Application of Microsatellite DNA Variation to Estimation of Stock Composition and Escapement of Skeena River Sockeye Salmon (*Oncorhynchus nerka*)

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Abstract: Microsatellite loci can be used to estimate spawning escapements of individual Pacific salmon populations returning to remote spawning locations throughout large river systems by analysis of appropriately weighted samples from test fisheries near the river mouth. Variation at six microsatellite loci (*Omy77*, *Ots3*, *Ots3*, *Ots3*, *Ots3*, and *Ots3*) was surveyed from approximately 1,700 sockeye salmon (*Oncorhynchus nerka*) from 17 populations in the Skeena River drainage in northern British Columbia, as well as from 1,400 fish in test fisheries conducted in the lower river during 1996–1999. Simulated mixed-stock samples suggested that the six microsatellite DNA loci should enable relatively accurate and precise estimates of stock composition when utilized for fishery management applications within the river. Analysis of the test fishery samples indicated that sockeye salmon from Babine Lake comprised a substantial portion of the returning fish. We also compared population structure of sockeye salmon from both the Skeena and Nass rivers. Simulated and actual mixed-stock samples suggested that accurate estimates of stock composition of sockeye salmon from these two major production areas in northern British Columbia should be obtained in analysis of samples from mixed-stock marine fisheries.

INTRODUCTION

Sockeye salmon (*Oncorhynchus nerka*) populations returning to the Skeena River in northern British Columbia comprise an important group of populations, second only to those of the Fraser River in British Columbia in terms of sockeye salmon production (Sprout and Kadowaki 1987). The Skeena River drainage contains one very large lake (Babine Lake), and a number of smaller lakes throughout the drainage (Smith and Lucop 1966). Sockeye salmon spawn in over 70 distinct sites and rear in 27 lakes in the watershed, but over 90% of the production has been attributed to Babine Lake and its tributaries (Larkin and McDonald 1968; West and Mason 1987). Production from the Babine Lake system is enhanced by two spawning channels at Fulton River and one at Pinkut Creek. As these facilities can produce over four million returning salmon per year (Wood et al. 1998), fishery managers ideally would target higher exploitation rates on these enhanced populations than on less productive populations within the drainage. However, there is currently no ability to differentiate enhanced populations from unenhanced populations within the drainage.

Estimation of the number of fish returning to spawn (escapement) is a key aspect of management and assessment of Pacific salmon. In theory, it should be possible to estimate the relative escapements of individual spawning populations by mixed-stock analysis of appropriately weighted samples from test (assessment) fisheries near the river mouth. In practice, the feasibility of this approach has usually been limited by practical difficulties in identifying closely related populations within the same watershed. For the Skeena River, a test fishery has been conducted annually since 1955 near the river mouth at Tyee, and the test fishery provides managers with an index of daily sockeye salmon escapement to the drainage (Jantz et al. 1990). Babine Lake escapement has been estimated at a counting fence since 1949 (Wood et al. 1998), but escapements to other lakes in the system are generally estimated by visual surveys. Previous estimates of stock compositions in the lower river test

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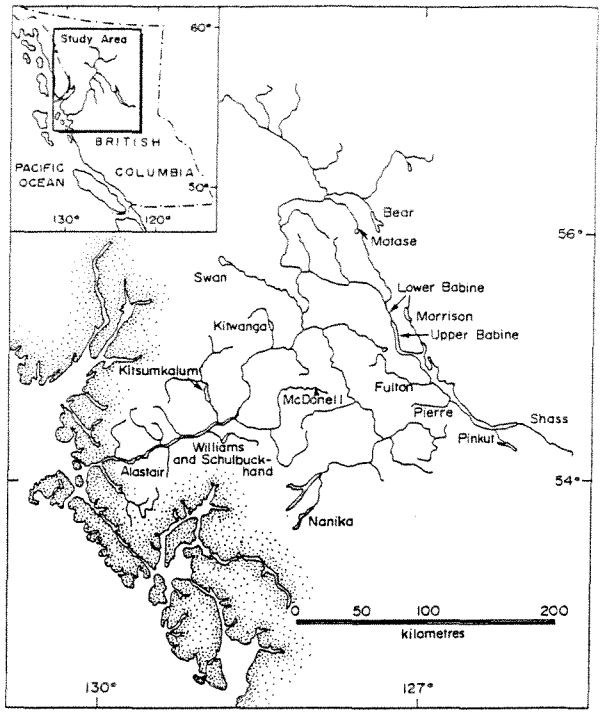
Fig. 1. Locations of spawning sampling sites in the survey.



fishery derived from analysis of allozymes, age composition, and parasite prevalence characters indicated that Babine Lake populations comprised 70–85% of total drainage escapement during 1987–97 (Rutherford et al. 1999), whereas comparison of escapement surveys suggested that 95% of drainage escapement was derived from Babine Lake populations. It is uncertain whether this discrepancy in the relative abundance of Babine Lake populations is due to errors in stock identification, unrepresentative sampling of the test fishery, or underestimation of the non-Babine component due to visual survey techniques. If representative samples are obtained from the test fishery and an accurate estimate of the Babine Lake stock component is available, then perhaps more accurate estimates of the escapement of the non-Babine Lake component of the returns can be derived than that currently provided by the sum of the visual estimates.

Wood et al. (1994) found that the allozyme variation in the sockeye salmon populations of British Columbia was not strongly regionally structured, and concluded that it would be of limited use for estimation of stock composition in coast-wide mixture problems. However, estimation of stock composition in marine sockeye salmon fisheries remains a continuing area of interest for fisheries managers. Compared with allozyme variation, variation at the more polymorphic, faster-evolving microsatellite loci (Banks et al. 1999) may reveal regional structure in sockeye salmon populations that can be used to provide estimates of stock composition in marine fisheries.

Analysis of microsatellite DNA variation has improved the ability to identify specific populations of sockeye salmon within major (Beacham and Wood 1999) and minor river systems (Nelson et al. 1998; Beacham et al. 1998, 2000). For Pacific salmonids, microsatellite variation has provided the ability to determine fine-scale population structure (Nielsen et al. 1997; Wenburg et al. 1998) and to provide estimates of stock composition on either a local or regional basis (Small et al. 1998; Beacham et al. 1999; Shaklee et al. 1999). Given the continuing issues in management of the Skeena River sockeye salmon stock complex to which an analysis of microsatellite variation may prove to be beneficial, we initiated a survey of microsatellite variation and evaluated some potential management applications.

Specifically, the objective of the present study was to analyze variation at six microsatellite DNA loci in Skeena River sockeye salmon to describe population structure and evaluate applications for stock identification. We then applied microsatellite DNA technology to estimate stock composition of sockeye salmon sampled during 1996, 1998, and 1999 in a test fishery near the mouth of the Skeena River. We also compared the Skeena River populations with those of the Nass River, another major river system in northern British Columbia, to determine if there was a regional population structure. We evaluated whether microsatellite variation can provide accurate and precise estimates of stock composition for sockeye salmon from the two watersheds when they occur together in marine mixed-stock fisheries.

