A Microsatellite DNA Analysis of Snake River fall-run Chinook (2002 & 2003)

By

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Introduction

Genetic characteristics of Chinook salmon within the Snake River and Columbia River basins have been examined extensively (Blankenship et al. 1997, Blankenship and Mendel 1994, Bugert et al. 1995, LaVoy and Mendel 1996, Marshall et al. 1995, Marshall et al. 2000, Utter et al. 1982, and Utter et al. 1995). A spring/summer-run of Chinook salmon and a fall-run were determined to be in separate ESUs (Waples 1991) and Snake River fall-run Chinook salmon were listed as threatened under ESA in 1992 (NMFS 1992). Management and conservation of these stocks have, therefore, been of interest to biologists in the Snake River Basin.

Returns of Chinook salmon trapped at Lyons Ferry Hatchery (LFH) include adipose clipped CWT fish that are determined to be hatchery broodstock, unmarked/untagged fish that volunteer to the hatchery, and marked/tagged strays from other hatcheries. The unmarked/untagged fish could be of hatchery origin or naturally reared origin. Reading scales allows biologists to differentiate hatchery-produced from naturally produced ("wild") fish but will not determine the specific origin of those hatchery fish because of similar sizes at release and scale patterns. Straying of hatchery origin salmon into the Snake River has been documented at Lyons Ferry Hatchery (Milks et al. 2003, Bugert et al. 1991). Scale patterns also allow for the identification of Chinook released from the hatchery as subyearlings and yearlings (Connor et al. In Press).

The unmarked/untagged hatchery origin subyearling Chinook that return to Lyons Ferry Hatchery are thought to be predominantly from the Nez Perce Tribe (NPT) acclimation sites (Lyons Ferry Hatchery origin fish; Debbie Milks,

WDFW personal communication). The unmarked/untagged hatchery origin yearling Chinook that return to Lyons Ferry Hatchery (included in the samples from 2002 and 2003) are thought to be out-of-basin strays because all of the yearling releases from Lyons Ferry Hatchery are adipose clipped, coded wire tagged, and VIE (visual implanted elastomer) tagged.

In 2001 and 2002, the run of fall Chinook at Lower Granite Dam, in conjunction with large steelhead runs, effectively shut down the adult trap at times, which allowed hatchery origin stray fish to pass the dam. As a result, it is unknown at what level strays have been infused into natural production in the Snake River Basin. Historically, the Umatilla Hatchery program was the major contributor of stray fall-run Chinook to the Snake River. Genetic comparison of the Umatilla Hatchery broodstock to the Lyons Ferry Hatchery broodstock would help determine how effectively the Lyons Ferry Hatchery program is maintaining the genetic integrity of the Snake River stock. Additional analysis of the naturally produced Chinook collected at Lower Granite Dam and of the Umatilla Hatchery broodstock would indicate if strays from the Umatilla Hatchery are impacting the naturally spawning Snake River stock.

A growing number of studies have used variation at microsatellite DNA loci to investigate stock structure (Small et al. 1998, Beacham et al. 1999, Shaklee et al. 1999, Balloux and Lugon-Moulin 2002, Beacham et al. 2003, and Beacham et al. 2004). Microsatellite markers typically exhibit high numbers of alleles and high heterozygosities, and are, therefore, statistically powerful markers to characterize stocks, estimate interrelationships among populations,

and analyze mixtures. Microsatellite loci are tandemly repeated arrays of short (commonly di-, tri-, and tetra-nucleotide) sequences and are considered to be non-coding in that they do not encode RNA or proteins, and, therefore, are assumed to be selectively neutral.

