Chum salmon stock identification in southern British Columbia and Puget Sound using microsatellites

Terry D. Beacham, K. Le, C. Wallace, and John R. Candy

Fisheries and Oceans Canada

Pacific Biological Station

3190 Hammond Bay Road

Nanaimo, British Columbia

Canada V9T 6N7

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Abstract

Chum salmon (*Oncorhynchus keta*) from 20 populations in southern British Columbia and Puget Sound were surveyed for variation at 28 microsatellite loci. The genotypic frequencies at 24 of the 28 loci examined conformed to those expected under Hardy-Weinberg equilibrium (HWE). Significant genetic differentiation was observed among chum salmon populations sampled in the different geographic regions surveyed. The F_{ST} value over all populations and loci was 0.011 (SD=0.001), with individual locus values ranging from 0.003 (*Omm1070*, *Omm1080*) to 0.034 (*Oke3*). Populations clustered into geographic regions. applications. Microsatellites with larger numbers of alleles were generally provided more accurate estimates of stock composition of single-population samples than did loci with smaller numbers of alleles. An average sample size of 300 fish was projected to be required for an accuracy of estimated stock compositions to be in excess of 90% to population consistently. The average accuracy of estimates of stock composition of the single-population samples was 75.2%, although estimates of individual populations ranged from 33.7% for the Cheakamus River population to 98.9% for the late-run Puyallup River hatchery population. The average regional accuracy of estimates of stock composition of the single-population samples was 88.6%, with estimates of individual populations ranging from 42.6% for the Cheakamus River population to 98.9% for the late-run Puyallup River hatchery population and the Hopedale Slough population in the Fraser River. Increasing the number of fish sampled in the baseline populations to at least 300 individuals should result in regional estimates of stock composition in excess of 90% for all 20 populations, as well as improving population-specific estimates of stock composition to at least 85% accuracy. Standard deviations about stock composition estimates were higher for population-specific estimates compared with regional estimates of stock composition, and the utilization of baselines with larger numbers of microsatellites for estimation of stock composition resulted in estimates with higher precision (lower standard deviations). Increased numbers of sampled fish per population also resulted in estimates of stock composition with higher precision.

Introduction

Microsatellites have been applied previously to estimate stock compositions in mixed-stock chum salmon fishery samples in southern British Columbia and Washington. These estimates were based upon 14 microsatellites as outlined by Beacham et al. (2009). These microsatellites had previously been standardized between DFO and WDFW in a preliminary project funded by the Southern Endowment Fund (Beacham et al. 2005, final report to PSC). Surveys of population genetic variation for these 14 microsatellites were conducted by DFO staff for both British Columbia and Washington populations. The baseline populations surveyed by DFO were used to provide estimates of stock composition in fisheries in Washington and British Columbia by Kirby (2008, 2011, Grant Kirby, Northwest Indian Fisheries Commission), with estimates provided by Fraser River, East Coast Vancouver Island, West Coast Vancouver Island, southern BC mainland, North Puget Sound, South Puget Sound, Hood Canal, Juan de Fuca Strait, and coastal Washington.

Staff at the Molecular Genetics Laboratory at the Pacific Biological Station in Nanaimo subsequently investigated over 100 additional microsatellites for finer population-specific or region-specific resolution, and up to 14 new microsatellites were chosen for subsequent evaluation. It is necessary to develop a baseline for microsatellite variation for possible subsequent applications for estimation of stock composition in mixed-stock fishery samples. Accordingly, an initial baseline was developed to survey microsatellite variation in salmon populations in southern British Columbia and Washington (Beacham et al. 2009). The next phase of the microsatellite baseline project was to increase the number of microsatellites in the baseline so that a common set of markers could be employed in studies concerning genetic stock identification of chum salmon.

A considerable baseline for stock ID analysis existed for the original 14 microsatellites in British Columbia and Washington (Beacham et al. 2009). In the current project, a standard set of 10 populations both from southern British Columbia and Washington was chosen as the standard reference populations. The approach for the microsatellite component of the current project had two aspects. The first aspect was to survey the original 14 microsatellites for all new reference samples available so that the baseline can be applied in a timely manner to mixed stock fishery samples. The second approach was to survey the additional 14 microsatellites for the reference populations chosen for evaluation in the study, so that an evaluation of the utility of specific microsatellites can be conducted.

The Southern Endowment Fund supported surveys of microsatellite variation in southern British Columbia and Washington chum salmon that were in addition to those reported by Beacham et al. (2009). The objectives of the project, as outlined in the project proposal, were:

“1. Assemble a standard set of 10 populations each from SBC and WA for reference populations (20 total). Distribute tissues to U.S. and Canadian laboratories.

2. Genotype individual chum salmon at SNP and microsatellite markers.

3. Evaluate the resolution of the SNP and microsatellite databases for genetic stock identification in the fisheries relevant to the Treaty.

The Chum Technical Committee will coordinate collection, cataloguing, and distribution of tissue samples to the analysis laboratories. The objective is to assemble a set of high quality tissues housed in both US and Canadian laboratories. For this study, we propose to genotype 20 populations from SBC and WA samples. Target samples size for each population for each analysis will be 96 individuals.”

The current report will outline the results of the survey of variation for 28 microsatellites in 20 populations of chum salmon from southern British Columbia and Washington. In fact, greater than 96 individuals were surveyed for microsatellite variation in the majority of the 20 populations surveyed.

Methods and Materials

Collection of DNA samples and laboratory analysis

Tissue samples were collected from mature chum salmon, generally preserved in 95% ethanol but previously frozen tissue initially collected for allozyme analysis was also accessed in some cases, and sent to the Molecular Genetics Laboratory at the Pacific Biological Station. Populations sampled, year of collection, and number of fish surveyed were outlined in Table 1. DNA was extracted from the tissue samples using a variety of methods, including a chelex resin protocol outlined by Small et al. (1998), a Qiagen 96-well Dneasy® procedure, or a Promega Wizard SV96 Genomic DNA Purification system. Once extracted DNA was available, surveys of variation at 28 microsatellite loci were conducted: Ots1, Ots3 (Banks et al. 1999), Oke3 (Buchholz et al. 2001), Oki2 (Smith et al. 1998), Oki100 (Beacham et al. 2008b), Omm1070, Omm1080, Omm1105, Omm1134 (Rexroad et al. 2001), Omy1011 (Spies et al. 2005), One101, One102, One104, One111, and One114 (Olsen et al. 2000), Ots103, Ots104 (Nelson and Beacham 1999), Ssa408, Ssa419 (Cairney et al. 2000), OtsG68, OtsG85 (Williamson et al. 2002), Omm1276 (Rexroad and Palti 2003), Cr373404 (Govoroun et al. 2006), Ots21esfu, Ots23esfu (Wright et al. 2007), Bhms176 (B. Hoyheim, Genbank accession number AF256765), Bhms313a (B. Hoyheim, Genbank accession number AF257052), and Ca368462 (Rexroad et al. 2003).