MATERIALS AND METHODS

Collection of DNA Samples and PCR

For the characterization of the baseline populations prior to 1998, DNA was extracted from liver samples of adult sockeye salmon that had been previously collected for protein electrophoretic studies and frozen at -20°C. The 1998 collections consisted of operculum punches from the Alastair Lake and Kitwanga River populations preserved in ethanol. Approximately 1,700 fish were sampled from 17 populations, with four of the populations sampled in two years (Table 1). Laboratory methods detailing DNA extraction procedures, details of the six loci amplified (*Omy77*, *Ots3*, *Ots100*, *Ots103*, *Ots107*, and *Ots108*), and PCR details were outlined in Beacham and Wood (1999).

Gel Electrophoresis and Band Analysis

PCR products were size fractionated on 16 cm by 17 cm non-denaturing polyacrylamide gels and visualized by staining with 0.5 mg/ml ethidium bromide

Table 1. Population, nursery lake, sample collection years, number of fish sampled per year, and total number of fish sampled for 17 populations of Skeena River sockeye salmon.

Population	Nursery Lake	Years	Number	Total
	Lower Skeena			
McDonell Lake	McDonell	1987, 1988	81, 75	156
Williams Creek	Lakelse	1988	98	98
Schulbuckhand Creek	Lakelse	1988	77	77
Alastair Lake	Alastair	1988, 1998	21, 83	104
Kitwanga River	Kitwanga	1998	98	98
Kitsumkalum River	Kitsumkalum	1994	77	77
	Upper Skeena			
Motase Lake	Motase	1987	50	50
Swan Lake	Swan	1988, 1994	100, 81	181
Bear	Bear	1988	71	71
	Bulkley River			
Nanika River	Morice	1988	75	75
	Babine Lake			
Lower Babine River	Babine	1994	100	100
Upper Babine River	Babine	1987	81	81
Pinkut Creek	Babine	1990	100	100
Fulton River	Babine	1990, 1994	100, 100	200
Morrison River	Babine	1988	76	76
Shass Creek	Babine	1987	78	78
Pierre Creek	Babine	1988	79	79

in water and illuminating with ultraviolet light. Nelson et al. (1998) and Beacham and Wood (1999) provided a more complete description of gel electrophoretic conditions. Gels were scanned at a 1024 x 1024 pixel density with a Kodak charge coupled device camera with low light capability and a yellow filter. Images were analyzed using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, Michigan), with the size of the amplified microsatellite alleles reported to the nearest bp based upon the molecular size grid created with 20-bp markers.

As there was some uncertainty in estimation of allele size as determined from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill et al. 1990). Peaks in the allele frequencies were used to identify main alleles, and bin widths generally corresponding to a repeat unit were set with the main allele occurring in the middle of the bin. Precision of estimation of allele size was evaluated with a standard fish analyzed for each locus.

Precision of Estimation of Allele Size

Standard deviations of the estimate of allele sizes for the heterozygous standard fish analyzed at each locus ranged from 0.34 to 0.89 with the larger alleles estimated with the least precision (Table 2). For the smaller allele at *Ots3*, 100% of the estimated sizes for the allele spanned a two-bp interval, as did 97% (35/36) of the size estimates for the larger allele. For the smaller allele at *Omy77*, 100% of the estimated sizes of the allele were in a two-bp interval, as were 89% (51/57) of the estimated sizes of the larger allele. Although *Omy77* is a dinucleotide microsatellite locus, the level of precision of estimated allele sizes for the standard fish indicated that alleles could not be consistently assigned correctly to 2-bp bins. Therefore, *Omy77* alleles were conservatively defined on the basis of 4-bp wide bins, which has the practical effect of pairwise pooling of alleles that differ in size by 2 bp. Estimated sizes of alleles of the standard fish analyzed at the other loci were all estimated within a four-bp bin interval.

Table 2. Precision of estimation of allele size (bp) for standard fish analyzed repeatedly at each locus, with the fish run only once on each gel. N is the number of gels where allele sizes for the fish were estimated. Standard deviation is in parentheses.

Locus	N	Allele size	Range	Allele size	Range
Ots3	24	93.6 (0.57)	93–95	74.1 (0.34)	74–75
	12	89.6 (0.51)	89–90	74.6 (0.51)	74–75
Omy77	57	116.0 (0.58)	114–117	105.3 (0.46)	105–106
Ots107	33	118.0 (0.56)	116–119	110.3 (0.52)	109–111
Ots108	25	185.8 (0.84)	185–187	111.8 (0.44)	111–113
Ots100	53	186.2 (0.57)	185–187	159.4 (0.53)	158–160
Ots103	41	214.0 (0.65)	213–215	175.8 (0.89)	175–177

Data Analysis

Each stock at each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) using GENEPOP version 3.1 (Raymond and Rousset 1995), as was temporal stability of allele frequencies. For populations sampled in more than one year, HWE was tested on samples pooled over years if no significant annual variation in allele frequencies was observed. For those populations in which significant annual variation in allele frequencies was detected at any locus, HWE was tested on an annual basis at all loci in the population. In this case, the lowest probability of conformance to HWE in the annual tests was considered. Tests of genetic differentiation utilizing pairwise comparisons between all population pairs at each locus were also conducted using GENEPOP. The dememorization number was set at 1,000, and 50 batches were run for each test with 1,000 iterations/batch. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989). A neighbor-joining analysis illustrating genetic relationships among populations was conducted with PHYLIP (Felsenstein 1993). The allele frequency matrix was resampled 1,000 times and Cavalli-Sforza and Edwards (1967) chord distance was used to estimate

distance among populations. θ values for each locus were determined with FSTAT (Goudet 1995). Estimation of variance components in allele frequencies among populations and years was determined with BIOSYS (Swofford and Selander 1981). Identification of individuals to specific populations was done with the DISCRIM procedure in SAS with a jackknife sampling procedure (SAS 1989). Classification functions were developed using all fish sampled except the one to be classified, with each fish tested individually in turn. This procedure should reflect the accuracy expected when applied to new, previously unsampled fish. Identification of individual fish was restricted to those fish for which data were available at all six loci.

Estimation of Stock Composition

Genotypic frequencies were determined at each locus for each stock, and the model of Fournier et al. (1984) was used to estimate stock composition. Each locus was considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies and used as model inputs. Each baseline population was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples before the estimation of stock composition of each mixture. Two mixture compositions were examined that would be typically expected in returning Skeena River sockeye salmon in order to evaluate accuracy and precision of the estimated stock compositions. Hypothetical fishery samples of 200 fish were generated by randomly resampling with replacement the baseline populations, and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined, with the whole process repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates. Estimated stock composition of each test fishery was determined as a point estimate of the sample, with standard deviations of individual stock estimates derived from bootstrap resampling of both the baseline populations and the mixture.

