Conservation Genetics **5:** 367–379, 2004.

Have native coho salmon (*Oncorhynchus kisutch*) persisted in the Nooksack and Samish rivers despite continuous hatchery production throughout the past century?

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Received 6 May 2003; accepted 5 July 2003

Key words: coho salmon, hatchery impact, microsatellites, native salmonids, *Oncorhynchus kisutch*

Abstract

For over a century, Washington State Department of Fish and Wildlife has implemented hatchery programs as a means to boost salmon abundance. Concerns have developed that native populations may be replaced by hatchery strains, decreasing the genetic diversity required to respond to environmental changes. We report a comparison of microsatellite DNA variation in wild-spawning and hatchery-strain coho salmon from the Nooksack and Samish rivers in northern Puget Sound. Significant heterogeneity in genotype frequencies was detected between wild-spawning coho salmon from the upper North Fork (NF) Nooksack River and hatchery-strain coho salmon from the Nooksack River (descendants of primarily Nooksack River broodstock). Little difference in genotype frequencies was detected between wild-spawning coho salmon from the Samish River and hatchery-strain coho salmon from the Nooksack River. The 13-locus suite provided high resolution: in assignment tests over 85% of wild-spawning coho salmon from the upper NF Nooksack River were assigned to source. Wild-spawning coho salmon collected below hatcheries in the Nooksack River and 50% of wild-spawning Samish River coho salmon were assigned to hatchery collections. The genetic divergence of wild-spawning coho salmon in the upper NF Nooksack River is remarkable given the extensive stocking history and proximity of a hatchery. We suggest that these upper river fish are native coho salmon and that wild spawners in the lower Nooksack and Samish River are descendants of hatchery productions. We attribute divergence to earlier run timing in upper NF Nooksack River wild spawners, availability of extensive spawning and rearing habitat upstream of a hatchery in the upper NF Nooksack River, and a longer stocking history in the Samish River.

Introduction

Coho salmon (*Oncorhynchyus kisutch*) abundance has declined in Washington State in both historic and recent times. Over one third of Washington State coho salmon stocks are rated as depressed: production is below levels expected with available habitat and estimated survival rates (WDF et al. 1993). In particular, coho salmon of the Puget Sound region have been identified as an evolutionarily significant unit (ESU) likely to become endangered in the near future (Weitkamp et al. 1995) due to high harvest rates, decrease in adult size (Ricker 1981), habitat degradation, changing oceanic conditions and interactions with hatchery productions (Beamish et al. 1997; Reisenbichler 1997). Washington Department of Fish and Wildlife (WDFW, formerly Washington Department of Fisheries) has included hatchery programs as an important component of the effort to boost coho salmon abundance. In the northern Puget Sounds' Nooksack River (Figure 1), hatcheries have been used for coho salmon production from 1950 until present at Kendall Creek Hatchery

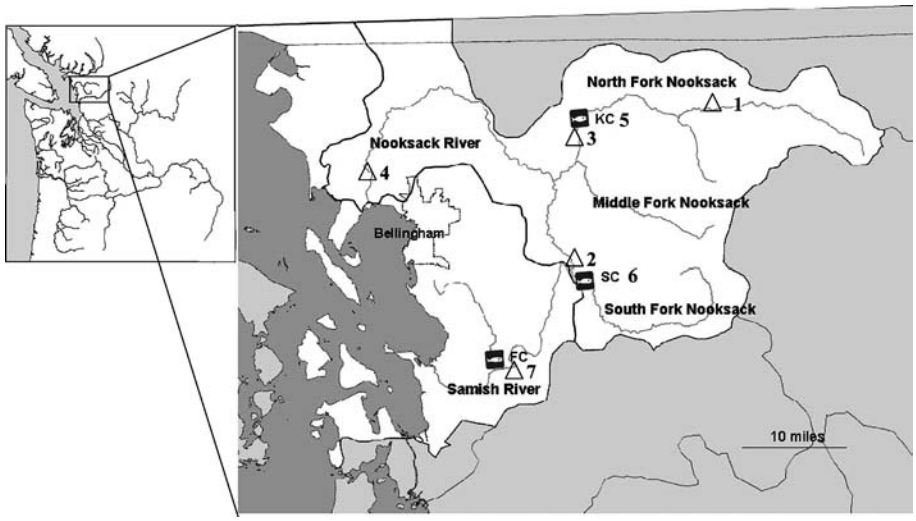

Figure 1. Right: map of the Nooksack and Samish drainages; left: map showing drainage location in Washington State. Triangles indicate collection sites for wild-spawning fish and fish emblems indicate hatchery locations (KC = Kendall Creek, SC = Skookum Creek, FC = Friday Creek). Numbers to the right of triangles and fish emblems identify sites for wild and hatchery fish collections: (1) 97NFWild, 01NFWild; (2) 97WildSF; (3) 97WildMain; (4) 97Wildlow; (5) 96NFHatch, 97NFHatch, 98NFHatch; (6) 97SFHatch, 98SFHatch; (7) 97Samish, 98Samish. Watershed boundaries are indicated by dark line, rivers are indicated by light line, and Bellingham city boundary is indicated by hatched line. High-resolution map outline was generated by Travis Butcher at Streamnet, Pacific States Marine Fisheries Commission. Map of major drainages in Washington State was generated by Shelly Snyder and Darrell Pruit at WDFW.

