A Comparison of Methods of Stock Identification for Sockeye Salmon (*Oncorhynchus nerka*) in Barkley Sound, British Columbia

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Sockeye salmon (*Oncorhynchus nerka*) returning to Barkley Sound on the west coast of Vancouver Island originate from three stocks: Sproat Lake, Great Central Lake, and Henderson Lake. Evaluations of four methods of stock identification were conducted, with the annual stability of the stock-discriminating markers also examined. Variation observed in parasite infection rates, at protein electrophoretic loci, minisatellite DNA loci, and microsatellite DNA loci was used to assess the accuracy and precision of estimates of stock composition for these three stocks. Significant annual variability in parasite infection rates was observed in two of six comparisons for the stocks sampled annually during 1977-84, and in two of four comparisons for two stocks sampled annually during 1993-95. No significant annual variability in allele frequencies was observed at three electrophoretic loci (two stocks sampled in two years, 1983 and 1990) or four microsatellite DNA loci examined (three stocks each sampled for three years during 1987-95 for two loci, two stocks sampled for two years, 1990 and 1992, for two loci). Highest accuracy and greatest precision of estimated stock compositions were observed using the prevalences of two myxosporean parasites (*Myxobolus arcticus* and *Henneguya salminicola*) observed in the stocks during 1977-84. However, changes in the prevalence of both parasites in Great Central Lake sockeye salmon during 1993-95 substantially reduced the precision of the estimates. The use of variation at four microsatellite loci (Ots3, Ots100, Ots103, and Omy77) resulted in the accuracy and precision of the stock composition estimates similar to that observed with parasites during 1977-84, but greater than that obtained during 1993-95. Stock composition analysis utilizing variation at minisatellite loci was less reliable than analysis based upon microsatellite loci, with minisatellite allele frequencies determined using three probes (Ssa1, Ssa-A34, and OtsPBS-1). Protein electrophoresis (variation at three polymorphic loci used : PGM-1, PGM-2, and PGDH) provided the least reliable estimates of the four methods evaluated, although data for all loci known to be polymorphic in sockeye salmon were not available for the Barkley Sound stocks.

INTRODUCTION

Sockeye salmon (*Oncorhynchus nerka*) returning to Barkley Sound on the west coast of Vancouver Island originate from three stocks: Sproat Lake, Great Central Lake, and Henderson Lake, and these stocks are exploited in a mixed-stock commercial fishery. The early development of this fishery, prior to 1940, could be characterized as largely a terminal beach seine and gillnet fishery that provided annual catches as high as 125,000 fish (Hyatt and Steer 1987), and because of its terminal nature, there was little requirement to determine the stock composition of the catch. During 1940-71, the fishery was characterized by a more diffuse gillnet fishery with an annual catch of up to 76,000 fish (Hyatt and Steer 1987). Since 1972, the fishery has been conducted by both gillnetters and purse seiners over a wide area in Barkley Sound and Alberni Inlet, and the annual catch has been highly variable, ranging from no catch

† deceased

(fishery closed) to about 1.2 million fish. Sockeye salmon from Sproat Lake and Great Central Lake have dominated the catch since 1980, comprising over 95% of the landings (Hyatt and Steer 1987). To prevent overexploitation of each of the component stocks, effective management of the current fishery, given its mixed-stock nature, requires accurate and precise estimates of stock composition to be available.

Lake fertilization has been used as a method to enhance the production of Barkley Sound sockeye salmon (LeBrasseur et al. 1978; Hyatt and Stockner 1985). Great Central Lake was fertilized annually from 1970-1973, and from 1977 to the present. Henderson Lake has been fertilized from 1976 to the present, and Sproat Lake from 1985-1987. The assessment of net changes in adult sockeye salmon production resulting from fertilization of nursery lakes is an integral part of evaluation of the utility of enhancing sockeye salmon production by this method (Hyatt and Stockner 1985).

