Impacts of supplementation: genetic diversity in supplemented and unsupplemented populations of summer chum salmon (*Oncorhynchus keta*) in Puget Sound (Washington, USA)

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Abstract: In supplementation programs, hatcheries employ wild-origin fish as brood stock and their offspring are allowed into wild spawning areas. Resource managers use supplementation to support imperiled salmonid populations, seeking to increase census size and possibly effective population size (N_e), while minimizing risks of genetic diversity loss and domestication from hatchery intervention. Here we document impacts of 5–10 years of supplementation on threatened summer-run chum salmon (*Oncorhynchus keta*) in Hood Canal (HC) and Strait of Juan de Fuca (SJF) in Washington State and compare them genetically with unsupplemented summer- and fall-run chum salmon from HC and South Puget Sound. Microsatellite allele frequencies identified four run-timing and geographic groups. HC and SJF summer chum salmon genetic relationships followed a metapopulation pattern of isolation by distance, similar to patterns prior to supplementation, suggesting that supplementation minimally impacted population structure. In most supplemented subpopulations, we detected no effects on diversity and N_e, but high variance in individual pairwise relatedness values indicated over-representation of family groups. In two subpopulations, hatchery impacts (decreased diversity and lower N_e) were confounded with extreme bottlenecks. Rebounds in census sizes in all subpopulations suggest that general survivorship has improved and that possible hatchery effects on genetic diversity will be overcome.

Résumé : Dans les programmes de supplémentation, les piscicultures utilisent des poissons d'origine sauvage comme stock reproducteur et les rejetons sont introduits dans des zones sauvages de fraie. Les gestionnaires des ressources utilisent la supplémentation pour soutenir les populations menacées de salmonidés, cherchant ainsi à augmenter la taille de la population recensée et, si possible, la taille effective de la population (N_e), tout en minimisant les risques de perte de la diversité génétique et de domestication dus à l'intervention en pisciculture. Nous étudions ici les impacts de la supplémentation pendant 5–10 ans sur des saumons kéta (*Oncorhynchus keta*) à montaison estivale menacés dans le canal de Hood (HC) et le détroit de Juan de Fuca (SJF) dans l'état de Washington; nous comparons génétiquement ces saumons à des saumons à montaisons estivale et automnale, mais sans supplémentation, de HC et de Puget Sound. Les fréquences des allèles des microsatellites mettent en évidence quatre groupes d'après le moment de la montaison et l'origine géographique. Les relations génétiques des saumons kéta d'été de HC et de SJF montrent un patron de métapopulation par isolement par la distance, semblable au patron trouvé avant la supplémentation, ce qui indique que la supplémentation a un impact minimal sur la structure de la population. Dans la plupart des sous-populations ayant connu la supplémentation, nous ne décelons aucun effet sur la diversité ni sur N_e, mais il y a une forte variance des valeurs appariées de parenté qui indique une surreprésentation des groupes familiaux. Dans deux sous-populations, les impacts de pisciculture (diversité et N_e réduites) se confondent avec des goulots d'étranglement extrêmes. Des remontées dans les tailles recensées des sous-populations laissent croire que la survie générale s'est améliorée et que les effets possibles de pisciculture sur la diversité génétique seront surmontés.

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Introduction

Fisheries managers are shifting towards supportive breeding or supplementation hatcheries as a means to boost population abundance in threatened populations while minimizing risks from domestication (Ford 2002; Goodman 2004). In supplementation programs, hatchery brood stocks are drawn from a portion of in-river spawners, and the offspring are raised in hatcheries for release into the wild. Upon return, some or all hatchery-origin offspring are allowed to spawn in natural spawning areas. Incorporating more spawners adapted to natural conditions into hatchery brood stocks is hypothesized to lessen overall domestication

selection in the population in comparison with using hatchery-origin brood stock (Lynch and O'Hely 2001; Ford 2002; Araki et al. 2007b). However, hatchery programs may still pose risks to genetic diversity and effective population size (N_e) if hatchery fish arise from small brood stocks and numerically overwhelm wild-origin fish on natural spawning grounds. This may increase overall variance in family sizes in the total population (Ryman–Laikre effects, Ryman and Laikre 1991) and decrease genetic diversity and N_e, the key parameters determining the adaptive potential of a population (Hedrick 2005). Loss of genetic diversity during hatchery programs or supplementation has been documented in Atlantic salmon (*Salmo salar*, Tessier et al. 1997) and brown trout (*Salmo trutta*, Hansen et al. 2000) when programs relied on hatchery-origin brood stock and founding numbers were small. In contrast, Heggenes et al. (2006) found slight reductions in genetic diversity after 20 years of supplementation in steelhead (anadromous rainbow trout, *Oncorhynchus mykiss*), and Eldridge and Killebrew (2008) found no loss of genetic diversity over 16 years of supplementation for a threatened Chinook salmon (*Oncorhynchus tschawytscha*) population. In this study, we explore impacts of supplementation through an examination of genetic diversity in supplemented and unsupplemented populations of summer chum salmon (*Oncorhynchus keta*) in the Puget Sound (PS) of Washington State, focusing primarily on threatened summer chum salmon in Hood Canal and the Strait of Juan de Fuca.

Chum salmon have the widest distribution of Pacific salmon, spawning along the Pacific Rim from Oregon to Japan and Korea and in tributaries along the Arctic Ocean (Groot and Margolis 1991). Chum salmon in Washington comprise three biogeographical groups based on genetic and ecological criteria: Washington Coast, Columbia River, and PS (Johnson et al. 1997). Chum salmon have a typical Pacific salmon life history, and similar to pink salmon (*Oncorhynchus gorbuscha*), they spend minimal time in fresh water. Chum salmon generally spawn in coastal areas or further up waterways below barrier falls, and juveniles migrate to estuaries within days of emergence (Johnson et al. 1997). Likewise, hatchery-origin chum salmon spend little time in artificial rearing environments and are released to the wild soon after emergence. Fall run-timed chum salmon are ubiquitous in PS watersheds. Summer chum salmon have a unique life history defined by spawning from early September through mid-October (Johnson et al. 1997; Tynan 1997). With the earliest run-timing in PS, Hood Canal (HC) and Strait of Juan de Fuca (SJF) summer chum salmon are adapted to a suite of ecological conditions (smaller drainages, warmer water, lower flow) associated with late-summer spawning (Johnson et al. 1997; Tynan 1997). Similar to other salmonids, chum salmon spawning habitat is spatially distributed in discrete patches in rivers. Natal homing creates localized subpopulations linked more or less by gene flow from straying, leading to a metapopulation structure within basins (Johnson et al. 1997; Schtickzelle and Quinn 2007).

