0	0	0	0	0.0053	0.0103	0	0	0	0.007	0	0
272	0	0	0	0	0	0	0	0	0	0	0	0.0055	0
276	0	0	0	0	0	0	0	0	0	0	0.007	0.011	0.0064
samples:	92	95	64	78	87	95	97	96	100	92	71	91	78

Ots-100	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
168	0	0	0.0923	0.0705	0.0385	0.0312	0.0561	0.0526	0.0594	0.0435	0.0366	0.04	0.1118
171	0	0	0	0	0.011	0	0	0	0	0	0	0	0
173	0.0604	0.0876	0.1692	0.1731	0.1758	0.1615	0.1071	0.1526	0.1485	0.1196	0.189	0.1667	0.2706
175	0	0	0	0	0	0	0.0102	0.0053	0	0	0	0	0.0294
177	0.0934	0.0567	0.0615	0.1154	0.1044	0.1562	0.0612	0.0474	0.0347	0.0543	0.0854	0.0867	0.0941
179	0	0	0	0	0.011	0	0.0102	0	0	0	0	0	0
181	0.2582	0.232	0.1077	0.1346	0.2857	0.25	0.2194	0.2053	0.2178	0.1902	0.3049	0.22	0.1588
183	0	0	0	0	0	0.0156	0	0	0.0297	0.0109	0	0	0
185	0.3077	0.2732	0.1154	0.0833	0.1264	0.1562	0.102	0.1579	0.0842	0.1576	0.1098	0.1	0.0647
187	0.033	0.0464	0.0615	0.0513	0.0165	0.0104	0.0204	0.0526	0.0446	0.0489	0.0305	0.0467	0.1235
189	0.1374	0.1701	0.1	0.0256	0.1154	0.1094	0.148	0.0842	0.0792	0.1413	0.0671	0.08	0
191	0.0055	0	0.0231	0.0385	0	0	0.0051	0.0158	0.0099	0	0.0122	0.0133	0.0059
193	0	0	0.0077	0.0064	0.0385	0.0365	0.0459	0.0211	0.0248	0.0435	0.061	0.0867	0
195	0.0275	0.0206	0	0.0064	0	0	0.0255	0.0158	0.0297	0.0272	0.0183	0.0133	0.0412
197	0.0055	0.0103	0	0.0962	0.033	0.0365	0.0867	0.0421	0.0396	0.0543	0.0488	0.08	0.0118
199	0.0055	0.0206	0.0077	0.0192	0	0	0.0102	0.0263	0.0495	0.0326	0.0122	0.0067	0.0824
201	0.011	0.0258	0.0615	0.0321	0.0385	0.0208	0.0459	0.0474	0.0545	0.0272	0.0183	0.0467	0
203	0.011	0.0052	0	0.0449	0	0.0052	0.0204	0.0368	0.0198	0.0272	0	0.0067	0
205	0	0	0	0	0	0.0052	0	0.0053	0.005	0	0	0	0
207	0.0385	0.0258	0.0462	0.0256	0	0	0	0.0053	0.0149	0.0054	0	0	0
209	0	0	0.1077	0.0064	0.0055	0.0052	0	0	0	0	0	0.0067	0
211	0	0.0052	0	0	0	0	0	0	0.0099	0	0	0	0.0059
215	0	0	0.0308	0.0577	0	0	0.0255	0.0211	0.0396	0.0163	0.0061	0	0
217	0	0.0052	0	0	0	0	0	0	0	0	0	0	0
219	0	0	0.0077	0.0128	0	0	0	0	0	0	0	0	0
224	0.0055	0.0155	0	0	0	0	0	0.0053	0.005	0	0	0	0
samples:	91	97	65	78	91	96	98	95	101	92	82	75	85

One-101	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
119	0.3191	0.4184	0.5538	0.609	0.7181	0.625	0.4433	0.4948	0.4059	0.456	0.6562	0.4653	0.2988
123	0	0	0	0	0	0	0	0	0	0.011	0	0	0
127	0.5904	0.5306	0.2615	0.3141	0.2606	0.3594	0.4588	0.4167	0.4851	0.4176	0.3333	0.4931	0.689
131	0	0	0	0	0	0	0	0	0	0	0	0	0.0122
139	0	0	0	0.0128	0	0	0	0	0	0	0	0	0
157	0	0	0	0.0064	0	0	0.0052	0.0208	0.005	0.0165	0	0.0069	0
166	0	0	0.0769	0.0192	0	0	0	0.0052	0.0099	0	0	0	0
170	0.0106	0	0	0	0	0	0.0206	0.0156	0.0149	0.0165	0	0	0

One-101cont	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
174	0.0532	0.0459	0.1	0.0321	0.0053	0	0.0052	0.0156	0.0149	0.0275	0	0.0069	0
178	0	0	0	0	0	0	0.0361	0.0156	0.0297	0.022	0.0104	0.0139	0
182	0	0	0	0	0	0	0.0052	0	0	0	0	0	0
186	0	0	0	0	0	0	0.0052	0	0.0149	0.0055	0	0	0
214	0	0	0	0	0	0	0.0155	0.0052	0.005	0.0165	0	0	0
219	0	0	0	0	0	0	0	0	0.005	0	0	0.0069	0
222	0	0	0	0	0.0053	0.0052	0	0	0	0	0	0	0
226	0	0	0	0.0064	0	0	0.0052	0.0104	0.005	0.0055	0	0	0
235	0.016	0	0	0	0	0	0	0	0	0	0	0	0
239	0.0106	0.0051	0.0077	0	0.0053	0.0052	0	0	0	0	0	0.0069	0
243	0	0	0	0	0.0053	0.0052	0	0	0	0	0	0	0
274	0	0	0	0	0	0	0	0	0.005	0.0055	0	0	0
samples:	94	98	65	78	94	96	97	96	101	91	48	72	82

One-108	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
164	0	0	0	0	0	0	0	0	0	0.0108	0	0	0.0316
169	0	0.0158	0.0379	0.0274	0.0054	0.0319	0.0263	0.0153	0.01	0.0054	0	0	0.0474
173	0.0211	0.0053	0.0455	0.0548	0.0054	0	0.0526	0.0204	0.1	0.0376	0	0	0.0158
177	0	0	0	0	0	0	0	0	0	0.0054	0.006	0.0183	0.0105
181	0.1316	0.1421	0.1439	0.1027	0.1344	0.1755	0.0368	0.0918	0.06	0.0591	0.0417	0.0854	0.0263
185	0.0316	0.0368	0.1061	0.0274	0.0753	0.0904	0.1368	0.1378	0.13	0.129	0.125	0.1463	0.1263
189	0.2579	0.3053	0.0758	0.1781	0.2151	0.1436	0.1158	0.1684	0.075	0.129	0.0952	0.122	0.0789
193	0.2316	0.1421	0.1742	0.0822	0.2097	0.2766	0.2632	0.25	0.2	0.2634	0.2738	0.2622	0.0947
197	0.0421	0.0316	0.0606	0.0959	0.0806	0.0372	0.1158	0.0714	0.085	0.0914	0.1071	0.0793	0.0684
201	0.0316	0.0053	0.0303	0.0411	0.0215	0.0053	0.0316	0.0663	0.045	0.0538	0.0179	0.0183	0.0263
205	0.0053	0.0474	0	0.0068	0.0376	0.0426	0.0316	0.0204	0.03	0.0269	0.0238	0.0061	0.0474
209	0.0053	0.0105	0.1364	0.1027	0.043	0.0426	0.0579	0.0408	0.055	0.043	0.0298	0.0183	0.1263
213	0.0263	0.0158	0.053	0.0822	0.0376	0.0266	0.0211	0.0408	0.05	0.0269	0.0595	0.0671	0.0053
217	0.0158	0.0263	0.0455	0.0479	0.043	0.0319	0.0316	0.0051	0.03	0.0376	0.0595	0.0122	0.0474
221	0.1368	0.1579	0.0227	0.0411	0.0269	0.016	0.0263	0.0102	0.035	0.0161	0.0179	0.061	0.2158
225	0.0053	0.0053	0	0	0	0.016	0.0105	0.0051	0	0.0054	0	0	0.0053
229	0	0	0	0.0205	0	0.0053	0	0.0153	0	0	0.006	0	0
233	0	0	0	0.0342	0.0054	0	0.0158	0.0255	0.045	0.0269	0.0179	0	0
237	0	0	0	0	0	0	0.0158	0	0.015	0.0054	0.0119	0.0061	0
241	0.0105	0.0211	0.0076	0	0.0054	0	0	0	0	0	0.0119	0.0366	0.0053
244	0.0053	0	0	0	0	0	0.0053	0.0102	0.005	0	0	0	0.0105
249	0.0316	0.0263	0.0455	0	0.0323	0.0426	0	0	0.015	0.0054	0.0774	0.0427	0
253	0	0	0	0	0	0	0	0	0.005	0.0054	0	0	0
257	0.0053	0	0	0	0	0	0	0	0	0.0054	0	0	0
261	0	0	0	0	0	0	0.0053	0	0	0	0.006	0.0061	0
265	0	0	0	0	0.0215	0.016	0	0	0	0	0	0	0
267	0	0	0	0	0	0	0	0	0	0	0	0.0061	0.0105
273	0	0	0	0	0	0	0	0	0	0	0	0.0061	0
317	0.0053	0.0053	0	0.0411	0	0	0	0	0.005	0	0.0119	0	0
324	0	0	0.0152	0	0	0	0	0	0	0	0	0	0
328	0	0	0	0.0068	0	0	0	0	0	0	0	0	0
332	0	0	0	0.0068	0	0	0	0.0051	0	0	0	0	0

337	0	0	0	0	0	0	0	0	0.005	0.0108	0	0	0
samples:	95	95	66	73	93	94	95	98	100	93	84	82	95

Ots-103	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
56	0.0316	0.0051	0.0352	0.0128	0	0	0.0213	0.0306	0.0396	0.0372	0	0.0598	0.0053
60	0	0	0.007	0	0.016	0	0.0053	0.0102	0	0.016	0	0.0109	0
74	0	0	0.0423	0.0192	0.0266	0.0469	0.016	0.051	0.0396	0.0266	0.0595	0.0652	0.0737
76	0	0	0	0	0	0	0.0266	0.0306	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0.0446	0.0372	0.0179	0	0.0526
82	0.9421	0.9745	0.9155	0.9615	0.8511	0.8802	0.8617	0.8367	0.8416	0.8777	0.8869	0.8261	0.8158
86	0.0211	0.0051	0	0.0064	0.0851	0.0521	0.0638	0.0408	0.0347	0.0053	0.0298	0.0272	0.0526
90	0.0053	0.0153	0	0	0.0213	0.0208	0.0053	0	0	0	0.006	0.0109	0
samples:	95	98	71	78	94	96	94	98	101	94	84	92	95

Oki-10	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
92	0.0052	0.0102	0	0	0.0068	0	0	0.0051	0	0	0	0	0
97	0	0	0	0	0	0	0.0152	0	0.0099	0.0054	0	0	0.0053
99	0.2083	0.398	0.2464	0.0649	0.4589	0.3764	0.2121	0.2245	0.2228	0.1957	0.1627	0.1789	0.1862
101	0.2188	0.2041	0.029	0.0714	0.1507	0.1461	0.0758	0.0918	0.0693	0.038	0.1325	0.1053	0.0213
103	0.0833	0.1173	0.1304	0.0844	0.0137	0.0506	0.0556	0.0153	0.0594	0.0543	0.253	0.2211	0.0426
105	0	0	0.0507	0.1169	0.0068	0.0393	0.0253	0.0153	0.0149	0.0054	0.012	0.0368	0
109	0.125	0.0357	0.2319	0.2727	0.1301	0.0787	0.1364	0.2041	0.2178	0.1576	0.1084	0.1316	0.1649
113	0.25	0.1888	0.1739	0.2078	0.2055	0.2416	0.3384	0.2806	0.2277	0.3261	0.1988	0.2316	0.0638
115	0	0	0.0072	0	0	0	0	0	0	0	0	0	0
117	0.0312	0.0153	0.0942	0.0779	0.0205	0.0506	0.0505	0.0663	0.0644	0.1196	0.0422	0.0316	0.1277
121	0	0.0051	0	0.013	0	0.0056	0.0303	0.0357	0.0594	0.0489	0.012	0.0158	0.0479
125	0	0	0.0072	0.0714	0	0.0056	0.0455	0.0357	0.0446	0.038	0.0361	0	0.0319
129	0.0729	0.0255	0	0.0195	0	0	0	0.0051	0	0	0.006	0	0.0053
133	0.0052	0	0.029	0	0	0.0056	0	0.0051	0	0.0054	0.0241	0.0316	0
137	0	0	0	0	0.0068	0	0.0152	0.0153	0.0099	0.0054	0.006	0.0158	0.2447
142	0	0	0	0	0	0	0	0	0	0	0.006	0	0.0479
172	0	0	0	0	0	0	0	0	0	0	0	0	0.0106
samples:	96	98	69	77	73	89	99	98	101	92	83	95	94

Omm-1128	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
211	0.0111	0	0.0072	0.0794	0.0571	0.02	0.0556	0.0667	0.0347	0.0591	0.0298	0.049	0
219	0	0	0	0	0	0	0	0	0	0	0	0	0.0163
223	0.0111	0.0266	0.0072	0.0476	0.0071	0	0.0505	0.0333	0.0149	0	0.0179	0.0343	0.0326
227	0.0167	0	0.029	0	0.0643	0.04	0.0556	0.0333	0.0743	0.0806	0.0595	0.0931	0.0217
231	0	0.0053	0	0	0.0071	0	0.0152	0.0111	0.0495	0.0806	0.0655	0.0343	0
233	0.0889	0.0426	0.087	0.0952	0.0143	0.0067	0.0606	0.0556	0	0	0	0	0
235	0	0	0	0.0079	0.0071	0	0.0354	0.0167	0.0842	0.0699	0.1012	0.0539	0.0272
237	0.0667	0.1223	0.0072	0.0873	0.0357	0.04	0.0455	0.0333	0	0	0	0	0
239	0.0056	0.0266	0.0072	0.0317	0.0357	0.0667	0.0505	0.05	0.0545	0.043	0.0595	0.0637	0
243	0.0667	0.0372	0.0362	0.0238	0.0214	0.0267	0.0253	0.0333	0.0594	0.0699	0.0298	0.0294	0.1087
247	0	0	0	0	0	0	0	0	0.0297	0.043	0.0298	0.0294	0
250	0.0278	0.0532	0.0362	0.0794	0.1	0.1467	0.1364	0.1111	0.0693	0.0753	0.0536	0.0637	0.1359
255	0.0056	0	0	0	0	0	0.0051	0.0167	0.0297	0.0591	0.0179	0.0392	0.0217