Management of the Barkley Sound sockeye salmon fishery, as well as assesssing the effects of longer-term fertilization of Great Central and Henderson lakes, required that reliable estimates of stock composition of the catch be determined. A survey of frequency of occurrence of two myxosporean parasites, *Myxobolus arcticus* (formerly identified as *M. neurobius* : see McDonald and Margolis (1995)) in the brain and *Henneguya salminicola* in the muscle, indicated that there were very substantial differences in the prevalence of these parasites among sockeye salmon in the three lakes during 1977-1984 (Quinn et al. 1987). These differences in prevalence were used to provide estimates of stock composition during this period (Steer et al. 1986, 1988). However, in the 1990s, it was observed that the prevalences of the parasites in Great Central Lake sockeye salmon changed substantially, with a marked decline in the ability to discriminate among stocks. Other methods of stock identification were required to provide effective management and assessment advice. In the present study, we evaluate four methods of stock identification of Barkley Sound sockeye salmon: prevalence of *Myxobolus* and *Henneguya* during 1977-84 and 1993-95, and three methods incorporating genetic variation, using allele frequency differences at protein electrophoretic, minisatellite DNA, and microsatellite DNA loci. Accuracy and precision of estimated stock compositions are evaluated, as well as temporal stability of either the parasite prevalences or allele frequencies.

MATERIALS AND METHODS

Samples and Laboratory Procedures

Parasites

Adult sockeye salmon were examined for the presence of two myxosporean parasites. *Myxobolus arcticus* was detectable in the brain as spores following digestion of the brain in a pepsin-hydrochloric acid solution, centrifugation, and microscopic examination of the resultant sediment. *Henneguya salminicola* was visually detectable in the musculature as macroscopic cysts (Boyce et al. 1985). The proportion of adults infected with *Myxobolus arcticus* was determined annually between 1977-1984 and between 1993-1995 for Sproat Lake and Great Central Lake sockeye salmon, and between 1978-1984 and in 1995 for Henderson Lake sockeye salmon (Fig. 1). *Henneguya salminicola* infection rates were recorded between 1979-1981 and 1993-1995 for Sproat and Great Central Lake sockeye salmon, and between 1978-1984 and in 1995 for Henderson Lake sockeye salmon.

Protein Electrophoresis

Heart, liver, muscle, and eye tissues were collected from all three stocks in 1983, and from Sproat Lake and Great Central Lake stocks in 1990. Samples were frozen, and variation at 35 protein-coding loci examined by starch gel electrophoresis (Aebersold et al. 1987). Levels of polymorphism at only 3 loci (PGM-1, PGM-2, and PGDH) were large enough to be evaluated for their utility in stock identification. Additional laboratory procedures have been outlined by Wood et al. (1994).