Within the PS region, HC and SJF summer-run chum salmon are genetically and ecologically distinct from the fall-run chum salmon in the region (Phelps et al. 1994) and are considered a separate evolutionarily significant unit (ESU) (Johnson et al. 1997). The ESU was listed as threatened under the Endangered Species Act in 1999. By the early 1990s, total spawner escapement had declined to under 1000 fish and remained depressed. Prior to declines, up to 20 spawning aggregations or subpopulations were identified in HC and SJF (Fig. 1), while up to nine extant subpopulations remain. As part of the effort to restore wild subpopulations of summer chum salmon, hatchery production in HC and SJF was initiated in 1992 for three subpopulations and in 1997, 1999, and in 2000 for three more subpopulations. Two subpopulations were not supplemented. Recovery efforts through supplementation were designed to reduce extinction risk and speed recovery while minimizing risks of deleterious genetic, ecological, and demographic effects to supplemented and unsupplemented subpopulations (Washington Department of Fish and Wildlife and Point No Point Treaty Tribes 2000). Supplementation programs were scheduled to run for a maximum of three generations (12 years). Brood stock were collected from streams where hatchery-propagated fish were to be released and mating employed partial factorial designs (Campton 2004; Busack and Knudsen 2007) to maximize genotypic diversity and effective subpopulation size (Waples and Do 1994; Withler and Beacham 1994). To monitor supplementation, most hatchery fish received unique hatchery-specific otolith marks (Volk et al. 1987) to identify the origin of returning hatchery spawners, and spawning streams were surveyed as adults returned (Washington Department of Fish and Wildlife and Point No Point Treaty Tribes 2000). Additionally, Washington Department of Fish and Wildlife (WDFW) developed a microsatellite genetic baseline of HC and SJF summer chum salmon and other PS chum salmon stocks (this study) to assess genetic changes.
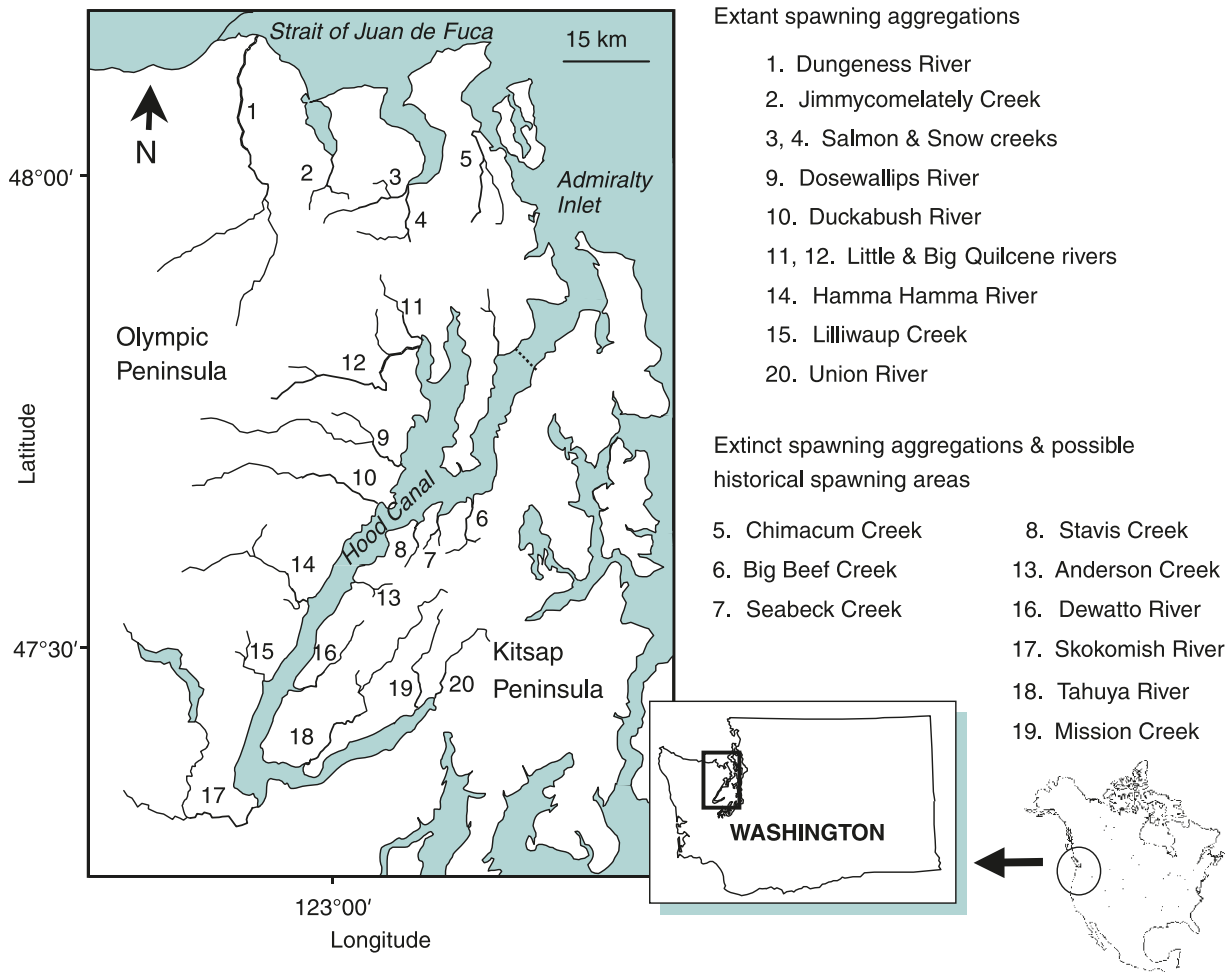
To look for evidence of loss of genetic diversity or decreased effective subpopulation size associated with supplementation, we compared genetic attributes over time between the six supplemented subpopulations and the two unsupplemented subpopulations. Likewise, in the supplemented subpopulations, we looked for differences before and after supplementation had been underway. Additionally, we compared diversity in HC and SJF summer chum salmon with diversity in nonthreatened fall chum salmon that co-occur in some of the streams with summer chum salmon in HC and elsewhere in PS. We also studied diversity of summer chum salmon from southern PS that have similar life history characteristics as HC and SJF summer chum but are genetically more similar to fall chum salmon. Further, since recovery within a metapopulation is dependent on connectivity among subpopulation components, we examined a hypothesis of isolation by distance (Wright 1943; Slatkin 1993) among HC and SJF summer chum salmon subpopulations and estimated impacts of supplementation on population structure.

Materials and methods

Collections

Collections consisted of summer chum salmon from HC, SJF, and South Puget Sound (SPS) and fall chum salmon from HC (see Fig. 1 for map and Table 1 for collection list). Tissue samples were collected from spawners in 16 rivers and creeks in HC, SJF, and SPS and from the Hoodsport Hatchery in HC from 1992 through 2003 (Table 1). The fol-

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Fig. 1. Map of Hood Canal, Strait of Juan de Fuca, and portions of Puget Sound. Rivers and streams are numbered. Tributary names associated with numbers and the status of the subpopulations are listed on the right. Map is modified from Sands et al. (2007).

Extant spawning aggregations

1. Dungeness River
2. Jimmycomelately Creek
3, 4. Salmon & Snow creeks
9. Dosewallips River
10. Duckabush River
11, 12. Little & Big Quilcene rivers
14. Hamma Hamma River
15. Lilliwaup Creek
20. Union River

Extinct spawning aggregations & possible historical spawning areas

5. Chimacum Creek
6. Big Beef Creek
7. Seabeck Creek
8. Stavis Creek
13. Anderson Creek
16. Dewatto River
17. Skokomish River
18. Tahuya River
19. Mission Creek

lowing summer chum salmon subpopulations had supplementation programs: Union, Hamma Hamma, Lilliwaup, Quilcene, Salmon, and Jimmycomelately (see Table 2 for fry releases). For supplemented tributaries in HC, we regressed total escapements (all spawners returning to tributary, Table 2) on all fry releases 3 and 4 years earlier in HC (e.g., escapement to Quilcene versus fry released in Quilcene, Lilliwaup, and Hamma Hamma) to test whether supplementation and possible hatchery-origin strays correlated with returns. For unsupplemented tributaries, we regressed total escapements on fry releases 3 and 4 years earlier in supplemented tributaries to assess contributions of hatchery strays. In SJF, we regressed only total escapements to Salmon Creek on fry releases 3 and 4 years prior in Salmon Creek, since strays from HC supplementation were negligible (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007) and the Jimmycomelately program started too recently (Table 2).

Summer chum salmon spawners from all subpopulations within HC and SJF were sampled annually to determine supplementation and natural-spawner contributions (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007). Hatchery-origin fish were identified by absence of adipose fins (Big Quilcene program only) or by otolith marks and aged via scale analysis (Washington

Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007).