Omm-1128cont	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
259	0	0	0.1087	0.0079	0	0	0.0051	0.0556	0.0099	0.0161	0.0357	0.0343	0.0761
263	0.0056	0.0213	0.1014	0.0317	0.0643	0.0467	0.0152	0.0222	0.0347	0.0215	0.0179	0.0147	0.0815
267	0.0667	0.0638	0.0362	0.0159	0.1143	0.0733	0.0303	0.0444	0.0149	0.0591	0.0298	0.0441	0
271	0.0056	0	0	0.1111	0.0714	0.04	0.0051	0.0167	0.0248	0.0161	0.0536	0.0343	0
275	0.0056	0.0106	0.0072	0	0.0643	0.04	0	0.0167	0.0149	0.0108	0.0774	0.0294	0.0978
279	0.05	0.0053	0.1377	0.0238	0.1071	0.1067	0.0909	0.0333	0.0198	0.0538	0.0357	0.0588	0.0272
282	0.0556	0.0213	0.029	0.0238	0.0071	0.0133	0.0455	0.0444	0.0495	0.043	0.0417	0.0343	0.0109
286	0.1444	0.1862	0.0725	0.0873	0.0214	0.1267	0.0354	0.0667	0.0495	0.0699	0.0119	0.0245	0.0217
290	0	0	0	0	0	0	0	0	0.0396	0.0108	0.0238	0.0147	0.0217
295	0.1389	0.1064	0.058	0.1349	0.0357	0.0333	0.0657	0.0556	0.0297	0.0161	0.0298	0.0196	0.0815
300	0.05	0.0532	0.0507	0.0159	0.1071	0.08	0.0556	0.0278	0.0743	0.0161	0.0119	0.0588	0.0435
304	0.0611	0.0904	0.058	0.0079	0.0071	0	0.0253	0.05	0.0248	0.0161	0.125	0.0735	0.038
308	0	0	0.0145	0	0.0071	0.0067	0.0253	0.0111	0.0297	0.0269	0.006	0.0245	0.0054
312	0.0167	0	0	0	0	0.02	0.0101	0.0389	0.0396	0.0161	0	0	0.0924
316	0.0056	0.0053	0	0.0397	0	0	0	0.0056	0.005	0	0	0	0.0163
320	0.0667	0.0798	0.0797	0.0079	0.0143	0.02	0.0152	0.0056	0.005	0	0	0	0
325	0.0167	0.0372	0.0072	0	0.0071	0.0467	0.0101	0.0167	0	0	0.0357	0.0147	0
329	0	0	0	0	0	0	0	0	0	0.0215	0	0.0049	0
332	0	0	0	0.0238	0.0143	0	0	0	0	0	0	0.0049	0.0054
337	0	0	0	0.0079	0	0	0	0.0056	0	0	0	0.0049	0
341	0.0111	0.0053	0.0072	0.0079	0	0	0.0101	0	0	0.0054	0	0.0098	0
345	0	0	0	0	0	0	0.0101	0.0056	0.005	0	0	0	0
350	0	0	0	0	0	0	0	0.0167	0.005	0	0	0.0049	0
353	0	0	0.0072	0	0	0	0	0	0	0	0	0	0
365	0	0	0.0072	0	0.0071	0	0.0101	0	0	0	0	0	0
373	0	0	0	0	0	0	0	0	0.0099	0	0	0	0
381	0	0	0	0	0	0	0	0	0.005	0	0	0	0
388	0	0	0	0	0	0	0	0	0.0099	0	0	0	0
404	0	0	0	0	0	0	0	0	0	0	0	0	0.0163
samples:	90	94	69	63	70	75	99	90	101	93	84	102	92

Omy-1001	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
163	0	0	0	0	0.0055	0	0	0.0105	0	0	0	0	0
167	0	0	0	0.02	0	0	0	0	0.005	0	0.0179	0.0147	0
171	0	0	0	0	0.0165	0.0217	0	0	0	0	0.006	0	0.0272
173	0.0156	0.0051	0.0217	0.04	0	0	0	0	0	0	0.006	0.0049	0
175	0	0.0306	0	0	0.011	0	0.03	0.0053	0.005	0.0053	0.0833	0.0196	0.0163
179	0.1458	0.1684	0.0942	0.0733	0.0385	0.0707	0.02	0.0211	0.0297	0.0479	0.0417	0.0686	0.0326
181	0.0156	0.0102	0.0145	0.0067	0.0934	0.0707	0	0.0053	0.005	0	0.0119	0.0147	0.0489
183	0.0104	0.0153	0.0145	0.0467	0.1429	0.087	0.075	0.0789	0.0693	0.0798	0.1369	0.049	0.0707
185	0	0	0	0	0	0	0.02	0.0053	0.005	0	0.006	0	0
187	0.0104	0.0306	0.0507	0.1133	0.0879	0.0924	0.18	0.1368	0.2129	0.117	0.119	0.1373	0.2772
189	0	0.0102	0.0362	0.0867	0.0604	0.0924	0.04	0.0421	0.0594	0.0691	0.1071	0.1078	0
191	0.0208	0.0153	0.0217	0.0333	0.0385	0.0326	0.175	0.1579	0.1188	0.1223	0.0833	0.1324	0.2772
193	0	0	0	0.0467	0.044	0.0326	0.02	0.0158	0.005	0.0266	0.006	0.0098	0.0054
195	0.0365	0.0102	0.1884	0.1133	0.0604	0.0598	0.075	0.1053	0.0842	0.0532	0.131	0.1127	0.0326
198	0.2135	0.1786	0.1159	0.12	0.0769	0.0924	0.045	0.0632	0.0347	0.0426	0.0833	0.1275	0

Omy-1001cont	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
200	0.1667	0.1429	0.0362	0.1467	0.0824	0.0761	0.045	0.0368	0.0545	0.0798	0.0238	0.0196	0.0326
202	0.0156	0.0204	0.0507	0	0.0055	0	0.045	0.0211	0.0347	0.0319	0	0.0147	0
204	0.2083	0.2194	0.1522	0.08	0.1374	0.1685	0.05	0.0684	0.0693	0.0798	0.0714	0.0882	0.0272
206	0	0	0	0	0	0	0	0	0	0	0.006	0.0245	0
208	0.0781	0.0663	0.0072	0.02	0.033	0.0272	0.06	0.1211	0.0842	0.0638	0.0179	0.0147	0.038
210	0	0	0	0	0	0	0	0.0053	0.005	0	0	0	0.0435
212	0.0052	0.0051	0.0072	0.0067	0.033	0.0163	0.035	0.0211	0.0099	0.0479	0.006	0.0049	0.0163
214	0	0	0	0	0	0	0	0.0053	0	0.0053	0	0	0
216	0.0104	0	0	0	0	0	0.01	0.0053	0.0198	0.0319	0	0	0
220	0	0	0	0.0133	0.0055	0.0217	0.025	0.0263	0.0297	0.0266	0.006	0	0
222	0	0	0.0072	0	0	0	0	0.0053	0	0.0053	0	0.0049	0
224	0.0417	0.0408	0.1739	0.0133	0.022	0.0217	0.05	0.0211	0.0545	0.0638	0.0298	0.0294	0.0543
226	0.0052	0.0306	0.0072	0	0.0055	0.0163	0	0.0158	0	0	0	0	0
228	0	0	0	0	0	0	0	0	0.005	0	0	0	0
249	0	0	0	0.02	0	0	0	0	0	0	0	0	0
samples:	96	98	69	75	91	92	100	95	101	94	84	102	92

One-18	00Topp	01Topp	00Ahtan	01Ahtan	00Satus	01Satus	00Roza	01Roza	03Roza	05Roza	04Naches	05Naches	01SkamHat
166	0	0	0	0	0.0053	0	0	0.0102	0	0	0	0	0
170	0.1753	0.1633	0.1304	0.109	0.3617	0.3777	0.2273	0.2143	0.1584	0.2021	0.2917	0.2206	0.1087
172	0	0	0	0.0064	0.0053	0	0.0051	0.0051	0	0.016	0.0238	0.0294	0.0326
174	0.4021	0.3929	0.3986	0.3782	0.2926	0.266	0.2071	0.1633	0.2673	0.2447	0.4048	0.3922	0.3587
176	0.1598	0.2194	0.0435	0.3141	0.1809	0.1915	0.2424	0.301	0.2822	0.2713	0.119	0.2108	0.212
178	0.0052	0	0.0217	0.0192	0	0	0.0051	0.0306	0.0347	0.0372	0.0179	0.0147	0.1359
180	0.0567	0.051	0.2609	0.0641	0.133	0.117	0.1768	0.1735	0.0941	0.1436	0.1131	0.0882	0.087
182	0.1856	0.1531	0.0942	0.0577	0.016	0.0479	0.0909	0.0765	0.0693	0.0638	0.0298	0.0343	0.0109
184	0	0	0	0	0	0	0	0	0	0	0	0	0.0543
186	0.0155	0.0204	0.0507	0.0513	0.0053	0	0.0455	0.0255	0.0941	0.0213	0	0.0049	0
195	0	0	0	0	0	0	0	0	0	0	0	0.0049	0
samples:	97	98	69	78	94	94	99	98	101	94	84	102	92

Chapter 5

A Reevaluation of Morphometric Comparisons between Hatchery-origin and Natural-origin Upper Yakima Spring Chinook

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Abstract

In our 2004 report (Busack et al. 2005) we characterized morphometric differences between hatchery-origin and natural-origin Upper Yakima spring chinook after one generation of hatchery culture. In examining the variation among years, however, we were concerned that variation seen might be attributable to inconsistent digitizing of landmark 7, the base of the tail. We redigitized landmark 7 for all fish, and redid the previous analysis with the corrected data. In this report we repeat key analyses with the redigitized data and compare the results with those of the previous report. We concluded that although the redigitization considerably reduced classification inaccuracy and perceived shape differences based on landmark 7, none of the previous conclusions were unjustified. Highly significant shape variation remains for all factors.

Introduction

In our 2004 report (Busack et al. 2005) we characterized morphometric differences between hatchery-origin and natural-origin Upper Yakima spring chinook after one generation of hatchery culture. Evaluating fish collected in 2002, 2003, and 2004, we found highly significant differences via MANOVA of partial warp scores for sex, origin (natural or hatchery), and collection year, as well as all possible interactions. In examining the variation among years, however, we noted that there seemed to be a pronounced variation in distances between the upper and lower caudal peduncle and the base of the tail (i.e., between landmarks 6 and 7, and 8 and 7). Because this type of variation was seen only in variation among years and not variation among sexes or origin types, we were concerned that part of the variation seen might be attributable to inconsistent digitizing of landmark 7. Examination of randomly chosen specimens did indicate an inconsistency, and all landmark 7 was redigitized for all fish. In this report we repeat key analyses with the redigitized data and compare the results with those of the previous report.

Materials and Methods

Because this report is merely a reanalysis of the earlier work using the same specimens, we refer the reader to the earlier report for further details of materials and methods. The analysis of the redigitized specimens is expanded in the next chapter.

Specimens

Hatchery-origin and natural-origin fish were photographed immediately after they were killed in September for spawning for production or monitoring purposes during September at the Cle Elum Supplementation Research Facility. All together, 935 fish (Table 1) were used in the study, 247 males (36 hatchery-origin and 211 natural-origin) and 688 females (126 hatchery-origin and 562 natural-origin).

Data Acquisition

Photographs were done on a light stand with camera in a fixed position, with fins pinned out to make landmarks more visible. The photographs were scanned for quality and then compiled into files for analysis, one per specimen, using the TPSUTIL program.[1] Images were digitized on a desktop computer using program TPSDIG[2]. We used virtually the same 13 landmarks (Fig. 1) as Hard et al. (2000), von Taubel et al. (2005) and Lang Wessel(in press): 1) tip of snout, 2) base of skull, 3) anterior insertion of

[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.sunsb.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.sunsb.edu/morph.

dorsal, 4) posterior insertion of dorsal, 5) anterior insertion of adipose, 6) dorsal insertion of caudal, 7) hypural plate, 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.

Analysis

The basic approach we have taken for morphometric analysis is thin-plate spline analysis. We used program TPSRELW[3] to generate partial warp scores and centroid values. Centroid values were regressed on fish length to identify possible digitizing errors. We then used SYSTAT,v.11 (SYSTAT Statistics, Inc.) to do multivariate analysis of variance and covariance (MANOVA and MANCOVA) and canonical discriminant analysis (CDA) of various groups. Differences in consensus shapes between groups were visualized and quantified using TPSREGR.[4] Fit to regression models used in TPSREGR was evaluated using Goodall's generalized F statistic (Goodall 1993). Distances were computed between various pairs of landmarks, using coordinates generated by TPSREGR, to qualitatively describe shape differences. For this study that involves three dimensions (sex, origin,year)of potential shape variation, numerous schemes of generating partial warp scores are possible. We used only two: the entire mixed sex sample, and the two sex-specific samples.

Table 1 lists the sample sizes by sex, origin type, and collection year:

Table 1. Aged 4-year old spring chinook used in morphometric analysis

		2002	2003	2004	Total
Males	Hatchery Origin	20	8	8	36
	Natural Origin	88	24	99	211
	Total	108	32	107	247
Females	Hatchery Origin	55	30	41	126
	Natural Origin	199	148	215	562
	Total	254	178	256	688

[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.sunsb.edu/morph.

[4] Rohlf, F.J. 2002. TPSREGR, version 1.26. Program for exploring the relationship between shape and one or more independent variables. Available from Department of Ecology and Evolution, State University of New York at Stony Brook through http:\\life.bio.sunsb.edu/morph.

Results and Discussion

The results presented below are only an overview, intended to show the difference in conclusions about shapes that resulted from the redigitizing. So, for example, we present the classification results without detailed information on exactly how the shapes differ. A complete analysis of the now-corrected data is presented in the next chapter, to which the reader should refer for these details.

Shape Differences between Males and Females

The redigitizing had only a trivial effect on the ability to distinguish males from females (Table 2 and Table 2(Revised)). Discrimination ability was still excellent, so there was still good support for doing further analyses of males and females separately.

Table 2. Jackknifed classification to sex of age-4 adult spring chinook (irrespective of origin) by canonical discriminant analysis. Values in parentheses indicate range over within-year analyses.

Sex	Classification		
	Male	Female	% Correct
Male	246	1	100(98-100)
Female	0	688	100
Total	246	689	100 (99-100)

Table 2(REVISED). Jackknifed classification to sex of age-4 adult spring chinook (irrespective of origin) by canonical discriminant analysis. Values in parentheses indicate range over within-year analyses.