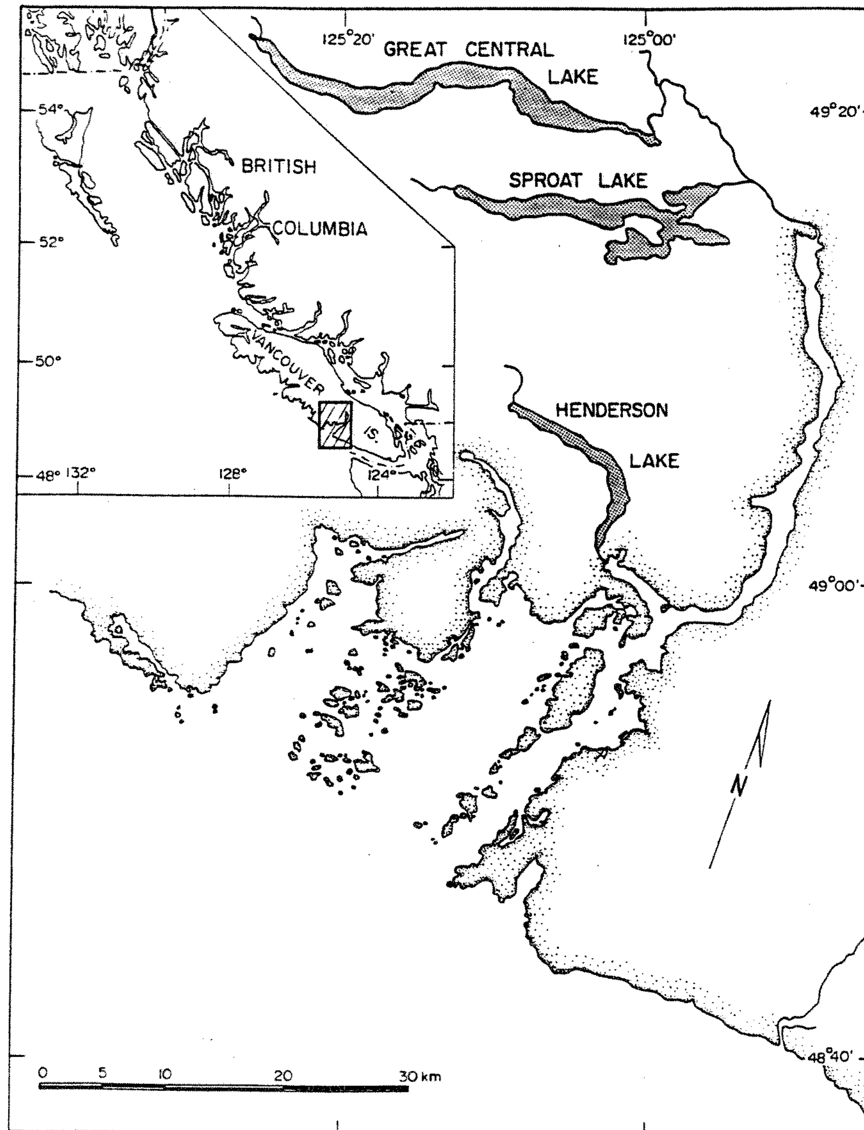
Minisatellite DNA Variation

Variation at minisatellite loci was examined with two single-locus (Ssa-1and Ssa-A34) and one multi-locus (OtsPBS-1) probes for Sproat Lake and Great Central Lake sockeye salmon sampled in 1992. Genomic DNA was restricted with either AluI or HaeIII, loaded on to an agarose gel where it was size fractionated by electrophoresis, transferred to a nylon membrane, denatured, and hybridized with the appropriate denatured probe. A more complete description of laboratory procedures was outlined by Beacham et al. (1995).

Microsatellite DNA Variation

Variation at four microsatellite loci was examined via the polymerase chain reaction (PCR) with the

Fig. 1 Location of study area, including Barkley Sound, Alberni Inlet, Great Central Lake, Sproat Lake, and Henderson Lake on Vancouver Island, British Columbia, Canada.



primer sets Omy77 (Morris et al. 1996), Ots100 (Nelson and Beacham in prep.), Ots3 (Olsen et al. 1996), and Ots103. The Ots103 locus has not been described previously. Summarized briefly, a tetranucleotide GACA repeat was observed at the locus. The sequence of the primers which were used to amplify Ots103 were: forward, 5'-AGGCTCTGGGTCCGTG-3' and reverse, 5'-TGATATGGTGTGATAGCTGG-3'. Samples were analyzed for Sproat Lake and Great Central Lake sockeye salmon sampled in 1987, 1990, and 1992, and Henderson Lake sockeye salmon sampled in 1988, 1993, and 1995. Genomic DNA was derived from either scales or liver tissue samples, the locus amplified via PCR using the appropriate primers, the amplified products loaded on to an acrylamide gel for size fractionation by electrophoresis, and allele size estimated. A more complete description of the laboratory analysis, primer development for Ots100, and estimation of allele size was provided by Nelson and Beacham (in prep.).

Data Analysis

Annual variation in parasite prevalence or allele frequencies at protein electrophoretic or microsatellite DNA loci was examined with the χ^2 test with 1,000 simulations conducted to determine the significance of the variability (Roff and Bentzen 1989). Significance levels were adjusted to account for the number of

multiple comparisons conducted (ie. 0.05/N, where N is the number of tests conducted) (Lessios 1992). Differences in parasite prevalence or allele frequencies were used to calculate a generalized distance (D), where $D^2 = \Sigma (X_A - X_B)^2$ (Pimentel 1979). This distance was calculated between each pair of stocks with sampling years combined, and a pairwise distance was calculated between all sampling years within each stock. Data on parasite prevalence was analyzed as two separate blocks, with the years 1977-84 as one block, and 1993-95 as the second block.