(WDFW) in the North Fork (NF) and Skookum Creek Hatchery (Lummi Tribal Nation) in the South Fork (SF). In the adjacent Samish River, Friday Creek Hatchery (WDFW) produced coho salmon from 1899 until 1978 (WDF et al. 1993, see Figure 1 for hatchery locations). Smolt production from these hatcheries has been prodigious, with an annual mean of nearly six million smolts released into the Nooksack basin from 1982 to 1991 (WDF et al. 1993). Hatcheries established runs from native coho salmon and used primarily returning hatchery-origin fish as broodstock, in addition to out-of-basin broodstock (Table 1) from northern and southern Puget Sound, the Strait of Juan de Fuca, Hood Canal, and Oregon.

The Samish River coho salmon stock has been considered a healthy population of composite hatchery ancestry, but the status of coho salmon in the Nooksack River is unknown due to uncertainties associated with escapement data (WDFW et al. in preparation). It is also unclear whether wild-spawning coho salmon in the Nooksack River are a hatchery composite, or if native coho salmon still exist within the Nooksack River (Nehlsen et al. 1991; WDF et al. 1993; WDFW et al. in preparation). The fishery has been managed as a hatchery production but management would likely change to protect a native component. In the upper NF Nooksack River, WDFW and USFS identified a group of wild-spawning coho salmon with a run timing different from wild spawners and hatchery fish in the lower river (Pete Castle and Doug Huddle, WDFW, unpublished data, and Brady Green, Aquatic Biologist, USFS, personal communication). These wild spawners enter the region starting in early October and spawning peaks around the third week in October, a month earlier than wild spawners and hatchery fish in the lower river. We suspect that these wild spawners are a native population.

Hatchery programs have been implemented for many salmonid species. With evidence accumulating that hatchery production has undesired effects upon native populations (Hindar et al. 1991; Waples 1991; Skaala et al. 1996; Beamish et al. 1997; Einum and Fleming 1997; Reisenbichler and Rubin 1999; Ford and Hard 2000; Lynch and O'Hely 2001), it will become increasingly important to identify native spawners. Locally adapted populations are repositories of adaptive variation (Taylor 1991), and some of this variation has a genetic basis. However, without information on the original genetic profile of a population, it is difficult to confirm whether a population is native or introduced

Table 1. Out-of-basin releases of fry (F) and yearlings (Y) at Washington State fish hatcheries from 1951 to 1994 in the Samish and Nooksack rivers (from Weitkamp et al. 1995 and Jeffrey Haymes, WDFW Biologist, personal communication)

River: hatchery	Stock	Years planted	Stages planted	Total planted	No. years planted
Samish: Friday Cr.	Big Beef Cr.	77	F	10,770	1
	Big Soos Cr.	56, 58	Y	32,041	2
	Green River	55–56, 59	F, Y	125,340	3
	Nooksack	81, 84	F	136,000	2
	Pilchuck	77	F	10,848	1
	Skagit	60, 72, 75–80	F, Y	2,976,168	8
	Skykomish	76, 80	F, Y	841,258	2
Nooksack: Kendall Cr.	Unknown	78–79	F	80,214	2
	Cascade (Oregon)	74	Y	192,000	1
	Green River	56–57, 63	F, Y	305,604	3
	Kalama Falls	76	Y	25,615	1
	Samish	52–57, 59, 61–63, 65, 73, 79, 80, 82			
			F, Y	1,239,144	15
	Skagit	60–62, 78–87, 91–92	F, Y	15,681,679	15
	Skykomish	80–81, 84, 92	Y	2,784,144	1
	Soleduck	63	F, Y	91,200	1
Nooksack: Skookum Cr.		Years received/planted	Eggs or fish		
	Kalama	73–74	E	2,040,400	2
	Green River	74	E	1,202,200	1
	Sandy River	74	E	550,250	1
	Cascade	74	E	1,623,750	1
	Quilcene	75	E	2,500,000	1
	Skagit	76, 78, 94	E	2,622,720	3
	Skagit	86, 87, 90	f	840,232	3
	Samish	77	E	1,827,171	1
	Lummi Bay	77–88, 90–95	E	21,512,612	17
	Skykomish	80, 90, 91, 94	E	8,287,400	4
	Skykomish	90	f	874,883	1
	Dungeness	81	E	501,600	1

Out-of-basin eggs (E) received and fish (f) planted at Skookum Creek Hatchery (Lummi Tribal Nation) in the SF Nooksack River (Steve Seymour, Lummi Tribal Biologist, personal communication).