Test Fishery

Samples were collected from sockeye salmon caught in a gillnet test fishery in 1996,1998, and 1999 near the mouth of the Skeena River (Jantz et al. 1990). We used weekly estimates of sockeye salmon escapement (CPUE) at the test fishery to construct an appropriately weighted random sample of approximately 200 fish representing the entire annual escapement for 1996 and 1998. For example, if 10% of the total run was estimated to have passed by the test fishery in a particular week, then the annual escapement sample of 200 fish included 20 fish picked randomly from fish sampled in that week. Increased analysis of samples collected during 1999 allowed biweekly estimates of stock composition to be made. The seasonal estimate of stock composition in 1999 was determined from a sample of 550 fish, with sampling based in proportion to weekly run abundance. DNA was derived from either a punch of operculum tissue or a single scale for each fish included in the analysis, and samples within a week were randomly selected from the available pool of samples. Escapement estimates for non-Babine stocks were calculated by using the annual stock composition estimates from the test fishery in conjunction with the Babine fence counts and reported catches in fresh water as outlined by Rutherford et al. (1999).

Comparison with the Nass River

The use of microsatellite DNA for estimation of stock composition in potential marine applications in northern British Columbia would require separate identification of Skeena River and Nass River sockeye salmon. The ability to discriminate between Skeena River and Nass River sockeye salmon using microsatellite variation was examined first by simulation analysis using mixtures of known proportions of Skeena River fish (0, 50, and 100%). The Nass River baseline populations used in the analysis included those populations for which at least 50 fish had been sampled, with the distribution of allele frequencies outlined by Beacham and Wood (1999). Six Nass River populations were included in the baseline (Meziadin Lake, Damdochax Lake, Bowser Lake, Bonney River, Kwinageese River, and Gingit Creek) along with the 17 populations surveyed in the Skeena River drainage. Test fishery samples from the Skeena River collected in 1996, 1998 and 1999 and the Nass River collected in 1996 (Beacham and Wood 1999) were also analyzed using the combined Skeena/Nass baseline.

RESULTS

Variation within Populations

Heterozygosity varied among both loci and populations surveyed. Observed heterozygosity at *Ots3* was 0.44 in the pooled samples (population range 0.12–0.89), *Omy77* 0.60 (0.40–0.87), *Ots100* 0.75 (0.57–0.87), *Ots103* (0.83 (0.53–0.94), *Ots107* 0.41 (0.03–0.67), and *Ots108* 0.76 (0.58–0.92). Mean heterozygosity of the lower Skeena River populations was 0.59, the same as the mean heterozygosity of the Nanika River (Bulkley drainage) population. Mean heterozygosity of upper Skeena River populations

was 0.68, with mean heterozygosity of the Babine Lake populations at 0.66. There was some tendency for more upriver populations to have higher levels of heterozygosity than those in the lower river.

Significant departures from genotypic frequencies expected under Hardy-Weinberg equilibrium were observed at *Omy77* for Williams Creek and Schulbuckhand (Scully) Creek (homozygote excess), at *Ots3* for Motase Lake, Nanika River (homozygote excess), and Pierre Creek (heterozygote excess), at *Ots107* for McDonell Lake and Kitsumkalum River (homozygote excess), and at *Ots108* for McDonell Lake, Bear Lake, Pinkut Creek, and Pierre Creek (homozygote excess). There was no evidence of a consistent null or nonamplifying allele at any of the six loci surveyed.

Significant annual variability in allele frequencies was observed at *Ots3* ($p = 0.0075$) and *Ots108* ($p = 0.0001$) for the Fulton River population, at *Ots3* ($p = 0.0000$) and *Ots107* ($p = 0.0047$) for the Alastair Lake population, and at *Ots3* ($p = 0.0004$) and *Ots108* ($p = 0.0000$) for the McDonell Lake population. No significant differences in allele frequencies were observed in the Swan Lake population (all nominal p values > 0.025).

Variation among Populations

There was clear genetic differentiation among sockeye salmon populations in the Skeena River drainage. The greatest differentiation at *Omy77* was observed among lower river populations, with the frequency of $Omy77^{94}$ ranging from 0.02 for Lakelse Lake populations (Williams Creek, Schulbuckhand (Scully) Creek) to 0.69 in Kitsumkalum River (Fig. 2). At *Ots3*, the greatest differentiation was again observed among lower river populations, with the frequency of $Ots3^{88}$ ranging from 0.18 (Williams Creek) to 0.94 (Kitwanga River). Some regional structuring of allele frequencies within the watershed was observed, with the frequency of $Ots100^{195} < 0.05$ in the lower river populations, 0.13–0.30 in the upper river populations, 0.31 in the Bulkley River (Nanika) population, and 0.03–0.12 in the Babine Lake populations. Similar regional structuring was observed at *Ots103*, with the frequency of $Ots103^{198} < 0.13$ in the lower river populations, 0.17–0.26 in the upper river populations, 0.20 in the Bulkley River stock, and <0.09 in the Babine Lake populations. Four main alleles were observed at *Ots107*, with a lower frequency of $Ots107^{113}$ in the upper river and Bulkley River populations (generally < 0.45) compared with higher frequencies in the lower river (generally > 0.80) and Babine Lake populations (generally 0.70–0.80)(Fig. 2). At *Ots108*, considerable variation in frequencies of the 122, 126, and 130 alleles was observed among lower river populations, similar to the pattern of variation at *Omy77* and *Ots3*.

Pairwise population comparisons of allele frequencies indicated that there was significant genetic differentiation between populations ($p < 0.0001$) with some exceptions. Non-significant differences were observed among populations in some comparisons between Babine Lake populations at all six loci surveyed. Similarly, in comparisons between the Schulbuckhand Creek and Williams Creek populations, both tributaries of Lakelse Lake, no significant differences were observed at any locus except *Ots3*, where a significant difference was observed ($p < 0.0001$). Other than the similarity between Scully Creek and Williams Creek, all lower river populations were genetically distinct at each locus from all other lower river populations. All three upper river populations were genetically distinct from each other at all loci except for *Ots3* allele frequencies between Motase Lake and Bear Lake ($p = 0.014$).

Stock Structure

Substantial differentiation was observed among populations within the Skeena River drainage, with θ values for the six loci as follows: *Omy77* 0.136 (SD = 0.028), *Ots3* 0.158 (0.045), *Ots100* 0.062 (0.014), *Ots103* 0.082 (0.040), *Ots107* 0.082 (0.025), and *Ots108* 0.086 (0.026). The average θ value for all six loci was 0.094 (0.013). Annual variation in allele frequencies within populations was always less than the differences among populations, with the ratios of the variance components for among population differences divided by between years within populations as follows: *Omy77* 6.72, *Ots3* 1.24, *Ots100* 32.80, *Ots103* 29.60, *Ots107* 8.37, and *Ots108* 4.30. The average variance component ratio for all six loci was 4.83. On average, differences among populations were about five times larger than annual variation within populations.