Estimation of Stock Composition

In order to estimate the stock composition of a mixture using the maximum likelihood method of Fournier et al. (1984), it is first necessary to determine the distributions of characters discriminating the stocks contributing to the mixture. For *Myxobolus* and *Henneguya*, the observed numbers of fish infected or not infected with each parasite in each stock were determined, and the resultant matrix (two rows by two colums for each stock) used to characterize the stocks in each of the two time blocks. Genotypic frequencies at each of three polymorphic protein-coding loci (PGM-1, PGM-2, PGDH) were determined from the reported allelic frequencies by assuming that the stocks were in Hardy-Weinberg equilibrium, as was reported by Wood et al. (1994). Three genotypes were possible at each locus, so each stock was characterized by a 3X3 matrix containing expected genotypic frequencies. The general procedures used to incorporate minisatellite DNA variation to characterize the baseline stocks have been outlined by Beacham et al. (1995). Summarized briefly, allele counts at the Ssal locus (nine varibles) and Ssa-A34 locus (two variables) and band counts observed with OtsPBS-1 (22 variables) were combined for each fish, and principal component analysis on the correlation matrix of the 33 variables was conducted to obtain uncorrelated variables or principal components. The continuous distributions of the principal component scores were summarized as 12-bin histograms, and 25 principal components were used in the analysis, so each stock was characterized by a matrix of 25 rows and 12 columns. Microsatellite DNA variation was incorporated as follows. Variation was scored at 10 alleles but subsequently condensed and summarized by eight alleles (36 genotypes) at the Omy77 locus, initially by 17 alleles and condensed to nine alleles (45 genotypes) at the Ots100 locus, initially by 16 alleles and condensed to 8 alleles (36 genotypes) at the Ots3 locus, and initially by 21 alleles and condensed to 10 alleles (55 genotypes) at the Ots103 locus. For example, 10 alleles at Omy77 were initially identified as follows: 91-93 bp (1), 94-96 bp (2), 97-99 bp (3), 100-102 bp (4), 103-106 bp (5), 107-109 bp (6), 110-112 bp (7), 113-115 bp (8), 116-118 bp (9), 119-121 bp (10). However, given the low observed frequency of allele 1 in any stock (<0.02), it was combined with allele 2, and alleles 9 and 10 were also combined, resulting in eight alleles used in determination of genotypic frequencies. Combining low frequency adjacent alleles in this example reduced the number of genotypic frequencies to be estimated with the available samples from 55 genotypes to 36 genotypes, with little or no loss in the ability to discriminate among stocks. Each locus was considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies. Each stock was characterized by an input of four rows, with the first row containing counts for 36 genotypes, the second row counts for 45 genotypes, the third row counts for 36 genotypes, and the fourth row counts for 55 genotypes. For all four methods, each baseline stock was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture.

Hypothetical fishery samples of 200 fish were generated by randomly resampling with replacement the three baseline stocks, and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined using the method of Fournier et al. (1984), with the whole process repeated 50 times to estimate the mean and standard deviation of the individual stock composition estimates.

RESULTS

Parasites

Substantial differences in the prevalence of *Myxobolus* were observed among Sproat Lake and Great Central Lake sockeye salmon sampled during 1977-84 (Table 1). Even though both lakes are part of the Somass River drainage, virtually all sockeye salmon in Sproat Lake were infected with *Myxobolus*, whereas an average of only 5% of Great Central Lake sockeye salmon were infected. Virtually all Henderson Lake sockeye salmon were infected, and small but statistically significant annual variation was observed ($P<0.001$). The high rates of prevalence limited the utility of this parasite in differentiating between the Sproat Lake and Henderson Lake stocks. Significant annual variation in the prevalence of *Myxobolus* in Great Central Lake was observed ($P<0.001$), but the prevalence of infection never reached 9% in any year during 1977-84 (Table 1). A

marked change in *Myxobolus* prevalence was observed in Great Central Lake sockeye salmon sampled during 1993-95, with an average 63% of fish sampled infected with the parasite. The parasite prevalence increased significantly in each of the three years sampled (P<0.001), with about 75% of the fish sampled in 1995 infected. If present trends continue, virtually all of Great Central Lake sockeye salmon may be infected by 1997, rendering *Myxobolus* prevalence of no value in discriminating among the three stocks.

Henneguya was virtually absent from Sproat Lake and Great Central Lake sockeye salmon sampled during 1977-84, but present in an average of 66% of Henderson Lake fish (Table 1). Significant annual variation was observed in *Henneguya* prevalence in Henderson Lake fish during 1977-84 (P<0.001), with from 55 to 75% of the fish examined infected. During 1993-95, the prevalence of *Henneguya* in Great Central Lake sockeye salmon was significantly higher (21%) than observed during 1977-84 (< 1%), but prevalence in the other two lakes was within the range observed in the earlier years. The prevalence in 1994 (2%) in Sproat Lake sockeye salmon was significantly higher (P<0.001) than in other years, when it was 0%.

During 1977-84, the mean distance between Sproat Lake and Great Central Lake Sockeye salmon, both stocks originating from the same drainage and dominating the catch in the fishery, was 0.99. The mean pairwise distance between all three stocks was 0.95 (range 0.66-1.19). During 1993-95, the change in prevalence of both parasites in Great Central Lake sockeye salmon reduced the previous differentiation observed among the three stocks (Table 1). The mean pairwise distance declined to 0.61 (range 0.42 to 0.74), with the distance between the Sproat Lake and Great Central Lake stocks being 0.42.