Sex	Classification		
	Male	Female	% Correct
Male	245	2	100(98-100)
Female	0	688	100
Total	246	689	100 (99-100)

Shape Differences due to Origin in Males

Redigitizing had little effect on the overall classification of males by production type (Table3 and Table 3(Revised), but did change the variation among years. This is probably not biologically meaningful, however, because the small sample sizes will make

Table 3. Jackknifed classification to origin of age-4 male spring chinook by canonical discriminant analysis. Values in parentheses indicate range over within-year analyses.			
Origin	Classification		
	Hatchery	Natural	% Correct
Hatchery	28	8	78 (60-88)
Natural	29	182	86 (76-95)
Total	57	190	85 (73-94)

Table 3 (REVISED). Jackknifed classification to origin of age-4 male spring chinook by canonical discriminant analysis. Values in parentheses indicate range over within-year analyses.			
Origin	Classification		
	Hatchery	Natural	% Correct
Hatchery	28	8	78 (50-78)
Natural	31	180	86 (77-95)
Total	57	190	85 (72-93)

a classification change of a single fish have a large effect on the percentage of correct classification.

Shape Differences due to Origin in Females

The same pattern seen in males was seen for females. Overall classification to origin was about the same with the redigitized data (Table 4 and Table 4(Revised)), and some variation was seen in assignment to within-year assignment to origin.

Table 4. Jackknifed classification to origin of age-4 female spring chinook by canonical discriminant function. Values in parentheses indicate range over within-year analyses.			
Origin	Classification		
	Hatchery	Natural	% Correct
Hatchery	106	20	84 (76-85)
Natural	75	487	87 (84-89)
Total	181	507	86 (83-89)

Table 4(REVISED). Jackknifed classification to origin of age-4 female spring chinook by canonical discriminant function. Values in parentheses indicate range over within-year analyses.			
Origin	Classification		
	Hatchery	Natural	% Correct
Hatchery	104	22	83 (75-87)
Natural	83	479	85 (82-88)
Total	187	501	85 (81-89)

Shape Differences due to Size

Natural-origin fish commonly are larger than hatchery-origin fish in this project (Knudsen et al. 2005), and this was also true of the fish used for this analysis (Table 5).

Table 5. Mean post-orbital hypural lengths (cm) of 4-year old spring chinook used in morphometric analysis

		2002	2003	2004	Overall
Males	Hatchery Origin	58.7	58.0	57.0	58.2
	Natural Origin	60.1	64.8	59.0	60.1
Females	Hatchery Origin	59.3	60.2	59.3	59.5
	Natural Origin	60.9	62.7	58.3	60.4

Because shape may be related to size, it was important to determine to what extent size might have influenced the earlier classification to origin. To investigate this, we compare the results of discriminant analyses using centroids only, partial warps only, and centroids + partial warps (Table 6 and Table 6(Revised)). Although the influence of size was not substantial before, digitizing seems to have reduced it somewhat. Discriminant analysis based entirely on in part on centroid was less effective in classifying fish to origin after redigitization.

Table 6. Percentage of correct classification to origin of male and female chinook, based on three different discriminant function schemes.

	Males			Females		
	Partial warps only	Centroid only	Warps + Centroid	Partial warps only	Centroid only	Warps + Centroid
Hatchery-origin	78	58	75	84	60	86
Natural-origin	86	59	86	87	58	87
Overall	85	59	85	86	58	86

Shape Differences among Years

The effect of redigitization was most strongly reflected in classification to years. For both males (Table 7 and Table 7(Revised)) and females (Table 8 and Table 8(Revised)) classification accuracy was markedly reduced for 2003 and 2004 after redigitization.

Table 6(REVISED). Percentage of correct classification to origin of male and female chinook, based on three different discriminant function schemes.

	Males			Females		
	Partial warps only	Centroid only	Warps + Centroid	Partial warps only	Centroid only	Warps + Centroid
Hatchery-origin	78	58	78	83	56	82
Natural-origin	86	60	85	85	52	85
Overall	85	60	84	85	52	85

Table 7. Jackknifed classification to year of age-4 male spring chinook, irrespective of origin, by canonical discriminant analysis. Parenthetical values indicate range of correct classification in within-origin analyses.

Year	Classification			
	2002	2003	2004	% Correct
2002	69	19	20	64 (60-66)
2003	9	21	2	66 (71-75)
2004	15	4	88	82 (75-83)
Total	93	44	110	72 (67-74)

Table 7(REVISED). Jackknifed classification to year of age-4 male spring chinook, irrespective of origin, by canonical discriminant analysis. Parenthetical values indicate range of correct classification in within-origin analyses.

Year	Classification			
	2002	2003	2004	% Correct
2002	69	22	17	64 (50-60)
2003	10	17	5	53 (50-67)
2004	21	14	72	67 (68-75)
Total	93	44	110	72 (56-64)

Table 8. Jackknifed classification to year of age-4 female spring chinook, irrespective of origin, by canonical discriminant analysis. Parenthetical values indicate range of correct classification in within-origin analyses.

Year	Classification			
	2002	2003	2004	% Correct
2002	142	57	55	56 (60-61)
2003	23	151	4	85 (75-84)
2004	44	6	206	80 (75-81)
Total	209	214	265	73 (67-75)

Table 8(REVISED). Jackknifed classification to year of age-4 female spring chinook, irrespective of origin, by canonical discriminant analysis. Parenthetical values indicate range of correct classification in within-origin analyses.

Year	Classification			
	2002	2003	2004	% Correct
2002	141	64	49	56 (57-69)
2003	40	114	24	64 (64-83)
2004	54	31	171	67 (66-67)
Total	209	214	265	62 (67-75)

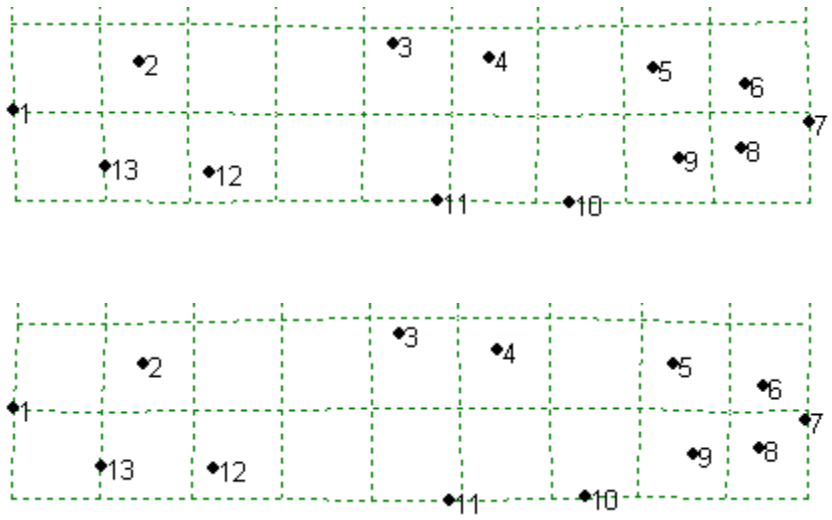
This indicates that among-year differences in shape were considerably less pronounced after redigitization than before.

The difference caused by redigitization is best seen by compared the consensus shape extrema among years. Figure 1 shows the consensus shape extrema over years for males, produced by the TPSRegr program. The two upper diagrams show clearly the previous enormous variation due to landmark 7; in the lower two diagrams there is no noticeable variation due to landmark 7. Females show a similar pattern.

Within-gender MANOVA results were not significantly different after redigitization. All three test statistics yielded p values of 0.000 for the effects of origin, year, origin x year, and centroid size before and after redigitization. We thus conclude that although the redigitization has considerably reduced classification inaccuracy and perceived shape differences based on landmark 7, none of the previous conclusions were unjustified. Highly significant shape variation remains for all factors.

In the next chapter we extend this analysis in two ways: by attempting to use the IMP Software package to correct for size variation, and by more fully describing the shape differences.

a)



b)

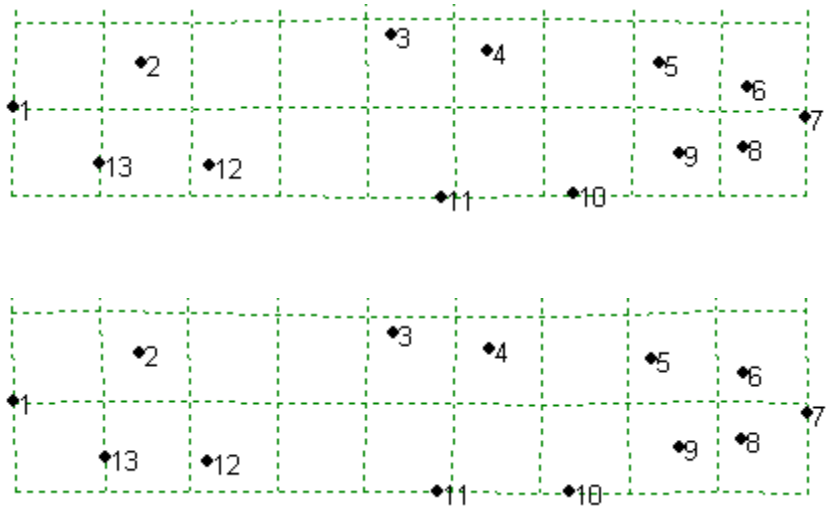


Fig. 1. Consensus shapes generated by TPSRegr of 4-yr old male spring chinook, showing extremes of variation due to year: a) shapes before redigitizing; b) shapes after redigitizing. Shape variation has been magnified threefold to aid in visualizing differences.

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Chapter 6

Differences in Body Shape Between First-Generation Hatchery and Wild Upper Yakima River Spring Chinook Salmon

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Abstract

Four-year old adult wild spring chinook at a supplementation hatchery were compared morphologically to their first-generation hatchery counterparts over three consecutive brood years using the method of thin-plate spline analysis on 12 digitized landmarks. Overall multivariate analysis of covariance of partial warp scores revealed highly significant differences for the effects of sex, origin (hatchery or wild), brood year, and centroid size. Overall sex-specific canonical discriminant analysis (CDA) was able to classify females to origin with about 75% accuracy (up to 84% accuracy for one brood year) and was able to classify males to origin with about 65% accuracy (up to 89% accuracy for one brood year). Classification to brood year was about 61% accurate for females and males (and up to 73% for wild fish). Adjustment for size resulted in virtually no improvement in classification accuracy for females but improved accuracy in males about 5%. CDA of brood-year specific partial warp scores in females resulted in overall accuracy of 75-84%, and 77-84% after size adjustment. Size adjustment in males resulted in overall accuracy of 69-89%, and 70-91%. Although wild fish were on average larger than hatchery fish, allometry clearly had a minor impact on classification accuracy. Consensus shapes suggested that hatchery fish had larger heads, were more slender than wild fish, and differed in dorsal and anal fin base widths; analysis of variance of traditional body proportion measurements bore this out. Body proportion differences between hatchery and wild females ranged between 0.27 and 0.41 standard deviations; differences in males were somewhat less. In terms of actual measurement these differences amounted to at most 2.7%. After one generation of artificial rearing hatchery and wild fish statistically differ in morphology, and these small differences may be biologically important, they allow only moderate classification power.

Introduction

Hatchery and natural environments differ in many ways. Compared to their wild counterparts, hatchery juveniles experience much higher fish densities, and a much less complex environment in terms of flow, temperature, food, and structure. Not only will the young fish react developmentally to the altered environment, the hatchery environment considerably may present a quite different selection regime than the natural environment, in some cases relaxing selection pressures, and in others reversing the direction of selection (Lynch and O'Hely 2001). Adults in the hatchery also face a very different environment from the wild. Natural spawners must select redd sites, dig redds, attract and compete for mates, engage in courtship, deposit gametes, and guard them. Hatchery broodstock need do none of these things, so natural selection for a large suite of reproductive behaviors is relaxed entirely. Fish subject to some level of hatchery culture would therefore be expected to differ phenotypically and genetically from their wild counterparts and progenitors in physiology, development, and behavior. In anadromous salmonids this has definitely been shown to be the case (Einum and Fleming 2001) The challenge in the future development of hatchery technology and methodology is in managing these changes so that the impacts to production and to natural productivity are kept within acceptable limits.

Morphological change is a well documented consequence of hatchery culture. There are two major areas of concern associated with morphological change. The first is the impact of shape change on swimming style and stamina. In coho salmon, fish with deeper bodies and larger median fins are better at burst swimming than more streamlined fish, and conversely, more streamlined fish are better at sustained swimming (Taylor and McPhail 1985a). Not surprisingly, morphological differences correlate with distance of freshwater travel to spawning sites in coho (Taylor and McPhail 1985b; Fleming and Gross 1989) and in Atlantic salmon (Riddell and Leggett 1981). Morphological change may also significantly affect secondary sexual characteristics, potentially disrupting normal sexual selection mechanisms. In coho, reproductive success in females is affected by caudal peduncle depth, and reproductive success in males is affected by kype length (Fleming and Gross 1994).

The existing research on morphological comparisons of wild and hatchery fish is typical of all wild-hatchery comparisons in that it is limited in species coverage and heterogeneous in approach. Most of the work has been done in Europe on Atlantic salmon (*Salmo salar*) and sea trout (*S. trutta*), with the bulk of the remainder having been done in North America on coho salmon (*Oncorhynchus kisutch*). Two basic patterns have been seen in hatchery fish, depending on the type of hatchery environment. "Farmed" fish, fish that reared to adulthood in captivity, tend to be deeper-bodied than wild fish (Webb et al. 1991; Fleming et al. 1994; Hard et al. 2000), but "sea-ranched" fish, hatchery fish that are released as juveniles into the natural environment tend to display the more streamlined phenotype typical of fish better adapted for sustained swimming (Taylor 1986; Fleming and Gross 1989; Swain et al. 1991; Wessel et al. 2006). Hatchery fish, both farmed and sea ranched, also show reduced snout development, and important secondary sexual characteristic (Fleming and Gross 1989; Petersson and Jarvi 1993; Hard et al. 2000).

The observed morphological differences between hatchery and wild fish have both environmental and genetic components. Aspects of morphology have high

heritabilities, at least in the hatchery environment (Hard et al. 1999), but the environment effects are large. Swain et al. (1991) and found that differences between hatchery and wild coho populations decreased when the fish were raised in the same hatchery environment, and von Cramon-Taubel et al. (2005) found the same effect for transplanted juvenile hatchery Atlantic salmon. A variety of hatchery-induced developmental changes have been noted, and should be expected due the high susceptibility of fish developmental processes to environmental influences. Such changes can range from gross changes in mid-body shape due to extreme feeding regimes (Currens et al. 1989), to changes in skull morphology in response to different food stuffs (Romanov 1984; Wintzer and Motta 2005), to changes in cerebellar development due to complexity of early rearing environment. Morphological change in response to environmental cues can be quite rapid. Juvenile brown trout and Atlantic salmon changed shape significantly within a month in response to altered water velocities (Pakkasmaa and Piironen 2000).

