



Northwest Indian Fisheries Commission

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SUBJECT: **Population structure in Chinook salmon from the South Fork of the Stillaguamish River.**

Summary

Genetic distance analysis indicates that the South Fork Stillaguamish population of Chinook salmon has absorbed significant Green River ancestry. Compared to other basins where Green River fish have been introduced, such as parts of the Snohomish basin or Nisqually River, the influence of Green River Chinook salmon is much less in the Stillaguamish, however. Although the data show contribution from Green River hatchery fish, the South Fork population remains genetically differentiated from the large complex of populations that are mostly Green River derived. The South Fork of the Stillaguamish population appears to have a healthy effective population size, as estimated from microsatellite DNA data and also from demographic data on the variation of run size through time. It is possible that the effective population size of the population has been inflated by strays, perhaps of Green River origin. Assignment testing of fish collected from the South Fork Stillaguamish suggests a substantial contribution from Snohomish basin fall Chinook, though no more than has been observed in Skagit basin fall collections or Snohomish basin summer collections. Completion of microsatellite datasets for populations from the Green River hatchery population and from the Snohomish basin should form a baseline to allow a finer scale resolution of the relationship of the South Fork Stillaguamish Chinook to populations from neighboring rivers, over both the long term and the more immediate scale of present-day gene flow.

Data

Unpublished allozyme and microsatellite DNA data from WDFW were used to address questions about the ancestry and effective population size of Chinook salmon from the South Fork of the Stillaguamish River, as well as the source of contribution from non-native Chinook salmon that may stray into the South Fork of the Stillaguamish. Additional populations of interest include Chinook salmon from the Snohomish River basin that may be historically ancestral to Stillaguamish Chinook as well as potentially providing current contribution from strays, Chinook from the Skagit River basin that may also be able to stray into the Stillaguamish basin, and,

finally, the Green River hatchery strain of Chinook salmon, which have been stocked into the Snohomish basin in the past.

Multilocus DNA genotypes (15 microsatellite loci) were taken from 585 Chinook salmon from the North Fork of the Stillaguamish from 1985 through 2001, and from 159 Chinook salmon from the South Fork over the period from 1992-2001. Until data are available from outside the Stillaguamish basin, the existing data are sufficient to estimate demographic parameters such as effective population size and distribution of genetic variation within and between the two forks of the Stillaguamish River, but they cannot be used to address questions involving relationship of Stillaguamish fish to those from populations in other basins. Data from a 31-locus suite of allozymes is available for populations from throughout Puget Sound (WDFW unpublished data, also Waples et al. 2004), and this dataset can be used to form a preliminary view of the relationship among Chinook salmon populations from the north and south forks of the Stillaguamish as well as the neighboring Snohomish and Skagit basins and the widely stocked Green River hatchery strain. Specific sample information (location and year of collection) is given in Table 1a (microsatellites) and 1b (allozymes).

Analyses

Variance effective population size (N_eV) was estimated from microsatellite data collected from samples representing brood years 1988-1999 (Table 1a), roughly 3 generations start to finish, using the program NeEstimator. Point estimates of N_e , calculated from temporal variation in allele frequencies, were 88 based on the method of moments described by Waples (1991), with 95% CI 49-235, and 205 from the pseudolikelihood method of Wang (2001), with 95% CI 111-1028. The point estimates ranged from 34-80% of the harmonic mean annual run size (255; SaSI 2002) over the time period sampled. The low end is fairly typical of salmonids, while the high end is definitely elevated relative to observations from Pacific salmon (Waples 2002; though not necessarily from Atlantic salmon, see Spidle et al. 2003, 2004). To the extent that the pseudolikelihood method's point estimate is correct, and the 95% confidence estimate far exceeds observed run size, it may reflect increased ancestry from a large population out of basin such as the Green River hatchery strain. It is also possible that redd counts may have underestimated actual spawning escapement, though obviously not to the degree implied by the width of the confidence interval. Additionally, inbreeding effective population size (N_eI) was calculated using the technique of Simon et al. (1986, see also Spidle et al. 2004). Variation in number of spawners from year to year was calculated from 1986-2004 escapement data (SaSI online at <http://wdfw.wa.gov/mapping/salmonscape/>), under the assumption of a sex ratio of 3 males : 2 females in the escapement. The inbreeding coefficient F_{IS} was estimated, using the program GenePop and the same microsatellite data used to estimate N_eV , to be 0.0175. Simon et al. (1986) had estimated that in hatchery coho salmon, variance in mean family size was 2-4 times the typical mean family size, a range that, with the demographic data from SaSI, lead to estimates of N_eI from 91 to 152, very similar to the range of estimates for N_eV .