Protein Electrophoresis

Although 35 loci were screened, only three loci were found where the frequency of a variant allele was >0.05. The greatest differentiation among stocks was observed at the PGM-1 locus, and the least differentiation at PGDH, but all differences were significant (P<0.001). Allele frequencies of Sproat Lake and Great Central Lake sockeye salmon were more similar to each other than to Henderson Lake

Table 1. Prevalence of the brain parasite *Myxobolus arcticus* and the muscle parasite *Henneguya salminicola* in three stocks of sockeye salmon from Barkley Sound, 1977-95. Results for 1977-84 have been adapted from Quinn et al. (1987). N is the number of fish sampled in each year.

	Sproat		Great Central		Henderson	
Year	N	%	N	%	N	%
			Myxobolus			
1977	15	100.0	15	0.0	0	-
1978	175	99.4	245	0.8	150	97.3
1979	760	99.6	900	2.7	211	98.1
1980	1330	99.9	1179	5.6	200	100.0
1981	1181	99.9	1337	8.4	300	98.3
1982	843	99.9	679	7.8	300	98.7
1983	1127	99.7	1034	2.4	299	100.0
1984	781	99.9	851	3.8	201	99.0
1977-84	6212	99.8	6240	5.0	1661	99.9
1993	650	99.7	632	52.4	0	-
1994	450	99.6	545	69.4	0	-
1995	225	100.0	274	75.5	100	100.0
1993-95	1325	99.7	1451	63.1	100	100.0
			Henneguya			
1978	0	-	0	-	188	54.8
1979	175	0.0	174	0.6	211	75.8
1980	1330	0.0	1165	0.6	200	71.5
1981	1181	0.0	1336	0.5	300	72.7
1982	0	-	0	-	300	63.3
1983	0	-	0	-	299	58.5
1984	0	-	0	-	201	65.7
1978-84	2686	0.0	2675	0.6	1699	66.0
1993	650	0.0	632	18.7	0	-
1994	450	2.0	545	23.4	0	-
1995	225	0.0	274	19.0	100	75.0
1993-95	1325	0.7	1451	20.6	100	75.0

sockeye salmon (Table 2). No significant annual variability in allele frequency was observed at any locus. The mean pairwise distance between stocks was 0.28 (range 0.17 to 0.42), and the distance between Sproat Lake and Great Central Lake stocks was 0.17.

Minisatellite DNA

Although three probes were used to examine variation at minisatellite DNA in Sproat and Great Central Lake sockeye salmon, a significant difference between stocks was observed only with AluI-restricted genomic DNA hybridized with Ssa1 (Table 3). The distance between these two stocks was 0.50.

Microsatellite DNA

Marked differences in allele frequencies were observed among stocks at the Omy77 locus ($P<0.001$), with the frequency of allele 1 ranging from 0.490 in Sproat Lake sockeye salmon to 0.008 in Henderson Lake sockeye salmon (Table 4). Conversely, the frequency of allele 4 was 0.223 in Sproat Lake sockeye salmon, but 0.556 in Henderson

Table. 2 Allele frequencies of the 100 allele at the PGM-1, PGM-2, and PGDH loci for Barkley Sound sockeye salmon. Results have been adapted from Wood et al. (1994). N is the number of fish sampled.

	Sproat Lake		Great Central Lake		Henderson Lake	
Year	N	Freq.	N	Freq.	N	Freq.
			PGM-1			
1983	132	0.617	111	0.761	138	0.964
1990	50	0.570	99	0.747	0	-
1983-90	82	0.605	210	0.752	138	0.964
			PGM-2			
1983	182	0.799	136	0.879	149	0.977
1990	650	0.840	99	0.383	0	-
1983-90	232	0.808	235	0.862	149	0.977
			PGDH			
1983	183	0.850	136	0.915	104	1.00
1990	50	0.880	100	0.900	0	-
1983-90	233	0.856	236	0.909	104	1.00

Table. 3 Allele frequencies at the Ssa1 and Ssa-A34 loci and mean band counts at 12 DNA fragment intervals for the probe OtsPBs-1 for Sproat Lake and Great Central Lake sockeye salmon. Results have been adapted from Beacham et al. (1995). Genomic DNA was restricted with either AluI or HaeIII before bybridization with Ssa1. Sample sizes range from 34-37 fish sampled for Sproat Lake and 41-46 fish for Great Central Lake.