There is more than adequate evidence to consider morphological change a likely consequence of domestication in sea-ranching operations typical of hatchery programs in western North America, and a consequence that should be evaluated both phenotypically and genetically. Most of the work that has been done to date, like much of the research on any type of hatchery-wild differences, has been accomplished by studying different populations. Even though clear genetic differences between the populations may have been established, different populations may respond in different ways due to their different genetic backgrounds. More studies of morphological change, as well as domestication in general, need to be done on fish with the same genetic background. The morphological studies of Fleming et al. (1994) and Wessel et al. (2006) have done this, but in populations with substantial cultural histories. No study to date has addressed the development of morphological change as domestication proceeds in a previously wild population. Such an opportunity existed in a new integrated supplementation program (Goodman 2004; Mobrand et al. 2005) for spring chinook on the Yakima River in central Washington. The Yakima program is being studied intensively in an attempt to understand the environmental and genetic components of domestication from its beginning. In a companion paper (Knudsen et al. 2006) we discussed first-generation life history differences between hatchery and wild fish in the Yakima spring chinook population, and here we extend this work to morphology. We assess our ability to classify adult fish according to origin, assess the impact of allometry on classification accuracy, and describe the shape differences between hatchery and wild fish.

Materials and Methods

Specimens

Fish for this study were all four-year old adult spring chinook salmon from the Upper Yakima River population in central Washington state. The Yakima/Klickitat Fishery Project (YKFP) began a spring chinook hatchery program at the Cle Elum Supplementation Research Facility (CESRF) on the upper Yakima (rkm 297; Figure 1) in 1997. This program is a supplementation effort targeting the Upper Yakima population, and is designed to test whether artificial propagation can be used to increase natural production and harvest opportunities while limiting ecological and genetic impacts. It is an integrated hatchery program in that only natural origin broodstock are used and returning hatchery origin adults are allowed to spawn in the wild. The program includes

a domestication monitoring effort that compares the supplemented population at several traits to a hatchery only control line founded from first-generation hatchery returns, and to a wild control line (the unsupplemented population in another Yakima tributary). See Knudsen et al. (2006) for further details.

Under this supplementation program, only natural origin fish are collected for hatchery broodstock, and hatchery-origin returnees are allowed to spawn freely in the wild. Hatchery-origin age 3 males began returning in 2000, and hatchery origin age 4 fish of both sexes began returning in 2001 (Table 1). Because of the age of the program and previous lack of hatchery activity in the basin, all hatchery-origin fish in this study were the progeny of wild parents. All natural origin fish in the study were also the progeny of wild parents, except for the possibility that some of the 2004 fish could have been sired by age 3 hatchery-origin males that returned in 2000.

A note on our use of the term "wild". As integrated hatchery programs proceed, it is inappropriate to call fish resulting from natural spawning wild, because they may be the progeny of naturally spawning hatchery fish. These fish are more appropriately called "natural origin" fish. We call the naturally produced fish in this study, representing the 1998, 1999, and 2000 brood years, wild because they occurred before significant numbers of naturally produced fish of hatchery ancestry were possible (Table 1). There may have been some contribution of hatchery origin age 3 males from the 1997 brood year to the 2000 brood, but this influence was probably slight, as these fish accounted for only 5% of the natural spawning population (Yakama Nation unpublished data). The natural-origin upper Yakima spring chinook in this study can be considered wild also because this population had been subjected to only negligible levels of hatchery activity in the past. With the possible exception noted above, the only difference between the natural-origin and hatchery-origin adults returns are the phenotypic effects of hatchery rearing and genetic effects of a single generation of hatchery culture.

Specimens were collected over the course of the spawning migration (late April to September) in 2002, 2003, and 2004 at the Roza Adult Monitoring Facility (RAMF) at Roza Dam (Fig. 1). Fish collected were intended for use as broodstock for the supplementation project, the hatchery control line, or for other research uses. At RAMF scale samples were taken for aging and each fish was injected in the pelvic girdle with a PIT tag for later identification at CESRF. All fish were photographed immediately after they were killed for spawning or monitoring purposes during September at CESRF.

Our study was limited to four-year old fish primarily because of the limited number of other-aged fish available and the possibility that shape varied with age and or size. This was especially important in the case of age 3 males, which appeared upon casual visual inspection to differ in shape from age 4 males, but also were considerably (~20 cm) smaller. Ages were determined by examining acetate impressions of scales using a microfiche reader. Two scale analysts independently aged all scales and resolved disagreements. Only fish that could be aged were included in the study. Because of the requirement that only natural-origin fish be used for production broodstock, far more natural-origin fish were available for the study than hatchery-origin fish. In total, 935 fish (Table 2) were used in the study, 247 males (36 hatchery-origin and 211 natural-origin) and 688 females (126 hatchery-origin and 562 natural-origin). We have presented data on a brood-year rather than a return-year basis to maintain consistency with a

companion paper (Knudsen et al. 2006) on wild-hatchery differences in life history traits in this population.

Data Acquisition

Fish were photographed 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. In many cases, multiple photographs were made of a single specimen. All photographs were taken by PH.

The photographs were scanned for quality and then compiled into files for analysis, one per specimen, using TPSUTIL (Rohlf 2004c). Images were digitized on a desktop computer using program TPSDIG (Rohlf 2004a). Digitizing involves clicking the cursor on body-shape landmarks to create a series of coordinates representing a fish's shape in the XY-plane. We initially used 13 landmarks (Fig. 1): 1) tip of snout, 2) base of skull, 3) anterior insertion of dorsal fin, 4) posterior insertion of dorsal fin, 5) anterior insertion of adipose fin, 6) dorsal insertion of caudal fin , 7) base of caudal fin (transition to fin membrane), 8) ventral insertion of caudal fin, 9) posterior insertion of anal fin, 10) anterior insertion of anal fin, 11) anterior insertion of pelvic fin, 12) anterior insertion of pectoral fin, and 13) posterior tip of maxillary. We later dropped landmark 5, the adipose fin landmark, from the analyses. The landmarks used were either identical or very similar to landmarks used in other recent morphological analyses of salmonids (Hard et al. 2000; von Cramon-Taubadel et al. 2005; Wessel et al. 2006). All digitizing was done by GH.

All hatchery fish in this study were adipose-clipped. This made landmark 5, the anterior insertion of the adipose fin, much more distinguishable on wild fish than hatchery fish, and created the possibility for bias. Visual inspection of Procrustes-superimposed landmark data in IMP:PCAGen6 (Sheets 2002; Zelditch et al. 2004) demonstrated a clear difference between groups in digitizing of this landmark: digitizing was anteriorly in hatchery fish relative to that in wild fish, and was considerably more variable. Although this could have reflected in part real differences between the two groups, it seemed more likely to be a difficulty in precisely locating the landmark. We concluded the possibility of bias was real and serious, and thus dropped the landmark from further comparisons of shapes of hatchery and wild fish. However, we did compare shape information based on all 13 landmarks with shape information omitting landmark 5 in wild fish- in which the landmark could be unambiguously digitized- to evaluate the loss of information caused by the loss of the landmark.

TPSDig allows the user to digitize points on a ruler in the photograph. Because the distance from camera may differ from fish to fish and day to day, this results in individual-specific scaling factors being built into the resulting file of landmark data. The scaling factor is ignored by the IMP programs, which then will erroneously assume all specimens are scaled identically. To eliminate this problem, the TPS data files were run through a simple editing program which multiplied the coordinates of each landmark by the scaling factor. This correctly sized all specimens, eliminating further need for the scaling factor. The edited files could then be run through both suites of programs.

Analysis

The basic approach we have taken for morphometric analysis is thin-plate spline analysis. The thin-plate spline approach decomposes 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 are 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 (Zelditch et al. 2004) for a good overview of the method). We used program TPSRELW (Rohlf 2004b) to generate partial warp scores and centroid sizes. Before applying TPSRELW we regressed centroid sizes were regressed on fish length to identify possible gross digitizing or scaling errors. Three obvious outliers were identified (one male and two females) and excluded from further analysis (Table 2 reflects sample size after this exclusion).

Partial warp scores of various groups were analyzed by standard multivariate methods: multivariate analysis of variance and covariance (MANOVA and MANCOVA) and canonical discriminant analysis (CDA) (SYSTAT,v.11 ,SYSTAT Statistics, Inc.). We used Cohen's kappa statistic (Titus et al. 1984) to compare the performance of discriminant analyses over what would be expected from chance. This statistic is especially useful when groups to be classified are very uneven in size, as they are in this study. Differences in consensus shapes between groups were visualized and quantified using TPSREGR (Rohlf 2002). Distances were computed between various pairs of landmarks, using coordinates generated by TPSREGR, to qualitatively describe shape differences. These "traditional" distances were then compared between hatchery and wild fish using ANCOVA, using length as a covariate, and testing for effects of origin, brood year, and origin x brood year interaction.

Partial warp scores are size-standardized measures of departure from the consensus shape of fish in the sample, so are relative rather than absolute measures. For this study that involves three dimensions (sex, origin, year)of potential shape variation, numerous schemes of generating partial warp scores are possible. We first generated and evaluated partial warps for the entire mixed-sex sample, then generated partial warps separately for the two sexes, and finally generated partial warps for sex-and-year specific samples.

Age-4 hatchery and wild fish differed significantly in length in the 1998 and 1999 brood years by as much as 0.5 standard deviations in this population (Knudsen et al. 2006). The subset of fish used in this study mirrored this observation (Table 3). If warp scores varied significantly with fish size, there was a possibility that allometry rather than true shape differences were responsible for observed differences between hatchery and wild fish. To investigate this possibility we used IMP:Standard6 (Sheets 2002; Zelditch et al. 2004; Zelditch et al. 2006) to remove linear dependencies of shape on centroid size for selected analyses and compared the size-adjusted with non-adjusted results. The actual method is described fully in Regression on size and subsequent size-adjustment of coordinates was done within- rather than across groups being compared because of the sensitivity of size-adjustments to differences in slope between groups. In adjusting for size, it is important to adjust to the same size; otherwise size adjustment

may result in spurious enhancement of shape differences. Here we adjusted to the mean log centroid size of all the fish of the same sex.

Shape differences due to origin were characterized by visual examination of consensus shapes using TPSRegr (Rohlf 2002). Traditional morphological distances were constructed among selected landmarks to corresponding to the variation seen between the consensus shapes using IMP:TMorphGen6 (Sheets 2002; Zelditch et al. 2004). Differences between origin types in body proportions were then examined by analysis of covariance of these morphological distances, using length (landmark1-landmark6) as a covariate.

Results

Overall Evaluation of Shape Differences

We began statistical analysis of shape differences with an overall multivariate analysis of covariance (MANCOVA) that included the main effects of sex, origin, and year; all possible two-way and the three-way interactions; and centroid size as a covariate to include possible allometry. Not surprisingly, the effect of sex was highly significant, but so were all other effects (maximum $p=0.010$; identical results from Wilks' Lambda, Pillai Trace, and Hotelling-Lawley Trace). These results indicated that detailed exploration of differences due to sex, origin, year, and size were warranted. Despite the complexity of effects, canonical discriminant analysis (CDA) was quite successful (Table 4). Overall 2 fish of each sex were misclassified; when the CDA was run within years, one fish of each sex was misclassified in brood year (BY) 1998, and none in the other two brood years. Males appeared to have larger heads and consequently longer maxillaries, but also shallower bodies and shorter pectoral-pelvic distances. The large shape differences between the sexes made sex-specific analyses of shape differences between origins and years seem most logical course of action in further analyses, and identical analyses were done separately on males and females from this point forward: first analysis based on partial warps over all brood years, and then brood-year specific partial warps. Parallel analyses were done for data adjusted for size.

Analysis by Sex , Brood Years Combined

A MANCOVA of female partial warp scores from all brood years combined was again highly significant for the effects of origin, brood year, and centroid size ($p<0.001$) by all three statistics, as above), but the origin x brood year interaction was not significant ($p=0.062$). For males, the effects of origin, brood year, origin x brood year interaction, and centroid size ($p\leq0.001$ by all three statistics). An overall CDA correctly classified 74% of the females to origin (kappa = 0.34), but within-brood year CDA's using the same overall partial warp scores were somewhat more successful, ranging from 75 to 84% (Table 4). The same pattern held with males, with the overall CDA correctly classifying 65% to origin (kappa = .15), and within-brood year CDA's being more successful, ranging from 76 to 89%. To put this classification accuracy in perspective in view of the significance of brood year in the MANCOVA, we also used CDA to classify by brood year, and did within-origin CDA's as well, using the same overall partial warp scores (Table 5). The overall CDA correctly classified 62% of the females to brood year (kappa = 0.43), and the within-origin CDA's were somewhat more successful, ranging

from 62 to 73%. For males, the overall CDA correctly classified 61% of the fish to brood year (kappa = 0.37), and the within-origin CDA's were also somewhat more successful, ranging from 58 to 73%. As was the case for females, shape varied significantly over origin and brood year, but the variation due to origin was larger. However, this difference was less in males. Based on classification accuracy, it appeared that although shape varied significantly over origin and brood year, the variation due to origin was larger. Kappa values were higher for the classification to brood year, so classification to brood year appears more reliable than classification to origin.

The same series of MANCOVA's and CDA's was repeated using size-adjusted data. In both sexes the two main effects and interaction were highly significant ($p \leq 0.001$) in the MANCOVA, but as expected, centroid size was not ($p \geq 0.534$). The overall CDA was trivially more successful than the non-adjusted CDA at classifying females to origin (Table 6), and there was a small improvement in the within-brood year CDA classification accuracy. The same pattern held for males except that larger variation was seen in the within-brood year CDA classification accuracy, reducing the lower range of classification accuracy. The size-adjusted overall CDA was no better than the unadjusted at classifying females to brood year, and the within-origin CDA's were actually slightly less accurate in classification than the CDA without size-adjustment (Table 7). In males, the size-adjusted overall CDA was somewhat better at classifying fish to brood year, and the within-origin CDA results were more variable than the unadjusted results, with the BY 1999 classification accuracy improving considerably, and with an increase in kappa from 0.37 to 0.47.

Analysis by Sex , Brood Years Combined

The last stage of statistical analysis involved calculating partial warp scores separately for each brood year on both unadjusted and size-adjusted data, then doing within-brood year MANCOVA and CDA. In females the effect of origin was highly significant for all three brood years ($p<0.001$), as was centroid size ($p \leq 0.008$). In males significance of effects varied considerably over brood years. Origin and centroid size were both highly significant for BY 2000 ($p<0.001$), both were significant in BY 1998 ($p=0.012$ and $p=0.004$, respectively), and neither was significant in BY 1999 ($p=0.081$ and $p=0.095$, respectively). This sharp contrast with the female results was undoubtedly caused by the small sample size (total sample size in BY 1999 was 32 fish). Correct classification of females by CDA ranged over brood years from 75 to 84% , with kappa values ranging from 0.36 to 0.50(Table 8), exactly the same classification accuracy as that using the partial warp scores calculated with brood years combined (Table 4). In males, correct classification by CDA ranged over brood years from 63 to 89%(with kappas from 0.00 to 0.44), considerably better than the classification accuracy using the partial warp scores calculated with brood years combined. The results from the 1999 brood year demonstrate the value of the kappa statistic quite well. Classification, although it appears to be 63% accurate, is exactly what would be expected by chance.