Regional structuring of populations within the Skeena River drainage was evident. The consensus neighbor-joining dendrogram indicated that Babine Lake populations were reasonably distinct and well-defined from other populations in the drainage, clustering together in 76% of the 1,000 trees used to construct the consensus tree (Fig 3). Lower river and upper river/Bulkley River populations formed less cohesive units, as they were grouped together in identifiable clusters about 40% of the time. The diversity observed among the lower river populations (Fig. 2) was reflected in the lower proportion of times that the populations grouped together compared with the more homogeneous Babine Lake populations.

Regional structuring of populations between the Nass River and Skeena River drainages was also ob-

Fig. 2. Population or regional allele frequencies of sockeye salmon in Lakelse Lake (Schulbuckhand (Scully) Creek and Williams Creek), Alastair Lake, Kitwanga River, Kitsumkalum River, McDonnell Lake, Bulkley River (Nanika River), upper Skeena River populations, and Babine Lake populations.

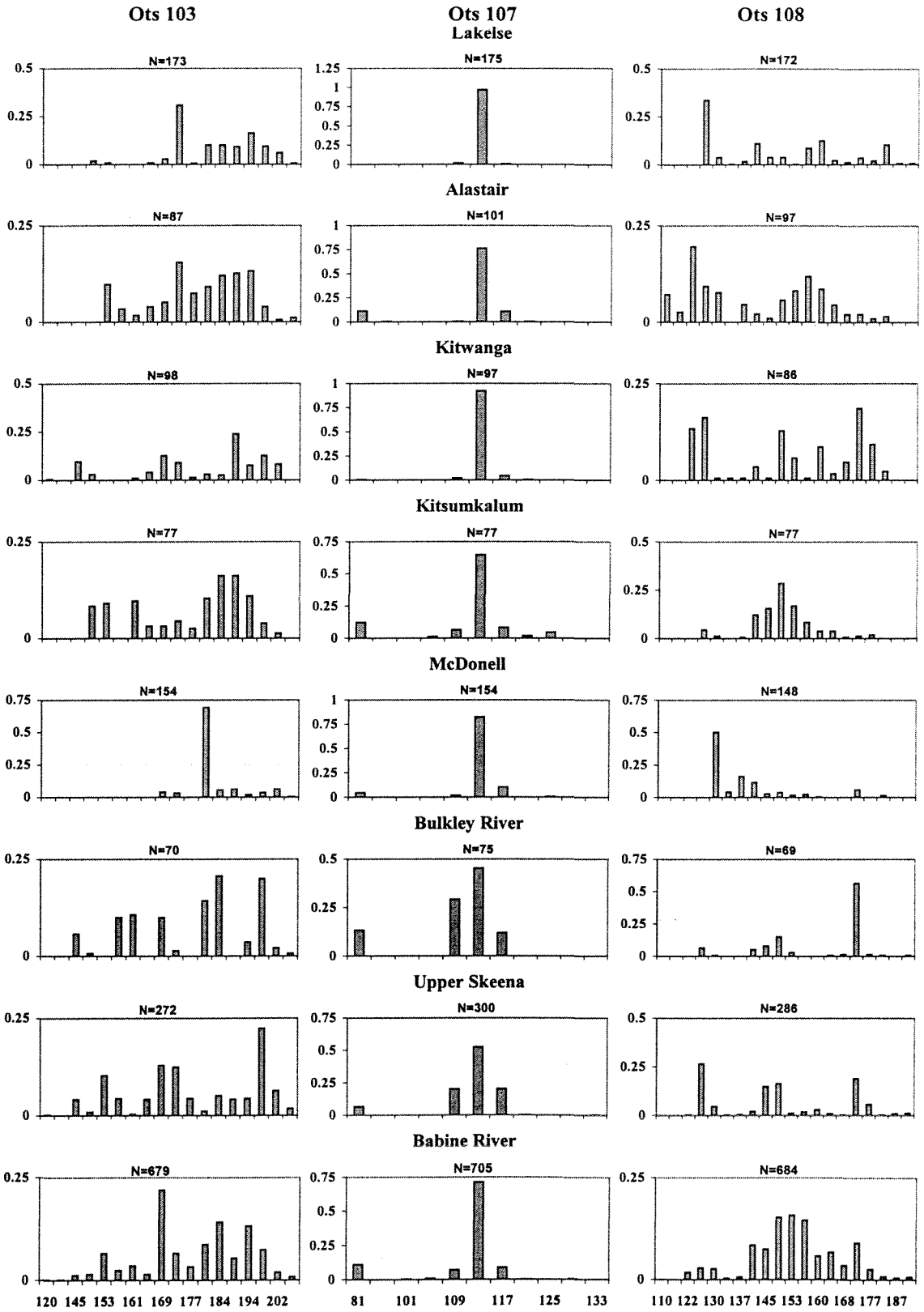


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Fig. 2. Continued.