(Spidle et al. 2001). Hendry et al. (1996) compared genetic profiles of sockeye salmon in the Lake Washington watershed to stocking sources. Lake Washington sockeye salmon had been presumed extinct prior to stocking, although Seeb and Wishard (1977) suggested that small native populations persisted. Hendry et al. (1996) found that some groups allied closely with stocking sources and other were related to native kokanee, and suggested that the latter were remnant native sockeye salmon. Spidle et al. (2001) conducted a study of wild-spawning Atlantic salmon in the Penobscot River in Maine. Salmon spawning in the heavily supplemented mainstem and two unsupplemented tributaries were clearly differentiated, but since samples prior to production were unavailable, Spidle et al. (2001) were unable to establish whether wild spawners were of native origin.

If native coho salmon still exist in the Nooksack and Samish rivers, there is an opportunity to protect some of the original biodiversity in the Puget Sound ESU. Genetics may tell us whether wild spawners are native or hatchery-origin coho salmon. If wild-spawning coho salmon in these

rivers are genetically distinct from hatchery stocks and differ in life history or adaptive traits, then they likely represent native coho salmon populations. If wild-spawning coho salmon are indistinguishable from hatchery stocks, then the wild spawners are likely a composite of hatchery and native stocks. In this study, we compare the genetic composition of wild-spawning coho salmon in the Nooksack and Samish rivers with hatchery coho salmon in the Nooksack River to investigate the possibility that native coho salmon populations exist and remain reproductively isolated from hatchery stocks in these systems.

Methods

Collections, DNA extraction, PCR and fragment analysis

Adult coho salmon tissue samples (scales or finclips) were collected from seven sites in northwestern Washington State: six sites in the Nooksack River and from one site in the neighboring Samish River (Figure 1). In this study, 'wild' refers to coho salmon spawning in a natural setting as opposed to a hatchery and does not presuppose purely native ancestry. Wild spawners are distinguished from straying hatchery fish by having intact adipose fins: these are removed from hatchery fish before release. Within the Nooksack River, three collections consisted of wild spawners. Two collections were made in the upper NF Nooksack River (Figure 1) above river mile (RM) 57 in 1997 (collection name: 97WildNF, 29 fish) and in 2001 (01WildNF, 98 fish). In 1997, an additional 18 wild spawners were collected throughout the Nooksack River downstream of the Kendall and Skookum Creek hatcheries: one from the SF (97WildSF), three from the lower mainstem near Bellingham (97WildMain) and 14 from the lower NF (RM 40.8) above the mouth of the Middle Fork and below Kendall Creek Hatchery (97WildLow, Figure 1). Since 97WildSF and 97WildMain included less than four fish each, only 97WildLow was included in analyses and 97WildSF and 97WildMain were included as unknown fish in the assignment test (see below). Nooksack River hatchery coho salmon collections included three from Kendall Creek Hatchery (NF Nooksack River, RM 45) collected in 1996 (96NFHatch, 96 fish), 1997 (97NFHatch, 100 fish), and 1998 (98NFHatch, 99 fish). Two hatchery coho salmon collections were made at Skookum Creek Hatchery in SF Nooksack River (RM 50) in 1997 (97SFHatch, 50 fish) and 1998 (98SFHatch, 88 fish). In the Samish River, tissues were collected from wild spawners trapped at RM 10.5 in 1997 (97Samish, 95 fish) and 1998 (98Samish, 82 fish), roughly two miles above the confluence with Friday Creek, which housed Friday Creek Hatchery.

Genomic DNA was extracted from tissues with chelex resin (Small et al. 1998). Microsatellite alleles at 13 loci (Table 2) were amplified using fluorescently labeled primers and the polymerase chain reaction (PCR, see Table 2 for detailed PCR information). PCR's were conducted on a MJResearch PTC-200 thermocycler in 10 μl volumes employing 1 μl template with final concentrations of 1.5 mM $MgCl_2$ and 1X Promega PCR buffer. Samples were run on an ABI 377 automated sequencer and alleles were sized to basepairs using an internal lane size standard (GS500 by Applied Biosystems), and computer programs GeneScan Analysis 3.7, and Genotyper 3.7. Raw allele mobilities were binned into discrete allele size bins according to allele frequency histograms in Excel.