In general, PCR DNA amplifications were conducted using DNA Engine Cycler Tetrad2 (BioRad, Hercules, CA) in 6µl volumes consisting of 0.15 units of Taq polymerase, 1µl of extracted DNA, 1x PCR buffer (Qiagen, Mississauga, Ontario), 60µM each nucleotide, 0.40µM of each primer, and deionized H_2O. Specific PCR conditions for a particular locus were outlined in Table 2. PCR fragments were initially size fractionated in denaturing polyacrylamide gels using an ABI 377 automated DNA sequencer, and genotypes were scored by Genotyper 2.5 software (Applied Biosystems, Foster City, CA) using an internal lane sizing standard. Later in the study, microsatellites were size fractionated in an ABI 3730 capillary DNA sequencer, and genotypes were scored by GeneMapper software 3.0 (Applied Biosystems, Foster City, CA) using an internal lane sizing standard. Allele scores derived from Genotyper or GeneMapper were verified by either one or two laboratory personnel. Allele identification between the two sequencers were standardized by analyzing approximately 600 individuals on both platforms and converting the sizing in the gel-based data set to match that obtained from the capillary-based set.

Baseline populations

The baseline survey consisted of analysis of 5,446 chum salmon from 20 populations from southern British Columbia and Puget Sound. The sampling sites or populations surveyed in each geographic region are outlined in Table 1. Weir and Cockerham's (1984) F_{ST} estimates for each locus over all populations were calculated with FSTAT version 2.9.3.2 (Goudet 1995).

Estimation of stock composition in single-population samples

Two software packages were utilized in estimation of stock composition of single-population mixtures: Statistical Package for the Analysis of Mixtures software program (SPAM version 3.7) (Debevec et al. 2000) and ONCOR (Kalinowski et al. 2007). SPAM was used to evaluate the accuracy and precision of estimated stock compositions both on a population and regional basis for an individual locus for all 28 loci surveyed. Genotypic frequencies were determined for each locus in each population and were used to estimate stock composition of simulated single-population samples. The Rannala and Mountain (1997) correction to baseline allele frequencies was used for SPAM analyses in order to avoid the occurrence of fish in the mixed sample from a specific population having an allele not observed in the baseline samples from that population. This correction incorporated Bayesian modelling of baseline allele frequency distributions. All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies. Reported stock compositions for simulated single-population samples are the bootstrap mean estimate of each mixture of 150 fish analyzed, with mean and variance estimates derived from 1000 bootstrap simulations. Each baseline population and simulated single-population sample was sampled with replacement in order to simulate random variation involved in the collection of the baseline and fishery samples. When ONCOR was used to estimate stock compositions, the Rannala and Mountain (1997) correction to baseline allele frequencies was again implemented, with precision of the stock compositions calculated by bootstrapping (100 simulations) over observed baseline population sample sizes and a mixture size of 200 fish. For both SPAM and ONCOR, allocations to individual baseline populations were summed to provide estimates of stock compositions for regional stock groups (Table 1). ONCOR was used to evaluate accuracy of estimated stock compositions both on a population and regional basis for the set of 14 microsatellites originally outlined by Beacham et al. (2009). These loci were *Ots3, Oke3, Oki2, Oki100, Omm1070, Omy1011, One101, One102, One104, One111, One114, Ots103, OtsG68*, and *Ssa419*. Next, the set of loci that were in Hardy-Weinberg equilibrium were added to the suite of loci used in estimation of stock composition. These loci were: *Bhms313a, Ca368462,* *Cr373404*, *Omm1080*, *Omm1105*, *Omm1134*, *Omm1276*, *Ots1*, *Ots23esuf*, and *OtsG85*. Finally, the set of four loci (*Bhms176*, *Ots104*, *Ots21esuf*, and *Ssa408*) not in Hardy-Weinberg equilibrium were added to the set of 24 loci evaluated previously to evaluate whether these four loci provided any additional resolution in estimates of stock composition.

Results and Discussion

Population structure

The genotypic frequencies at 24 of the 28 loci examined conformed to those expected under Hardy-Weinberg equilibrium (HWE) (Table 3). Large alleles (up to 700 bp) were observed at two loci (*Ssa408*, *Ots104*), and analysis of degraded tissue may have resulted in large allele "dropout", and thus lower levels of heterozygosity than expected. Null alleles may have accounted for genotypes at the other two loci (*Bhms176*, *Ots21esfu*) not being in Hardy-Weinberg equilibrium. Significant genetic differentiation was observed among chum salmon populations sampled in the different geographic regions surveyed. The F_{ST} value over all populations and loci was 0.011 (SD=0.001), with individual locus values ranging from 0.003 (*Omm1070*, *Omm1080*) to 0.034 (*Oke3*) (Table 3). Dendrogram analysis indicated that there was typically clustering of populations within a geographic region. For example, the two populations sampled from Hood Canal (Big Beef Creek, Lilliwaup River) clustered together, the two normal-timed populations from South Puget Sound (Kennedy Creek, Skookum/Mill creeks) clustered together, as did three populations from North Puget Sound (Skagit, Stillaguamish, Skykomish rivers), two populations from the Fraser River (Hopedale Slough, Squakum Creek), and the east coast of Vancouver Island (Puntledge and Big Qualicum rivers) (Figure 1). A regional clustering of populations is important in order to support regional estimates of stock composition in mixed-stock fishery sampling.

Estimation of stock composition

Substantial variation was observed in the number of alleles at the 28 microsatellite loci surveyed in the study. The fewest number of alleles was observed at *Ssa419* (20 alleles), and the greatest number of alleles observed at *One111* (85 alleles) (Table 4). Microsatellites with larger numbers of alleles were generally provided more accurate estimates of stock composition of single-population samples than did loci with smaller numbers of alleles (Table 4).

The effect of baseline population sample size on accuracy of estimated stock compositions for single-population mixtures was evaluated for the 20 populations surveyed. Population-specific accuracy values were determined from the ONCOR simulations involving 28 loci and single-population samples with observed baseline sample sizes as indicated in Table 1. Substantial variation in accuracy was observed with respect to individual population sample size. An average sample size of 300 fish was projected to be required for an accuracy of estimated stock compositions to be in excess of 90% to population consistently (Figure 2). Accurate estimation of regional contributions is a less demanding objective. Larger sample sizes were required to obtain the same level of population-specific accuracy compared with region-specific accuracy.

ONCOR simulations incorporating estimation of stock composition of single-population samples was conducted for the original set of 14 microsatellites as outlined by Beacham et al.