Because these DNA markers offer the potential of higher resolution analyses, WDFW initiated a study of microsatellite DNA variation in the Snake River fall-run Chinook to characterize groups of fish relevant to the Lyons Ferry Hatchery: Lyons Ferry Hatchery broodstock, unmarked/untagged adults from yearling and subyearling releases that volunteered to Lyons Ferry Hatchery, unmarked/untagged adults of natural origin from collections at Lower Granite Dam in 2002 and 2003, and Umatilla Hatchery broodstock to conduct the following analyses:

a. Pairwise analyses from collections made in 2002: adults from Lyons Ferry Hatchery (LFH) broodstock, unmarked/untagged hatchery adults volunteering to Lyons Ferry Hatchery (yearling and sub-yearling releases), and adults of natural origin sampled at Lower Granite Dam (LGD).

b. Pairwise analyses from collections made in 2003: adults from Lyons Ferry Hatchery (LFH) broodstock, unmarked/untagged hatchery adults volunteering to Lyons Ferry Hatchery (yearling and sub-yearling releases), and adults of natural origin sampled at Lower Granite Dam (LGD).

c. Pairwise analyses of Umatilla Hatchery broodstock 2003 to the collections made in 2002 and 2003.

Microsatellite DNA loci are valuable genetic markers not only because of their high levels of genetic variability but also because they (like other DNA markers) can be analyzed using fin clip and other non-lethal biopsy samples. Non-lethal methods may prove to be essential for this application because of the critically low abundance of the Snake River fall-run Chinook stock.

Material and Methods

Collections

In 2002, staff from Snake River Lab collected samples of Lyons Ferry Hatchery broodstock and unmarked/untagged adult volunteers to Lyons Ferry Hatchery. In addition, staff from NOAA collected scales from unmarked/untagged adults as fish were passed upstream at Lower Granite Dam (Table 1).

In 2003, the collection of LFH broodstock samples was repeated because of a change in spawning protocol to include unmarked/untagged subyearlings in LFH broodstock.

In addition, sampling was expanded to include a random sample of Umatilla broodstock. Samples consisted of operculum punches, fin clips, and scales. Tissue samples were stored in 100% ethanol, and scales were stored dry on scale cards.

DNA Extraction Methods

Genomic DNA was extracted by digesting a small piece of fin tissue or one or more scales using silica membrane based kits obtained from Clontech Incorporated using the following conditions: incubate tissue fragment or scale 6 hours to overnight at 56^{o}C in 200 µL Proteinase K solution, add 200 µL Buffer B3 and 200 µL 100% ethanol, mix and transfer the supernatant into a Tissue Binding Plate containing the silica binding membranes, centrifuge 10 minutes, add 500 µL Buffer BW, centrifuge 2 minutes, add 700 µL Buffer B5, centrifuge 4 minutes, place Tissue Binding Plate on a collection rack, incubate 10 minutes at 70^{o}C to remove residual ethanol, add 100 µL Buffer BE (elution buffer) at 70^{o}C, incubate 1 minute, centrifuge 2 minutes, dispose of Tissue Binding Plate, refrigerate eluted DNA or store at –20^{o}C.

PCR Methods

The polymerase chain reaction mixture contained the following for a 10 µL reaction: approximately 25 ng template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$,

200 µM each of dATP, dCTP, dGTP, and dTTP, 0.09 – 0.42 µM of each oligonucleotide primer (concentrations for each primer are in Table 2), and 0.05 units *Taq* polymerase (Promega). Amplification was performed using an MJ Research PTC-200 thermocycler. The thermal profile was as follows: an initial denaturation step of 3 minutes at 95^{o}C; 30 - 35 cycles of 15 seconds at 95^{o}C, 30 seconds at 50 - 63^{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.

Fifteen microsatellite DNA loci of interest were amplified via the polymerase chain reaction (PCR; see Saiki et al., 1988) using fluorescently labeled primers with vector-based tails (obtained from Applied Biosystems or Integrated DNA Technologies).

Data were collected using an ABI-3730 semi-automated sequencer. Applied Biosystems software (ABI-Collection, Genemapper v.3.0) was used to collect and analyze the raw data to determine genotypes at each locus (based on estimated size in base pairs using an internal size standard). The output tables from Genemapper were imported into MS Excel where allele calling was accomplished using size bins. Allele binning and naming were accomplished using MicrosatelliteBinner 1.f (S.F. Young, WDFW pers. com., available from the author). MicrosatelliteBinner creates groups (bins) of alleles with similar mobilities (alleles with the same number of repeat units). 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.