Data analysis

Statistical tests were conducted on loci and collections to assess conformity with Hardy–Weinberg expectations (Hardy–Weinberg equilibrium, HWE), linkage disequilibrium and genotypic heterogeneity using GENEPOP version 3.3 (Raymond and Rousset 1995). Loci and collections were tested for deficits of heterozygotes and homozygotes (HWE) across all loci and across all collections using GENEPOP. Collection statistics including number of alleles, allelic richness (number of alleles corrected for sample size), and gene diversity (expected heterozygosity corrected for sample size) were calculated per collection using FSTAT 2.9.3 (Goudet 2001). Collections were also combined into three groups: wild collections from the upper NF Nooksack (97WildNF, 01WildNF); wild collections from the Samish River (97Samish, 98Samish); and hatchery collections from the Nooksack River (96NFHatch, 97NFHatch,

Table 2. Loci names, PCR conditions and primer citation (Source)

Multiplex	Locus	Primer conc (μM)	Range in basepairs	Anneal temp	# cycles	Taq (units/rxn)	# alleles	H_e	Source
Oki-A	*Ots-101*	0.17	92–231	46	34	0.07	29	0.894	Small et al. (1998)
	Ogo-2	0.09	234–264				10	0.669	Olsen et al. (1998)
	p53	0.2	161–201				15	0.846	Linda Parks (personal communication)
	Ots-3M	0.09	124–161				14	0.769	Banks et al. (1999)
	One-2	0.12	185–275				42	0.907	Scribner et al. (1996)
Oki-B	*Ots-2M*	0.04	133–147	46	31	0.1	8	0.662	Banks et al. (1999)
	Ocl-8	0.05	97–135				20	0.839	Condrey and Bentzen (1998)
	One-13M	0.07	194–230				18	0.867	Scribner et al. (1996)
	Ogo-1a	0.06	110–154				7	0.521	Olsen et al. (1998)
Oki-C	*Omm-1121*	0.07	187–198	54	35	0.05	3	0.308	Rexroad III et al. (2001)
	Omm-1128	0.12	199–319				28	0.837	Rexroad III et al. (2001)
	Oki-1	0.1	84–161				16	0.73	Smith et al. (1998)
	Omy-1011	0.1	177–225				12	0.78	Paul Bentzen (personal communication)

The number of alleles and expected heterozygosity (H_e) were tabulated for each locus using FSTAT (Goudet 2001).

98NFHatch, and 97WildLow), and tested with 1000 permutations for differences among groups in characteristics including HWE, allelic richness, gene diversity and F_{st} values using FSTAT. Our first analyses had indicated that 97WildLow was strongly associated with Nooksack hatcheries, thus we grouped 97WildLow with the hatchery collections in this and subsequent analyses.

Relationships among collections were examined with pairwise tests. Collections were tested for differences in genotypic distributions at each locus and across all loci using GENEPOP with 200 batches and 2000 iterations. Pairwise multi-locus F_{st} values were assessed using ARLEQUIN 2.001 (Schneider et al. 2000). A series of AMOVA (analysis of molecular variance) tests (Excoffier et al. 1992) was performed using ARLEQUIN to calculate Wright's F statistics (Wright 1978) and determine how molecular variance was partitioned in the data set. Collections were arranged in various groupings (all collections in a single group; upper NF Nooksack River wild collections grouped versus all Samish River, hatchery and 97WildLow grouped, etc.) and tested for which grouping resulted in the greatest amount of molecular variance among groups, giving an indication of genetic structure within the data set. Results in all tests were corrected for multiple simultaneous tests to an overall alpha level of 0.05 (Rice 1989).

Genetic relationships among collections were also explored with ordination and dendrogram analyses. We presume that the collections in this study differ due to founder effects and genetic drift rather than mutations accumulating within populations within the past 50–100 years, so we calculated pairwise geometric chord distances (Cavalli-Sforza and Edwards 1967, CSE). Pairwise CSE distances were generated from allele frequency data using GENDIST in the computer software PHYLIP (Felsenstein 1993). CSE distances were plotted in a multi-dimensional scaling (MDS) analysis using NTSYSpc-2.02j (Rohlf 1993). PHYLIP was also used to construct a neighbor-joining (NJ) tree of chord distances in the program NEIGHBOR. To test the repeatability of tree branching, 10,000 bootstrap replicates of the allele frequency file were generated in SEQBOOT and analyzed in GENDIST. Tree topologies were created for replicates using NEIGHBOR and a consensus tree was generated in CONSENSE.

The program WHICHRUN 4.1 (Banks and Eichert 2000) was used to assign each fish to a collection. The program implements a jackknife procedure where each fish in turn is removed from the dataset, allele frequencies of baseline samples (all the collections in the study) are calculated, and the fish is assigned to the most likely group based upon its genotype and the allele frequencies of the groups. Assignments for the 97WildLow collection were conducted both with the collection in the baseline and with the collection as unknown fish. When 97WildLow was included in the baseline, the small sample size distorted allele frequencies such that high numbers of fish from other collections were assigned to 97WildLow. Thus, assignments for other collections are reported without 97WildLow in the baseline. Other wild spawners collected in 1997 within lower portions of the Nooksack drainage (97WildMain, 97WildSF) were also tested for assignments as unknown fish.