The corresponding size-adjusted MANCOVA of female data showed origin to be highly significant for all three brood years ($p<0.001$). In males, the corresponding size-adjusted MANCOVA's varied over brood year, with the effect of origin being significant

in BY 1998 and BY 2000 ($p=0.009$ and $p<0.001$, respectively) and insignificant in BY 1999 ($p=0.069$). As expected, there was no effect of centroid size ($p=1.000$) in either sex in any brood year. Size-adjusted CDA correct classification of females ranged from 77 to 83%, with kappa ranging from 0.43 to 0.50 (Table 9), almost exactly the same accuracy as the CDA based on overall partial warp scores (Table 6). In males, size-adjusted CDA correct classification ranged from 70 to 91%, with kappa ranging from 0.27 to 0.63, almost exactly the same accuracy as the CDA based on overall partial warp scores.

Impact of Deleting Adipose Landmark

We attempted a simple evaluation of the impact of deleting the adipose landmark (landmark 5) by comparing shape information in wild fish, in which we assumed the landmark could be unambiguously digitized. We derived sex-specific principal and partial warps using the full set of 13 landmarks and the set of 12 (with adipose deleted), and then did a CDA using each partial warp set to classify fish to brood year. The first four principal warps cumulatively accounted for 59% of the variation when the adipose landmark was included, and 61% when it was not included. Classification accuracy was 62% when the landmark was included, and 62% when it was not. So for wild females, the landmark seems to add little or no value. The situation was much the same in males. The first four principal warps cumulatively accounted for 60% of the variation when the adipose landmark was included, and 62% when it was not included. Classification accuracy was overall 64% when the landmark was included, and 62% when it was not. Although there seemed to be some benefit in including the adipose landmark in males, it was slight.

Characterizing Shape Differences

Consensus shapes on D'Arcy Thompson grids produced by TPSRegr for females and males for brood-year specific analysis are presented in Figs. 3 and 4, respectively. Although the male shapes vary far more between years than the female shapes, probably due to the much smaller sample sizes for males, two trends are fairly conspicuous: hatchery fish had proportionately larger heads and narrower bodies that wild fish. Other less conspicuous differences were also suggested in at least one sex, but were much more obvious in sliding between hatchery and wild shapes with the TPSRegr software than they are in the figures. These included the width of the dorsal and anal fin bases (landmarks 3-4 and 9-10), and length and depth of caudal peduncle (landmarks 7-8 and 5-7). These differences were evaluated quantitatively by constructing nine "traditional" distances between the landmarks involved and evaluating differences between wild and hatchery fish using ANCOVA with length (landmarks 1-7) as a covariate (Table 10). In females, comparisons of all measurements except peduncle depth were significant for the effect of origin ($p<0.01$), of these, all except posterior dorsal-pelvic insertion (4-11), also exhibited significant variation ($p < 0.05$) among brood years, and four of traits were significant ($p < 0.05$) for the interaction. Examination of the least squares means for the traits with significant interaction effects showed that for all traits except the snout-maxillary length (1-13) the interaction was caused more by strong versus weak effect rather than strong effects in opposite directions. Thus, for females, there seemed to be statistically significant evidence for hatchery fish having longer heads, narrower bodies in

the dorsal to pelvic region, narrower dorsal fin bases, wider anal fins bases, and shorter caudal peduncles than wild fish. The pattern for males was quite different, with only three traits significant ($p < 0.05$) for the effect of origin. This was probably more a reflection of small sample size than actual lack of difference. Two of the traits for which males showed significant origin effects were also significant in females; the other was caudal peduncle depth, which was highly significant ($p < 0.001$). Caudal peduncle depth also was highly significant for the interaction effect, but examination of the least squares means indicated that this interaction was due to differences among brood years in intensity, and not direction. Thus, it appears to be a real effect. Despite the contrast between males and females in number of significant tests, there was agreement in direction of difference (i.e., H>W or W>H) at seven of eight traits. There was fairly good agreement as well on magnitude of the hatchery-wild differences expressed in standard deviation units. Assuming that these deviations in males are evidence of real effects and that the lack of statistical significance was due to sample size, the conclusions about hatchery-wild differences in females may hold for both sexes. Based on statistical tests, hatchery-wild differences seemed to vary considerably between the sexes. Detailed examination of least squares means, however, showed that the two sexes responded similarly in all three brood years. In BY 1998 wild fish had proportionately longer maxillaries than hatchery fish (0.09 SD for males and 0.41 SD for females), but in both BY 1999 and BY 2000 hatchery fish had proportionately longer maxillaries than wild fish (males 1.0 SD and 0.576 SD; females 0.691 SD and 0.534 SD).

In summary, for both sexes there was statistically significant evident of hatchery fish having narrower dorsal fin bases and wider anal fin bases than wild fish. For females only there was also statistically significant evidence of hatchery fish having longer heads (measured from snout to base of skull), narrower mid-bodies, and shorter peduncles (measured ventrally). Agreement in direction and magnitude of deviation suggests that despite lack of statistical power to detect it, the same differences hold for males. There was also statistically significant evidence for hatchery fish having deeper caudal peduncles than wild fish. Morphological differences between wild and hatchery fish varied significantly over brood years for several characteristics.

Putting these differences in biological perspective, at traits in which tests were significant ($p<0.05$), the absolute difference between female hatchery and wild means ranged from 0.272 to 0.412 standard deviations, with a mean of 0.327 (Table 10). The same type of evaluation for males is made more speculative by the lack of significance, but the mean for the two significant tests was 0.423 standard deviations. Male absolute differences at traits that were significant for origin effects in females ranged from 0.122 to 0.429, with a mean of 0.259. In Table 10 we also attempted to put the differences in "practical" terms by computing the percentage deviation from the wild measure. The differences just summarized as standard deviations translate in females to an absolute percentage deviation range of 1.2 to 2.7, with a mean of 1.85; and 0.8 to 3.3 in males, with a mean of 1.52.

Discussion

The study was limited by the availability of aged males. It is likely that the small samples available to us were unable to capture the full range of morphological variation

in males, and if so, our shape comparisons may have underestimated or overestimated differences. So although the analyses we have presented show that differences between hatchery and wild fish are smaller in males than in females, this could easily be an artifact of the small sample size. The same situation holds for qualitative differences between the sexes attributable to origin. Further work would obviously benefit from the inclusion of more males, particularly hatchery-origin males.

The study may also have been limited by discarding the adipose landmark. In our limited analysis, this landmark seemed of little value overall in contributing to shape information or in use in distinguishing brood years of wild females and males. It may have had some real value, however, in distinguishing hatchery and wild fish. Unfortunately, unclipped hatchery fish are needed to evaluate this, because even if clipped fish are reexamined very carefully, it is possible that growth in the clipped area is affected by the clipping, and thus the clip itself rather than the morphology of clipped fish may be the cause of the morphological distinction.

The adjustment for size was quite interesting. The effect of centroid size was highly significant in the MANCOVA's indicating that fish shape significantly changed with size, but the size adjustment had little impact on classification accuracy. Presumably classification accuracy using unadjusted data and adjusted data would have differed more with greater differences than the 2 cm length differences in mean size of hatchery and wild fish in this study. There did seem to be an effect at 5 cm (the difference between hatchery and wild males in the BY 1999 sample).

Research on morphological differences between adult sea-ranched hatchery and wild salmonids is limited; our results can be easily compared to only three. All three found as we did that hatchery fish have shallower bodies than wild fish: Fleming and Gross (1989) in coho; and von Cramon-Taubadel et al. (2005) and Fleming et al. (1994), both in Atlantic salmon. Shallower bodies have also been noted in juvenile hatchery coho (Taylor 1986; Swain et al. 1991) and chinook (Wessel et al. 2006). von Cramon-Taubadel et al. (2005) also noted that adult hatchery Atlantic salmon had longer caudal peduncles than wild fish, another observation that has been made in juvenile coho and chinook (Taylor 1986; Wessel et al. 2006). Our ability to characterize the caudal peduncle shape was limited by loss of the adipose landmark, but all indications are that our hatchery fish had shorter caudal peduncles than wild fish. Shorter dorsal and anal fin bases were seen in female hatchery coho (Fleming and Gross 1989); we noted shorter dorsal fin bases but longer anal fin bases. The coho literature frequently mentions the relationship of median fin sizes to body depth (Taylor and McPhail 1985b; Fleming and Gross 1989; Swain and Holtby 1989; Swain et al. 1991). Our results indicating differences between hatchery and wild fish in dorsal and anal fin base widths suggest research into median fin size in chinook may reveal similar relationships in this species.

We found that hatchery fish had longer heads (measured from snout to base of skull) than wild fish. This appears to be a hitherto unreported phenomenon. Juvenile studies have always noted smaller heads in hatchery fish, and head observations on adults have been limited to snout development as a secondary sexual character. Evidence of reduced snout development has been observed in sea ranched sea trout (Petersson and Jarvi 1993), coho (Fleming and Gross 1989), and also in coho caught as wild juveniles and raised to adulthood in captivity (Hard et al. 2000). In this study our focus was on

general body shape, and the suite of landmarks we chose was inadequate to distinguish snout development from general head development . Chinook are considerably less sexually dimorphic than coho, but at least two landmarks (mideye and most distal point of operculum) could be added to the current samples to do a more in depth analysis of sexual dimorphism in head morphology. Such a study will likely still be limited by the availability of hatchery males, however.

Comparisons of our results with the study of Wessel et al. (2006) are important because it is the only other hatchery-wild morphological comparison in chinook. Hatchery fish in the Wessel et al. study were more compressed in body depth, and had narrower heads, shorter maxillae, and longer and narrower peduncles. In our study we did find shallower bodies in the hatchery-origin fish, but our results were otherwise the opposite of what Wessel et al. found. Our hatchery fish had shorter and possibly deeper peduncles, although the results were ambiguous. Our results on the maxillary length varied, but in two out of three years, hatchery fish had longer maxillaries than wild fish. There are several possibilities for the discrepancies between our results and those of Wessel et al. (2006). One that immediately has to be considered is the life stage sampled. Perhaps the manifestation of hatchery rearing is different in adults than in juveniles. Another possibility is the number of generations of hatchery influence. Wessel et al. (2006) compared juveniles from a chinook hatchery population five generations old with juveniles of a "wild" hatchery stock only one generation removed from the same wild progenitor population, and a second-generation hybrid stock. The hatchery fish Wessel et al. sampled had been under hatchery influence for four generations, so their morphology may reflect a substantial genetic change due to domestication. All their study groups were products of at least one generation of hatchery culture, whereas our wild fish were free of hatchery influence. Our hatchery fish were the result of a single generation of culture first-generation fish, and may thus reflect mainly the phenotypic effects of hatchery rearing, and little genetic change. A final consideration for this comparison and all others is shared environments. Wessel et al.'s fish shared the same hatchery rearing environment their entire lives. As juveniles wild fish were never reared in a hatchery environment, and the hatchery fish were in it for only about 40% of their lives. As adults, both our hatchery and wild groups spent from the time they arrived at RAMF to the time of spawning in a hatchery holding pond, a period of anywhere from a few days to four months.

This study demonstrated, as did that of Fleming et al. (1994), that hatchery-wild differences can vary significantly over time. In our study cases of hatchery and wild fish differing in opposite directions in different years were rare, cases of significant differences in intensity of effect were fairly common (Table 10). Perhaps in later generations differences will increase to the point where temporal variation is not significant, but there is no doubt that sampling multiple years is critical to correctly characterizing morphological differences due to a single generation of hatchery rearing.

It is often assumed that any changes observed as a result of hatchery culture are genetic, when they may be the result of a mix of genetic and environmental causes. At this point we have no idea of the degree of genetic determination of the morphological differences we observed, but given the plasticity noted by previous researchers (Swain et al. 1991; Pakkasmaa and Piironen 2000; von Cramon-Taubadel et al. 2005) we would not be surprised to learn that they are primarily environmental in origin, especially given the

variation over broods we observed. Comparing future generations of returns with the project hatchery and wild control lines (see Knudsen et al. 2006) should allow us to partition the effects into genetic and nongenetic causes.

Although the morphological differences we observed were highly significant, in a practical sense, the morphological differences between hatchery and wild fish were small. This is demonstrated quite well by the classification results. A common rule of thumb for classification accuracy is that it should be at least half again as accurate as that expected by chance. Thus, for a two-way classification, Cohen's kappa should be at least 0.5. Classification by CDA over all brood years attained a maximum kappa of 0.35. Within brood years, and with size adjustment, however, the 0.5 level was exceeded in BY 1999 and BY 2000 for both sexes. The kappas attained in this study were comparable to those in other studies: Fleming et al. (1994) achieved a kappa of 0.43 for distinguishing hatchery and wild female coho salmon, and the kappa for Wessel et al.'s (2006) classification of chinook juveniles was 0.53. The discriminant analyses demonstrated that year-to year effects could be substantial, and that the differences between hatchery and wild fish, although highly significant statistically, were in practical terms quite small. As we have shown, they amounted to a change in a single body proportion of a maximum of 2.5%.

Despite the small magnitude of the differences as measured in absolute terms or by the discriminant analysis, it is possible that the morphological change has some fitness consequence. According to Lande's (1976) model of stabilizing selection, differences of 0.5 to 1 standard deviations can have fitness impacts of 1 to 5% (Knudsen et al. 2006). The seemingly small shape differences we have observed, although they are somewhat smaller than the size and timing differences, could in aggregate have a small fitness impact if they are subject to stabilizing selection. However, we have no hypothesis for a functional relationship between these types of morphological divergence and fitness. We would suspect that the fitness costs of the morphological change would be dwarfed by the potential fitness consequences of smaller size and earlier arrival times in hatchery-origin fish observed in this population (Knudsen et al. 2006).

Echoing Knudsen et al. (2006), perhaps the most important observation to be derived from this study at this point is that hatcheries do not produce fish that are identical to wild fish, even in a program designed to minimize differences between the two production types. The early hatchery and natural rearing environments experienced by upper Yakima spring chinook differ sufficiently to cause differences of up to 0.5 standard deviations after 2.5 years of growth in a common environment. The significance of these differences is unclear, but their implications for population productivity clearly need to be better understood as we proceed with the use of conservation hatcheries to sustain salmon and steelhead production.