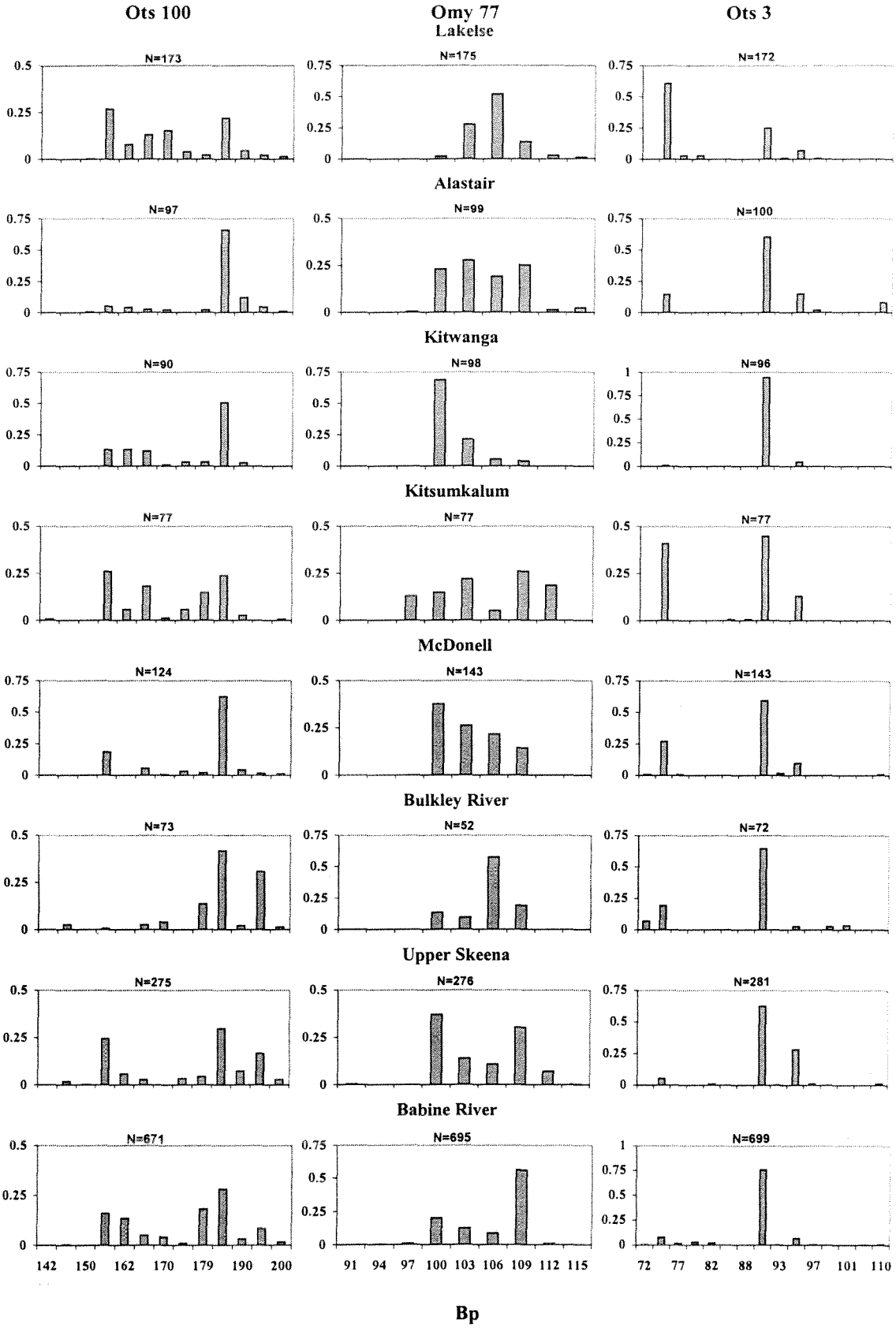
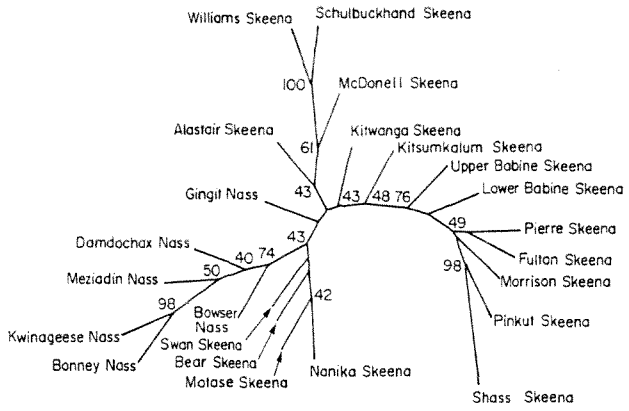


Fig. 3. Neighbor-joining tree of genetic (chord) distance relationships among sockeye salmon spawning sites in the Skeena River (n = 17) and Nass River (n = 6).



served. Populations in the Nass River in which juveniles rear in lakes (Kwinageese, Bonney, Meziadin, Damdochax, Bowser) formed a reasonably distinct group that clustered together in 74% of the 1,000 trees examined. The Nass River population in which juveniles rear in rivers without access to lake habitat (Gingit) was less distinct from the Skeena River populations than the lake-rearing populations. The analysis indicated that there was sufficient genetic differentiation between Nass River and Skeena River sockeye salmon populations to enable their distinction in marine mixed-stock samples.

Estimation of Stock Composition within the Skeena River Watershed

Simulated mixtures

We evaluated whether the level of genetic differentiation observed among sockeye salmon populations within the Skeena River drainage can be applied to practical issues of stock identification. Two simulated test fishery mixtures were developed that might be representative of the relative abundance of spawning escapements within the drainage. As Babine Lake populations are considered to account for 95% of returns, the proportion of Babine Lake fish in the simulated mixture samples was set at 80% and 95%. The estimated contributions of Babine Lake populations averaged 83% and 92%, respectively (Table 3). The estimated proportion of Babine Lake sockeye was estimated with a reasonably high degree of accuracy, reflecting their relative genetic distinctiveness (Fig. 3). Other regional stocks of sockeye salmon in the drainage were estimated with reasonable levels of accuracy. For example, the mean estimated proportion of lower river sockeye salmon was within 3% of the true value in both simulated mixtures. The simulations indicated that accurate estimates of regional stock composition should be derived when applied to samples collected from the Skeena River drainage.

Application to the test fishery

Analysis of the test fishery samples indicated that sockeye salmon returning to Babine Lake comprised 86% of the returns in 1996 and 73% of the returns in 1998. Sockeye salmon from lower river populations accounted for an additional 10% and 23%, respectively, of the returns in the two years (Table 4). With an observed count of 558,000 fish at the Babine River fence in 1998, drainage escapement may have been nearly 770,000 fish. The Kitsumkalum River population was the most abundant lower river population, with an estimated escapement of 87,000 fish. Less than 5% of the drainage escapement was attributed to upper river populations.

Increased analysis of sampled sockeye salmon during the 1999 return allowed estimates of stock composition to be made on a biweekly basis and seasonal basis. Sockeye salmon from Lakelse Lake comprised over 30% of the returns prior to June 26, but salmon from this population were virtually absent after July 11 (Table 5). Upper Skeena populations were more prevalent in the early portion of the returns compared with the later portion. Babine Lake sockeye salmon were estimated to have comprised 81% of the total returns in 1999, intermediate in relative abundance compared with returns in 1996 and 1998.

Comparisons between Nass River and Skeena River

Identification of Individuals

Accuracy of estimated stock composition is correlated with the ability to identify individual fish in mixtures to specific stocks. We evaluated the ability of microsatellite variation to identify individual sockeye salmon as originating from either the Nass River or Skeena River drainages, with the individuals tested excluded from the development of the discriminant functions. Correct assignment to river drainage was achieved for 84% (n = 1758) of Nass River and 83% (n = 1643) Skeena River sockeye salmon. With this relatively accurate classification of individual fish to correct river drainage, estimated stock compositions of mixtures of these fish should also be accurate.