Genotyping

Genotypes were assessed for 1342 individuals from 26 collections (Table 1) at 16 microsatellite loci (Table 3). DNA was extracted with a silica membrane protocol following manufacturer's instructions (Macherey-Nagel). Microsatellite loci were amplified as outlined in Table 3, and PCR products were run on ABI-3100 and ABI-3730 automated sequencers. A subset of samples was run on both sequencers to standardize allele mobility data generated by the two different platforms. Microsatellite alleles were scored and binned using GENOTYPER and GENEMAPPER software, both from Applied Biosystems (Applied Biosystems Inc., Foster City, California).

Statistical tests

We calculated basic statistics for collections to examine whether collections met expectations of random sampling and used these statistics to explore differences between supplemented and unsupplemented collections and to look for indications of Ryman–Laikre effects. Collections were tested for departures from Hardy–Weinberg equilibrium (HWE) at each locus and across all loci using FSTAT 2.9.3 (Nei 1987;

Table 1. Statistics for chum salmon (*Oncorhynchus keta*) collections.

Region–run	River	Name	N	Gene div.	Rich.	% link	F_{IS}	P	Escape.	N_e	N_e/N
HCS	Union	00Union	54	0.8040	10.94	2.21	0.019	0.1214	744	74.5	0.10
		03Union	48	0.8029	10.53	0.74	−0.002	0.1534	11 916	118.6	0.01
	Dosewallips	00Dose	56	0.8343	12.88	0.00	0.042	<u>0.0036</u>	1 260	65	0.05
		03Dose	46	0.8345	12.88	0.00	0.030	<u>0.0313</u>	7 066	101.2	0.01
	Duckabush	00Duck	48	0.8296	12.73	0.74	0.030	<u>0.0355</u>	466	81.3	0.17
		03Duck	47	0.8223	12.25	2.94	0.018	0.1274	1 869	86.1	0.05
	Hamma Hamma S	01Ham(S)	56	0.8253	12.71	0.74	0.000	0.5030	1 227	152.6	0.12
		03Ham(S)	48	0.8228	12.00	1.47	0.001	0.4742	854	96.3	0.11
	Quilcene S	92Quil(S)	50	0.8330	12.60	0.74	−0.019	0.8963	743	236.7	0.32
		97Quil(S)	54	0.8229	12.02	0.00	0.022	0.0705	7 903	119.6	0.02
	Lilliwaup	01Lilli	53	0.8240	12.07	3.68	0.001	0.4859	92	34.4	0.37
		02Lilli	48	0.7870	8.83	38.24	−0.013	0.7664	858	3.2	0.00
SJFS	Salmon	00Salmon	60	0.8114	10.86	2.94	0.026	<u>0.0388</u>	876	118.5	0.14
		03Salmon	48	0.8147	11.02	1.47	−0.005	0.6255	5 955	56.9	0.01
	Jimmycomelately	01Jim	60	0.7908	9.25	10.29	−0.009	0.8707	260	21.8	0.08
		03Jim	41	0.7468	7.52	13.97	−0.017	0.6699	446	7.6	0.02
HCF	Quilcene F	Quil(F)	47	0.8313	13.07	0.00	0.018	0.1017			
	Hoodsport Hatchery	98Hood	51	0.8497	12.99	0.00	0.029	<u>0.0239</u>			
		03Hood	46	0.8514	13.67	0.00	0.001	0.4633			
	Dewatto	Dewat	57	0.8388	13.50	0.00	0.021	0.0786			
	Mission	Miss	78	0.8415	13.20	1.47	0.045	**<u><0.0001</u>**			
	Hamma Hamma F	Ham(F)	45	0.8395	13.40	0.74	0.041	<u>0.0066</u>			
SPSS	John's Creek	John	54	0.8251	12.88	0.00	0.070	**<u><0.0001</u>**			
	Sherwood	Sher	55	0.8242	12.71	1.47	0.092	**<u><0.0001</u>**			
	Coulter	Coult	54	0.8460	13.47	0.00	0.086	**<u><0.0001</u>**			
	Blackjack	Black	38	0.8226	11.53	1.47	0.072	**<u><0.0001</u>**			
Average				0.8222	11.98	3.28	0.023				
Median				0.8247	12.65	0.74	0.020				
SD				0.0223	1.56	7.83	0.031				

Note: Regions and run type abbreviations: HCS, Hood Canal summer; SJFS, Strait of Juan de Fuca summer; HCF, Hood Canal fall; SPSS, South Puget Sound summer. If collections were made in 2 years, collection names are preceded by two-digit year. If two run types were collected in a river, these are indicated with "S" or "F" for summer and fall, respectively. Statistics include gene diversity (Gene div.), allelic richness (Rich.), the percentage of locus pairs (out of 136 pairs) in linkage disequilibria (% link), the Hardy–Weinberg equilibrium value (F_{IS}) and its associated P value (underlined values are significant ($P \leq 0.05$) before Bonferroni corrections; bold values are significant after corrections). Escapement (Escape.) from Washington Department of Fish and Wildlife and Western Washington Treaty Indian Tribes (2002) was calculated from an area under the curve method. Effective population size (N_e) was calculated using linkage disequilibrium, and N_e/N is the ratio of N_e to escapement (N).

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Table 2. Number of fry (for brood year) released into supplemented tributaries and number of summer chum salmon (*Oncorhynchus keta*)

| | Hood Canal | | | | | | |
| | Union | | Hamma Hamma | | Quilcene | | Lilliwaup |
Year	Hatchery; total	Fry	Hatchery; total	Fry	Hatchery; total	Fry	Hatchery; total
1987	—; 497		—; 26		—; 79		—; 32
1988	—; 629		—; 440		—; 297		—; 275
1989	—; 450		—; 16		—; 2		—; 43
1990	—; 275		—; 90		—; 6		—; 2
1991	—; 208		—; 71		—; 50		—; 30
1992	—; 140		—; 123		—; 743	216 441	—; 99
1993	—; 251		—; 69		—; 148	24 784	—; 77
1994	—; 738		—; 370		—; 722	343 550	—; 111
1995	—; 721		—; 476		—; 4 574	441 167	—; 79
1996	—; 494		—; 774		—; 9 515	612 598	—; 76
1997	—; 410		—; 104	12 000	—; 7 903	340 744	—; 28
1998	—; 223		—; 127	2 800	—; 3 057	343 530	—; 24
1999	—; 159		—; 255	51 600	—; 3 237	181 711	—; 13
2000	—; 744	75 876	—; 229	55 400	384; 5 898	414 353	—; 22
2001	—; 1 491	73 472	72; 1 227	49 500	3 325; 6 373	351 709	51; 92
2002	—; 872	82 636	1 278; 2 328	61 000	1 276; 4 487	272 017	822; 858
2003	4 010; 11 916	35 343	318; 854	75 356	1 993; 12 733	92 559	326; 353
2004	2 378; 5 976	0	282; 2 691	57 000	2 315; 38 153	0	881; 1 017

Note: Data is from Washington Department of Fish and Wildlife and Western Washington Treaty Indian Tribes (2002) and Washington Department of