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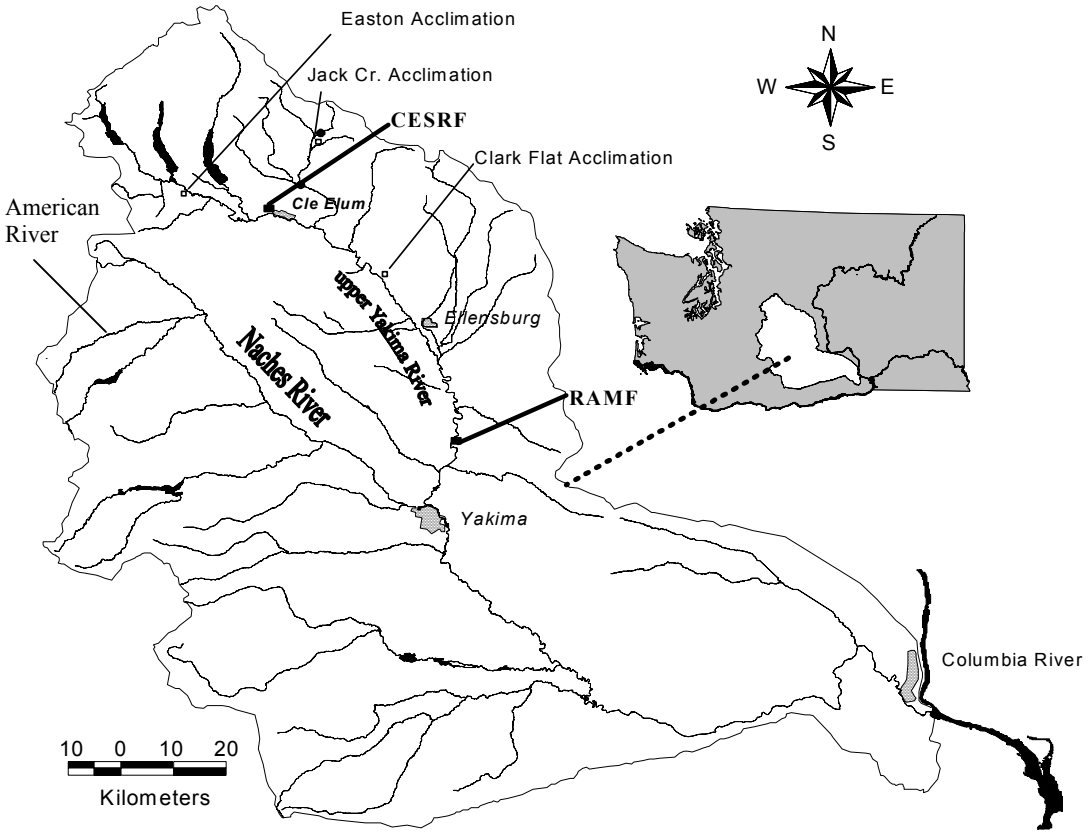
Fig. 1. Map of the Yakima River Basin, showing the locations of culture and trapping facilities.

Fig. 2. Female spring Chinook laid out for digitizing 13 landmarks used in this study. Lamdmarks are marked by dots. Image is a screen capture from TPSDIG program. The adipose landmark (5) was dropped from analysis because it could not be unambiguously marked in hatchery fish, all of which had clipped adipose fins. Image is a screen capture from TPSDIG program.

Fig. 3. Consensus shapes for hatchery and wild female spring Chinook for three brood years, generated by TPSRegr. Shape distortions have been magnified three-fold to accentuate shape differences.

Fig. 4. Consensus shapes for hatchery and wild male spring Chinook for three brood years, generated by TPSRegr. Shape distortions have been magnified three-fold to accentuate shape differences.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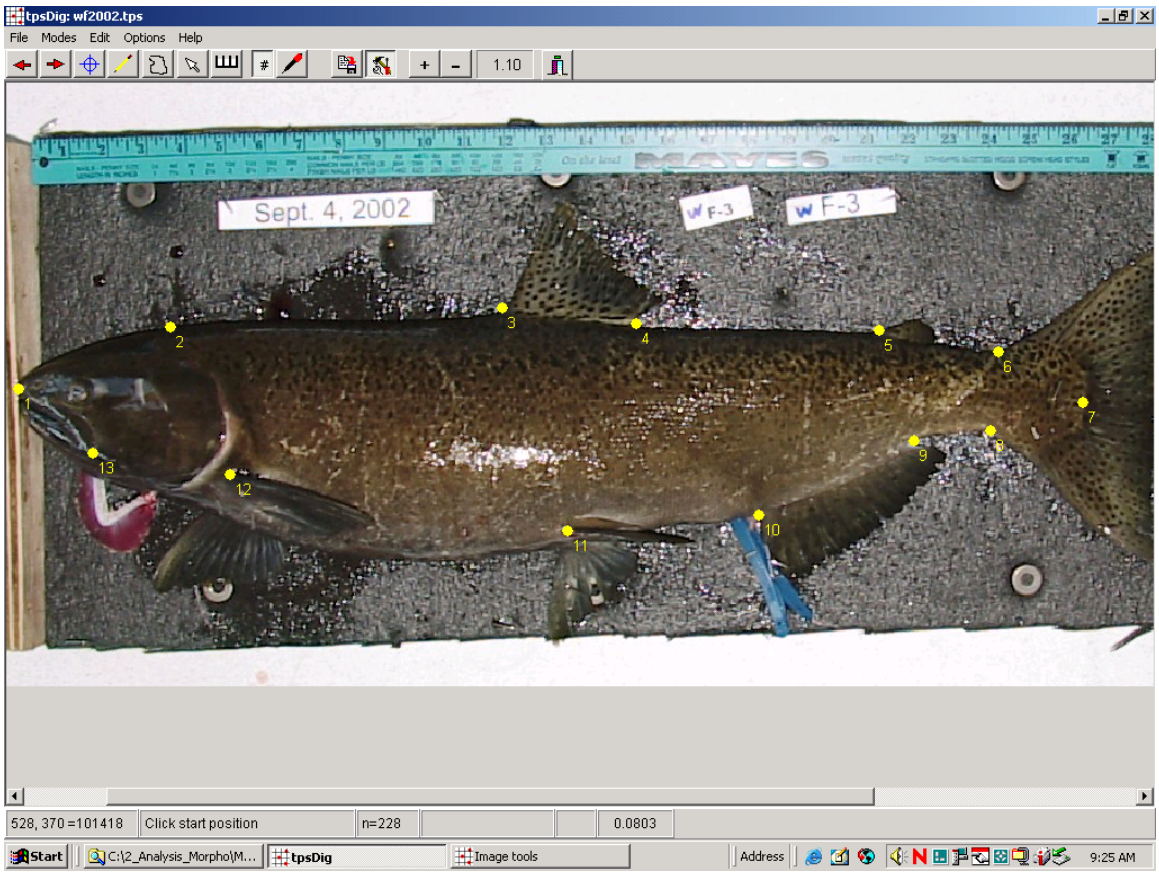
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Fig. 2.

Wild Females

Hatchery Females

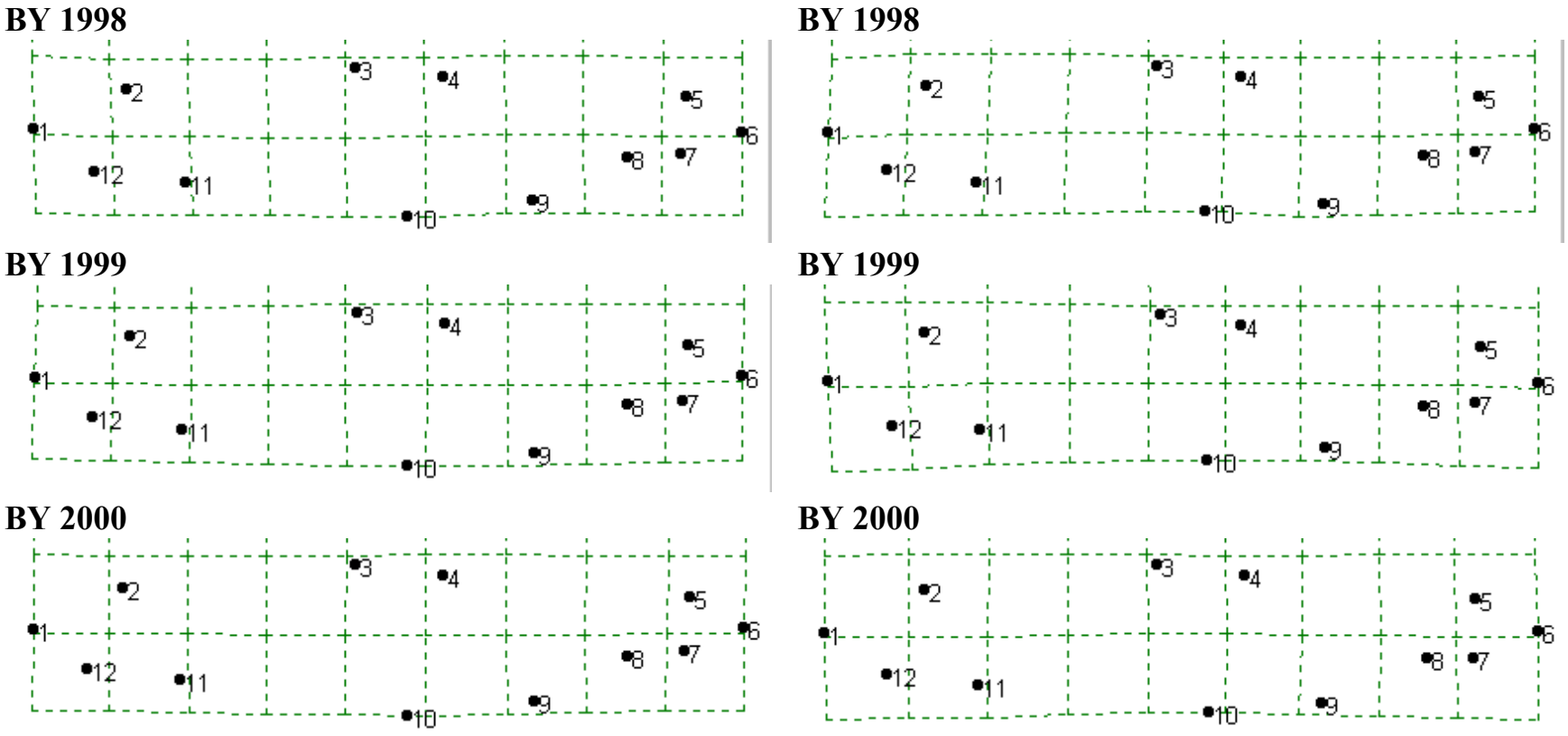

Figure 3

Wild Males

Hatchery Males

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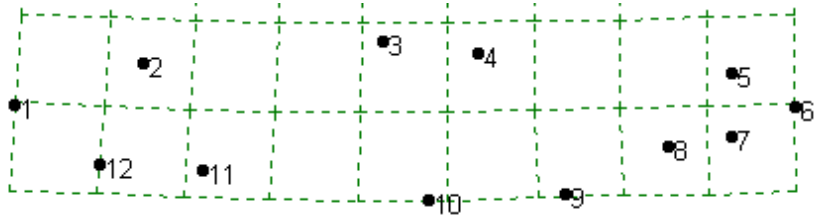

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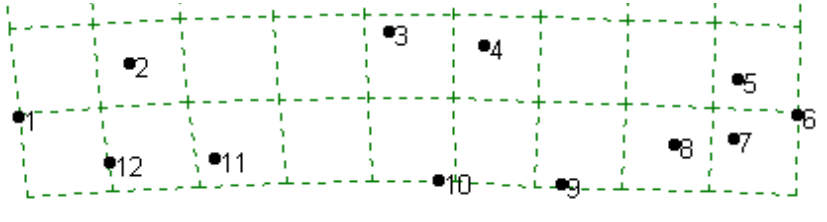

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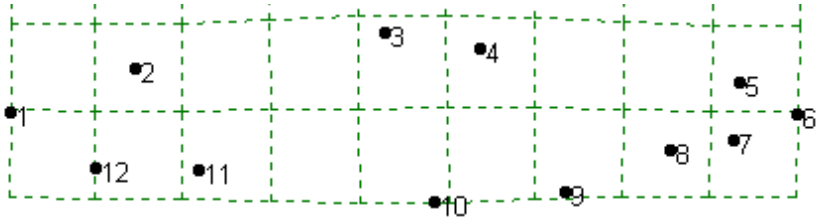

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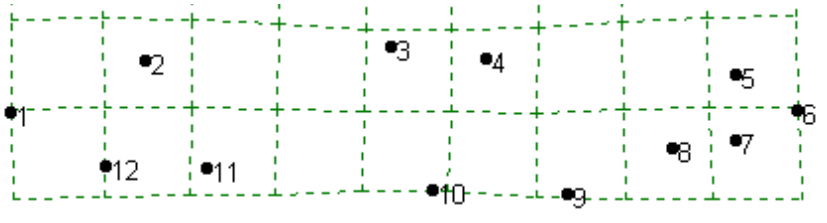

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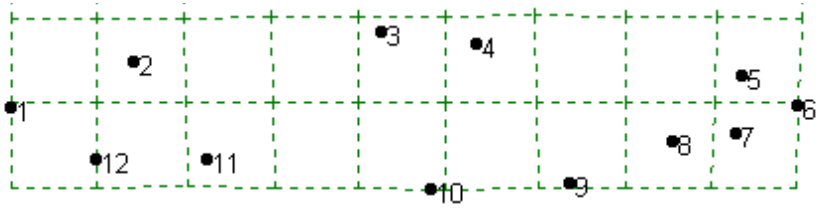

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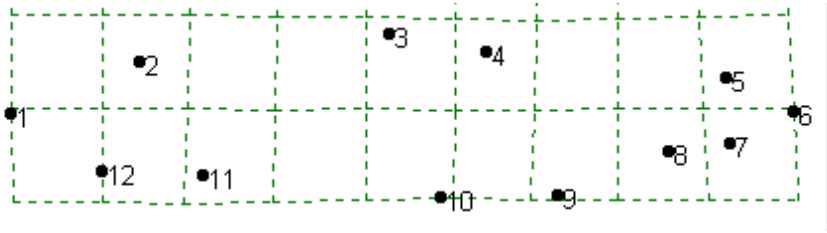

Figure 4

Chapter 7

Natural Production and Domestication Monitoring of the Yakima Spring Chinook Supplementation Program: December 2005 Revision

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Abstract

An expanded domestication monitoring plan for Upper Yakima spring chinook was developed in 2002 in response to concerns raised by the ISRP. The plan was first described in 2002, then somewhat revised in subsequent years. Here we present a major revision, with much expanded detail on protocols and analyses for all traits and incorporation of results to date. More important, however, are revisions to include two major efforts to address concerns raised in a recent issue paper on supplementation monitoring by the ISRP/ISAB (2005) and a comprehensive overview of supplementation by Goodman (2004). The first is a pedigree study called *Target Population Natural Replacement Rate,* in which the reproductive success in the wild of natural-origin and hatchery-origin fish can be compared. The second critical change is an expansion of trait A1, now called *Productivity: Female Recruits Produced per Naturally Spawning Female.*

Introduction

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

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

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

Experimental Lines and General Hypotheses

A. *Supplementation line (S):* the Upper Yakima spring chinook population, supplemented annually by production from 16 raceways at CESRF and associated acclimation sites at Jack Creek, Easton, and Clark Flat. Broodstock collection is at the Roza Adult Monitoring Facility (RAMF) at Roza Dam. In contrast to most hatchery programs,

broodstock are collected randomly throughout run, and consist of 100% natural origin fish. Other aspects of the program are as already described in numerous project documents.