Simulated samples

Three simulated samples of Nass River and Skeena River sockeye salmon were constructed and their composition estimated with a 23-stock Nass/Skeena baseline to evaluate the accuracy and precision

Table 3. Estimated percentage composition of two mixtures of Skeena River sockeye salmon in simulations using observed variation at six microsatellite DNA loci. Each mixture of 200 fish was generated 100 times. To control stock composition, desired contributions for each stock were resampled with replacement from the baseline data, then combined. Stock composition of the mixture was estimated by resampling each baseline stock with replacement to obtain the original sample size and a new distribution of allele frequencies. The individual estimates for all populations within each geographic area outlined in Table 1 have been summed to provide an estimate for each area. Standard deviation is in parentheses.

	Mixture 1			Mixture 2	
Stock	Correct	Estimated	Stock	Correct	Estimated
Swan	5.0	4.3 (2.1)	Σ upper Skeena	0.0	1.6 (1.4)
Σ upper Skeena	5.0	6.0 (2.7)	Schulbuckhand Creek	5.0	2.8 (1.7)
Alastair	5.0	4.2 (2.1)	Σ lower Skeena	5.0	6.5 (2.3)
Kitsumkalum	5.0	1.8 (1.3)	Σ Bulkley	0.0	0.3 (0.6)
Σ lower Skeena	10.0	7.2 (2.8)	Upper Babine	5.0	4.2 (4.8)
Nanika	5.0	3.9 (1.8)	Lower Babine	15.0	13.3 (9.0)
Σ Bulkley	5.0	3.9 (1.8)	Pinkut Creek	25.0	28.5 (10.0)
Pinkut Creek	30.0	31.7 (10.5)	Fulton River	20.0	9.2 (7.9)
Fulton River	25.0	8.3 (6.2)	Morrison	10.0	6.8 (5.9)
Pierre Creek	25.0	27.3 (5.3)	Pierre	20.0	23.3 (4.4)
Σ Babine	80.0	82.8 (3.6)	Σ Babine	95.0	91.6 (2.5)

Table 4. Estimated percentage stock composition of 1996 and 1998 Skeena River test fishery samples and resulting escapement estimate (number of fish) for sockeye salmon in four areas in the Skeena River drainage. Standard deviation is in parentheses. Escapement estimate from Babine Lake is the actual fence count.

	1996		1998	
Stock	% Composition	Escapement	% Composition	Escapement
N	207		201	
Motase Lake	0.66 (0.76)		0.00 (0.24)	
Swan Lake	2.12 (1.50)		2.50 (1.41)	
Bear	0.00 (0.38)		1.85 (1.48)	
Σ upper Skeena	2.78 (1.68)	66,400	4.35 (1.98)	23,300
McDonell	0.00 (0.17)		0.59 (0.77)	
Lakelse	1.75 (1.17)		3.66 (1.67)	
Alastair	2.12 (1.70)		5.37 (1.96)	
Kitwanga	0.76 (0.72)		2.04 (1.62)	
Kitsumkalum	5.37 (3.63)		11.31 (3.31)	
Σ lower Skeena	10.00 (3.76)	261,000	22.97 (4.18)	142,500
Nanika	1.44 (1.23)		0.00 (0.16)	
Σ Bulkley	1.44	32,500	0.00 (0.16)	0
Upper Babine	19.45 (8.20)		16.52 (6.29)	
Lower Babine	0.00 (4.38)		0.00 (0.16)	
Pinkut Creek	5.78 (6.18)		9.83 (6.97)	
Fulton River	17.19 (9.49)		19.22 (10.73)	
Morrison	29.37 (9.91)		18.38 (8.90)	
Pierre	0.00 (0.43)		0.00 (1.52)	
Shass	13.99 (6.47		8.73 (4.21)	
Σ Babine	85.78 (4.40)	2,056,205	72.68 (4.10)	558,873

Table 5. Estimated percentage stock composition of the 1999 Skeena River Tyee sockeye salmon test fishery samples for 6 periods in 1999. N is number of fish analyzed in each period. Standard deviation is in parentheses. The seasonal estimate of 550 fish is based on sampling in proportion to weekly run abundance.

Stock	June 6–26	June 27–July 10	July 11–24	July 25–Aug. 7	Aug. 8–21	Aug. 22–Sept. 18	Seasonal
N	122	205	200	225	213	62	550
Motase	0.0 (0.0)	1.1 (0.5)	0.0 (0.2)	0.6 (1.3)	1.7 (1.1)	1.4 (1.2)	0.4 (0.8)
Swan	3.3 (3.2)	5.5 (3.2)	14.2 (3.4)	2.1 (1.9)	0.0 (1.6)	0.0 (3.9)	5.5 (1.9)
Bear	0.0 (1.3)	0.9 (2.2)	1.9 (1.2)	2.3 (2.3)	6.2 (2.9)	0.0 (1.5)	1.3 (0.9)
Σ upper Skeena	3.3 (3.2)	7.5 (3.7)	16.1 (3.6)	5.0 (3.0)	7.9 (4.1)	1.4 (4.2)	7.2 (2.3)
McDonell	0.0 (1.2)	1.5 (1.7)	1.5 (1.6)	0.5 (0.3)	0.0 (1.1)	1.3 (3.5)	1.0 (0.6)
Lakelse	32.5 (5.5)	9.5 (2.2)	0.0 (1.4)	1.6 (1.2)	1.2 (0.7)	0.0 (4.0)	3.3 (0.9)
Alastair	1.3 (0.8)	6.7 (2.6)	0.0 (1.4)	4.0 (2.6)	4.4 (2.2)	7.3 (6.8)	2.7 (1.3)
Kitwanga	2.8 (2.4)	1.0 (1.5)	0.0 (1.8)	1.7 (0.9)	1.7 (1.5)	2.7 (2.8)	2.0 (0.7)
Kitsumkalum	2.1 (3.9)	0.8 (2.7)	2.8 (2.5)	2.0 (1.2)	5.1 (2.1)	7.4 (6.3)	2.8 (1.6)
Σ lower Skeena	38.7 (7.7)	19.5 (4.5)	4.3 (4.1)	9.8 (3.4)	12.4 (3.7)	18.7 (9.9)	11.8 (2.7)
Nanika	0.0 (2.6)	0.2 (1.1)	0.0 (0.8)	0.6 (0.8)	0.2 (0.9)	0.0 (0.3)	0.0 (0.2)
Σ Bulkley	0.0 (2.6)	0.2 (1.1)	0.0 (0.8)	0.6 (0.8)	0.2 (0.9)	0.0 (0.3)	0.0 (0.2)
Σ Babine	58.0 (8.3)	72.8 (5.3)	79.6 (4.8)	84.7 (4.3)	79.6 (5.0)	80.0 (10.4)	81.0 (3.4)

of estimated stock compositions for possible marine applications. Mean estimated stock composition of samples of pure (100%) Skeena River sockeye salmon was 97.6% (SD = 2.0%) Skeena origin, and that of samples comprising 50% Skeena River origin fish was 48.9% (SD = 2.0%) Skeena origin (Table 6). Mean estimated stock composition of samples containing no (0%) Skeena River fish was 2.8% (SD = 1.4%) Skeena origin. These simulations demonstrate that the microsatellite loci surveyed could be used to provide relatively accurate and precise estimates of stock composition for mixtures comprising only Skeena River or Nass River sockeye salmon.