(2009) (Set 1, Table 5), adding ten new microsatellites in Hardy-Weinberg equilibrium to the original suite of 14 microsatellites (Set 2, Table 6), and finally adding four loci out of Hardy-Weinberg equilibrium to the previous set (Set 3, Table 7). Simulations were conducted using the observed baseline sample sizes for each population (Table 1), and with population sample size standardized to 300 fish as suggested by Figure 2. The simulations indicated that adding more loci to the suite of microsatellites used for estimation of stock composition improved estimates of stock composition, regardless of whether the loci were in Hardy-Weinberg equilibrium or not (Table 8, Table 9). Standardizing to a population sample size of 300 individuals generally improved accuracy of estimates of stock composition for populations where less than 300 individuals were sampled (eg. Cheakamus River), but decreased accuracy for populations in which more than 300 individuals were sampled (eg. Squakum Creek)(Tables 5, 6, 7).

The results from the simulations involving 28 loci will be reviewed in some detail. The average accuracy of estimates of stock composition of the single-population samples was 75.2% (Table 8), although estimates of individual populations ranged from 33.7% for the Cheakamus River population to 98.9% for the late-run Puyallup River hatchery population. Lower accuracy of estimated stock compositions was generally observed in those populations with lower numbers of fish sampled for microsatellite variation. For example, the Cheakamus River population displayed the lowest accuracy of population-specific stock composition, and it also had the fewest number of fish surveyed in the population (Table 1).

Regional estimates of stock composition, derived from the 11 regions defined in Table 1, were, as expected, higher than those for populations. The average regional accuracy of estimates of stock composition of the single-population samples was 88.6% (Table 9), with estimates of individual populations ranging from 42.6% for the Cheakamus River population to 98.9% for the late-run Puyallup River hatchery population and the Hopedale Slough population in the Fraser River (Table 7). Lower regional accuracy of estimated stock compositions was generally observed in those populations with lower numbers of fish sampled for microsatellite variation. Regional estimates of stock composition were below an approximate 90% value only for three populations from the southern British Columbia mainland coast (Cheakamus River, Phillips River, and Lang Creek) and the Nooksack River in northern Washington. However, increasing the number of fish sampled in these baseline populations to at least 300 individuals should result in regional estimates of stock composition in excess of 90% for these populations, as well as improving population-specific estimates of stock composition to at least 85% accuracy (Table 7).

Standard deviations of the population-specific estimates of stock composition declined as the number of loci used in estimation increased. For example, average standard deviations for estimates of specific populations were 4.1% for the 14-locus baseline, 3.4% for the 24-locus baseline, and 3.1% for the 28-locus baseline (Tables 5, 6, 7). Corresponding values for the regional estimates of stock composition were 3.1%, 2.5%, and 2.2%, respectively. With population sample size standardized to 300 individuals per population, average standard deviations for population-specific estimates were 3.2%, 2.5%, and 1.8%, respectively. Corresponding standard deviations of regional estimates of stock composition in the standardized 300 individuals per population baseline were 2.6%, 2.0%, and 1.4%, respectively. As expected, standard deviations about the estimates were higher for population-specific estimates compared with regional estimates of stock composition, and the utilization of baselines with larger numbers of microsatellites for estimation of stock composition resulted in estimates with higher precision (lower standard deviations). Increased numbers of sampled fish per population also resulted in estimates of stock composition with higher precision.

Stock compositions were estimated for mixtures of 200 fish containing individuals from four of the 20 populations in the baseline with the baseline population sample size at observed levels (Table 10) and with baseline sample size standardized to 300 fish per population (Table 11). Regional estimates of stock composition were typically within 2% of actual values, except when populations of ECVI or Nooksack River dominated the mixtures. Standardization of baseline samples sizes to 300 fish per population generally improved estimates of stock composition.

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Table 1. Chum salmon spawning locations, sample collection years, and total number of fish sampled (N) for 20 populations in 11 geographic areas in southern British Columbia and Washington. N is the average sample size over all loci scored.

Region	Population	Years	N
Johnstone Strait	Nimpkish River	2002, 2004, 2010	539
Southern mainland	Southgate River	2003, 2004	162
	Phillips River	2004, 2006, 2011, 2012	143
	Lang Creek	2008, 2009, 2011	258
	Cheakamus River	1992, 2002, 2008	88
Fraser River	Hopedale Slough	2005, 2007, 2008, 2009, 2010, 2011	230
	Squakum Creek	2000, 2004, 2005, 2007, 2008, 2009, 2010, 2011	871
East coast Vancouver Island	Big Qualicum River	1992, 2007, 2009, 2010	540
	Puntledge River	1991, 2007, 2009, 2010	542
West coast Vancouver Island	Nitinat River	1992, 2004, 2010	347
Nooksack River	Nooksack River	1998, 2010, 2011	174
North Puget Sound	Skagit River	1994, 1998, 2010	151
	Stillaguamish River	2010, 2012	303
	Skykomish River	2007, 2010, 2012	278
Central Puget Sound	Grovers/Chico	2010, 2011, 2012	169
South Puget Sound	Kennedy Creek	2003, 2010, 2011	183
	Skookum/Mill	2010, 2011	103
South Puget Sound Late	Puyallup River	2011, 2012	174
Hood Canal	Big Beef Creek	2010, 2011	84
	Lilliwaup River	2002, 2010	107

Table 2. Microsatellite loci surveyed in chum salmon and their associated annealing and extension temperatures and times (seconds), as well as the number of cycles used in polymerase chain reaction amplifications.

Locus	Annealing	Extension	Cycles
Bhms176	52 ^{o}C/60s	68^{o}C/60s	35
Bhms313a	50 ^{o}C/60s	68^{o}C/60s	45
Ca368462	50 ^{o}C/60s	72^{o}C/60s	40
Cr373404	47^{o}C/60s	68^{o}C/60s	35
Oke3	62 ^{o}C/45s	72 ^{o}C/45s	38
Oki100	50 ^{o}C/60s	72^{o}C/60s	33
Oki2	47 ^{o}C/60s	72^{o}C/60s	33
Omm1070	65 ^{o}C/60s	72^{o}C/60s	40
Omm1080	50 ^{o}C/60s	68^{o}C/60s	35
Omm1105	48 ^{o}C/60s	68^{o}C/60s	40
Omm1134	50^{o}C/60s	72^{o}C/60s	45
Omm1276	38 ^{o}C/60s	64^{o}C/60s	45
Omy1011	50 ^{o}C/30s	72^{o}C/30s	35
One101	52 ^{o}C/60s	68^{o}C/60s	28
One102	52 ^{o}C/60s	72^{o}C/60s	33
One104	52 ^{o}C/30s	70^{o}C/30s	40
One111	52 ^{o}C/30s	68^{o}C/60s	30
One114	47 ^{o}C/30s	68^{o}C/60s	33
Ots1	48 ^{o}C/60s	64^{o}C/60s	45
Ots103	61 ^{o}C/60s	72^{o}C/60s	28
Ots104	47 ^{o}C/60s	68^{o}C/60s	45
Ots21e	55 ^{o}C/60s	68^{o}C/60s	35
Ots23e	58^{o}C/60s	68^{o}C/60s	30
Ots3	48^{o}C/60s	72^{o}C/60s	40
OtsG68	50^{o}C/60s	72^{o}C/60s	37
OtsG85	50 ^{o}C/60s	68^{o}C/60s	40
Ssa408	48 ^{o}C/60s	68^{o}C/60s	45
Ssa419	57 ^{o}C/30s	68^{o}C/60s	33