Statistical Methods

Tests for conformance to Hardy Weinberg expectations were calculated using GENEPOP (version 3.3, Raymond and Rousset 1995) to determine if any loci should be excluded from subsequent analyses. Pairwise tests of genotypic differentiation were calculated using FSTAT (version 2.9.3, Goudet 2001). A non-sequential Bonferroni correction for multiple tests was used to adjust alpha values to determine significance levels for the pairwise comparisons (Rice 1989)

for both the Hardy Weinberg tests and genotypic differentiation tests. The Bonferroni correction is a conservative approach to determine significance levels versus identifying all P-values less than 0.05 as significant.

Results

Two of the fifteen loci screened were excluded from any statistical analyses. One locus (Ots-G474) was not resolved for all samples and a second locus (Omy-1011) did not meet Hardy-Weinberg expectations for all collections. Observed heterozygosity ranged from 0.628 – 0.969 (Ots-9 and Omm-1080 respectively) among the thirteen loci that were scored (Table 2). The number of alleles observed ranged from 11 – 50 (Ots-9 and Omm-1080 respectively) and the observed allele size range at each locus is shown in Table 2.

Hardy Weinberg Tests

Tests for conformance to Hardy Weinberg expectations revealed few significant deviations. Deviation for Ots-201b occurred in the unmarked/untagged hatchery adults from sub-yearling releases volunteering to Lyons Ferry Hatchery 2003 while deviation for Ots-212 occurred at one collection of adults from Lyons Ferry Hatchery broodstock 2003**.**

Tests of Population Differentiation

Analyses were conducted on the Lyons Ferry Hatchery broodstock, hatchery unmarked/untagged adults (subyearlings and yearlings) volunteering to Lyons Ferry Hatchery, and unmarked/untagged adults at Lower Granite Dam. Analysis of the hatchery unmarked/untagged adults (yearlings) volunteering to Lyons Ferry Hatchery included samples sizes that were small in both 2002 (N = 17) and 2003 (N = 43). The results for the unmarked/untagged hatchery yearling volunteers were different for the 2002 and 2003 collections (Table 4 (A and B)). In 2002, the unmarked/untagged hatchery yearling volunteers were not significantly different from the unmarked/untagged hatchery subyearling volunteers or Lower Granite Dam samples while in 2003 they were significantly

different. All other results were consistent between the 2002 and 2003 collections. The Lyons Ferry Hatchery broodstock were significantly different from the unmarked/untagged hatchery yearling volunteers and not significantly different from the unmarked/untagged hatchery subyearling volunteers, as was expected. The unmarked/untagged hatchery subyearling volunteers were not significantly different from the Lower Granite Dam collections, also expected.

A collection of samples from Umatilla Hatchery broodstock in 2003 were compared to the Lyons Ferry Hatchery broodstock, unmarked/untagged hatchery yearling and subyearling volunteers, and Lower Granite Dam samples (Table 4 (C-1 and C-2)). The Umatilla Hatchery broodstock was significantly different from Lyons Ferry Hatchery broodstock and from unmarked/untagged hatchery subyearling volunteers while not significantly different from unmarked/untagged hatchery yearling volunteers or from the Lower Granite Dam samples.

An analysis was also conducted on a combined collection of unmarked/untagged hatchery yearling volunteers from both 2002 and 2003 to compare to Umatilla Hatchery broodstock 2003 and Lyons Ferry Hatchery broodstock 2003 (Table 4 (C-3)). The results were the same as with the individual collection of unmarked/untagged hatchery yearling volunteers from 2003. The Lyons Ferry Hatchery broodstock in 2003 was significantly different while the Umatilla Hatchery broodstock in 2003 was not significantly different.

Discussion

Genetic characterization of hatchery and natural origin fall-run Chinook salmon in the Snake River is an important component of conserving genetically different stocks in the Columbia River Basin. A management goal of the stocks within the Snake River is to allow for a sustainable and harvestable resource, while also protecting the individual genetic stocks. The microsatellite analysis of the Lyons Ferry Hatchery collections (broodstock and volunteers) in conjunction with scale analysis has provided a means to evaluate the stocking program and influence of strays on natural origin Chinook in the Snake River.