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

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

By comparing the supplemented line to both controls, we will address two key questions: 1) how much domestication is incurred by a population undergoing YKFP-style supplementation?; 2) how much less domestication is incurred under YKFP-style supplementation than would be incurred under continuous hatchery culture?. As already mentioned, because the wild control line is not an internal control we know at the outset that there will be differences in mean performance at several traits. As supplementation proceeds, if there is no discernible effect of domestication, the differences in mean trait values between the two lines should not change except for random fluctuations. If domestication does occur, however, the S line means will change and should continue to change over generations as domestication changes proceed directionally. The net effect will be a trend of increasing or decreasing differences between the supplemented and wild control line over generations. Comparisons between the hatchery control and

supplemented lines will be somewhat different. Performance in the two lines should be equivalent initially because the hatchery control is an internal control. If domestication does not occur, performance of the two lines should remain the same except for random fluctuations and a small amount of drift due to the relatively low effective size of the hatchery control line. If domestication does occur, both lines will be affected, and the hatchery control line should be more affected. Thus performance at any trait should change in the same direction in both lines, but change should be greater in the hatchery control line. The rate at which the two lines diverge will be a reflection of the extent to which domestication can be retarded by the regular cycling of hatchery fish into the wild environment facilitated by the exclusive use of natural-origin broodstock. Details on expectations for individual traits are found below.

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

Experimental Power Concerns

Hatchery Ancestry and Power

The fact that the Yakima spring chinook program has complete control over broodstock composition and has a policy of 100% natural-origin broodstock makes this a well controlled, low variability system for monitoring cumulative effects of hatchery operations. We will deal first with the issue of control of hatchery effects. Simple modeling based on Ford (2002) and Lynch and O'Hely (2001) shows that the genetic dynamics of an integrated hatchery program is controlled by two gene flow rates: the proportion of natural-origin fish in the hatchery broodstock (pNOB), and the proportion of natural spawners comprised by hatchery-origin fish (pHOS). The proportion of time the population spends in the hatchery, called proportionate hatchery influence (PHI) is given by $PHI = \frac{pHOS}{pHOS + pNOB)}$. Simulations of integrated systems show that after the initial generation or two, the rate of increase of hatchery ancestry (generations of exposure to the hatchery environment) in the natural-origin fish in the population is equivalent to the program's PHI. For a program like the Yakima spring chinook program, in which all broodstock are natural-origin fish (pNOB=1.00) and the proportion of hatchery-origin fish on the spawning grounds is approximately 50% (pHOS=0.5), PHI=.33. ISRP/ISAB stress the need for control of the proportion of hatchery fish on the spawning grounds, something that is typically unacceptable to project managers. It is important to point out in this regard that although the Yakima spring chinook has no control of hatchery fish on the spawning grounds except for a small selective sport fishery, because of the natural-origin only broodstock rule, the PHI of the population is likely to fluctuate only between 0.33 (pHOS=0.5), and 0.44 (pHOS=0.8). Any other program having a fixed pNOB will have a similarly limited PHI range, but fixed-PHI

programs are rare. Thus even without explicit controls on pHOS, the Yakima spring chinook program is fairly well "controlled".

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

Precocious Males

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

Selective Fishery Impacts

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

SH and HC recoveries at RAMF to determine if selection is occurring and adjust our RAMF recovery data accordingly.

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

Trait, Protocol, and Analysis Overview

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

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

The number of traits to be evaluated can be misleading. Many of the traits are measured on the same fish with no difference in protocol except for the measurement. Thus, the "effective" number of traits in terms of logistics and cost is considerably lower. The best example of this is the set of traits A7-A9, which are all measurements of reproductive traits on the same specimens. We list the measurements as separate traits because we consider them all important, and because we want to insure they are all done. Some traits require considerable effort and cost, whereas others will be measured in the course of ordinary fish culture operations. Our guiding philosophy was to take advantage

of the opportunities offered by the CESRF and other facilities in the basin to measure as many traits relevant to domestication as feasible while minimizing impacts to the supplementation effort and the wild control population.

Nomenclature for Experimental Groups

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

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

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

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

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

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

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

Table 7. Tasks required for use in the adult and juvenile domestication traits.

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

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

2001 – A10, A11, J3, J4.
2002 – A1, A3-A9, J5, J6, J9, J11, J12.
2003 – A12, J13 (HC, SH), J14 (HC, SH).
2004 – J1, J2.
2005 – J13 (HC, SH, WC), J14 (HC, SH, WC).
2006 – A2 (proposed), A13, A14, J7, J8, J10, J15.

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

A2 – Data collection over two or more generations with the possibility of some flexibility as to how many years within a generation need to be sampled. Analysis can occur later as funds become available.
A13 – Annually for four years.
A14 – Annually for four years.

A1. Productivity: Female recruits produced per naturally spawning female *(revised 12/21/05)*

Background and Justification

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

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

Groups Compared WC, SN, and SH

Protocol

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

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

Expectations/Hypotheses

If domestication does not occur, differences in productivity of naturally spawning females among groups will remain constant over time after adjusting for inter-annual density effects. Conversely, if domestication does occur we would expect the productivity of SH females naturally spawning to decrease over time reducing the productivity of the aggregate mixture of naturally spawning

females. The reduction will be a function of the effects of domestication and the proportion of SH females on the spawning grounds. Thus, the proportion of females of SH origin naturally spawning each year must be estimated. HC fish will be intercepted at RAMF and not allowed to naturally spawn.
Analytical/Statistical Methods and Issues
Within brood years no statistical analysis will be done. However, over brood years, analysis of covariance will be used to evaluate differences in trends. Trend analysis will take into account year-to-year spawner density effects and the proportion of SH females on the spawning grounds.
Findings To Date
No analyses have been completed to date. This productivity metric has just been added to the Domestication plan.

A2. Target Population Natural Spawning Replacement Rate

(revised 12/21/05)

Background and Justification

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

Part B. Genetic Decline in Fitness

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

Location(s) RAMF

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

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings to Date

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

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

Background and Justification

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

Location RAMF, CESRF, Naches spawning grounds

Groups Compared WC, HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

No significant differences were observed between SH and SN origin adults based on age composition within return years 2001 to 2004 (X^2-test; $p>0.05$; Knudsen et al. 2002, 2003, 2004, 2005), except 2003 (X^2-test; $p<0.01$), primarily due to a strong component of SN age-4's and SH age-5's. This indicates that supplementation has not significantly reduced the age-of-maturity of SH returns based on analysis of return years. WC returns were significantly older on average compared to SN returns (X^2-test; $p<0.01$; Knudsen et al. 2002, 2003b, 2004b, 2005b; Knudsen et al. in prep.). The age composition data are currently being reanalyzed on a brood year basis with the expectation that variance between years will decrease and the power to detect differences between populations and over time will increase.

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

Background and Justification

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

Location RAMF, CESRF, and Naches spawning grounds

Groups Compared WC, HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

SN origin adults have been larger than SH adults each year between 2000 and 2004 (Knudsen et al. 2002; Knudsen et al. 2003b, 2004b, 2005). SN age-3 returns were significantly larger than SH returns each year, as well (mean difference = 2.7 cm and 0.3 kg; ANOVA; $p<0.01$). Age-4's were significantly larger in 2001, 2002, 2003 and 2005 (mean difference 1.7 cm and 0.3 kg, ANOVA; $p<0.01$). SN origin age-5 returns were also significantly larger (mean difference 2.7 cm and 0.8 kg, ANOVA; $p<0.01$). There were no significant temporal trends in size over the period 2001 to 2005 for SN and SH returns (ANCOVA slopes = 0; $p>0.13$; Knudsen et al. 2005).

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

Background and Justification

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

Location RAMF, CESRF, and Naches spawning grounds

Groups Compared WC, HC, SN, and SH

Protocol

Protocol same as for trait A3 (same fish)

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

Based on return year data gathered so far, there have been no significant differences between SH and SN origin adult fish between 2001 and 2004 (X^2-tests; $p>0.213$; Knudsen et al. 2002, 2003b, 2004b, 2005). We are in the process of reanalyzing these data based on broodyear, rather than return year.

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

Background and Justification

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

Location RAMF

Groups Compared HC, SN, and SH

Protocol

Fish moving through the Roza Adult Monitoring Facility (RAMF) will be inspected for tags and marks making it possible to record the origin and date of passage of each fish.

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

Significant differences have been observed in the temporal distributions of SH and SN fish passing Roza Adult Monitoring Facility (Kruskal-Wallis ANOVA; $p<0.05$; Knudsen et al. 2006), but there is no consistent trend in these temporal differences. However, both SH and SN returns have shown consistent significant differences in median passage timing at RAMF by age (i.e., age-5 earliest<age-4<age-3 19 days later; Kruskal-Wallis ANOVA $p<0.05$; Knudsen et al. 2006). Four-yr-old HC fish will arrive at Roza in 2006 so their arrival dates will be included in future analyses.

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

Background and Justification

When spring Chinook reach maturation and spawn is strongly affected by the water temperatures they encounter and the water temperatures their offspring are likely to experience (Brannon et al. 2004). Clearly, time of spawning is a fitness related trait as the offspring of fish that spawn too early or late can suffer significant incubation and post-emergence mortality (Brannon 1987; Hendry et al. 1998; Smoker et al. 1998; Einum and Fleming 2000). We have found that natural spring Chinook populations in the Yakima River Basin exhibit differences in spawn timing that have evolved to maximize fitness (Knudsen et al. in prep.). Given this situation, an obvious question to ask is whether exposure to hatchery conditions will alter traditional maturation timing in Yakima spring Chinook. As in many of the other adult traits examined, the presence of HC, SH, SN fish as well as natural controls, will allow this question to be addressed.

Location CESRF, Upper Yakima and Naches spawning grounds

Groups Compared WC, HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

SH fish matured 7 days earlier than SN fish on average at CESRF in 2001, 2002 and 2004 (Kruskal-Wallis ANOVA; $p<0.01$; Knudsen et al. 2002, 2003b, 2004b, 2005b). In contrast, there were no significant differences in carcass recovery temporal distributions each of these years (Kruskal-Wallis ANOVA $p>0.48$; Knudsen et al. 2006). In 2006, 4-yr-old HC fish will return to the Yakima and their maturation timing will be used to examine how two generations of hatchery exposure may have affected this trait.

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

Background and Justification

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

Location CESRF

Groups Compared HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

Based on differences in size-at-age and fecundity/size relationships, the fecundities of age-4 SH females were lower than SN origin females by 8% on average. Due to low sample sizes, estimates of age-5 fecundity were of poor quality. No significant differences between body size/fecundity relationships of SH and SN origin females were observed (ANCOVA equal slopes; $p>0.15$; Knudsen et al. 2002, 2003c, 2004c, 2005d). Fecundity has decreased at a linear rate of approximately 70 eggs/year in both SN and SH age-4 females (Knudsen et al. in prep.). In SH females, this linear relationship was not significant ($p=0.081$), while in SN females it was ($p<0.001$), but the SN regression explained only 1% of the total variation in fecundity over time.

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

Background and Justification

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

Location CESRF, Naches spawning grounds

Groups Compared WC, HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

No consistent difference in mean egg weights of SH and SN origin females has been observed (Knudsen et al. 2002, 2003c, 2004c, 2005d, in prep.). In both SN and SH females, eggs of age-5 fish are significantly larger than age-4's (ANOVA; $p<0.01$). Trends in age-specific egg weights over time were not significant for either group ($p>0.35$). Eggs of WC females are significantly heavier than eggs of SH and SN females of the same size ($p<0.05$; Knudsen et al. 2005d). In 2006, 4-yr-old females from the HC line will be available for the first time making it possible to evaluate how two generations of exposure to hatchery conditions may affect egg size.

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

Background and Justification

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

Location CESRF

Groups Compared HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

From 2001 through 2005 there were no differences between SH and SN origin age-4 females (2-way ANOVA; Origin effects p=0.64; Knudsen et al. 2002, 2003c, 2004c, 2005d, in prep.). Male RE exhibited no significant difference between SH and SN fish in 2003 (p=0.54; Knudsen et al. 2004c). The trend over time (2001 to 2005) in age-4 female RE was positive and significant (p=0.01; Knudsen et al. in prep.), but explained less than 1% of the total variation in RE over time.

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

Background and Justification

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

Location CESRF

Groups Compared HC, SN, and SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

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

A12. Adult morphology at spawning *(revised 12/21/05)*

Background and Justification

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

Location(s) CESRF and possibly some effort on Naches spawning grounds

Groups Compared WC,HC,SN, SH

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Progress to Date

Initial analysis showed males to differ significantly from females, hatchery-origin fish of both sexes to differ significantly from natural-origin fish, and Naches fish of both sexes to differ significantly from Upper Yakima fish (Busack et al. 2004). A more in depth comparison of hatchery-origin and natural-origin Upper Yakima fish over three return years revealed significant differences between sexes, origins, and years (Busack et al. 2005), but comparison of shapes revealed digitizing problems at one landmark. Fish have been redigitized and reanalysis is underway. We intend to produce a MS for publication on this work.

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

Background and Justification

A critical assumption associated with supplementation is that hatchery-origin adults possess behavioral traits that allow them to spawn under natural conditions at a level that is comparable to natural-origin fish. Previous work that examined the spawning behavior of wild and first generation hatchery spring Chinook at CESRF showed that hatchery-origin fish were not as successful at producing offspring as wild fish. Such a comparison does not allow the relative importance of environmental and genetic effects to be evaluated. Here the reproductive behavior of first- and second-generation hatchery spring Chinook will be compared. In this instance, the early-life history of the fish will be similar (both will have been reared in a hatchery) and thus any differences observed can be attributed to genetic changes caused by inadvertent domestication. Such differences can also be linked to a single generation of additional exposure to hatchery conditions. Documenting the magnitude of any genetic changes observed will significantly increase our understanding of the biological costs associated with supplementation programs that rely on hatcheries prior to release.

Location Observation stream located at the Cle Elum Supplementation Research Facility

Groups Compared HC and SH

Protocol

Homogenous spawning populations consisting of pure SH or HC adults will be introduced into 4.9 m wide by 15.2 m long sections of an observation stream while still in an immature state. Fish will be weighed, measured, tagged, and DNA sampled prior to being liberated into their designated locations. The fish placed into the observation stream will be observed multiple times per day until they perish. Eight fish of each sex will be placed into each section and all sections will be filled on the same day. Behavioral traits measured in females will include total life time in the observation stream (longevity), date they became territorial, length of time they were sexually active, length of post-spawn guarding period, number of redd locations, location of their redds in relationship to water depth and velocity, redd size, redd tenure, and egg retention at death. Male traits will include longevity, multiple assessments of social status, their color patterns, and frequency of agonistic and courting behavior.