Table 3. Microsatellite loci surveyed in chum salmon, total number of fish scored per locus, as well as expected and observed heterozygosity, and Fst values. Set 1 is the 14 microsatellites outlined by Beacham et al. (2009), Set 2 is a set of 10 new microsatellites in Hardy-Weinberg equilibrium, and Set 3 is a set of four new microsatellites considered out of Hardy-Weinberg equilibrium.

Locus	N	He	Ho	Fst
Set 1				
Oke3	5632	0.709	0.677	0.034 (0.009)
Oki100	5375	0.874	0.836	0.028 (0.006)
Ots3	5429	0.740	0.726	0.010 (0.003)
Oki2	5068	0.846	0.815	0.025 (0.005)
Omy1011	5306	0.886	0.863	0.008 (0.002)
One104	5472	0.947	0.911	0.009 (0.002)
Ots103	5245	0.947	0.931	0.008 (0.002)
Ssa419	5413	0.818	0.794	0.009 (0.003)
One101	5132	0.937	0.921	0.012 (0.004)
Omm1070	5102	0.957	0.948	0.003 (0.001)
One114	5253	0.927	0.910	0.008 (0.002)
One102	5276	0.921	0.914	0.003 (0.001)
OtsG68	5256	0.965	0.948	0.007 (0.002)
One111	5137	0.938	0.925	0.007 (0.002)
Set 2				
Ots23esfu	5385	0.747	0.725	0.009 (0.002)
OtsG85	5610	0.901	0.889	0.008 (0.002)
Cr373404	5648	0.851	0.825	0.009 (0.002)
Omm1134	5638	0.836	0.804	0.012 (0.004)
Ots1	5596	0.836	0.799	0.011 (0.004)
Bhms313a	5682	0.734	0.692	0.007 (0.002)
Omm1105	5505	0.954	0.910	0.004 (0.001)
Ca368462	5590	0.940	0.893	0.016 (0.008)
Omm1276	5368	0.963	0.927	0.009 (0.002)
Omm1080	4998	0.978	0.947	0.003 (0.001)
Set 3				
Bhms176	5389	0.926	0.849*	0.015 (0.001)
Ssa408	5040	0.897	0.759*	0.017 (0.007)
Ots104	4722	0.942	0.841*	0.008 (0.002)
Ots21esfu	5569	0.951	0.845*	0.007 (0.001)

* Locus considered out of Hardy-Weinberg equilibrium

Table 4. Number of alleles observed at a locus, as well as mean percentage and standard deviations (SD) of estimated percentage compositions of single-population mixtures of chum salmon from the 20 populations listed in Table 1. The region designation includes the sum of percentage allocations to all populations in the region as outlined in Table 1. Simulations for each locus individually were conducted with SPAM with a 20-population baseline, 150 fish in the mixture sample, and 100 resamplings in the mixture sample and baseline samples.

Locus	Number	Population		Region	
	of alleles	Estimate	SD	Estimate	SD
Ca368462	60	79	9	84	8
One111	85	78	8	84	7
Ots21e	80	78	9	83	8
Ssa408	54	77	1	81	10
Omm1276	69	73	10	80	9
Ots104	66	73	11	78	10
Bhms176	40	72	12	79	10
Ots1	49	72	13	78	11
Bhms313a	49	71	12	78	11
Omm1134	41	71	15	78	12
One101	43	70	11	76	10
Oki100	23	70	14	76	12
Omm1105	59	70	11	76	11
One104	37	69	12	75	11
OtsG68	55	69	11	76	10
Cr373404	31	69	15	74	14
Ots103	40	68	12	76	10
One114	37	67	13	74	12
Omm1080	81	66	10	70	10
Omy1011	28	66	15	73	13
Oki2	21	65	14	71	13
OtsG85	24	64	14	72	13
Omm1070	41	64	13	70	12
Ots23e	21	63	17	69	16
One102	33	62	15	69	14
Ssa419	20	58	19	66	17
Ots3	25	57	21	70	16
Oke3	23	56	21	67	18

Table 5. Mean accuracy and standard deviation of estimated stock compositions of single-population samples of 200 fish for the original suite of 14 microsatellites as outlined by Beacham et al. (2009) (Set 1 of Table 3) estimated from ONCOR over 20 populations with observed individual population sample sizes as outlined in Table 1 and with a simulated baseline where population sample sizes have been standardized to 300 individuals per population. ECVI is east coast of Vancouver Island, WCVI is west coast of Vancouver Island, N. Puget Sound is North Puget Sound, C. Puget Sound is Central Puget Sound, and S. Puget Sound is South Puget Sound, and S. Puget Sound L. is South Puget Sound Late.

		Observed baseline		Simulated baseline	
Region	Population	Population%	Regional %	Population%	Regional%
Johnstone Strait	Nimpkish River	95.9 (1.8)	95.9 (1.8)	92.2 (2.3)	92.2 (2.3)
Southern mainland	Southgate River	89.9 (2.7)	94.1 (2.1)	96.0 (1.5)	97.9 (1.2)
	Phillips River	45.9 (5.6)	53.4 (6.0)	74.8 (4.4)	80.1 (4.5)
	Lang Creek	60.7 (5.5)	66.6 (5.3)	79.9 (4.1)	85.7 (3.9)
	Cheakamus River	39.8 (4.4)	47.2 (4.8)	89.4 (2.5)	92.1 (2.3)
Fraser River	Hopedale Slough	59.5 (4.5)	96.9 (1.9)	82.0 (3.8)	93.6 (2.4)
	Squakum Creek	91.0 (3.6)	95.6 (2.1)	78.4 (4.1)	89.0 (3.3)
ECVI	Big Qualicum River	71.2 (5.8)	90.2 (3.8)	68.1 (4.8)	80.7 (4.3)
	Puntledge River	69.2 (5.4)	90.5 (3.7)	72.5 (4.9)	85.5 (3.9)
WCVI	Nitinat River	95.1 (1.8)	95.1 (1.8)	93.9 (2.2)	93.9 (2.2)
Nooksack River	Nooksack River	73.4 (4.4)	73.4 (4.4)	85.6 (2.9)	85.6 (2.9)
N. Puget Sound	Skagit River	59.6 (4.2)	88.7 (3.0)	81.8 (3.7)	92.6 (2.7)
	Stillaguamish River	52.9 (6.2)	87.1 (3.8)	70.0 (4.5)	87.9 (3.6)
	Skykomish River	50.1 (6.0)	87.0 (3.2)	71.1 (4.9)	83.8 (3.7)
C. Puget Sound	Grovers/Chico	91.1 (2.7)	91.1 (2.7)	95.8 (1.8)	95.8 (1.8)
S. Puget Sound	Kennedy Creek	89.5 (3.2)	96.6 (1.4)	95.2 (2.0)	98.4 (1.1)
	Skookum/Mill	52.5 (4.3)	80.4 (2.8)	91.6 (2.5)	95.4 (1.9)
S. Puget Sound L.	Puyallup River	96.3 (1.7)	96.3 (1.7)	98.1 (1.1)	98.1 (1.1)
Hood Canal	Big Beef Creek	51.8 (4.4)	78.3 (3.8)	91.0 (2.7)	94.5 (2.1)
	Lilliwaup River	75.5 (3.8)	90.1 (2.5)	93.3 (2.5)	96.7 (1.6)