Results

Loci and population characteristics

Loci were highly polymorphic with up to 42 alleles per locus and expected heterozygosity ranged from 0.3 to 0.9 (Table 2). Allelic richness ranged from lows of 6.12 for 01WildNF and 6.69 for 97WildNF to a high of 8.06 in 98Samish, with a mean richness of 7.94 over all collections. Gene diversity was comparable in all collections (mean gene diversity 0.748).

Gametic disequilibrium

Linkage disequilibrium was detected in four locus pairs in 01WildNF: *Ocl-8* and *Ots-101*, *One-2* and *p53*, *Ocl-8* and *Omm-1121*, and *Ots-101* and *Omm-1128*. One locus pair was in linkage disequilibrium in the following collections: 97NFWild, *One-2* and *One-13M*; 97Samish, *Ots-3M* and *One-13M*; 98Samish, *Ots-2M* and *Ots-101*. Since linkage disequilibrium was inconsistent among collections, disequilibrium was perhaps due to small effective population sizes rather than physical linkage of loci on the same chromosomes.

HWE tests

In HWE tests of loci across collections, two loci were out of equilibrium, *Ocl-8* and *One-2*, with significant deficits of heterozygotes ($P = 0.001$ for both). In HWE tests within collections, heterozygote deficits were detected in 97SFHatch, 98SFHatch and 98NFHatch at *One-2*, and 97WildNF at *One-13M* ($P < 0.05$ for each). Since most loci were in HWE in tests within collections, we left all loci in the analysis. The combined value for all loci showed a deficit of heterozygotes in the data set ($P = 0.001$), indicating some tendency towards departure from HWE assumptions. This was probably a result of population subdivision (see results below) since there was no locus with apparent null alleles (a locus with few heterozygotes where several individuals failed to amplify although they amplified at other loci). In multilocus tests within populations, significant and positive F_{is} values for 97WildNF and 97Samish (0.069, $P = 0.033$; 0.03, $P = 0.011$ respectively) suggest that these populations have experienced some inbreeding or recent admixture.

In three-way group tests, (upper NF Nooksack collections combined, etc.), the upper NF Nooksack group had significantly lower allelic richness (6.41) and greater structure ($F_{st} = 0.053$) than the Samish River group (richness = 7.96, $F_{st} = 0.001$) and Nooksack hatcheries-97WildLow group (richness = 7.62, $F_{st} = 0.009$). P values for tests of differences among the three groups equal 0.004 (allelic richness) and 0.012 (F_{st}).

Collection differentiation

Pairwise genotypic tests indicated heterogeneity in genotype distributions among most collections. Comparisons displaying overlapping genotype distributions include hatchery collections from 1997 to 1998, the Samish River collections, and several comparisons with 97WildLow (Table 3). Pairwise F_{st} tests show a lack of differentiation in several additional comparisons among 97WildLow, hatchery and Samish River collections (see Table 3). Pairwise test results indicated little gene flow between the upper NF Nooksack collections and any of the hatchery, 97Wildlow, or Samish River collections.

Significant genetic structure in the data set was also indicated by AMOVA tests. The tests showed

Table 3. Pairwise test results

	01WildNF	97WildNF	96NFHatch	97WildLow	97SFHatch	97NFHatch	98SFHatch	98NFHatch	97Samish	98Samish
01WildNF	–	0	0	0	0	0	0	0	0	0
97WildNF	0.0514	–	0	0	0	0	0	0	0	0
96NFHatch	0.0298	0.0285	–	0	0	0	0	0	0	0
97WildLow	0.0300	0.0290	**0.0017**	–	0.576	0.106	0	0	0.026	0.072
97SFHatch	0.0321	0.0328	0.0127	**–0.0056**	–	0.127	0	0	0	0
97NFHatch	0.0385	0.0363	0.0095	**–0.0038**	**0.0001**	–	0	0	0	0
98SFHatch	0.0267	0.0275	**–0.0109**	**–0.0023**	**–0.0010**	**–0.0072**	–	0.0036	0	0
98NFHatch	0.0341	0.0332	0.0128	**0.0057**	0.0142	0.0105	**–0.0055**	–	0	0
97Samish	0.0309	0.0304	**–0.0021**	**–0.0008**	**–0.0010**	**–0.0048**	0.0062	0.0078	–	0.0196
98Samish	0.0333	0.0313	0.0065	**–0.0017**	0.0061	**0.0030**	**–0.0085**	0.0075	**–0.0045**	–

Pairwise F_{st} test results from ARLEQUIN (Schneider et al. 2000) are in lower triangular matrix. 50,000 permutations were conducted and non-significant pairwise F_{st} values are in bold type. *P* values from pairwise genotypic test results from GENEPOP (Raymond and Rousset 1995) are in upper triangular matrix. Abbreviations include NF for North Fork, SF for South Fork and Hatch for hatchery.

low but significant genetic structure ($F_{st} = 0.0168$ over all loci) and suggested that the collections formed three groups: 97WildNF, 01WildNF, and a combination of the 97WildLow, Samish River and Nooksack hatchery collections (Table 4). This arrangement of collections yielded the greatest molecular variance among groups (3.01), the lowest variance (0.24) among collections within groups and the hightest F_{st} value (0.032, Table 4).