Allele/Band	Sproat Lake	Great Central Lake
	Ssa1 (AluI)	
1	0.632	0.370
2	0.132	0.315
3	0.235	0.315
	Ssa1 (HaeIII)	
1	0.786	0.707
2	0.186	0.283
3	0.028	0.010
	Ssa-A34	
1	0.500	0.565
	OtsPBS-1	
1	0.00	0.05
2	0.14	0.41
3	0.30	0.29
4	0.03	0.02
5	0.05	0.12
6	0.03	0.00
7	0.00	0.12
8	0.05	0.05
9	0.08	0.05
10	0.03	0.05
11	0.84	0.68
12	0.05	0.10

Table. 4 **Allele frequencies at the Omy77, Ots100, Ots3, and Ots103 microsatellite loci for Barkley Sound sockeye salmon. Results for Omy77 and Ots100 have been adapted from Nelson and Beacham (in prep.). Allele sizes at Omy77 were: 91-96 bp (1), 97-99 bp (2), 100-102 bp (3), 103-106 bp (4), 107-109 bp (5), 110-112 bp (6), 113-115 bp (7), and 116-121 bp (8). Allele sizes at Ots100 were: 129-152 bp (1), 153-156 bp (2), 157-161 bp (3), 162-166 bp (4), 167-179 bp (5), 180-184 bp (6), 185-187 bp (7), 188-191 bp (8), and 192-205 bp (9). Allele sizes at Ots3 were: 74-75 bp (1), 76-87 bp (2), 88-90 bp (3), 91-92 bp (4), 93-94 bp (5), 95-98 bp (6), 99-100 bp (7), and 101-106 bp (8). Allele sizes at Ots103 were: 130-167 bp (1), 168-171 bp (2), 172-175 bp (3), 176-179 bp (4), 180-183 bp (5), 184-187 bp (6), 188-191 bp (7), 192-195 bp (8), 196-199 bp (9), and 200-227 bp (10). N is the number of fish sampled.**

Stock	Year	N	1	2	3	4	5	6	7	8	9	10
						Omy77						
Sproat	1987	78	0.481	0.013	0.128	0.224	0.058	0.064	0.000	0.032		
Lake	1990	91	0.467	0.016	0.143	0.231	0.022	0.060	0.011	0.049		
	1992	95	0.522	0.011	0.121	0.216	0.042	0.037	0.000	0.052		
	1987-92	264	0.490	0.013	0.131	0.223	0.040	0.053	0.004	0.046		
Great	1987	89	0.197	0.000	0.247	0.371	0.000	0.017	0.135	0.034		
Central	1990	98	0.168	0.020	0.240	0.372	0.010	0.077	0.077	0.036		
Lake	1992	116	0.215	0.000	0.224	0.375	0.004	0.065	0.065	0.052		
	1987-92	303	0.194	0.007	0.236	0.373	0.005	0.054	0.089	0.041		
Henderson	1988	97	0.005	0.000	0.289	0.567	0.000	0.134	0.000	0.005		
Lake	1993	94	0.016	0.000	0.330	0.569	0.000	0.074	0.011	0.000		
	1995	114	0.004	0.000	0.298	0.561	0.004	0.096	0.013	0.022		
	1988-95	305	0.008	0.000	0.305	0.566	0.002	0.102	0.008	0.010		
						Ots100						
Sproat	1987	51	0.078	0.020	0.284	0.176	0.020	0.343	0.059	0.000	0.020	
Lake	1990	76	0.047	0.013	0.283	0.178	0.033	0.408	0.020	0.007	0.013	
	1992	87	0.086	0.011	0.322	0.155	0.023	0.328	0.040	0.011	0.022	
	1987-92	214	0.069	0.014	0.299	0.168	0.025	0.360	0.037	0.007	0.019	
Great	1987	91	0.011	0.055	0.341	0.077	0.060	0.225	0.088	0.049	0.093	
Central lake	1990	99	0.010	0.020	0.343	0.071	0.071	0.242	0.076	0.056	0.111	
	1992	118	0.012	0.025	0.403	0.059	0.047	0.237	0.047	0.021	0.148	
	1987-92	308	0.011	0.032	0.365	0.068	0.058	0.235	0.068	0.041	0.120	
Henderson	1988	93	0.016	0.032	0.548	0.145	0.049	0.145	0.022	0.027	0.016	
Lake	1993	70	0.000	0.007	0.479	0.136	0.100	0.236	0.021	0.029	0.007	
	1995	118	0.000	0.013	0.504	0.157	0.055	0.237	0.013	0.008	0.012	
	1988-95	281	0.006	0.018	0.512	0.148	0.064	0.206	0.018	0.020	0.012	
						Ots3						
Sproat	1990	44	0.114	0.011	0.568	0.000	0.239	0.011	0.057	0.000		
Lake	1992	46	0.109	0.000	0.587	0.011	0.272	0.000	0.043	0.000		
	1990-92	90	0.111	0.006	0.578	0.006	0.256	0.006	0.050	0.000		
Great	1990	87	0.121	0.006	0.391	0.029	0.362	0.011	0.080	0.000		
Central	1992	48	0.135	0.010	0.333	0.010	0.469	0.000	0.031	0.001		
Lake	1990-92	135	0.126	0.007	0.370	0.022	0.400	0.007	0.063	0.004		
Henderson Lake	1995	94	0.112	0.026	0.080	0.000	0.527	0.027	0.229	0.000		
						Ots103						
Sproat	1990	43	0.197	0.012	0.000	0.023	0.012	0.035	0.058	0.302	0.256	0.105
Lake	1992	48	0.136	0.010	0.021	0.010	0.042	0.021	0.073	0.271	0.313	0.104
	1990-92	91	0.164	0.011	0.011	0.016	0.027	0.027	0.066	0.286	0.286	0.104
Great	1990	92	0.092	0.005	0.043	0.027	0.082	0.076	0.168	0.255	0.158	0.092
Central lake	1992	48	0.061	0.042	0.000	0.021	0.021	0.125	0.125	0.250	0.167	0.146
	1990-92	140	0.081	0.018	0.029	0.025	0.031	0.093	0.154	0.254	0.161	0.129
Henderson Lake	1995	96	0.031	0.000	0.016	0.042	0.078	0.052	0.063	0.359	0.224	0.145