Table 6. Mean accuracy and standard deviation of estimated stock compositions of single-population samples of 200 fish for a suite of 24 microsatellites as outlined by Beacham et al. (2009) (Set 1 and Set 2 of Table 3) estimated from ONCOR over 20 populations with observed individual population sample sizes as outlined in Table 1 and with a simulated baseline where population sample sizes have been standardized to 300 individuals per population. ECVI is east coast of Vancouver Island, WCVI is west coast of Vancouver Island, N. Puget Sound is North Puget Sound, C. Puget Sound is Central Puget Sound, and S. Puget Sound is South Puget Sound, and S. Puget Sound L. is South Puget Sound Late.

		Observed baseline		Simulated baseline	
Region	Population	Population%	Regional %	Population%	Regional%
Johnstone Strait	Nimpkish River	97.9 (1.4)	97.9 (1.4)	95.1 (1.7)	95.1 (1.7)
Southern mainland	Southgate River	91.7 (2.1)	95.5 (1.9)	96.1 (1.7)	96.1 (1.7)
	Phillips River	42.3 (3.4)	50.8 (4.1)	79.5 (3.8)	83.2 (3.5)
	Lang Creek	58.3 (4.7)	63.8 (4.6)	81.1 (3.4)	85.7 (3.1)
	Cheakamus River	34.7 (4.1)	43.7 (4.8)	91.7 (2.5)	93.8 (2.1)
Fraser River	Hopedale Slough	56.4 (4.7)	97.2 (1.4)	87.1 (2.9)	94.7 (1.0)
	Squakum Creek	93.8 (2.7)	96.9 (1.6)	83.2 (3.4)	89.1 (2.8)
ECVI	Big Qualicum River	68.6 (5.0)	90.7 (3.1)	72.3 (4.0)	83.8 (3.8)
	Puntledge River	71.4 (4.7)	93.2 (2.5)	75.1 (3.5)	86.8 (3.3)
WCVI	Nitinat River	97.6 (1.1)	97.6 (1.1)	97.4 (1.0)	97.4 (1.0)
Nooksack River	Nooksack River	78.2 (3.7)	78.2 (3.7)	91.8 (2.4)	91.8 (2.4)
N. Puget Sound	Skagit River	60.0 (4.2)	94.0 (2.0)	82.0 (3.5)	94.1 (2.1)
	Stillaguamish River	56.3 (5.1)	89.9 (3.3)	76.7 (3.8)	90.1 (2.9)
	Skykomish River	58.0 (4.6)	89.5 (2.7)	77.6 (4.1)	90.5 (2.5)
C. Puget Sound	Grovers/Chico	95.1 (1.7)	95.1 (1.7)	98.6 (1.0)	98.6 (1.0)
S. Puget Sound	Kennedy Creek	89.5 (2.8)	98.2 (1.0)		
	Skookum/Mill	55.1 (4.0)	85.3 (2.8)	96.1 (1.5)	98.0 (1.2)
S. Puget Sound L.	Puyallup River	98.2 (1.0)	98.2 (1.0)	99.0 (0.8)	99.0 (0.8)
Hood Canal	Big Beef Creek	61.8 (4.0)	81.8 (3.2)	95.4 (1.8)	96.5 (1.6)
	Lilliwaup River	85.2 (2.5)	94.2 (1.8)	96.4 (1.5)	97.9 (1.0)

Table 7. Mean accuracy and standard deviation of estimated stock compositions of single-population samples of 200 fish for a suite of 28 microsatellites (Set 1, Set 2, and Set 3 of Table 3) estimated from ONCOR over 20 populations with observed individual population sample sizes as outlined in Table 1 and with a simulated baseline where population sample sizes have been standardized to 300 individuals per population. ECVI is east coast of Vancouver Island, WCVI is west coast of Vancouver Island, N. Puget Sound is North Puget Sound, C. Puget Sound is Central Puget Sound, and S. Puget Sound is South Puget Sound, and S. Puget Sound L. is South Puget Sound Late.

		Observed baseline		Simulated baseline	
Region	Population	Population %	Regional %	Population%	Regional%
Johnstone Strait	Nimpkish River	98.5 (0.9)	98.5 (0.9)	97.1 (1.3)	97.1 (1.3)
Southern mainland	Southgate River	92.4 (2.3)	95.6 (1.9)	98.6 (0.9)	99.2 (0.7)
	Phillips River	49.5 (3.8)	57.7 (3.8)	87.6 (2.8)	89.5 (2.6)
	Lang Creek	64.6 (4.2)	68.7 (4.5)	89.5 (2.7)	91.7 (2.6)
	Cheakamus River	33.7 (4.0)	42.6 (4.2)	94.6 (1.7)	95.9 (1.5)
Fraser River	Hopedale Slough	59.0 (4.0)	98.9 (1.0)	94.3 (1.2)	98.1 (1.1)
	Squakum Creek	95.6 (2.1)	97.9 (1.2)	88.5 (2.7)	91.0 (2.4)
ECVI	Big Qualicum River	72.0 (4.9)	93.1 (2.6)	86.7 (3.3)	91.1 (2.6)
	Puntledge River	76.2 (3.9)	94.9 (2.1)	85.7 (3.4)	92.2 (2.5)
WCVI	Nitinat River	98.6 (0.9)	98.6 (0.9)	98.9 (0.8)	98.9 (0.8)
Nooksack River	Nooksack River	81.0 (3.3)	81.0 (3.3)	96.4 (1.5)	96.4 (1.5)
N. Puget Sound	Skagit River	61.7 (4.7)	95.5 (1.8)	93.0 (2.1)	97.1 (1.3)
	Stillaguamish River	57.7 (4.0)	90.9 (2.4)	88.1 (3.0)	94.0 (0.2)
	Skykomish River	60.4 (4.3)	91.7 (2.3)	87.6 (2.6)	94.7 (2.1)
C. Puget Sound	Grovers/Chico	96.5 (1.5)	96.5 (1.5)	99.3 (0.5)	99.3 (0.5)
S. Puget Sound	Kennedy Creek	91.9 (2.2)	98.6 (0.9)	98.9 (0.8)	99.6 (0.4)
	Skookum/Mill	58.9 (4.0)	89.3 (2.1)	97.7 (1.1)	98.6 (0.9)
S. Puget Sound L.	Puyallup River	98.9 (0.7)	98.9 (0.7)	99.7 (0.3)	99.7 (0.3)
Hood Canal	Big Beef Creek	68.6 (3.6)	87.5 (3.1)	98.3 (1.2)	98.6 (1.0)
	Lilliwaup River	88.0 (3.0)	95.2 (1.8)	98.9 (0.8)	99.2 (0.7)