Population structure

When pairwise CSE distances were employed in a MDS analysis, the hatchery and Samish River collections formed a cloud of points in the center of the three-dimensional array with the wild collections distant from the cloud along the first, second and third axes (Figure 2). When CSE distances were plotted in the neighbor-joining dendrogram, some strong groupings were indicated (tree not shown). The upper NF Nooksack wild collections grouped with 93% bootstrap support, 97WildLow and the 1997 hatchery collections grouped with 99% bootstrap support and the 1998 hatchery collections grouped with 87% bootstrap support, the latter two suggesting year class associations. Some distances in the MDS concurred with pairwise tests: upper NF Nooksack wild collections were distinct from each other and from collections in the hatchery–Samish River cluster, and the hatchery and Samish River collections were similar to each other. However, although distances were large between 97WildLow and the 97 hatchery collections, they formed a branch with 99% support in the dendrogram analysis. Further, 97WildLow was mostly undifferentiated from hatchery and Samish River collections in pairwise tests. Genetic distances involving 97WildLow were likely distorted by its small sample size.

Assignment tests

In assignment tests, 89% of 97WildNF and 85% of 01WildNF were assigned to their collection of origin, in the rest of the collections assignments to origin ranged from 21 to 53% (Table 5). Although 97WildNF was a small sample, the high percentage of correct assignments suggests that the collection is well characterized, similar to the more robust 01WildNF collection. Most Samish River

Table 4. AMOVA results from ARLEQUIN (Schneider *et al*. 2000)

		Var components	% Var	F_{ct}	F_{sc}	F_{st}	P
1 Group	Among collections	0.0551	1.25				
	Within collections	4.3553	98.75			0.0168	0
2 Groups (WildNF, 97Wild-Low + Samish + Hatch)							
	Among groups	0.08706	1.95	0.0195			0.004
	Among collections within groups	0.02746	0.61		0.00626		0
3 Groups (WildNF, 97WildLow, Samish + Hatch)							
	Within collections	4.35532	97.44		0.0256		0
	Among groups	0.0756	1.69	0.0169			0.0089
	Among collections within groups	0.0285	0.64		0.00065		0
	Within collections	4.3553	97.67			0.0233	0
4 Groups (97WildNF, 01WildNF, 97WildLow, Samish + Hatch)							
	Among groups	0.1208	2.69	0.0269			0.0087
	Among collections within groups	0.0113	0.25		0.0026		0
	Within collections	4.4874	97.06			0.0294	0
3 Groups (97WildNF, 01WildNF, 97WildLow + Samish + Hatch)							
	Among groups	0.1353	3.01	0.03			0.024
	Among collections within groups	0.0106	0.24		0.002		0
	Within collections	4.355	96.76			0.032	0

Collections were grouped in different arrangements and data were analyzed for partitioning of molecular variance. In groups, WildNF included both upper NF Nooksack River collections, Samish included both Samish River collections and Hatch included all hatchery collections. P is the probability that a random value would be larger than the observed variance (Var) and F values.

fish were assigned to the other Samish River year class or to hatchery collections, indicating minimal genetic divergence among Samish River and Nooksack hatchery collections. Similarly, most of the fish in each of the Nooksack hatchery collections were assigned to other Nooksack hatcheries or to the Samish River. If 97WildLow was included in the baseline, half of the fish were assigned back to origin and the rest were assigned to hatchery collections (Table 5). When 97WildLow, 97WildSF and 97WildMain were tested as unknown fish, all were assigned to hatchery collections, suggesting that they were more closely related to hatchery collections than to the 97WildNF population (Table 5).

Discussion

The Nooksack–Samish system provided an interesting opportunity to address an important question: have native coho salmon populations persisted in this system despite extensive hatchery productions, inter-river transfers and the infusion of non-native hatchery stocks? Our study demonstrated that wild-spawning coho salmon in the upper NF Nooksack River were genetically distinct from hatchery coho salmon in the Nooksack River and from wild-spawning coho salmon in the lower NF Nooksack River, and that wild spawners collected below the hatchery in the lower NF Nooksack River were indistinguishable from

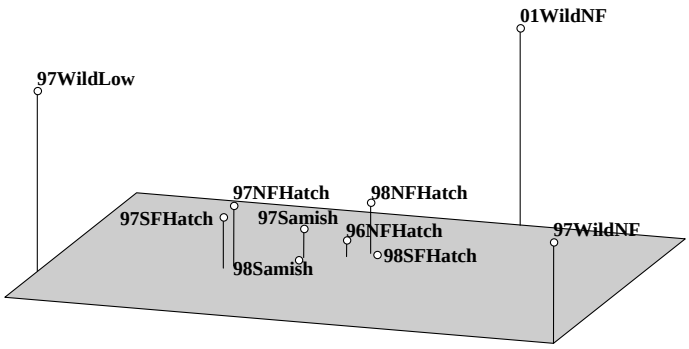

Figure 2. MDS analysis of Cavalli-Sforza and Edward pairwise distances between collections. South Fork is abbreviated as 'SF', North Fork is abbreviated as 'NF' and hatchery is abbreviated as 'Hatch'.