Analyses of collections from 2002 and 2003 were consistent between years. As expected the Lyons Ferry Hatchery broodstock was not significantly different than the unmarked/untagged hatchery volunteers (subyearlings) trapped at LFH. The unmarked/untagged hatchery subyearling volunteers are thought to be predominantly from the Nez Perce Tribe acclimation sites (that is, they are Lyons Ferry Hatchery origin fish). Interestingly, the Lyons Ferry Hatchery broodstock was significantly different from the natural origin Lower Granite Dam samples while the unmarked/untagged hatchery volunteers (subyearlings) were not significantly different from the natural origin Lower Granite Dam samples. The hatchery origin volunteers (subyearlings) that are unmarked/untagged could include genotypes shared with the Lyons Ferry Hatchery broodstock while having different genotypes that were shared with the natural origin samples from Lower Granite Dam. The Lyons Ferry Hatchery volunteers (subyearlings) would, therefore, not be significantly different to either the broodstock or the Lower Granite Dam samples, but those two collections would be significantly different to each other.

Analysis of the unmarked/untagged hatchery subyearling and yearling volunteers at Lyons Ferry Hatchery revealed different results for the two groups between the 2002 and 2003 collections. The analysis of the unmarked/untagged hatchery yearling volunteers from 2002 resulted in a significant difference to the Lyon Ferry Hatchery broodstock only. The different results for the two years could simply be due to the increased statistical power due to the larger sample size in the 2003 sample or due to genetic differences between the two different years' samples.

The collection of unmarked/untagged hatchery yearling volunteers is thought to consist of out-of-basin origin fish. It is not surprising then, that this group is significantly different from all of the collections in the Snake River Basin. Analyses of fall-run Chinook in the Columbia River Basin and Snake River Basin have documented genetic differences between the populations in these two basins (Marshall et al. 2000).

The Umatilla Hatchery broodstock origin is from the Columbia River, therefore the Umatilla Hatchery broodstock would be genetically different from collections in the Snake River. The Umatilla Hatchery program is considered to be the primary source of stray Chinook to Lyons Ferry Hatchery. If fall-run Chinook from the Umatilla Hatchery were straying into the Snake River and being included with the Lyons Ferry Hatchery broodstock then the two populations might be indistinguishable or at least exhibit some similarity. The Umatilla Hatchery broodstock and Lyons Ferry Hatchery broodstock were significantly different in both the 2002 and 2003 collections suggesting the infusion of strays from the Umatilla has neither swamped nor significantly altered the genetic structure of the Lyons Ferry Hatchery broodstock. The unmarked/untagged hatchery subyearling volunteers that originated from the Lyons Ferry Hatchery are also significantly different from Umatilla Hatchery again suggesting that any strays from Umatilla Hatchery have not had a large impact on the genetics of the Lyons Ferry Hatchery stock. The unmarked/untagged hatchery yearling volunteers from out of the Snake River basin and natural origin samples from Lower Granite Dam are not significantly different from the Umatilla Hatchery broodstock suggesting these samples are similar and may reflect the presence of Umatilla Hatchery progeny in these collections.

The analysis comparing the combination of unmarked/untagged hatchery yearling volunteers from both 2002 and 2003 to Lyons Ferry Hatchery broodstock from 2003 and Umatilla Hatchery broodstock from 2003 reveals a similar result for the 2003 collection to earlier analyses. The unmarked/untagged hatchery yearling volunteers are significantly different from Lyons Ferry Hatchery broodstock 2003, but not to Umatilla broodstock 2003. It appears the larger sample size from the combined collection supports the results for the 2003 collection instead of the results for the 2002 collection.

Conclusions

Snake River fall-run Chinook from Lyons Ferry Hatchery broodstock appear to be genetically distinguishable from the out-of-basin samples

(unmarked/untagged hatchery yearling volunteers and Umatilla Hatchery broodstock) that were analyzed. Chinook that volunteer to Lyons Ferry Hatchery that are from unmarked/untagged hatchery subyearling releases and identified as hatchery origin appear to be similar to Lyons Ferry Hatchery broodstock and could be used to supplement the broodstock. Identification of the hatchery or natural origin and subyearling or yearling status would be necessary for inclusion into Lyons Ferry Hatchery broodstock. Natural origin fall-run Chinook collected at Lower Granite Dam appear to have some out-of-basin influence based on the lack of difference to the unmarked/untagged hatchery yearling volunteers and Umatilla Hatchery broodstock.