Lake sockeye salmon, the dominant allele in the stock. Initially, for the three stocks, a significant annual difference in allele frequencies was observed for Great Central Lake sockeye salmon ($P=0.02$), but after correction of the significance level to account for the number of simultaneous tests ($0.05/10=0.005$), the difference was not significant. Significant differences in allele frequencies among stocks were also observed at the Ots100 locus ($P<0.001$) (Table 4). No significant annual variation in allele frequencies, even before correction of the significance level, was observed at the Ots100 locus

for any stock. There were marked differences among stocks in the allele frequencies at the Ots3 locus, with the frequency of allele 3 ranging from 0.58 to 0.08 among stocks (Table 4). No significant annual variability in allele frequencies was observed. Finally, at the Ots103 locus, substantial variation in allele frequencies among stocks was observed ($P<0.001$) (Table 4). Initial significant annual variablity was observed in Great Central Lake sockeye salmon ($P=0.02$), but as with Omy77 locus, the difference was not significant after consideration of the number of tests conducted. The mean pairwise distance between stocks was 0.66 (range 0.53-0.92). Analysis of microsatellite DNA variation indicated that there was substantial differentiation of allele frequencies among stocks relative to the annual variability within stocks.

Variation Among and Within Stocks

In order to compare the relative variation among and within stocks, we used the distance metric previously outlined for two sets of comparisons. Pairwise distances between stocks were determined, as were pairwise differences between sampling years within stocks. The mean pairwise distance within each stock was calculated. As the utility of a particular set of characteristics for stock identification is a function of both the differentiation among and within stocks, we compared the distance between a pair of stocks with the larger within-stock distance of the pair. The first set of comparisons involved comparing the differences between and within Sproat Lake and Great Central Lake stocks. The second set involved determining the minimum pairwise distance among the three stocks, and comparing it with the larger within-stock distance of the pair. The greatest separation between Sproat Lake and Great Central Lake stocks was obtained with parasite prevalences observed during 1977-84, with microsatellite DNA variation next in separation (Table 5). Although significant annual variation was observed in the prevalence of *Myxobolus* in Great Central Lake during this period, the magnitude of the difference between the two stocks was about 33 times greater than the within-stock variation of the more variable stock, which suggests that in this case the annual variation was of no practical significance. Conversely, although there was no significant annual variation in allele frequency at the protein-coding loci, there was also little differentiation between the two stocks, so that stock separation was only 2.4 times greater than annual separation (Table 5). The ranking of the different techniques (relative to among- and within-stock separation) indicated that parasite prevalence during 1977-84 resulted in the greatest relative differentiation, and protein electrophoresis resulted in the least relative differentiation.