Table 5. Assignment test results from WHICHRUN (Banks and Eichert 2000)

	n	01WildNF	97WildNF	96NF-Hatch	97SF-Hatch	97NF-Hatch	98NF-Hatch	98SF-Hatch	97Sam-ish	98Sam-ish	97Wild-Low
01WildNF	98	**83**	1	2	2	–	6	4	–	–	
97WildNF	29	–	**26**	–	2	–	1	–	–	–	
96NFHatch	96	2	2	**51**	11	4	3	10	6	7	
97SFHatch	50	1	3	1	**14**	19	1	2	8	1	
97NFHatch	100	–	11	2	25	**35**	5	5	8	9	
98NFHatch	99	3	6	8	3	3	**45**	21	5	5	
98SFHatch	88	3	9	3	5	9	18	**33**	3	5	
97Samish	95	3	8	6	12	11	7	9	**29**	10	
98Samish	82	1	9	5	10	11	9	5	15	**17**	
97WildLow(U)	14	–	–	2	6	6	–	–	–	–	
97WildLow(B)	14	–	–	1	1	5	–	–	–	–	**7**
Other unknowns											
97WildSF	1	–	–	–	–	1	–	–	–	–	
97WildMain	3	–	–	–	–	2	1	–	–	–	

Individual fish were given the most likely assignment to a collection based upon the genotype of the fish and the allele frequencies in the collections. The table presents numbers of fish from a single collection assigned to various collections (n = number of fish analyzed in collection) with assignments to origin in bold type. Since 97WildLow was a small group, they were analyzed first as unknown fish (U) and then were included in the baseline (B) to estimate assignments back to collection of origin. Other unknowns include one from SF Nooksack River (97WildSF) and three from lower mainstem Nooksack River (97WildMain).

hatchery fish. Our survey also showed that wild-spawning Samish River coho salmon were nearly as similar to the Nooksack hatchery collections as they were to each other. However, if native fish persist in the upper Samish River, they were not included in study sampling. We hypothesize that wild spawners in the upper NF Nooksack are native fish and that wild spawners in lower NF Nooksack River and Samish River are composite groups derived from hatchery releases and native coho salmon. We offer that characteristics of the Nooksack and Samish River drainages, run timing differences, and different histories of hatchery production may have allowed native fish to persist in the upper NF Nooksack River and allowed hatchery fish to predominate in the lower Nooksack and Samish rivers.

The Nooksack River system is larger and more diverse than the Samish River system (Figure 1). In the Nooksack drainage, coho salmon utilize

much of the available habitat in 1325 lineal miles of mainstem and 654 tributaries. The two major drainages, NF and SF, differ environmentally and geologically (Marshall et al. 1995) providing diverse opportunities for local adaptation. The NF is glacial-fed, originating in volcanic rock, sandstone and siltstone, the SF drainage is fed by snowfields and rain and is mainly a low elevation drainage, originating at a basaltic mountain (Marshall et al. 1995 and references therein). In the Samish River, a low, rain-dominated drainage, coho salmon utilize habitat in approximately 42 lineal miles of mainstem and 11 tributaries (Williams et al. 1975). The extensive habitat in the Nooksack River offers greater potential for spatial isolation between hatchery and native juveniles, and greater potential for spatial isolation between adult native coho salmon and hatchery strays in spawning habitat. With roughly 30 times as much available habitat in the Nooksack drainage, hatchery-origin coho salmon may have failed to displace or introgress into native coho salmon in at least a portion of the Nooksack drainage. Since a few upper NF wild spawners were assigned to hatchery collections, some hatchery fish may have strayed into the upper NF and interbred with wild fish. These assignments could also reflect their relatively common ancestry and the use of in-river coho salmon for hatchery brood stocks.

In addition to spatial isolation, run timing and environmental heterogeneity may have contributed to temporal isolation of Nooksack River coho salmon populations. The headwaters of the NF Nooksack River are fed by glacial run-off, creating colder conditions and higher flows later in the summer, favoring early spawners (Reisenbichler et al. 2003). With peak run timing a month in advance of hatchery fish, putative native coho salmon spawning in the upper NF Nooksack River were temporally isolated from hatchery coho salmon straying into spawning grounds above Kendall Creek Hatchery. Earlier freshwater entry might also have provided an opportunity for native coho salmon to escape fisheries targeting hatchery productions (Pella and Milner 1987; Utter and Ryman 1993). These fisheries have generally had harvest rates considered unsustainable by wild production (Ricker 1976).