Goudet 2001) with 1000 permutations. If collections depart from HWE, this can be an indication that collections contained family groups or a strong year class, included more than one subpopulation, or that some parents were related in the previous generation. We tested whether genotypes at each locus were independent with the genotypic disequilibrium test in GENEPOP3.3 (Raymond and Rousset 1995) with 500 batches and 3000 iterations. If a collection has several pairs of loci in disequilibrium, this can be an indication that the collection contains family groups, that the population is under selection, or that alleles have drifted because of a small subpopulation size. We calculated basic diversity measures (Nei's (1987) estimate of heterozygosity and allelic richness (based on minimum 30 individuals)) using FSTAT. In general, populations that have smaller N_e have fewer alleles and lower heterozygosity and allelic richness (Naish et al. 2008). Because abundance of threatened subpopulations decreased to very low numbers before recent recovery (see Table 2), we tested collections for bottleneck signals using the program BOTTLENECK (Piry et al. 1998). Results for all tests were adjusted for multiple comparisons (sequential Bonferroni correction, Rice 1989) to an alpha level of 0.05. We estimated effective subpopulation sizes using linkage disequilibrium (Waples 2006) in the program LDN_e (Waples and Do 2008). To look for evidence of Ryman–Laikre effects, we estimated family structure within HC and SJF summer chum salmon collections using IDENTIX (Belkhir et al. 2002). We calculated pairwise relatedness values (proportion of shared, identical by descent alleles) among individuals using Queller and Goodnight's (1989) Q value ($Q = 0.5$ for full siblings) and computed the mean and variance of Q for each collection. We assessed their significance by comparing calculated values with mean and variance of Q values in a panmictic population of 1000 multilocus genotypes generated from original data by random sampling without replacement. For 2002 Lilliwaup and 2003 Jimmycomelately creeks collections, we clustered individuals with $Q \geq 0.45$ into hypothetical full-sibling families and compared them with sibling groups estimated with maximum likelihood implemented in ML-RELATE (Kalinowski et al. 2006). While both methods identified the same basic groups, we accepted pairwise full-sibling relationships that had 95% likelihood with 1000 permutations executed in ML-RELATE.

Subpopulation comparisons

We explored whether strays from supplementation programs had perturbed population structure and whether supplementation had induced temporal variance in genetic attributes. We tested for significant differences in genotypic distributions between temporal collections within tributaries and among tributaries using GENEPOP 3.3 and examined partitioning of variance with pairwise F_{ST} and analysis of molecular variance (AMOVA) tests in ARLEQUIN 2.001 (Schneider et al. 2000). In pairwise F_{ST} tests, we assessed whether variance was significantly different from zero with 10 000 permutations. We estimated variance among and within run-timing and regional groups (HC summer and fall, SJF summer, and SPS summer) with the AMOVA.

We also examined supplementation effects by testing for significant differences in heterozygosity and allelic richness between unsupplemented and supplemented collections using Student's t tests. In supplemented subpopulations, we also tested for differences before and during supplementation (if presupplementation collections existed) or earlier and later during supplementation.

Genetic distance

We examined basic subpopulation structure using a den-

escapements (hatchery, if available, and total) into tributaries in Hood Canal and Strait of Juan de Fuca.

| Fry | Dosewallips, hatchery; total | Duckabush, hatchery; total | Strait of Juan de Fuca | | | |
| | | | Salmon | | Jimmycomelately | |
			Hatchery; total	Fry	Hatchery; total	Fry
	—; 9	—; 12	—; 1 527		—; 464	
	—; 661	—; 497	—; 2 638		—; 1 052	
	—; 16	—; 60	—; 215		—; 173	
	—; 8	—; 42	—; 278		—; 63	
	—; 250	—; 102	—; 184		—; 125	
20 000	—; 655	—; 617	—; 454	19 200	—; 616	
12 000	—; 105	—; 105	—; 463	44 000	—; 110	
15 000	—; 225	—; 263	—; 163	2 000	—; 15	
0	—; 2 787	—; 825	—; 616	38 808	—; 223	
15 000	—; 6 976	—; 2 650	—; 1 054	620 002	—; 30	
14 200	—; 47	—; 475	59; 901	718 212	—; 61	
17 200	—; 336	—; 226	529; 1 171	678 322	—; 98	
17 400	—; 351	—; 92	367; 528	346 802	—; 7	3 880
14 800	—; 1 260	—; 464	412; 876	904 352	—; 55	25 900
38 000	233; 990	280; 942	1 470; 2 792	90 980	9; 260	54 515
96 000	314; 1 627	175; 530	1 772; 6 049	118 347	55; 57	20 887
103 913	556; 7 066	269; 1 869	1 866; 5 955	88 610	378; 446	49 897
99 500	1 265; 11 549	789; 8 639	1 918; 6 021	0	613; 1 662	76 982

Fish and Wildlife and Point No Point Treaty Council Tribes (2007).

drogram. Pairwise chord distances (Cavalli-Sforza and Edwards 1967) among collections were generated from allele frequencies using GENDIST in PHYLIP (Felsenstein 1993). A dendrogram illustrating genetic relationships was constructed from pairwise chord distances using the neighbor-joining algorithm in the program NEIGHBOR in PHYLIP. To test the repeatability of tree branching, we made 10 000 bootstrap replicates of the pairwise chord distances using SEQBOOT, tree topologies were created for all replicates using NEIGHBOR, and a consensus tree was produced using CONSENSE in PHYLIP.

Assignment tests

To investigate genetic structure from a different perspective, we used assignment tests with the Rannala and Mountain (1997) algorithm in GeneClass2 (Piry et al. 2004). The program calculates the likelihood that an individual fish originated in the subpopulation in the tributary where it was sampled based on the genotype of the fish and allele frequencies in collections (with the fish removed from its original collection). High assignments back to run group in the tributary of origin indicate that genetic structure occurs at the level of run group in individual rivers; high assignment back to region (but not specific to a river) indicates that genetic structure is at the regional level. In assignments, likelihood values for assignment to temporal collections were grouped by tributary, and we calculated relative assignment likelihood by dividing the highest likelihood by the sum of all likelihoods. We accepted assignments for individuals with relative likelihood scores above 50%.

Isolation by distance

We examined data from HC and SJF summer chum salmon for evidence of isolation by distance. If supplementation had perturbed metapopulation structure, we expected a random pattern rather than isolation by distance. After genotypic tests indicated no significant differences among years within sample locations (except for Lilliwaup collections), we combined temporal data within tributaries (analyses were conducted with and without Lilliwaup collections combined). Although the data were unlikely to meet assumptions of equilibrium between mutation and migration, we estimated migrants per generation, Nm, from pairwise F_{ST} values using Nm = $(1 - F_{ST})/4F_{ST}$ as a rough approximation. Nm values for F_{ST} near zero were arbitrarily set to 250 to avoid undefined values. Geographical distances (kilometres) between mouths of streams were calculated using the most direct passage over open water. Mantel tests for association between Nm and distance and reduced major axis regressions were performed using IBD 1.4 (Bohonak 2002). Regression confidence limits were constructed from 1000 bootstrap regressions over all points.

Results

Supplementation and escapement

Fry, tabulated by parental brood year, were released in spring the following year and mainly returned 3 to 4 years later. We conducted multiple regressions (not shown) of total escapements within tributaries (no breakdown by age structure) on hatchery fry releases 3 and 4 years prior within tributaries (see Table 2) and also releases 3 and 4 years prior in other tributaries to explore possible effects of straying. Number of fry released per year varied by orders of magnitude among tributaries. Fry releases in Lilliwaup and Hamma Hamma tributaries were positively related to adult returns within and between their tributaries and also related to adult returns in Quilcene, Duckabush, and Dosewallips rivers. However, because of extreme variance and limited

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Table 3. Microsatellite loci and multiplex information.