Expectations/Hypotheses

Second generation hatchery fish are expected to be less competent at spawning than first-generation or SH individuals. Fleming et al (1996) and Fleming et al (2000), for example, found that fifth generation hatchery Atlantic salmon were 20 to 40% less effective than wild cohorts at reproducing under natural conditions. If significant domestication occurs, second generation hatchery females are expected to have shorter life-times, greater egg retentions, marginal redd locations, and are more likely to abandon or be evicted from their redds. Second generation males are also expected to have shorter life-times and to be less aggressive toward rivals and less attentive to sexually active females. Fleming et al. (1996) discovered that hatchery Atlantic salmon males ignored key behavioral signals provided by females as they approached ovi-deposition and consequently their ability to fertilize eggs was severely compromised.

Analytical/Statistical Methods and Issues

Both non-parametric and parametric analyses will be utilized. For example, longevity data will be ordinal in nature because of how it is collected and consequently Kruskal-Wallis one-way ANOVAs or Mann-Whitney U tests will be used to examine whether differences exist in the longevity of SH and HC adults. In those instances where the response variable is at the interval or ratio scale, nested ANOVAs will be employed. The fixed treatment in these analyses will be adult type (SH or HC), the first order group would be the year that the experiment was performed, subgroups would be the sections in the observation stream that were used, while the items in the subgroups would be values obtained from the individuals placed into a section. An example of this type of analysis would be a comparison between the activity levels of SH and HC males. The random variable in this case would be the number of times a male was observed courting females or fighting rivals divided by the total times he was observed. Work by Fleming et al (1996) would suggest that HC males are more likely to be passive and therefore this ratio would be lower in HC males than in SH individuals. The Nested ANOVA design would be used to test this expectation

after the arc sin square root transformation was used on the raw data.

Sokal and Rohlf (1995) suggest that variance estimates at each level of a nested ANOVA be used to help design an experiment. Previous work in the observation stream indicates that the greatest variation is likely to occur among individuals placed into each section of the channel. Although not as variable, year-to-year differences in water temperature and other environmental factors may also occur. Finally, the least variable of the factors examined will likely be the sections used since they will all possess the same gravel, water flow, and fish densities. Consequently we will maximize the number of individual fish placed into each section. For females, that appears to be between 8 and10 individuals. When numbers get higher than this intrasexual competition among females becomes intense enough to prevent some of them from spawning. For males this number could be higher, however, we have decided to release 8 fish of each sex into every channel section. Results from the first year of this study will be used to make any necessary adjustments in fish numbers.

Findings To Date

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

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

Background and Justification

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

Location(s) Observation stream located at the Cle Elum Supplementation Research Facility

Groups Compared HC and SH

Protocol

Homogenous populations consisting of pure SH or HC spring Chinook adults will be introduced into 4.9 m wide by 15.2 m long sections of an observation stream just prior to becoming mature. Fish will be weighed, measured, tagged, and DNA sampled prior to being liberated into their designated sections. Eight fish of each sex will be placed into each section and all sections will be filled on the same day. The fish in each section will be allowed to spawn naturally. An estimate will be made of the fecundity of each female to predict her potential egg deposition (PED) and her actual egg deposition (AED) will be estimated by subtracting any eggs she retained at death from her predicted fecundity. Modified fyke nets with floating live boxes will be installed at the end of every section to capture juveniles as they emerge and begin to migrate downstream. The fry traps will be checked daily, the number of fry caught will be counted and 10% of them will be preserved in 100% ethanol for later micro-satellite DNA analyses. At the end of the emergence period, electro-shocking gear and seines will be used to remove any remaining juveniles. A pedigree analysis will be performed using DNA samples from the adults and juveniles to estimate the number of offspring each adult produced. Results from the pedigree assessments will allow us to estimate the egg-to-fry survival rates (both PED and AED) of each female placed into a channel section. The capacity to produce offspring depends on the ability of females to choose appropriate nest sites, to construct and guard their nests, and on the ability of males to successfully match their gamete releases to when a female spawns her eggs. If either sex is unable to complete a specific series of tasks productivity will decrease. That is why we will be looking at two egg-to-fry survival measures. The first one (PED-to-fry survival) is a measure of how successfully a female was able to convert the eggs she brought into a spawning ground to fry. The second one (AED-to-fry survival) looks at how successful the eggs deposited by a female are converted into juveniles.

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

Mixed model Nested ANOVAs will be used to compare the productivity of HC and SH

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

The number of adults that will be placed into each population was based on previous studies in the observation stream from 2000-2005. This work suggests that eight spring Chinook females are able to spawn simultaneously in 4.9 m wide x 15.2 m long stream sections. When higher numbers are present, significant intrasexual competition among females for space occurs. In most instances, instantaneous densities of spawning females in supplemented populations will be low. Consequently, eight females represent a compromise between the need for replication and the desire to mimic natural spawning densities. Refinements to nested ANOVA designs are based on assessments of how much variation exists in each of the random groups and subgroups. As in the Trait 12A we expect that the most variable portion will be the individual values obtained from the females placed into the observation stream. If necessary, adjustments to the number of fish used in each population will be made after the first study year has been completed. For example, up to twelve females could be placed into each section. However, at these loading densities, a number of females may be prevented from spawning or might only be able to partially spawn. Consequently the desire for replication would actually increase variance and subsequently reduce power.

Findings To Date

Beginning in 2001 we created heterogeneous populations of wild- and hatchery-origin spring Chinook and allowed them to spawn naturally in the observation stream. Micro-satellite DNA was used in pedigree analyses to estimate the number of offspring each adult produced. These assessments showed that reproductive success in males is often twice as variable as that found in females. For example, the coefficient of variation in male success ranged from 90 to 200% whereas for females it varied from 34% to 77%. In the five populations where pedigree information is available, wild-origin males (ages 4 and 5) produced the most offspring. Comparable hatchery males were on average 85% as effective at producing offspring. Hatchery and wild males maturing as 3-yr-olds (jacks) and as 1- and 0-yr-olds (precocious males) were also included in these populations. In general jacks were 32% and precocious males 17% as effective as wild 4 and 5-yr-old males. In four of the five populations we have pedigree data for, females were allotted about 12 square meters of space. Under this density, hatchery-origin females were less successful at depositing eggs (4 to 18%) and the eggs they did deposit had lower egg-to-fry survival rates (3 to 10%). In three other populations, females were allotted over 20 square meters to determine if reduced instantaneous densities would enhance the ability of hatchery females to produce offspring. Pedigree results from one of these populations have been analyzed. In this low-density population, hatchery females were more successful at depositing their eggs (+12.5%) and achieved comparable AED-to-fry survival rates. Whether, this is a consistent phenomena remains to be seen. Looked at it holistically, it appears that first generation hatchery fish are slightly less capable of producing offspring than comparable wild fish when allowed to spawn under natural conditions. The goal of work described above is to determine how much of an effect on reproductive success will occur when fish are subjected to another generation of hatchery exposure. For a complete description of these results *see* Schroder et al. 2003a, 2003b, 2004, and 2005.

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

Background and Justification

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

Location Cle Elum Supplementation and Research Facility incubation room

Groups Compared WC_p, SH, SH_p, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

Results from 2002 and 2003 were reported in Knudsen et al. 2003c and 2004c. However, due to problems with uncontrolled water temperatures during those years we believe our earlier analyses were compromised. We have now developed a method to control water temperature across vessels using a single mixing head box delivery system.

J2. K_D at emergence *(revised 12/21/05)*

Background and Justification

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

Location Cle Elum Supplementation and Research Facility incubation room

Groups Compared WC_p, SH, SH_p, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

There was a significant positive relationship between KD values and egg weight for both SH and SN fry ($R2>0.42$, $p<0.001$; Knudsen et al. 2003c, 2004c, 2005d). The ANCOVA of KD and Egg weight for 2002, 2003 and 2004 all showed that SH and SN relationships had equal slopes ($p>0.26$), but significantly different means adjusted for egg weight ($p<0.02$; Knudsen et al. 2003c, 2004c, 2005d). The differences in KD means are very small and may not be biologically meaningful. However, SH origin samples (KD means ranged from 1.911 to 1.916) were consistently greater than SN samples (KD means ranged from 1.892 to 1.895). Analysis of temporal trends has not been completed.

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

Background and Justification

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

Location Cle Elum Supplementation and Research Facility incubation room

Groups Compared SH, SH_p, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

In comparisons of SH and SN origin single-pair inter se matings there have been no significant differences in egg-to-fry survival between groups (Kruskal-Wallis ANOVA; $p>0.13$; Knudsen et al. 2003c, 2004c, 2005d). Analysis of temporal trends has not been completed.

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

Background and Justification

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

Location Cle Elum Supplementation and Research Facility incubation room

Groups Compared SH, SH_p, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

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

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

Background and Justification

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

Location Cle Elum Supplementation and Research Facility

Groups Compared SH and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

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

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

Background and Justification

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

Location(s) HC Acclimation site

Groups Compared ***SH, HC***

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings to Date

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

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

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

Background and Justification

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

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

Groups Compared a) HC and SH from Clark Flats
b) SN, SH, HC from RAMF to Chandler, McNary, and John Day Dams
c) SH, HC, SN, and WC from Chandler to McNary and John Day Dams

Protocol

a) HC and SH pre-smolts reared at Clark Flats will receive PIT tags prior to being released. PIT tag detectors will monitor their passage through Chandler, McNary, and John Day dams. Tag recovery will be downloaded and analyzed to compare the survival rates of HC and SH smolts.
b) A sub-sample of SN, SH, and HC fish will receive PIT tags at Roza (RAMF). Survival rate comparisons of SN, SH, and HC fish will only occur among individuals that passed through the Roza juvenile trap during the same time period. WC smolts do not migrate past the RAMF and therefore will not be included in this analysis.
c) Additional fish will be tagged at Chandler, including Naches and American smolts (identified by DNA micro-satellites). Comparisons of survival rates among these fish will be based on PIT tag recoveries at monitoring sites located at McNary, John Day, and any other suitably equipped downstream sites.

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

None.

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

Background and Justification

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

Location Chandler Smolt Facility

Groups Compared WC, SN, SH, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

No data have yet been collected for this trait

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

Background and Justification

Previous studies (Fleming and Petersson 2001; Fleming et al. 1996; Fleming et al.1997; Fleming et al. 2000) have shown that populations that have been repeatedly recycled through a hatchery are more likely suffer from inadvertent domestication than those that have not been continuously exposed to hatchery conditions. Moreover, salmonids with prolonged hatchery rearing periods are more likely to undergo domestication than those that are reared for shorter periods. Because spring Chinook are kept in culture for over a year they may be susceptible to inadvertent domestication, particularly if they are continuously recycled back into a hatchery environment. In this trait, we examine whether the smolt-to-adult survival of HC fish differs from SH individuals. Any difference detected will reflect a genetic change caused by hatchery exposure as both populations will have been incubated and reared in comparable hatchery environments prior to release into the upper Yakima River.

Location Clark Flat Acclimation Site to RAMF

Groups Compared SH and HC

Protocol

Prior to release, every SH and HC fish will be tagged so that its origin can be identified. An estimate of the number of smolts leaving each raceway will be made via continuous PIT tag monitoring. The numbers of adult fish produced from each raceway returning to Roza will be recorded by inspecting fish for tags and marks. Scale samples will be taken to assign an age to each returning adult. The survival of fish by age class will be calculated for each raceway by broodyear. This will be done by dividing the number of 3, 4, or 5 year-olds originating from a raceway/broodyear combination by the total number of fish released from that raceway. WC fish will not be included for reasons outlined under J5.

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

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

J10. Smolt out-migration timing and rate *(revised 12/21/05)*

Background and Justification

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

Location From the Clark Flat Acclimation site to downstream monitoring sites

Groups Compared SN, SH, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

None, study will begin in 2006

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

Background and Justification

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

Location(s) Cle Elum Supplementation and Research Facility and smolt acclimation sites

Groups Compared SH and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings to Date

Data are available, but have not yet been analyzed.

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

Background and Justification

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

Location CESRF and smolt acclimation sites

Groups Compared SH and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings To Date

ANCOVA of 2002 and 2003 juvenile weights and lengths collected during mid-January showed there were significant interaction effects when both Low and High growth SN and HC groups were analyzed together. When the data were analyzed separating the two growth treatment groups the SN and HC fish had the same length/body weight slopes in all comparisons ($p>0.09$). In both 2002 and 2003 samples, after accounting for differences in body length, there was no significant difference between the SN and HC High growth groups ($p>0.241$). However, there were significant differences in means of SN and HC Low growth groups ($p<0.001$). In both years, Low growth HC fish were heavier than SN fish at a standardized length.

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

a) Contest competition
b) Scramble competition
c) Aggression

Background and Justification

Competition and aggression has been demonstrated to be influenced by domestication

Location(s) Cle Elum Supplementation and Research Facility

Groups Compared WC_p, SH, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings to Date

Offspring of wild origin fish dominated 4% more contests than offspring of hatchery origin fish ($P \leq 0.05$). Dominance was not significantly different in the scramble competition trials ($P > 0.05$). Wild fish initiated more agonistic interactions than hatchery fish in both contest and scramble trials. There were no differences in the frequency of different types of agonistic interactions that were used by hatchery and wild fish. We also found that dominant fish grew more than subordinate fish in both contest and scramble trials ($P \leq 0.05$). Detailed descriptions can be found in Pearsons et al. (2004 and 2005).

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

Background and Justification

Predation has been demonstrated to be influenced by domestication

Location(s) Cle Elum Supplementation and Research Facility

Groups Compared WC_p, SH, and HC

Protocol

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

Expectations/Hypotheses

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

Analytical/Statistical Methods and Issues

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

Findings to Date

There was no significant difference in survival between the SH and HC fry during 2003 (P=0.051) or 2004 (P=0.122). SH fry were found to have a 2.15% survival advantage over HC fry when 2003 and 2004 data were combined to increase statistical power (P=0.016). Detailed description can be found in Pearsons et al. (2004 and 2005). Trials for the 3-line comparison were conducted in 2005 and results will be available in the spring of 2006.

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

Background and Justification

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

Location(s) Clark Flat Acclimation site

Groups Compared SH and HC

Protocol

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

Expectations/Hypotheses

If domestication does not occur, we would expect HC and SH fish to have equivalent rates of precocial development. If domestication does occur, we would expect HC fish to have a lower incidence of precocialism.

Analytical/Statistical Methods and Issues

Within year analysis will use one-way ANOVAs. Analysis of covariance will be used to ascertain if trends in the production of precocious males in these two lines diverge over time

Findings to Date

Mean precocity rates of male progeny from first generation hatchery parents were 14% (brood year 2002) and 11% (brood year 2003). Mean precocity rates of male progeny for natural origin parents were 40% (brood year 2002) and 21% (brood year 2003).

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