Application to test fisheries

The simulations demonstrated that it should be feasible to obtain accurate estimates of stock composition from actual fishery samples. The validity of the simulations was investigated by estimating stock compositions of sockeye salmon samples from test fisheries conducted at the mouth of the Nass River in 1996 and the mouth of the Skeena River in 1996, 1998, and 1999. The estimated composition of the Nass River sample was 97.6% (SD = 1.5%) Nass-origin fish, and that of the 1996 Skeena River sample was 91.5% (SD = 3.9%), the 1998 Skeena sample 94.4% (SD = 3.5%), and for the 1999 sample used to estimate seasonal stock composition 93.5% (SD = 3.5%) Skeena-orgin fish (Table 7). When the 1996 samples from both rivers were combined, creating a sample of 44.9% Skeena-origin fish, the estimated stock contribution of Skeena River was 41.2% (SD = 3.0%). When the 1996 Nass and 1998 Skeena samples were combined, creating a sample of 44.2% Skeena-origin fish, the estimated Skeena River contribution was 42.2% (SD = 2.9%). When the 1996 Nass and 1999 Skeena seasonal samples were combined, creating a sample of 68.4% Skeena-origin fish, the estimated Skeena River contribution was estimated at 65.8% (SD = 2.6%). Thus, accurate estimates of Nass River and Skeena River sockeye salmon were obtained over a wide range of relative proportions in samples from fisheries in which only Nass River and Skeena River sockeye salmon occur.

DISCUSSION

Microsatellites are becoming an increasingly powerful tool for characterizing population structure in natural populations. In salmonids, microsatellites are generally characterized by high levels of variability and differentiation among spawning populations (Nielsen et al. 1997; Seeb et al. 1998; Small et al. 1998). The high heterozygosities observed at microsatellite loci allows a substantial amount of genetic differentiation to be detected and exploited for stock identification applications, which was the main focus of our current study.

Significant annual variation in allele frequencies

Table 6. Estimated percentage composition of three mixtures of Skeena River and Nass River sockeye salmon in simulations using observed variation at six microsatellite DNA loci. Each mixture of 200 fish was generated 100 times with replacement, and stock compositions of the mixtures estimated by resampling each baseline stock with replacement to obtain the original sample size and a new distribution of allele frequencies. Seventeen populations were used in the Skeena River baseline, and six populations in the Nass River baseline. Standard deviation is in parentheses.

Mixture 1			Mixture 2			Mixture 3		
Stock	Correct	Estimated	Stock	Correct	Estimated	Stock	Correct	Estimated
Lakelse	5.0	2.8 (1.3)	Gingit	10.0	10.4 (1.9)	Bonney	10.0	11.0 (3.1)
Upper Babine	5.0	4.4 (4.1)	Meziadin	30.0	29.1 (1.2)	Gingit	10.0	11.1 (1.6)
Lower Babine	15.0	12.0 (8.9)	Damdochax	10.0	10.4 (1.7)	Kwinageese	5.0	5.6 (3.8)
Pinkut Creek	25.0	27.6 (11.4)	Pinkut	25.0	17.9 (4.2)	Meziadin	50.0	48.6 (2.0)
Fulton River	20.0	8.3 (7.9)	Fulton	10.0	10.5 (4.3)	Damdochax	10.0	11.1 (2.6)
Morrison	10.0	6.1 (5.7)	Bear	5.0	4.0 (1.2)	Bowser	10.0	10.0 (1.6)
Pierre	20.0	24.0 (4.9)	Kitsumkalum	10	9.0 (1.5)	Brown Bear	5.0	0.0 (0.1)
Σ Skeena	100.0	97.6 (2.0)	Σ Skeena	50.0	48.9 (2.0)	Σ Skeena	0.0	2.8 (1.4)
Σ Nass	0.0	2.4 (2.0)	Σ Nass	50.0	51.1 (2.0)	Σ Nass	100.0	97.2 (1.4)

Table 7. Estimated percentage stock composition of the 1996,1998, and 1999 Skeena River test fishery samples, the 1996 Nass River test fishery sample outlined in Beacham and Wood (1999), and combined test fishery samples derived from the 23 stock baseline used for the simulated mixtures in Table 5. Standard deviation is in parentheses. N is the number of fish sampled in each mixture.

		Skeena River		Nass River	
Sample	N	Correct	Estimated	Correct	Estimated
1996 Skeena test fishery	207	100.0	91.5 (3.9)	0.0	8.5 (3.9)
1998 Skeena test fishery	201	100.0	94.4 (3.5)	0.0	5.6 (3.5)
1999 Skeena test fishery	550	1000.0	93.5 (3.5)	0.0	6.5 (3.5)
1996 Nass test fishery	254	0.0	2.4 (1.5)	100.0	97.6 (1.5)
1996 Skeena, 1996 Nass	461	44.9	41.2 (3.0)	55.1	58.8 (3.0)
1998 Skeena, 1996 Nass	455	44.2	42.2 (2.9)	55.8	57.8 (2.9)
1999 Skeena, 1996 Nass	804	68.4	65.8 (2.6)	31.6	34.2 (2.6)

was observed at some loci in some populations surveyed in the Skeena River drainage, but on average, differences among populations were about five times larger than annual variation within populations. Generally, differentiation among sockeye salmon populations within a region is greater than annual variation within populations. For example, differentiation among Nass River populations was about 11 times greater than annual variation within populations (Beacham and Wood 1999), and about 12 times greater for Barkley Sound populations on the west coast of Vancouver Island (Beacham et al. 2000). Although temporal changes in allele frequencies can affect mixed-stock fishery analysis (Waples 1990), the relative magnitude of population differentiation versus within population variation suggests that annual variation in allele frequencies will have minimal effects on estimates of stock composition. For practical estimation of stock composition, annual sampling of populations contributing to a sockeye salmon fishery is not required, but clearly some level of monitoring of allele frequencies over time is appropriate to ensure that allele frequencies characterizing each population are still relevant.

The value of a particular microsatellite locus in stock identification can differ among regions. Previous analyses of population differentiation at the six microsatellite loci surveyed in this study indicated that *Ots103* provided the least population differentiation for Nass River (Beacham and Wood 1999) and Barkley Sound populations (Beacham et al. 2000). However, in the Skeena River drainage, its value in population differentiation was considerably enhanced, with θ values up to seven times larger than in previous studies. Surveys of microsatellite variation in each geographic region of interest will probably be necessary to determine which loci are the most effective in identifying local populations in stock identification applications.