The association between hatchery collections and wild spawners from the lower Nooksack Rivers suggests that hatchery-origin coho salmon have had considerable impact on native populations in the lower Nooksack River. We hypothesize that impacts occurred via hatchery juveniles and adults: juveniles released from hatcheries moved primarily downriver, displacing native juveniles (McGinnity et al. 1997), thereby reducing potential native adult returns. Native adult numbers would be diminished further if run timings overlapped and large hatchery returns masked low native abundance so that native fish were overharvested in a mixed hatchery-wild fishery (Ricker 1976; Pella and Milner 1987; Utter and Ryman 1993). Some hatchery-origin adults must have strayed into wild spawning grounds, so that their descendants are returning as wild spawners (Crozier 1993; Clifford et al. 1998). Survival of hatchery juveniles is reduced under natural conditions (Reisenbichler and Rubin 1999) and hatchery-origin adults are less successful than native salmon on spawning grounds (Fleming and Gross 1993). However, overtime, repeated hatchery releases and escapement of hatchery fish apparently allowed hatchery-origin fish to dominate in the lower Nooksack River.

In the Samish River, limited homogeneous habitat and long-term hatchery planting may have promoted more thorough displacement of native juveniles and adults by hatchery productions. We suggest that Samish River fish lacked the run timing heterogeneity that may have allowed native coho salmon to persist in the upper NF Nooksack River. However, despite the apparent hatchery derivation of the Samish River wild spawners, they are now adapted since the run is healthy (WDFW et al. in preparation).

Our results suggest that hatchery practices have had a strong influence on the genetic composition of coho salmon in the Nooksack–Samish system. Given the proximity of the river mouths, the native populations may have been related originally through straying and common ancestry. However, now Samish River wild spawners are closely related genetically to Nooksack River hatchery fish as well as to lower Nooksack River wild spawners, which we suspect are hatchery descendants. We speculate that hatchery production homogenized inter-population genetic diversity (Waples 1991) in this system. The gene pools in the Samish and lower Nooksack rivers may have been homogenized by releasing Samish River hatchery produc-

tions into the Nooksack River for 15 years and by using other broodstock in common (Table 1). In particular, broodstock from the Skagit River, the next drainage south of the Samish River, may have thrived.

If wild spawners in the upper NF Nooksack River are native coho salmon, we might expect these collections to form a group distinct from hatchery collections. Yet, although they formed a distinctive group with high support in the neighbor-joining analysis, they were significantly different in pairwise tests. If the wild spawning population has a low N_e, as suggested by the F_{is} value for 97WildNF, these differences may be due to random variation expected in small populations. The differences may also be influenced by year-class differentiation as suggested by year-class associations in the neighbor-joining analysis. With the predominant 3 year coho salmon life cycle in Washington State (Williams et al. 1975; Weitkamp et al. 1995), a closer genetic relationship might be found between parent and returning offspring collections such as 97WildNF and an adult 2000 WildNF collection. However, related year classes were not collected.

The task of distinguishing native populations from hatchery-derived stock is difficult without information on the system prior to hatchery production (Spidle et al. 2001). We could strengthen our understanding of the upper NF Nooksack River spawning group by analyzing genetic variation over a longer time and by examining mitochondrial DNA variation within this system. Smith et al. (2001) detected regional mtDNA variation within Pacific Northwest coho salmon as well as variation within rivers. Mitochondrial DNA analysis might support the genetic uniqueness of the upper NF Nooksack River wild spawners and provide an opportunity to estimate introgression between hatchery and putative native fish. Adding out-of-basin hatchery sources (particularly Skagit River) to the analysis would also improve our understanding and mtDNA might link an out-of-basin source population with the upper NF Nooksack River wild spawners. For example, the upper NF wild-spawners may have descended from an out-of-basin hatchery group with an affinity for spawning in glacial-fed headwaters and then differentiated due to drift. However, out-of-basin juveniles survive poorly (Allendorf and Ryman 1987) and it is unlikely that a hatchery group founded the upper NF Nooksack River population yet failed to introgress into the hatchery population. The hatchery collections included over 400 fish, sampling much of the genetic variation therein and the upper NF Nooksack River coho salmon displayed little genetic affinity to the hatchery collections.

Acknowledgements

We thank Cherril Bowman for laboratory support, and Doug Huddle, Bruce Baker, Norm Switzler, Zack Hess, Darren Sahlfield and other WDFW staff for collecting tissues, and thank Jeffrey Haymes for information on WDFW hatchery stocking. Steve Seymour provided information on hatchery stocking at Skookum Creek Hatchery. The development of this study was enhanced by discussions with Carol Aubry and Brady Green and this manuscript was improved by reviews from Carol Aubry, Janet Loxterman, Anne Marshall, Jim Shaklee, Fred Utter, editor Bob Vrijenhoek, and two anonymous reviewers. Funding was provided by the US Forest Service, the Pacific Salmon Commission and the State of Washington General Fund.

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