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Literature Cited

Balloux, F. and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11:155-165.

Beacham, T.D., S. Pollard, and K.D. Lee. 1999. Population structure and stock identification of steelhead in southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. Transactions of the American Fisheries Society 128:1068-1084.

Beacham, T. D., K. J. Supernault, M. Wetklo, B. Deagle, K. Labaree, J. R. Irvine, J. R. Candy, K. M. Miller, R. J. Nelson and R. E. Withler. 2003. The geographic basis for population structure in Fraser River Chinook salmon (Oncorhynchus tshawytscha). NOAA Fisheries Bulletin 101: 229-242.

Beacham, T.D., M. LaPointe, J.R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L. Deng, K.M. Miller, and R.E. Withler. 2004. Stock identification of Fraser River sockeye salmon using microsatellites and major histocompatibility complex variation. Transactions of the American Fisheries Society 133:1117-1137.

Blankenship, H.L. and G. Mendel. 1994. Upstream passage, spawning, and stock identification of fall chinook salmon in the Snake River, 1992. Annual report FY 92-93 (Contract DE-BI 79-92 BP60415) of Washington Department of Fisheries to Bonneville Power Administration, Portland, Oregon.

Blankenship, H.L., and five coauthors. 1997. Stock identification of Snake River fall chinook salmon. Pages 76-95 *in* H.L. Blankenship and G.W. Mendel, editors. Upstream passage, spawning, and stock identification of fall Chinook in the Snake River, 1992 and 1993. Final Report to Bonneville Power Administration, Project 92-046, Portland, Oregon.

Bugert, R., and six coauthors. 1991. Lyons Ferry fall chinook salmon hatchery evaluation program. 1990 annual report to Washington Department of Fisheries to U.S. Fish and Wildlife Service, Lower Snake River Compensation Plan Office, Boise, Idaho.

Bugert, R., C.W. Hopley, C. Busak, and G. Mendel. 1995. Maintenance of stock integrity in Snake River fall chinook salmon. Pages 267-276 *in* H.L. Schram, Jr., and R.G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Connor, W.P., J.G. Sneva, K.F. Tiffan, R.K. Stienhorst, and D. Ross. In Press. Two alternating juvenile life history types for fall chinook salmon in the Snake River basin. Transactions of the American Fisheries Society.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 293). Updated from Goudet (1995) Available from http://www.unilch/izea/softwares/fstat.html.

LaVoy, L.W. and G. Mendel. 1996. Stock composition of fall chinook at Lower Granite Dam in 1995. Washington Department of Fish and Wildlife, Columbia River Laboratory Report 96-13, Battleground, WA.

Marshall, A.R., C. Smith, R. Brix, W. Dammers, J. Hymer, and L. LaVoy. 1995. Genetic diversity units and major ancestral lineages for chinook salmon in Washington. Pages D1-D62 *in* C. Busak and J. Shaklee, editors. Genetic diversity units and major ancestral lineages of salmonid fishes in Washington. Washington Department of Fish and Wildlife, Technical Report RAD 95-02, Olympia, Washington.

Marshall, A.R., H.L. Blankenship, and W.P. Connor. 2000. Genetic characterization of naturally spawned Snake River fall-run Chinook salmon. Transactions of the American Fisheries Society 129:680-698.

Milks, D., M. Varney, and M. Schuck. 2003. Lyons Ferry Hatchery Evaluation: Fall chinook salmon annual report 2000. Washington Department of Fish and Wildlife, Science Division Report # FPA 03-04 to U.S. Fish and Wildlife Service, Boise, Idaho.

NMFS (National Marine Fisheries Service). 1992. Threatened status for Snake River spring/summer chinook salmon, threatened status for Snake River fall chinook salmon. Final rule, Federal register 57:78(22 April 1992):14653.

Raymond. M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-490.

Shaklee, J.B., T.D. Beacham, L. Seeb, and B.A. White. 1999. Managing fisheries using genetic data: Case studies from four species of Pacific salmon. Fisheries Research 43:45-78.