The question of the ancestry of the present-day South Fork Stillaguamish population of Chinook salmon was addressed by estimating genetic distances among allozyme datasets from the north and south forks of the Stillaguamish, from the Soos Creek Green River hatchery population, and wild and hatchery populations from the Snohomish basin, while using collections from the south and north forks of the Nooksack River as outgroups, and from the Nisqually River as an 'ingroup' to show the relatedness of a population known to have been heavily influenced by the Green River strain. A genetic distance matrix (Da, Nei et al. 1983) was estimated from the allozyme dataset (Table 1b) using the program *dispan*. From there, two separate analyses were conducted: a multidimensional scaling plot of each population was made, in *Systat*, to illustrate the relatedness of each population and subpopulation (Figure 1), and then the subpopulations from rivers were combined into pools and a neighbor-joining tree was calculated from the matrix using the program 'neighbor' from the *Phylip* package. The tree was bootstrapped (2000 replicates sampling over loci) using the program *NJBPOP*. Populations that significantly (as indicated by bootstrapping of the tree) were highlighted on the MDS plot (Figure 1).

The origin of potential strays into the gene pool of the South Fork Stillaguamish Chinook population was inferred by using individual assignment tests where the likelihood of an individual genotype being drawn from its gene pool of origin (or some other gene pool) is measured. Only populations that could feasibly contribute strays to the Stillaguamish basin were used as potential sources of origin. Genetic distance analysis suggested similarity between collections with the same run timing across tributaries within a basin, so populations were pooled by run time across basins with more than one tributary sampled (Snohomish, Skagit). Table 2 gives the rates at which individuals from known populations were assigned back to each of the populations in the dataset. Salmon from the South Fork of the Stillaguamish River were incorrectly assigned back to the Snohomish basin fall pool at a slightly greater rate than they were correctly assigned back to the South Fork. For comparison's sake, the Snohomish summer Chinook were assigned back to the Snohomish fall origin pool at the same rate as South Fork Stillaguamish fish were. Individuals from both the Snohomish summers and the South Fork Stillaguamish falls were correctly identified at similar rates. Interestingly, Skagit basin fall Chinook were also mis-assigned to the Snohomish basin fall pool at a similar rate as South Fork Stillaguamish and Snohomish summers. Snohomish fall Chinook, North Fork Stillaguamish summers, and Skagit spring Chinook were most often assigned to their actual collection of origin rather than to any other collection. Skagit summers were most often mis-assigned to either North Fork Stillaguamish summers or to Skagit springs. Green River influence in the South Fork Stillaguamish, therefore, can be inferred to be comparable to that in summer run Snohomish basin Chinook, and fall run Skagit basin Chinook, but less than has been estimated for fall run Snohomish basin Chinook.

References

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Table 1a Origin of microsatellite samples collected from north and of the Stillaguamish River.

Collection location	Year	N
NF Stillaguamish mainstem	1985	20
NF catch area 786	1985	17
Upper NF spawning survey	1985	52
NF spawning ground	1987	69
NF spawning ground	1988	26
NF spawning ground	1996	108
NF hatchery broodstock	2001	118
NF spawning ground carcasses	2001	79
Total North Fork N		488
SF Jim Creek catch area 322	1992	13
SF Jim Creek catch area 322	1993	7
SF Jim Creek catch area 322	1994	19
SF Jim Creek catch area 322	1995	16
SF Jim Creek catch area 322	1996	60
SF Jim Creek catch area 322	1997	7
SF catch area 324	1997	6
SF catch area 350	2001	1
SF catch area 1	2001	3
SF Jim Creek catch area 322	2001	27
Total South Fork N		159

Table 1b Origin of allozyme samples used in the present analysis. South Fork Stillaguamish collections are the same as from Table 1a (through 1996).

Collection location, run timing	Year	N
NF Nooksack Hatchery	1988, 1993	201
SF Nooksack River	1993-1995, 1998	104
Total Nooksack basin springs		305
Suiattle River	1986-1990, 1998	505
Upper Sauk River	1986, 1994, 1998	163
Cascade Hatchery	1990, 1993-1994, 1996	428
Upper Cascade River	1993-1994	83
Total Skagit basin springs		1179
Upper Skagit River	1986, 1994-1995	284
Lower Sauk River	1986	74
Total Skagit basin summers		358
Lower Skagit River falls	1986-1988, 1998	128
NF Stillaguamish summers	1987-1988, 1996	184
SF Stillaguamish falls	1992-1996	113
Wallace River	1988-1989	94
Skykomish River	1988-1989, 1993, 1996	182
Bridal Veil Creek	1987-1988	87
Wallace Hatchery	1987-1988, 1996	259
Total Snohomish basin summers		919
Sultan River	1987-1989	95
Snoqualmie River	1988	101
Wallace Hatchery	1987	106
Total Snohomish basin falls		302
Nisqually River falls	1998	30
Green River Hatchery falls	1987-1998	399

Table 2. Result of individual assignment tests. Each cell indicates the percentage of fish from a given row assigned to the river represented in each column. Percentages sum to 1.00 across rows. Grayed cells indicate percentage of fish correctly assigned to their population of origin. Bold indicates the collection (column) to which fish from the row in question were most frequently assigned.