Table 8. Population percentage accuracy of estimated stock compositions of single-population samples of 200 fish for a suite of 14, 24, and 28 microsatellites (Set 1, Set 2, and Set 3 of Table 3) estimated from ONCOR over 20 populations with observed baseline sample sizes. ECVI is east coast of Vancouver Island, WCVI is west coast of Vancouver Island, N. Puget Sound is North Puget Sound, C. Puget Sound is Central Puget Sound, and S. Puget Sound is South Puget Sound, and S. Puget Sound L. is South Puget Sound Late.

		Number of microsatellites		
Region	Population	14	24	28
Johnstone Strait	Nimpkish River	95.9	97.9	98.5
Southern mainland	Southgate River	89.9	91.7	92.4
	Phillips River	45.9	42.3	49.5
	Lang Creek	60.7	58.3	64.6
	Cheakamus River	39.8	34.7	33.7
Fraser River	Hopedale Slough	59.5	56.4	59.0
	Squakum Creek	91.0	93.8	95.6
ECVI	Big Qualicum River	71.2	68.6	72.0
	Puntledge River	69.2	71.4	76.2
WCVI	Nitinat River	95.1	97.6	98.6
Nooksack River	Nooksack River	73.4	78.2	81.0
N. Puget Sound	Skagit River	59.6	60.0	61.7
	Stillaguamish River	52.9	56.3	57.7
	Skykomish River	50.1	58.0	60.4
C. Puget Sound	Grovers/Chico	91.1	95.1	96.5
S. Puget Sound	Kennedy Creek	89.5	89.5	91.9
	Skookum/Mill	52.5	55.1	58.9
S. Puget Sound L.	Puyallup River	96.3	98.2	98.9
Hood Canal	Big Beef Creek	51.8	61.8	68.6
	Lilliwaup River	75.5	85.2	88.0
	Mean	70.5	72.5	75.2

Table 9. Regional percentage accuracy of estimated stock compositions of single-population samples of 200 fish for a suite of 14, 24, and 28 microsatellites (Set 1, Set 2, and Set 3 of Table 3) estimated from ONCOR over 20 populations with observed baseline sample sizes. ECVI is east coast of Vancouver Island, WCVI is west coast of Vancouver Island, N. Puget Sound is North Puget Sound, C. Puget Sound is Central Puget Sound, and S. Puget Sound is South Puget Sound, and S. Puget Sound L. is South Puget Sound Late.

		Number of microsatellites		
Region	Population	14	24	28
Johnstone Strait	Nimpkish River	95.9	97.9	98.5
Southern mainland	Southgate River	94.1	95.5	95.6
	Phillips River	53.4	50.8	57.7
	Lang Creek	66.6	63.8	68.7
	Cheakamus River	47.2	43.7	42.6
Fraser River	Hopedale Slough	96.9	97.2	98.9
	Squakum Creek	95.6	96.9	97.9
ECVI	Big Qualicum River	90.2	90.7	93.1
	Puntledge River	90.5	93.2	94.9
WCVI	Nitinat River	95.1	97.6	98.6
Nooksack River	Nooksack River	73.4	78.2	81.0
N. Puget Sound	Skagit River	88.7	94.0	95.5
	Stillaguamish River	87.1	89.9	90.9
	Skykomish River	87.0	89.5	91.7
C. Puget Sound	Grovers/Chico	91.1	95.1	96.5
S. Puget Sound	Kennedy Creek	96.6	98.2	98.6
	Skookum/Mill	80.4	85.3	89.3
S. Puget Sound L.	Puyallup River	96.3	98.2	98.9
Hood Canal	Big Beef Creek	78.3	81.8	87.5
	Lilliwaup River	90.1	94.2	95.2
	Mean	84.7	86.6	88.6

Table 10. Percentage estimated stock compositions from mixture simulations (four populations 200 fish/mixture) in ONCOR for chum salmon populations genotyped with 28 microsatelite loci. Mixture percentages were estimated to population and to region.