Small, M.P., R.E. Withler, and T.D. Beacham. 1998. Population structure and stock identification of British Columbia coho salmon, Oncorhynchus kisutch, based on microsatellite DNA variation. Fisheries Bulletin 96:843-858.

Utter, F.M., W.J. Ebel, G.B. Milner, and D.J. Teel. 1982. Population structures of fall chinook salmon, Oncorhynchus tshawytscha, of the mid-Columbia and Snake Rivers. National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Processed Report 82-10, Seattle, Washington.

Utter, F.M., D. Chapman, and A.R. Marshall. 1995. Genetic population structure and history of chinook salmon of the upper Columbia River. Pages 149-165 *in* J.L. Nielsen, editor. Evolution and the aquatic ecosystem; defining unique units in population conservation. American Fisheries Society, Symposium 17, Bethesda, Maryland.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus spp.*, and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service, Marine Fisheries Review 53(3):11-22.

Table 1. Collections analyzed, number anlayzed, and the tissue used for the analysis.

Collection Location	Collection Code	# Analyzed	Tissue collected
2002 Lyons Ferry Hatchery broodstock	02GL	96	Fin
2003 Lyons Ferry Hatchery broodstock	03BR	96	Fin
2002 Unmarked/Untagged adults volunteering to Lyons Ferry Hatchery - subyearling releases	02GK	96	Fin
2002 Unmarked/Untagged adults volunteering to Lyons Ferry Hatchery - yearling releases	02GK	17	Fin
2003 Unmarked/Untagged adults volunteering to Lyons Ferry Hatchery - subyearling releases	03BQ	96	Fin
2003 Unmarked/Untagged adults volunteering to Lyons Ferry Hatchery - yearling releases	03BQ	43	Fin
2002 Unmarked/Untagged adults collected at Lower Granite Dam	02PH	70	Scales
2003 Unmarked/Untagged adults collected at Lower Granite Dam	03HC	127	Scales
2003 Umatilla Hatchery broodstock	03BS	100	Fin

Table 2. Microsatellite DNA loci, measures of variability, and PCR conditions used to analyze collections of fall-run Chinook from the Snake River and Umatilla Hatchery.

Locus	Repeat Length (bp)	Number Alleles	Ho[b] (observed heterozygosity)	Allelic Size Range	Primer Conc [uM]	Anneal Temp ^{o}C	Number Cycles	$MgCl_2$ Conc [mM]	Taq [units/rxn]
Ogo-2 V3	2	18	0.823	228 - 280	0.09	60°	35	1.5	0.05
Ogo-4 V2	2	15	0.742	158 - 190	0.2	60°	35	1.5	0.05
Oki-100 V1	4	40	0.927	188 - 375	0.3	50°	35	1.5	0.05
Omm-1080 V1	4	50	0.969	187 - 389	0.25	50°	35	1.5	0.05
Ots-3M V2	2	14	0.790	152 - 183	0.2	63°	30	1.5	0.05
Ots-9 V3	2	11	0.628	121 - 160	0.2	63°	30	1.5	0.05
Ots-201b V2	4	48	0.901	123 - 351	0.42	50°	35	1.5	0.05
Ots-208b V3	4	46	0.954	178 - 372	0.1	50°	35	1.5	0.05
Ots-211 V3	4	30	0.949	219 - 349	0.25	60°	35	1.5	0.05
Ots-212 V2	4	32	0.893	145 - 276	0.18	63°	30	1.5	0.05
Ots-213 V3	4	48	0.965	202 - 386	0.25	60°	35	1.5	0.05
Ssa-197 V3	4	35	0.938	174 - 307	0.25	60°	35	1.5	0.05
Ssa-408 V3	4	31	0.924	204 - 326	0.18	50°	35	1.5	0.05
Omy-1011 V1	2	43		153 - 385	0.2	50°	35	1.5	0.05
Ots-G474 V3	4				0.1	60°	35	1.5	0.05

[b] = Observed heterozygosity was calculated using FSTAT (Goudet 1995).

Loci excluded from analysis.