Population of origin	Percentage assigned to each population						
	Snohomish summers	Snohomish falls	SF Stillaguamish	NF Stillaguamish	Skagit springs	Skagit summers	Skagit falls
Snohomish summers	0.24	0.34	0.10	0.09	0.08	0.06	0.09
Snohomish falls	0.13	0.52	0.11	0.07	0.03	0.06	0.09
SF Stillaguamish	0.09	0.33	0.25	0.11	0.10	0.03	0.11
NF Stillaguamish	0.10	0.16	0.05	0.32	0.17	0.14	0.07
Skagit springs	0.11	0.15	0.09	0.14	0.36	0.10	0.06
Skagit summers	0.13	0.12	0.10	0.18	0.18	0.15	0.12
Skagit falls	0.16	0.31	0.12	0.15	0.09	0.10	0.08

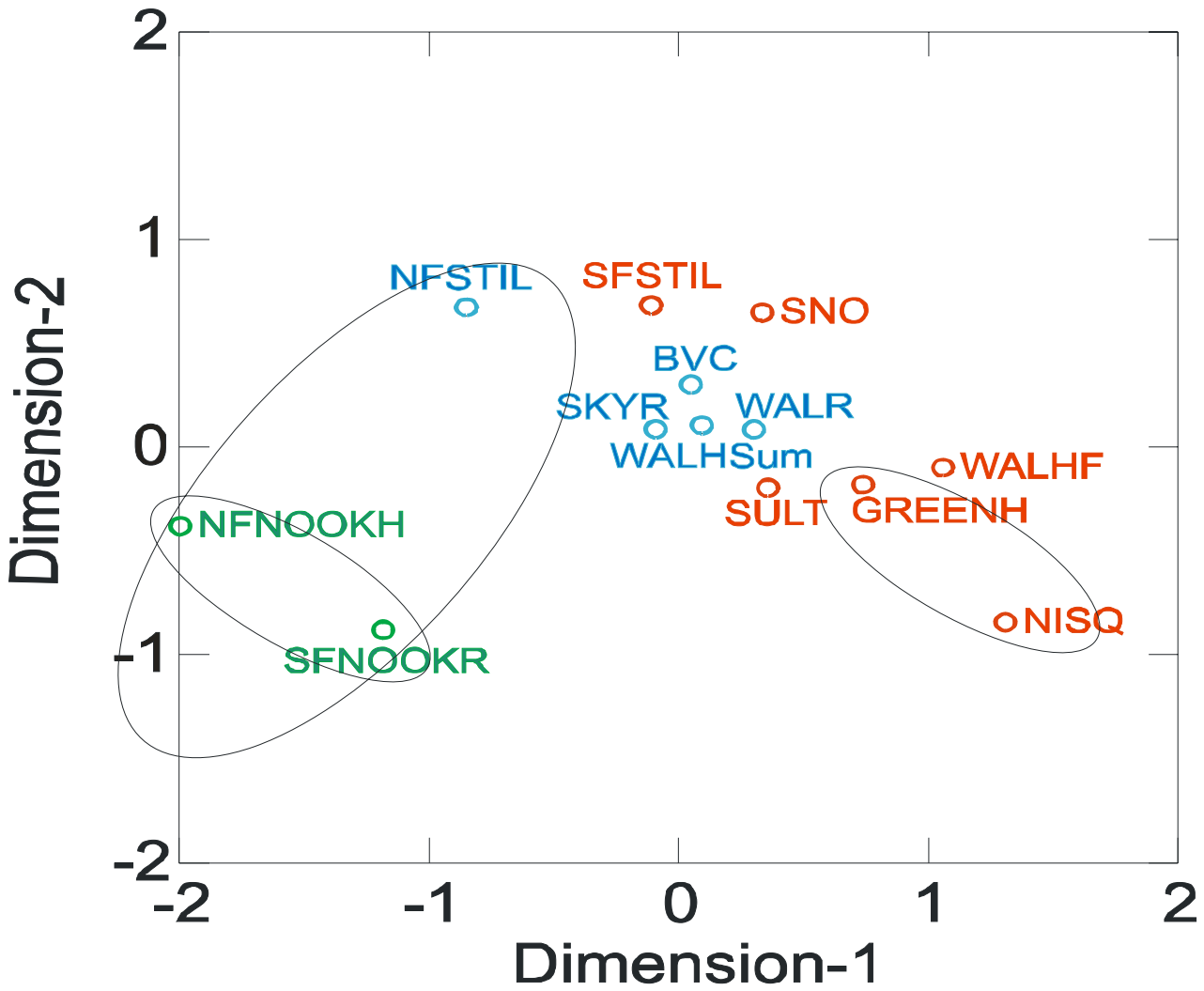


Figure 1. Multidimensional scaling plot of genetic distance (D_a) between Chinook salmon populations from Stillaguamish and Snohomish basins. Nooksack, Green River hatchery, and Nisqually River collections of Chinook were used as outgroups. Circles indicate populations that cluster together on a neighbor-joining tree of genetic distance with greater than 45% bootstrap support. Color-codes indicate run timing (green = spring, blue = summer, red = fall).