Multiplex	Locus	Conc. (μmol·L^{-1})	Anneal temp. (°C)	Size range	No. of alleles	Citation
OkeA	*Ots*-G311	0.4	50	240–485	53	Williamson et al. 2002
	Oke-3	0.4		340–442	8	Buchholz et al. 2001
	Omy-1011	0.1		183–242	14	Rexroad et al. 2002
OkeB	*One*-102	0.5	50	215–300	21	Olsen et al. 2000
	One-114	0.4		176–292	29	Olsen et al. 2000
	Ots-3M	0.1		130–160	13	Banks et al. 1999
OkeC	*Ots*-1	0.15	50	115–240	17	Banks et al. 1999
	One-101	0.07		117–264	33	Olsen et al. 2000
OkeE	*One*-106	0.1	53	177–333	48	Olsen et al. 2000
	Ssa-419	0.05		258–306	13	Cairney et al. 2000
	One-18	0.04		160–177	6	Scribner et al. 1996
OkeF	*One*-111	0.2	53	169–333	60	Olsen et al. 2000
	Oki-1	0.1		174–246	17	Smith et al. 1998
	Ots-2M	0.12		143–158	6	Banks et al. 1999
OkeG	*One*-108	0.1	45	154–331	45	Olsen et al. 2000
	Ots-103	0.28		96–282	36	Small et al. 1998

Note: Polymerase chain reactions were conducted for 35 cycles in 10 μL volumes using 1 μL template DNA with a final concentration of 1.5 mmol·L^{-1} MgCl$_2$, 0.05 units of *Taq* DNA polymerase in 1× Promega DNA polymerase buffer. Literature source for primer sequences is under "Citation".

data, all regression coefficients were insignificant ($P >$ 0.05), and only the regression of adult returns in Lilliwaup Creek on fry releases within Lilliwaup Creek was significant ($F = 13.44$, $P = 0.002$, $R^2 = 75\%$). Fry releases in Quilcene were uncorrelated (flat or negative regression line and insignificant regression coefficients, $P > 0.05$) with adult returns in Quilcene River and in all other tributaries. Fry releases in Salmon River were positively correlated with adult returns, but the regression and regression coefficients were not significant ($P > 0.05$). Few HC summer chum salmon strayed to Salmon River (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007).

Supplementation programs contributed average 17% to 90% of adults to escapements in supplemented HC and SJF subpopulation (Table 2; Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007). Strays averaged 12% of adults to unsupplemented subpopulations (Table 2). Year-by-year estimates of straying by program of origin and stream of recovery are reported in Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes (2007). Natural-origin recruits per spawner increased following supplementation releases (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007), suggesting that supplementation contributed to spawners.

Subpopulation statistics and comparisons among subpopulations

In tests for HWE, nine tests at four loci in the SPS summer and HC fall chum salmon collections were significant after corrections. In HWE tests over all loci within collections, all SPS summer and some HC fall chum salmon collections showed significant deficits of heterozygotes (Table 1).

We looked for evidence that supplementation had eroded diversity over time by comparing heterozygosity and allelic richness in earlier and later collections (1 to 5 years after

first collection) from the same tributary. We detected no differences between earlier and later collections (t test $P =$ 0.17 and 0.13 for lower average heterozygosity and richness in later collections, respectively; see Table 1). In individual tests, the second collection years for Lilliwaup and Jimmycomelately creeks had significantly lower heterozygosity (t test $P = 0.005$ and 0.001, respectively) and allelic richness (t test $P = 0.0001$ and 0.001, respectively), and tests in other tributaries were not significant. Heterozygosity values were lower in HC summer chum salmon than in HC fall chum salmon (t test $P = 0.0007$) and not different between HC and SPS summer chum salmon (t test $P = 0.09$). Allelic richness patterns were similar, with lower richness in HC summer chum salmon than in HC fall chum salmon (t test $P = 0.0018$) and no difference between HC and SPS summer chum salmon (t test $P = 0.06$).

Genotypic disequilibrium tests indicated that genotypes at each locus were independent, with the exception of high disequilibrium in the 2002 Lilliwaup Creek and 2003 Jimmycomelately Creek collections (Table 1). All collections had positive bottleneck signals under the infinite allele model, but only 2002 Lilliwaup Creek gave a significant bottleneck signal under the two-phase model (proportion of stepwise mutations set at 80%).

We calculated N_e and its 95% confidence interval (1000 bootstraps) for each temporal HC and SJF summer chum collection using a linkage disequilibrium method (Waples 2006; Waples and Do 2008), with the lowest frequency allele set at 5% to avoid bias introduced by small collections (Fig. 2). N_e values were significantly lower only in the later collections from Lilliwaup, Jimmycomelately, and Salmon creeks. Since we lacked brood year information on some collections and thus analyzed them as single collection years, the values we present as N_e may be intermediate between N_e and the number of breeders (N_b) (Waples 2005).

Relatedness tests yielded information on how supplementation might be impacting subpopulations. To look for dif-

Fig. 2. Graph of effective subpopulation size (N_e, Waples linkage disequilibrium method) and 95% confidence intervals for Hood Canal and Strait of Juan de Fuca summer chum salmon (*Oncorhynchus keta*). Interval was too small to see for 2002 Lilliwaup Creek (±0.3). Sample names with asterisks were either collected before supplementation or there was no supplementation in the tributary. Note that samples were not decomposed into brood years.
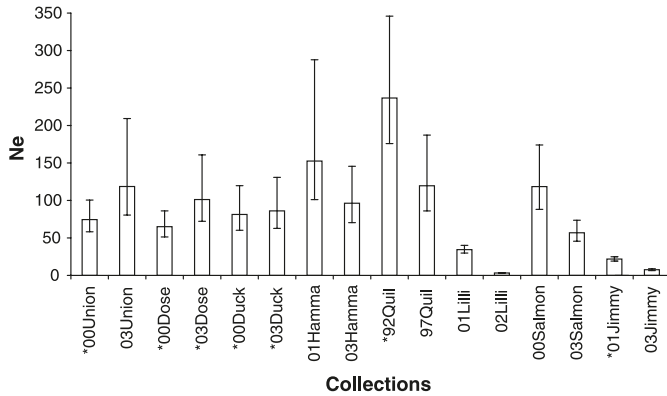
ferences between supplemented and unsupplemented collections, we examined collection years separately in relatedness tests (Table 4). We calculated means and standard deviations for full-sibling relationships over all collections with and without 2002 Lilliwaup and 2003 Jimmycomelately creeks collections, since their values were extremely high. In 2002 Lilliwaup Creek, we estimated six full-sibling groups totaling 35 offspring (one family had approximately 15 offspring) and 13 unrelated individuals. In 2003 Jimmycomelately Creek, we estimated nine full-sibling groups (largest family had approximately nine offspring) and 15 unrelated individuals. Mean Q values were not significantly different from means of permuted Q values. But subpopulation Q values were negative, indicating a tendency in individual pairwise tests for one member to have alleles at a frequency lower than the estimated frequency for the collection, which may arise when the collection includes a high proportion of related individuals (Gardner and West 2004; Munshi-South 2008). In supplemented collections, variance in relatedness values was significantly higher than variance of permuted random values. This suggested family groups in supplemented collections, since individuals will be either closely related to family members or unrelated, thereby increasing variance. Among unsupplemented collections, 1999 Jimmycomelately Creek and 2000 Union River collections had significantly high variance in relatedness values (Table 4). However, the Jimmycomelately Creek subpopulation had endured severe bottlenecks (Table 2), and the collection had a relatively high percentage of full-siblings (Table 4). This suggested that some parents may have been related or that there were family groups in the collection. In the 2000 Union River collection, there may have been some nonrandom components in the collection, such as family groups.

Genetic variance patterns within and among subpopulations

Pairwise genotypic and F_{ST} tests indicated temporal stability within summer chum salmon subpopulations from HC and SJF (Table 5), with the exception of collections from

Table 4. Relatedness values and percentage of full-sibling relationships in Hood Canal and Strait of Juan de Fuca summer chum salmon (*Oncorhynchus keta*) collections.