Table 3. Pairwise comparisons of fall-run Chinook salmon collected from Lyons Ferry Hatchery, Lower Granite Dam, and Umatilla Hatchery calculated using FSTAT. Pairwise comparisons that were significantly different are highlighted in black with white type. Pairwise comparisons were defined as significant after implementation of Bonferonni correction for multiple tests (Rice 1989; 36 comparisons; alpha = 0.05/36 = 0.001389).

	LFH V02 SY	LFH B02	LGD 02	LFH V03 Y	LFH V03 SY	LFH B03	Umatilla 03	LGD 03
LFH V02 Y	0.06453	0.00056	0.04900	0.39689	0.05719	0.00736	0.57350	0.74731
LFH V02 SY		0.06731	0.19767	0.00078	0.37614	0.42400	0.00128	0.15800
LFH B02			0.00003	0.00003	0.07900	0.05461	0.00003	0.00011
LGD 02				0.00042	0.01017	0.00003	0.03464	0.21753
LFH V03 Y					0.00003	0.00003	0.01194	0.00008
LFH V03 SY						0.36914	0.00003	0.18886
LFH B03							0.00003	0.00011
Umatilla 03								0.02356

Pairwise comparisons with Lyons Ferry Hatchery volunteers (yearling releases) from 2002 and 2003 combined.

	LFH V02 SY	LFH B02	LGD 02	LFH V03 SY	LFH B03	Umatilla 03	LGD 03
LFH V02/03 Y	0.00009	0.00002	0.00104	0.00002	0.00002	0.03340	0.00367

LFH = Lyons Ferry Hatchery
V = unclipped/untagged adults volunteering to Lyons Ferry Hatchery
B = Lyons Ferry Hatchery broodstock
Y = adult returns that were released as yearlings (identified by scale analysis)
SY = adult returns that were released as sub-yearlings (identified by scale analysis)

Table 4. Population differentiation results for collections from Lyons Ferry Hatchery broodstock (LFH B), unmarked/untagged adults volunteering to Lyons Ferry Hatchery (yearling and subyearling releases, LFH V), unmarked/untagged adults from Lower Granite Dam (LGD), and Umatilla Hatchery broodstock (Umatilla).

A: How similar are LFH B, LFH V (subyearling and yearling releases), and samples taken at LGD in 2002?

Collection	Significantly Different	Not Significantly Different
LFH B02	LFH V02 Y LGD 02	LFH V02 SY
LFH V02 Y	LFH B02	LFH V02 SY LGD 02
LFH V02 SY		LFH V02 Y LFH B02 LGD 02
LGD 02	LFH B02	LFH V02 Y LFH V02 SY

B: How similar are LFH B, LFH V (subyearling and yearling releases), and samples taken at LGD in 2002?

Collection	Significantly Different	Not Significantly Different
LFH B03	LFH V03 Y LGD 03	LFH V03 SY
LFH V03 Y	LFH V03 SY LFH B03 LGD 03	
LFH V03 SY	LFH V03 Y	LFH B03 LGD 03
LGD 03	LFH V03 Y LFH B03	LFH V03 SY

C-1: Compare Umatilla broodstock 2003 with 2002 samples from Lyons Ferry Hatchery and Lower Granite Dam.

Collection	Significantly Different	Not Significantly Different
LFH B02	Umatilla 03	
LFH V02 SY	Umatilla 03	
LFH V02 Y		Umatilla 03
LGD 02		Umatilla 03

Table 4. continued.

C-2: Compare Umatilla broodstock 2003 with 2003 samples from Lyons Ferry Hatchery and Lower Granite Dam.

Collection	Significantly Different	Not Significantly Different
LFH B03	Umatilla 03	
LFH V03 SY	Umatilla 03	
LFH V03 Y		Umatilla 03
LGD 03		Umatilla 03

Question C-3: Are LFH V02/V03 yearlings more similar to Umatilla than LFH B03?

Collection	Significantly Different	Not Significantly Different
LFH V02 Y N = 17		Umatilla 03 LFH B03
LFH V03 Y N = 43	LFH B03	Umatilla 03
LFH V02/03 Y N = 60	LFH B03	Umatilla 03