Population	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD
Nimpkish		0.2	0.38		0.1	0.21		0.1	0.32		0.1	0.27		0.2	0.29
Southgate		0.0	0.09		0.0	0.08		0.0	0.14		0.0	0.08		0.0	0.12
Phillips		0.7	0.75		0.3	0.56		0.6	0.86		0.9	1.08		1.0	1.01
Lang Creek		0.7	0.83		0.4	0.55		0.6	0.86		1.3	1.14		0.7	0.82
Cheakamus		0.1	0.18		0.0	0.14		0.1	0.22		0.1	0.20		0.1	0.19
Hopedale Slough	25	14.4	2.10	70	40.7	3.35	10	5.7	1.36	10	5.7	1.44	10	5.9	1.44
Squawkum		12.6	2.80		29.5	3.30		6.2	2.02		5.6	1.93		7.4	2.49
Big Qualicum		6.5	2.33		2.2	1.59		3.8	1.95		14.1	3.71		4.0	2.07
Puntledge	25	20.6	2.84	10	8.4	1.74	10	10.2	2.19	70	54.0	3.60	10	9.3	1.91
Nitinat		0.0	0.15		0.0	0.04		0.0	0.07		0.1	0.16		0.1	0.14
Nooksack	25	18.2	1.85	10	7.2	1.11	70	54.4	2.79	10	7.0	1.41	10	7.4	1.37
Skagit		2.4	1.52		1.1	1.09		2.1	1.42		1.0	1.05		5.8	2.12
Stillaguamish	25	16.5	2.97	10	7.1	1.96	10	11.5	2.79	10	7.0	2.08	70	41.3	4.33
Skykomish		7.1	2.26		2.9	1.74		4.5	2.00		3.1	1.78		17.0	4.01
Grovers/Chico		0.0	0.03		0.0	0.05		0.0	0.05		0.0	0.07		0.0	0.00
Kennedy		0.0	0.00		0.0	0.05		0.0	0.03		0.0	0.05		0.0	0.01
Skookum/Mill		0.0	0.05		0.0	0.04		0.0	0.00		0.0	0.01		0.0	0.08
Puyallup Hatchery		0.0	0.08		0.0	0.06		0.0	0.02		0.0	0.00		0.0	0.02
Big Beef		0.0	0.05		0.0	0.00		0.0	0.06		0.0	0.05		0.0	0.03
Lilliwaup		0.0	0.03		0.0	0.00		0.0	0.07		0.0	0.05		0.0	0.09
Region															
Johnstone Strait		0.2	0.38		0.1	0.21		0.1	0.32		0.1	0.27		0.2	0.29
South Coast		1.5	1.00		0.8	0.77		1.4	1.20		2.3	1.42		1.8	1.38
ECVI	25	27.1	2.33	10	10.6	1.55	10	14.0	2.15	70	68.1	2.33	10	13.3	2.18
WCVI		0.0	0.15		0.0	0.04		0.0	0.07		0.1	0.16		0.1	0.14
Fraser	25	27.1	1.76	70	70.2	1.47	10	11.9	1.36	10	11.3	1.40	10	13.2	1.85
Nooksack	25	18.2	1.85	10	7.2	1.11	70	54.4	2.79	10	7.0	1.41	10	7.4	1.37
N. Puget Sound	25	26.0	2.57	10	11.1	1.76	10	18.1	2.96	10	11.1	1.86	70	64.1	2.90
Central Puget Sound		0.0	0.03		0.0	0.05		0.0	0.05		0.0	0.07		0.0	0.00
S. Puget Sound		0.0	0.05		0.0	0.07		0.0	0.03		0.0	0.06		0.0	0.08
S .Puget Sound late		0.0	0.08		0.0	0.06		0.0	0.02		0.0	0.00		0.0	0.02
Hood Canal		0.0	0.06		0.0	0.00		0.0	0.09		0.0	0.07		0.0	0.10

Table 11. Percentage estimated stock compositions from mixture simulations (four populations 200 fish/mixture) in ONCOR for chum salmon populations genotyped with 28 microsatelite loci with population sample sizes standardized to 300 fish per population. Mixture percentages were estimated to population and to region.

Population	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD	Value	Avg	SD
Nimpkish		0.1	0.29		0.1	0.20		0.1	0.22		0.3	0.45		0.2	0.30
Southgate		0.0	0.13		0.0	0.11		0.0	0.10		0.0	0.15		0.1	0.17
Phillips		1.8	1.30		1.0	0.97		0.9	0.89		2.8	1.67		1.6	1.28
Lang Creek		1.2	1.04		0.6	0.67		0.8	0.87		2.2	1.51		1.2	0.94
Cheakamus		0.4	0.61		0.2	0.35		0.4	0.48		0.5	0.64		0.4	0.54
Hopedale Slough	25	21.6	1.94	70	61.1	2.57	10	8.7	1.20	10	8.6	1.22	10	8.8	1.32
Squawkum		3.1	1.65		6.3	2.34		1.7	1.19		1.8	1.41		2.2	1.55
Big Qualicum		3.9	1.92		1.7	1.00		1.9	1.34		9.1	2.91		2.8	1.62
Puntledge	25	19.8	2.39	10	8.3	1.53	10	8.2	1.59	70	52.6	3.78	10	8.8	1.75
Nitinat		0.0	0.09		0.0	0.07		0.0	0.13		0.1	0.15		0.1	0.15
Nooksack	25	22.8	1.54	10	9.2	1.14	70	64.2	1.96	10	9.2	1.14	10	9.6	1.26
Skagit		1.7	1.45		0.8	0.83		1.3	1.05		0.9	1.14		3.6	1.93
Stillaguamish	25	20.0	2.38	10	8.7	1.80	10	9.2	1.97	10	9.1	1.92	70	53.4	3.88
Skykomish		3.4	1.99		2.0	1.47		2.4	1.41		2.6	1.55		7.4	2.79
Grovers/Chico		0.0	0.06		0.0	0.12		0.0	0.06		0.0	0.09		0.0	0.07
Kennedy		0.0	0.09		0.0	0.03		0.0	0.06		0.0	0.10		0.0	0.07
Skookum/Mill		0.1	0.19		0.0	0.15		0.0	0.09		0.0	0.12		0.1	0.19
Puyallup Hatchery		0.0	0.11		0.0	0.08		0.0	0.04		0.0	0.04		0.0	0.02
Big Beef		0.0	0.11		0.0	0.11		0.0	0.13		0.0	0.12		0.1	0.18
Lilliwaup		0.0	0.10		0.0	0.13		0.0	0.08		0.0	0.06		0.1	0.15
Region															
Johnstone Strait		0.1	0.29		0.1	0.20		0.1	0.22		0.3	0.45		0.2	0.30
South Coast		3.5	1.61		1.8	1.30		2.1	1.18		5.6	2.06		3.2	1.62
ECVI	25	23.7	2.16	10	10.0	1.56	10	10.1	1.66	70	61.7	2.71	10	11.6	1.86
WCVI		0.0	0.09		0.0	0.07		0.0	0.13		0.1	0.15		0.1	0.15
Fraser	25	24.6	1.45	70	67.4	1.87	10	10.4	1.17	10	10.5	1.28	10	11.1	1.61
Nooksack	25	22.8	1.54	10	9.2	1.14	70	64.2	1.96	10	9.2	1.14	10	9.6	1.26
N, Puget Sound	25	25.1	2.30	10	11.4	1.98	10	12.9	2.00	10	12.6	2.01	70	64.3	2.72
Central Puget Sound		0.0	0.00		0.0	0.00		0.0	0.00		0.0	0.00		0.0	0.00
S. Puget Sound		0.1	0.21		0.0	0.15		0.0	0.12		0.1	0.15		0.1	0.20
S. Puget Sound late		0.0	0.11		0.0	0.08		0.0	0.04		0.0	0.04		0.0	0.02
Hood Canal		0.0	0.00		0.0	0.00		0.0	0.00		0.0	0.00		0.0	0.00