Collections	% full	Mean Q	P	Variance Q	P
*00Union	0.57	–0.020	>0.05	0.018	**<0.0010**
03Union	0.45	–0.021	>0.05	0.020	**<0.0010**
*00Dose	0.46	–0.020	>0.05	0.020	**0.0150**
*03Dose	0.00	–0.024	>0.05	0.014	0.0590
*00Duck	0.09	–0.024	>0.05	0.017	0.2800
*03Duck	0.38	–0.024	>0.05	0.015	0.0620
01Ham	0.26	–0.020	>0.05	0.014	**<0.0100**
03Ham	0.63	–0.021	>0.05	0.014	**<0.0010**
*92Quil	0.14	–0.021	>0.05	0.012	0.0690
97Quil	0.14	–0.019	>0.05	0.015	**0.0290**
01Lilli	1.45	–0.019	>0.05	0.021	**<0.0001**
02Lilli	**9.04**	–0.021	>0.05	**0.079**	**<0.0001**
00Salmon	0.33	–0.016	>0.05	0.017	**<0.0010**
03Salmon	0.72	–0.021	>0.05	0.017	**<0.0010**
*01Jim	1.81	–0.018	>0.05	0.032	**<0.0001**
03Jim	**4.76**	–0.029	>0.05	**0.051**	**<0.0001**
Mean (no Lilli, Jim)	1.33 (0.35)	–0.021		0.02 (0.018)	
SD (no Lilli, Jim)	2.36 (0.23)	0.003		0.02 (0.005)	

Note: Mean relatedness values for collections are Q values from Queller and Goodnight (1989). Means and standard deviations over all collections were calculated with (first number) and without (second number in parentheses) Lilliwaup and Jimmycomelately collections. High values for Lilliwaup and Jimmycomelately are in bold type. Significance (*P* values) for mean Q and variance of Q (H_0: mean and variance of relatedness values are indistinguishable from mean and variance of relatedness values in an unstructured population) were calculated using 1000 permutations, with significant *P* values in bold type. Abbreviations follow Table 1.

*Collections were not supplemented or were collected prior to supplementation returns.

Lilliwaup Creek. 2002 Lilliwaup Creek differed significantly from 2001 Lilliwaup Creek and all other collections, and F_{ST} tests showed that 2001 Lilliwaup Creek was weakly differentiated from Dosewallips, Duckabush, and Hamma Hamma rivers collections. Union River also differed from all other HC summer chum salmon collections. We found low differentiation among collections from Dosewallips, Duckabush, Hamma Hamma, and Quilcene rivers. The SJF collections were differentiated from each other and from HC summer chum salmon collections. Fall and summer runs within the same river were significantly different (not shown).

The AMOVA showed significant variance between summer chum salmon collections from HC and SJF (2.67%, $P < 0.001$). In the run-timing and regional analysis, 1.56% ($P < 0.001$) of the variance was partitioned between the two major groups (summer chum salmon from HC and SJF versus fall chum salmon from HC and summer chum salmon from SPS), and 2.33% ($P < 0.001$) of the variance was between the four run-timing and regional groups (with 1.66% variance among populations within the four groups, $P < 0.001$).

Genetic clusters identified in dendrogram

The consensus dendrogram identified two major clusters of subpopulations with bootstrap support of 100% (Fig. 3): one cluster included summer chum salmon from HC and SJF, and the other cluster included fall chum salmon from HC and summer chum salmon from SPS. These divided further into the four run-timing and regional groups, supporting

Table 5. Pairwise genotypic and F_{ST} test results for temporal comparisons in Hood Canal and Strait of Juan de Fuca summer chum salmon (*Oncorhynchus keta*) collections.

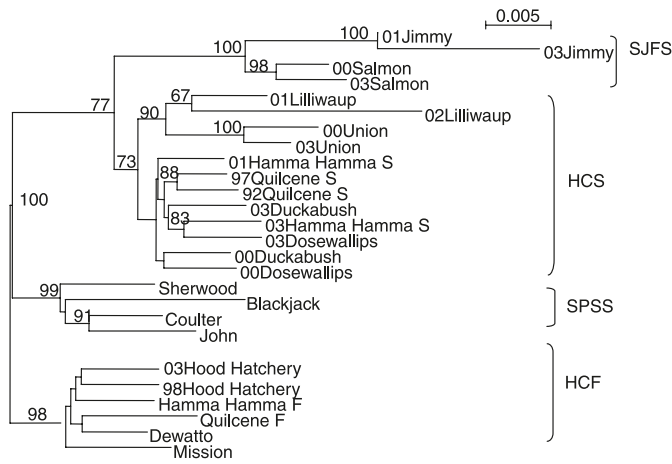|	00Union	03Union	00Dose	03Dose	00Duck	03Duck	01Ham	03Ham	92Quil	97Quil	01Lilli	02Lilli	00Salmon	03Salmon	01Jim	03Jim
00Union		0.84517	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
03Union	−0.0040		**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Dose	**0.0149**	0.0093		0.16178	0.5709	**0.00022**	0.00442	**0.00001**	**0.00067**	0.01151	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
03Dose	**0.0163**	**0.0173**	−0.0130		0.07067	0.03025	**0.0008**	0.18945	0.00108	0.02538	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Duck	**0.0192**	**0.0138**	−0.0008	−0.0066		0.03738	0.01599	**0.00018**	0.00366	0.39463	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
03Duck	**0.0248**	**0.0199**	−0.0023	0.0019	−0.0003		0.01065	0.02021	0.01649	0.12783	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
01Ham	**0.0225**	**0.0146**	−0.0084	−0.0023	0.0001	−0.0044		**0.00001**	0.02932	0.21778	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
03Ham	**0.0224**	**0.0203**	−0.0055	−0.0012	−0.0022	0.0011	0.0033		0.00316	**0.00066**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
92Quil	**0.0179**	**0.0159**	−0.0094	−0.0009	−0.0032	0.0006	−0.0015	0.0019		0.94416	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
97Quil	**0.0205**	**0.0159**	−0.0035	−0.0032	−0.0029	0.0002	−0.0021	0.0023	−0.0013		**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
01Lilli	**0.0171**	**0.0171**	0.0032	0.0069	**0.0096**	**0.0118**	0.0082	**0.0130**	0.0089	**0.0092**		**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.00001**
02Lilli	**0.0409**	**0.0391**	**0.0329**	**0.0374**	**0.0396**	**0.0389**	**0.0322**	**0.0450**	**0.0437**	**0.0419**	**0.0300**		**0.00001**	**0.00001**	**0.00001**	**0.00001**
00Salmon	**0.0393**	**0.0370**	**0.0164**	**0.0195**	**0.0185**	**0.0252**	**0.0263**	**0.0253**	**0.0282**	**0.0288**	**0.0338**	**0.0592**		0.44026	**0.00001**	**0.00001**
03Salmon	**0.0350**	**0.0320**	**0.0144**	**0.0191**	**0.0159**	**0.0211**	**0.0221**	**0.0237**	**0.0239**	**0.0246**	**0.0247**	**0.0544**	0.0009		**0.00001**	**0.00001**
01Jim	**0.0512**	**0.0440**	**0.0336**	**0.0327**	**0.0303**	**0.0278**	**0.0426**	**0.0342**	**0.0338**	**0.0350**	**0.0443**	**0.0722**	**0.0144**	**0.0102**		0.00087
03Jim	**0.0776**	**0.0725**	**0.0621**	**0.0641**	**0.0545**	**0.0539**	**0.0623**	**0.0632**	**0.0601**	**0.0620**	**0.0740**	**0.1005**	**0.0513**	**0.0425**	0.0122	

Note: Data above diagonal show *P* values for pairwise genotypic tests; data below diagonal show pairwise F_{ST} values. Significant values (corrected for multiple tests) for both tests are in bold type. Names follow Table 1.