An important aspect of fishery management applications of microsatellite variation within the Skeena River drainage is to be able to identify Babine Lake populations. The counting fence on Babine River provides a direct and presumably reliable count of escapement to Babine Lake. If representative samples from the test fishery at the mouth of the Skeena River and a reliable estimate of the proportion of Babine Lake populations in these samples are obtained, coupled with catch data from fisheries in the drainage, then an estimate of total escapement to the Skeena River drainage is possible.

There has been a continuing discrepancy in the estimated contribution of Babine Lake sockeye salmon in Skeena River drainage escapement between the approaches in which non-Babine escapements are estimated from visual surveys and those in which they are estimated from the test fishery at the mouth of the Skeena River (Rutherford et al. 1999). The proportion that Babine Lake populations contribute to drainage escapement is always higher when estimated from direct observation than when estimated from test fishery sampling. This discrepancy may arise from (1) stock identification errors made in the determination of the Babine Lake component of samples derived from the test fishery whereby the Babine Lake component is consistently underestimated, (2) the test fishery provides non-representative samples such that Babine Lake sockeye salmon are consistently underrepresented, perhaps because of their smaller average size or higher abundance (Cox-Rogers and Jantz 1993), or (3) visual estimates of escapement that seriously underestimate true escapement in rivers other than Babine.

Previous stock identification studies of Skeena River sockeye salmon have utilized variation at allozymes, frequency of occurrence of parasites, and age compositions to estimate the proportion of Babine Lake sockeye salmon (Rutherford et al. 1994, 1999). The estimated proportion of Babine Lake populations in the test fishery has ranged from 70-86% during 1989–1997, and the 1998 estimate derived from microsatellites was 73%, toward the lower end of these estimates. As samples from the 1996 test fishery were analyzed using both allo-zymes/parasites/scales (Rutherford et al. 1999) and microsatellites (this study), it was possible to compare stock compositions estimated by the two methods. There was a striking concordance in results, with both methods providing estimates of 85.8% Babine-origin fish, and 10% lower-river (downstream from the Skeena/Bulkley confluence) populations. Simulation studies for both stock identification methods indicate that reliable estimates of the proportion of Babine Lake sockeye salmon should be obtained. Since the two methods are independent but give strikingly similar results, the discrepancy in the estimated contribution of Babine Lake to Skeena River drainage escapement is not likely due to a systematic bias or underestimation of the Babine Lake component in the test fishery samples.

Are samples representative of the migrating fish obtained from the test fishery? During times of high abundance of migrating fish, the gillnet sampling gear could be saturated, such that the true abundance of migrating populations would not be adequately represented in the reconstructed sample. If so, the very abundant Babine Lake component would not be adequately represented in the reconstructed sample. One way to evaluate whether the gillnet test gear does become saturated is to compare the abundance index derived from the test fishery with the observed escapement of Babine Lake populations at the counting fence. If indeed the catchability of the gillnet changes, then large returns to Babine Lake should not

necessarily be associated with higher indices of abundance in the lower river test fishery. However, there is clearly a relationship between the lower river test fishery abundance index and the Babine Lake fence count (Rutherford et al. 1999), so the discrepancy in the relative contribution of Babine Lake populations is unlikely due primarily to unrepresentative sampling in the test fishery, although this may have some role.

How accurately do visual surveys of escapement estimate true abundance? Visual surveys in the Skeena River drainage traditionally underestimate abundance when compared with direct fence counts (Brett 1952), and in the case of the Sustut River, a relatively clear tributary in the upper Skeena River drainage, fence counts may be five times higher than visual counts (McKinnell and Rutherford 1994). Possibly a significant portion of the discrepancy between estimated and observed non-Babine Lake escapement is attributable to underestimation of escapement by visual observation in the absence of a fence. If the estimated stock composition of Babine Lake sockeye salmon is accurate and the sampling at the test fishery is representative, then the 1996 estimated escapement of non-Babine Lake sockeye salmon was about 360,000 fish and in 1998 the estimated escapement of non-Babine Lake sockeye salmon was approximately 166,000 fish. The estimate of 166,000 fish for 1998 contrasted with a visual estimate of 22,000 fish. If they are present, where are these "missing" fish? The stock identification analysis suggests that they are largely in the lower river tributaries. A large part of the discrepancy is attributable to the Kitsumkalum River population, which has an estimated escapement of 70,000 fish from stock identification analysis but only 5,000 from visual surveys. The Kitsumkalum River is glacially turbid with limited visibility, and would be a good candidate for more refined escapement estimation procedures. Another would be Alastair Lake (microsatellite estimate 33,000 fish in 1998) which has been historically reported to contain significant populations of sockeye salmon (Shepard and Withler 1958; Aro and Shepard 1967), but for which only 2,500 fish were observed in 1998. In 1999, the data indicate that Lakelse Lake may have had larger escapements than in 1996 or 1998.

Populations originating from Babine Lake were estimated to have comprised 73% of the drainage escapement in 1998, towards the lower end of the range derived from stock identification studies (Rutherford et al. 1999). Populations enhanced in the Babine Lake Development Project (primarily Pinkut Creek and Fulton River) account for most of the fry recruitment into Babine Lake (Wood et al. 1998). However, infection by the "ich" parasite (white spot disease) caused high prespawning mortality at both enhancement sites in 1994 and 1995 (Traxler et al. 1998), such that enhanced fry production from these two brood years was < 60% of the 1984–93 average (Wood et al. 1998). Total smolt production from the 1994 and 1995 brood years in the Babine Lake system was the lowest observed since production began from the enhancement facilities. Relatively poor returns from Babine Lake populations were thus expected in 1998, and this was reflected in the relatively low estimated proportion of Babine Lake sockeye salmon in the test fishery.

Wood et al. (1994) found that allozyme variation in the sockeye salmon populations of British Columbia was not strongly regionally structured, and concluded that it would be of limited value in coast-wide mixture problems. However, variation at the more polymorphic, faster-evolving microsatellite loci surveyed to date revealed regional structure between Nass and Skeena River sockeye salmon populations that allowed accurate estimates of stock composition when sockeye salmon from both river drainages occurred in mixed-stock samples. Elucidation of regional structure is important because successful application of genetic stock identification to mixed-stock salmon fisheries generally requires that geographically proximate populations share distinctive genetic characteristics. That makes it unnecessary to obtain baseline samples from all the individual populations that might contribute to a fishery. For regionally-structured species, that portion of a mixed-stock sample derived from unsampled populations is usually allocated to sampled populations from the same region, reducing the cost and complexity of establishing a sufficient baseline for mixture analysis. Although only Nass River and Skeena River populations were compared in the current study, additional regional structure exists in British Columbia sockeye salmon populations (Beacham et al. unpublished data), and once a suitable baseline has been established, microsatellites will likely prove very effective in providing estimates of stock composition of samples in high seas applications.

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