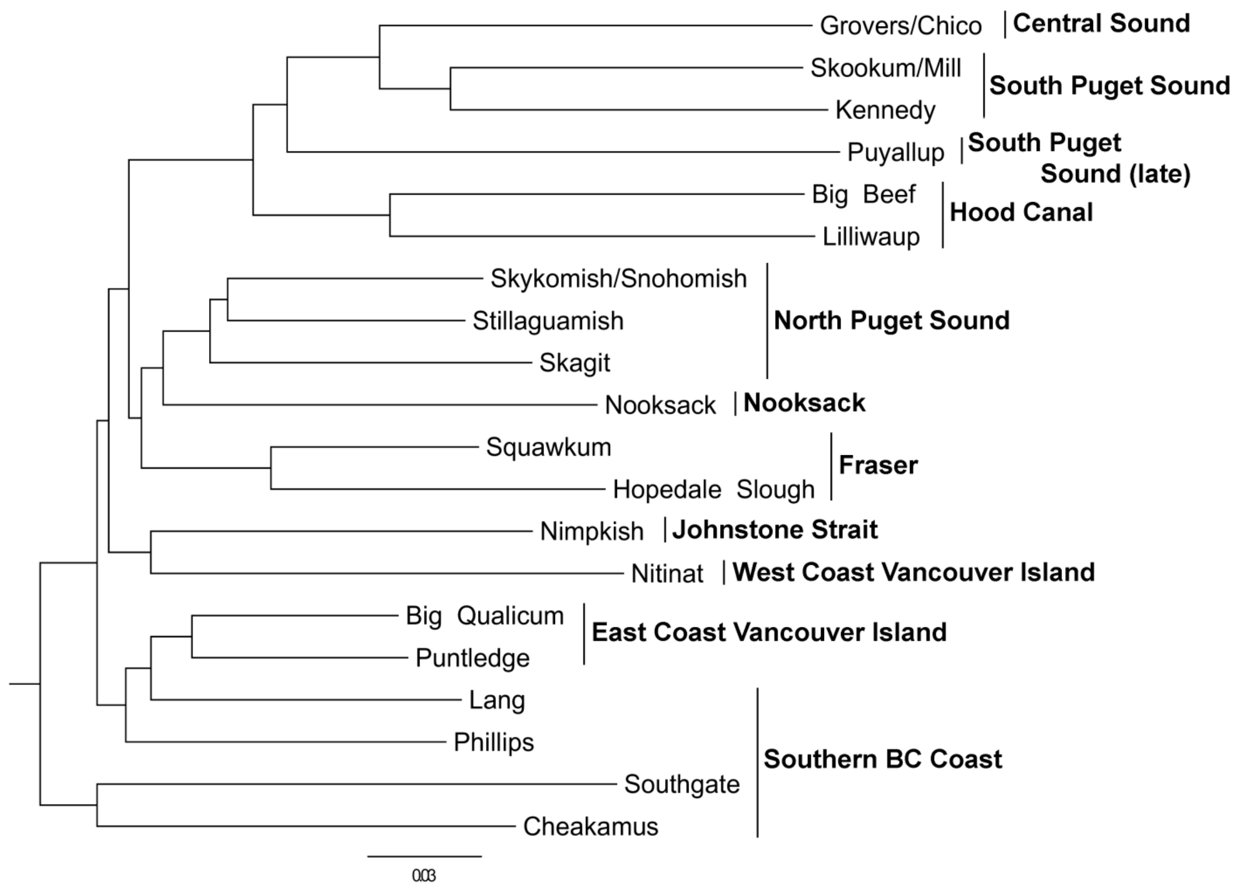

Figure 1: Neighbour-joining dendrogram of Cavalli-Sforza and Edwards (1967) chord distance for 20 southern British Columbia and Puget Sound populations of chum salmon (*Oncorhynchus keta*) surveyed at 28 microsatellites.

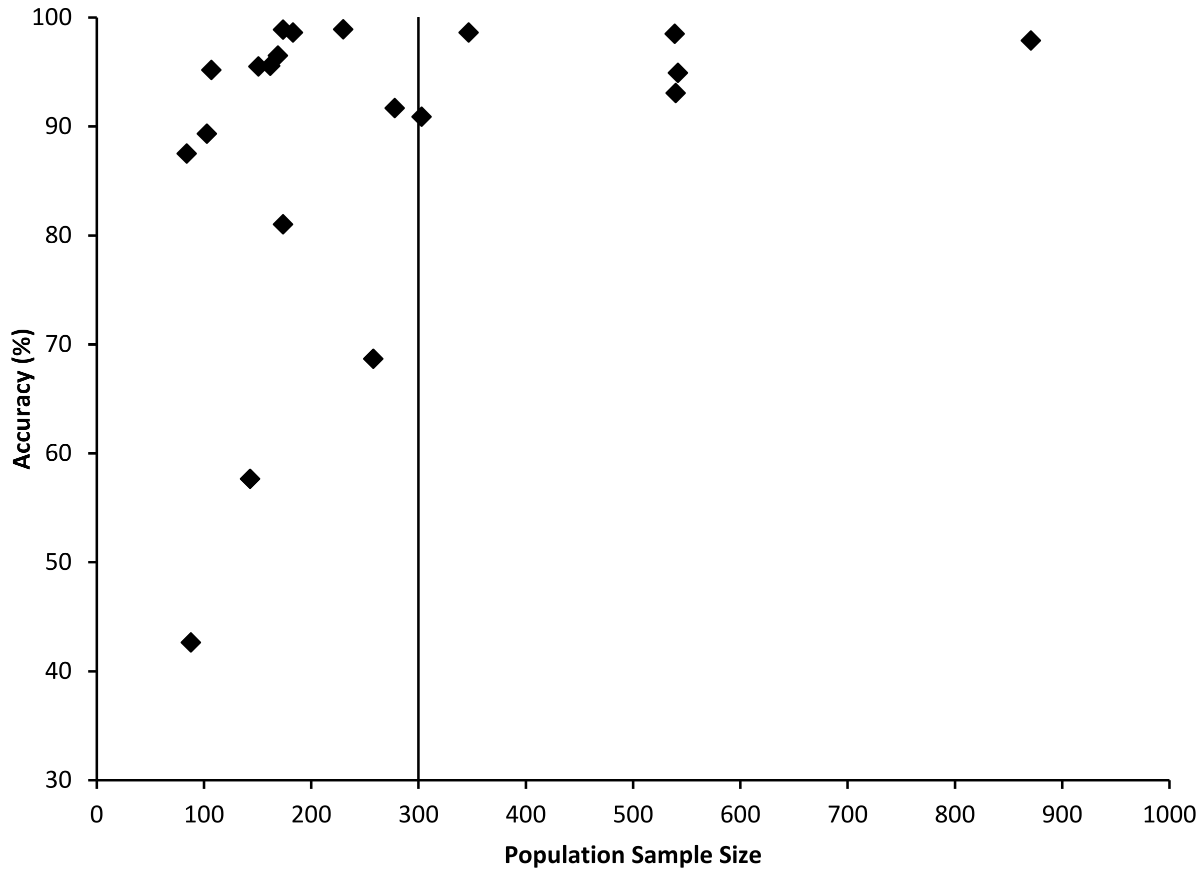

Figure 2. Relationship between the number of chum salmon surveyed for microsatellite variation in a specific population and the accuracy obtained for estimated population percentage of simulated single-population mixtures for 20 populations of chum salmon.