Table 6. Table of assignments using GeneClass2 with all collections in baseline.

Region–run		Union	Dose	Duck	Ham(S)	Quil(S)	Lilli	Salmon	Jim	John	Sherw	Coult	Black	Hood	Dewat	Quil(F)	Ham(F)	Miss	Total
HCS	Union	**93**	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	1	99
	Dose	1	**29**	23	18	13	2	1	0	0	1	0	0	0	1	1	0	1	91
	Duck	0	18	**20**	15	21	3	0	0	0	1	2	0	0	0	0	0	0	80
	Ham(S)	1	22	18	**34**	19	2	1	0	0	0	0	0	1	0	0	0	0	98
	Quil(S)	3	11	17	17	**44**	3	0	0	0	0	1	0	1	0	0	0	0	97
	Lilli	1	0	2	7	14	**75**	0	0	0	0	0	0	0	0	0	0	0	99
SJFS	Salmon	0	1	1	0	1	0	**100**	4	0	0	0	0	0	0	1	0	0	108
	Jim	0	0	0	0	0	0	7	**92**	0	0	0	0	0	0	0	0	0	99
SPSS	John	0	1	1	1	0	0	0	0	**29**	3	7	1	0	0	2	1	2	48
	Sherw	0	0	0	0	0	0	0	0	7	**30**	5	3	4	1	0	0	2	52
	Coult	0	0	1	1	1	0	0	0	10	9	**19**	1	1	4	1	2	1	51
	Black	0	0	0	0	0	0	0	0	3	2	2	**25**	2	0	0	0	0	34
HCF	Hood	0	1	1	0	0	0	0	0	0	1	1	0	**42**	17	3	12	14	92
	Dewat	0	0	1	0	0	1	0	0	0	3	1	0	15	**10**	9	7	2	49
	Quil(F)	0	0	0	0	0	0	1	0	0	1	2	0	9	8	**19**	3	2	45
	Ham(F)	0	0	1	0	0	0	0	0	0	0	1	0	13	7	3	**5**	7	37
	Miss	0	1	2	1	0	1	0	0	0	0	3	0	11	9	5	10	**30**	73
	Total	102	102	95	104	104	101	108	101	54	55	54	38	97	57	47	45	78	1342
	Total assigned	99	91	80	98	97	99	108	99	48	52	51	34	92	49	45	37	73	1252
	Unassigned	3	11	15	6	7	2	0	2	6	3	3	4	5	8	2	8	5	90
	Correct	93	29	20	34	44	75	100	92	29	30	19	25	42	10	19	5	30	696
	% correct	93.94	31.87	25.00	34.69	45.36	75.76	92.59	92.93	60.42	57.69	37.25	73.53	45.65	20.41	42.22	13.51	41.10	55.59

Note: Assignments for each collection are in rows (read across), with total fish analyzed in collection at side and bottom. Assignments back to collection of origin ("correct" assignments) are in bold type along diagonal. Unassigned fish had relative likelihood scores below 50 for highest assignment. Percent correct were correct assignments over total assigned. Abbreviations follow Table 1.

Fig. 3. Neighbor-joining tree showing chord distances among collections and bootstrap support for groupings on the tree. The numbers at the nodes are the percentage of 10 000 trees (over 65%) in which the collections beyond the node grouped together. Region and run group are on right, following Table 1.

Fig. 4. Relationship between migrants per generation (Nm) and geographic distance among Hood Canal and Strait of Juan de Fuca summer chum salmon (*Oncorhynchus keta*). Solid squares are Strait of Juan de Fuca collections; open diamonds represent Hood Canal collections. Lilliwaup Creek collections (circles) were divided by collection year: 2001 (open circles) and 2002 (solid circles). Mantel test indicated significant association between Nm and distance ($Z = 2390.72$; $P \leq 0.002$). Regression line using reduced major axis regression is $\log(Nm) = 2.15 - 0.0099$ km, $R^2 = 0.533$. Thinner solid lines show 95% confidence limits based on 1000 bootstrap regressions over all points.

that genetic variance was structured by run-timing and geography. Within HC summers, Union and Lilliwaup collections formed a sub-branch. Quilcene River collections formed a branch, but collections from Dosewallips, Duckabush, and Hamma Hamma rivers mixed within an undifferentiated branch.

Assignment test

Assignment tests supported a metapopulation structure with straying among physically close subpopulations. Self-assignments (assignment back to river and run group in which the spawner was collected) were high and moderate for fish collected in Union River and Lilliwaup Creek, respectively, and low in the Dosewallips, Duckabush, Hamma Hamma, and Quilcene rivers collections (Table 6). Although self-assignments for the Duckabush River collection was 25% (the value expected for random assignment to a baseline containing four undifferentiated subpopulations), assignments for Dosewallips, Hamma Hamma, and Quilcene rivers collections were higher than random expectations (threshold = 30% at $P < 0.05$, Waples and Gaggiotti 2006), suggesting cryptic differentiation. In all collections, assignments to run-timing and regional groups were high, supporting that run-timing and geography structure genetic variance.

Isolation by distance

Isolation by distance explains much of the genetic structure in summer chum salmon. We found a strong negative relationship between Nm and geographical distance (Fig. 4). Greatest genetic exchange was between aggregations in the Dosewallips, Duckabush, Hamma Hamma, and Quilcene rivers, with less differentiation than expected among these aggregations. The 2002 Lilliwaup Creek collection differed from 2001 Lilliwaup Creek and other HC and SJF summer chum salmon collections, with most values plotting outside the 95% confidence limits. If Lilliwaup Creek year classes were combined, most values for Lilliwaup Creek plotted outside the lower confidence limits for the line (not shown).

Discussion

Hatchery supplementation is an adaptation of traditional hatchery programs designed to buffer threatened salmonid populations from risks of extinction and genetic drift associated with small N_e while avoiding problems associated with traditional hatcheries. Traditional hatcheries often used only hatchery-origin fish with an out-of-basin origin. While traditional hatcheries generally achieved the goal of producing fish, negative impacts arose when hatchery fish interacted with wild spawners, bringing in out-of-basin or hatchery-selected traits (Lynch and O'Hely 2001) and depressing N_e through overabundance of few hatchery families on natural spawning grounds (Allendorf 1993; Wang and Ryman 2001; Ford 2002) or unequal sex ratios (Allendorf 1993). Supplementation protocols seek to minimize domestication selection with more natural rearing conditions and prevent the introduction of exotic alleles by utilizing in-river brood stocks composed of hatchery- and wild-origin fish. Negative impacts could occur, however, from unequal sex ratios and family sizes (Araki et al. 2007*a*), if small brood stocks include closely related fish, or if hatchery-origin fish are less productive (Araki et al. 2007*b*). However, if hatchery impacts arise from selection on juveniles during rearing, selection might be less in chum salmon, since they migrate shortly after emergence, thus spending minimal time in the hatchery environment.

Genetic structure in HC, SJF, and PS chum salmon is organized by region and run-timing, similar to presupplementation genetic patterns (Phelps et al. 1994). In HC and SJF,

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summer chum salmon genetic diversity appeared to follow a metapopulation structure in which the amount of genetic exchange depended on the distance between spawner groups. This information facilitates management planning for HC summer chum salmon, since we clarify a key component affecting recovery goals — connectivity within the metapopulation must be maintained by averting local extinctions.

Hood Canal summer chum salmon ESU

HC summer chum salmon are genetically and ecologically distinct, constituting an ESU, yet they utilize the same tributaries as HC fall chum salmon, members of the PS–Strait of Georgia ESU (Johnson et al. 1997). While fall chum salmon subpopulations in the same rivers remained healthy (Washington Department of Fish and Wildlife and Western Washington Treaty Indian Tribes 2002), HC summer chum salmon declined because of loss of spawning habitat, low river flows, possible competition in the juvenile stage, and incidental harvest in the coho salmon fishery (Johnson et al. 1997; Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2000). Although HC fall chum salmon probably suffered habitat loss and competition as well, they enter the SJF after the coho salmon fishery terminates and enter rivers after fall rains begin. Whereas the first three problems remain for HC summer chum salmon, incidental harvest decreased when the coho salmon fishery was restricted following declines in abundance of HC and SJF coho salmon (Johnson et al. 1997; Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2000). Concurrent with the coho salmon decline, supplementation was initiated for some HC summer chum salmon subpopulations. Regressions and spawner-origin analyses indicate that supplementation and terminating adult harvest contributed to increases in HC and SJF summer chum salmon escapements. Changing oceanic conditions appear uncorrelated with chum salmon abundance (T. Johnson, WDFW, unpublished data; E. Casillas, National Marine Fisheries Service-NOAA, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112, USA, personal communication). Chum salmon out-migrate at a very young age, and initial juvenile survival may be unaffected by changes associated with decadal oscillations.

Hatchery impacts

Preserving genetic diversity, the foundation for response to environmental variation, is a fundamental goal of conservation programs. While Heggenes et al. (2006) found that allelic richness had decreased after 20 years of supplementation in steelhead, this study found genetic diversity and effective subpopulation sizes mostly unaltered by up to 7 years of supplementation, with the exception of the Lilliwaup and Jimmycomelately collections (see discussion below). Genetic diversity in HC and SJF summer chum salmon was similar to that of SPS summer chum salmon and significantly lower than HC fall chum salmon, suggesting that habitat for summer chum salmon in the region limits subpopulation size and diversity. Alternatively, diversity may be similar to that in SPS summer chum salmon if gene flow supported diversity within the metapopulation (Duchesne and Bernatchez 2002). Family group signals suggested

hatchery impacts in supplemented collections (Ryman and Laikre 1991; Wang and Ryman 2001; Belkhir et al. 2002). Although unsupplemented collections experienced similar population declines and received some hatchery strays, they lacked family group signals. Perhaps natural-origin fish overwhelmed impacts of strays to unsupplemented tributaries. The suspected family groups in supplemented collections had no significant impact on average or variance in N_e/N ratios in comparison with unsupplemented collections (t test $P = 0.34$, F test $P = 0.79$).

Hatchery impacts appeared strongest in the Lilliwaup Creek subpopulation. In 2001 Lilliwaup Creek, diversity was similar to other HC collections, and 55% of spawners originated in hatcheries (half of these in Lilliwaup hatchery). In 2002, 96% originated in hatcheries and 84% of these were from Lilliwaup hatchery — we genetically analyzed only these samples. Scale ages indicated that 90% were offspring from 10 fish spawned in 1999. The few parents and unequal hatchery family sizes (mean 2.8 offspring, SD 3.7) yielded offspring expressing few allele combinations. This generated extreme linkage disequilibrium and decreases in diversity and N_e (Ryman and Laikre 1991; Hedrick 2005; Araki et al. 2007a) and produced substantial temporal genotypic differentiation.

Since chum salmon have overlapping generations, with the strongest return at age 3 or 4 years in this region, the generational N_e would be roughly 3.5 times the arithmetic average of breeders calculated over a generation (Waples 2004). We suspect that we detected a genetic bottleneck signal in the 2002 Lilliwaup collection, since low escapements in parental brood years were bracketed by years with similarly low returns. If N has recently increased from a bottleneck, N_e can be downwardly biased for a few generations (Waples 2006). Further, to overcome negative impacts, supplementation must substantially increase census size for several years (Wang and Ryman 2001). Census size is increasing in Lilliwaup Creek (average from 2001–2006 = 831 fish, harmonic mean = 344 fish), and increased N_e may follow. Natural straying and gene flow within the metapopulation may have prevented bottleneck signals in other subpopulations (Busch et al. 2007).

The situation causing temporal changes in diversity was somewhat different in Jimmycomelately Creek. Otolith analysis showed that the 2001 fish descended from natural parents, yet high linkage disequilibrium suggested differential reproductive success for year classes or family groups in the collection. In the 2003 spawners, 84% originated in Jimmycomelately hatchery; 33% of these descended from four fish spawned in 1999, and 61% descended from 37 fish spawned in 2000. Diversity and N_e decreased and linkage increased in the hatchery-dominated collection. However, similar to the Lilliwaup subpopulation, supplementation boosted census size (average from 2001–2006 = 741 fish, harmonic mean = 184 fish).

Increased straying is another source of concern and uncertainty for supplementation programs. Salmonids stray naturally (Quinn 1993), and as hatchery programs increase fish, they may increase abundance of strays or returns might exceed available habitat in target tributaries. However, to minimize this risk, programs in HC and SJF were sized so that the number of fry released would produce adult returns ap-

Table 7. Summary of results from analyses exploring supplementation effects.

Test or measure	Neutral	Negative
Heterozygosity	Most	02Lilli, 03Jim
Allelic richness	Most	02Lilli, 03Jim
Linkage	Most	02Lilli, 03Jim
HWE	All	—
N_e	Most	02Lilli, 03Jim, 03Salmon
Ratio of N_e/N	All	—
Mean relatedness	All	—
Variance relatedness	—	All
AMOVA	All	—
Population structure	All	—

Note: Supplementation was hypothesized as neutral or negative, based on changes during supplementation. Where results varied, differing collections are listed. Note that supplementation was considered one of several factors impacting subpopulations.

proximately matching habitat potential (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007). Given chum salmon generation time, subpopulations were exposed to hatchery-origin strays since 1995 (Lilliwaup hatchery generated few returns until 2002). However, evidence from the IBD analysis and other analyses implies that long-term straying was below panmictic levels, and subpopulations remain somewhat differentiated.

Isolation by distance

The isolation by distance relationship suggested that gene flow connects HC and SJF summer chum salmon aggregations in a single metapopulation. Although data for the SJF collections indicated fewer migrants per generation between SJF and HC subpopulations, the isolation by distance relationship for all SJF comparisons, including those at short distances, is similar to the relationship for summer chum salmon overall. Historically, HC and SJF summer chum salmon subpopulations may have been more connected by migration and gene flow via stepping stone subpopulations (spawning habitat occurs in discrete patches and most straying is to nearby streams) in streams such as Chimacum, Big Beef, Seabeck, and Stavis creeks in northern HC. Under stepping stone migration, gene flow would have limited the genetic differentiation that occurs at greater geographical isolation. Geographically intermediate subpopulations may also have been genetically intermediate because of the increased opportunity for gene flow, which would have limited the clustering observed in contemporary analyses.

Conservation implications

Supplementation had neutral and negative effects (Table 7). Although abundances increased over the lows sustained throughout the 1980s and 1990s, possibly aided by supplementation and restriction of the coho fishery, diversity and N_e mostly remained unchanged. While hatchery-origin fish contributed to spawner returns, without direct information on their reproductive success we offer hypotheses from genetic signals associated with hatchery impacts. Supplemented collections had a common signal, suggesting unequal representation of family groups. Lilliwaup and Jimmycomelately creeks remain of particular concern, since

diversity and N_e were substantially lower than other collections, likely a result both of collection anomalies and limitations in hatchery brood stocks imposed by previous and contemporary bottlenecks. All HC and SJF subpopulations will be sampled after supplementation ends and again later to assess whether subpopulations remain self-sustaining. While we can only speculate on the impact of three generations of supplementation on population trajectories in the future, extinction risks have decreased to moderate or low in all HC and SJF summer chum subpopulations (Washington Department of Fish and Wildlife and Point No Point Treaty Council Tribes 2007).

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