Table.5 Mean generalized distance (D) between Sproat Lake and Great Central Lake sockeye salmon stocks, the mean distance among years within stocks, and the ratio of the two distances for three methods of stock identification are indicated.

Method		Between	Within	Ratio
Parasites	1977-84	0.993	0.030	33.10
	1993-55	0.417	0.161	2.59
Electrophoresis		0.165	0.069	2.39
Microsatellite DNA		0.528	0.108	4.89

Estimation of Stock Composition

Three different mixtures were simulated, in which either Sproat Lake, Great Central Lake, or Henderson Lake sockeye salmon predominated in the mixture. The simulations indicated that parasite prevalence during 1977-84 was a very effective method of stock identification, with the estimates of the Great Central Lake component accurate to a mean error of 0.2% and a coefficient of variation (CV) less than 5% (Table 6). The precision of the Sproat Lake and Henderson Lake components was less than that of the Great Central component, primarily due to the inability to distinguish between Henderson Lake sockeye salmon not infected with *Henneguya* and Sproat Lake sockeye salmon. However, parasite prevalences observed during 1977-84 provided the most accurate and precise estimates of stock composition for all techniques investigated (Table 7).

The change in the parasite prevalence of Great Central Lake sockeye salmon observed during 1993-95 resulted in a substantial decline in the precision of the estimated stock compositions (Tables 6, 7). For example, the use of parasite prevalences during 1977-84 to estimate a true contribution of 20% Great Central Lake resulted in estimates with a CV of about 4%, whereas parasite prevalences during 1993-95 resulted in a CV of 28%. The greater uncertainity and increased bias in the estimated stock compositions, particularly when Great Central Lake sockeye salmon predominated in the mixture (Table 6), reduced the effectiveness of parasite prevalence in management of the fishery in the 1990s.

Variation at microsatellite loci provided the most accurate and precise estimates of stock composition of the three methods examined utilizing genetic variation. The separation observed between Great Central Lake and Sproat Lake sockeye salmon was $D=0.53$, between Sproat Lake and Henderson Lake fish $D=0.92$, and between Great Central Lake and Henderson Lake sockeye salmon $D=0.54$, similar to

Table 6. Estimated percentage composition of three mixtures of Barkley Sound sockeye salmon for five methods of stock identification. Each mixture of 200 fish was generated 50 times with replacement, and stock compositons of the mixtures estimated by resampling each baseline stock with replacement to obtain a new distribution of parasite prevalence or allele frequencies, with the same sample size in the new distribution as in the original one. For the minisatellite DNA analysis, mixtures evaluated were: (1) 75% Sproat, 25% Great Central, and (2) 25% Sproat, 75% Great Central as data for Henderson Lake sockeye salmon were not available. Standard deviation is in parenthesis.

Method	Sproat	Great Central	Henderson
True %	75.0	20.0	5.0
Parasites 1977-84	75.4 (1.8)	19.8 (0.8)	4.7 (1.4)
1993-95	74.4 (5.6)	18.5 (5.1)	7.2 (2.9)
Electrophoresis	84.3 (12.5)	15.4 (12.7)	0.3 (1.1)
Minisatellite DNA	67.8 (5.4)	32.2 (5.4)	-
Microsatellite DNA	71.6 (3.8)	23.9 (4.5)	4.5 (1.7)
True %	20.0	75.0	5.0
Parasites 1977-84	19.1 (2.0)	75.3 (1.4)	5.6 (1.2)
993-95	13.6 (9.3)	73.2 (11.1)	13.2 (4.4)
Electrophoresis	28.6 (21.9)	71.4 (21.9)	0.0 (-)
Minisatellite DNA	30.2 (6.0)	69.8 (6.0)	-
Microsatellite DNA	19.9 (3.6)	74.7 (4.8)	5.5 (2.2)
True %	10.0	10.0	80.0
Parasites 1977-84	10.6 (3.8)	9.8 (0.5)	79.6 (3.8)
1993-95	9.5 (4.3)	10.7 (7.1)	79.8 (6.4)
Electrophoresis	23.3 (6.9)	2.3 (3.4)	74.4 (5.4)
Microsatellite DNA	9.5 (2.0)	12.4 (3.4)	78.2 (2.8)

Table 7. Mean bias over all simulated mixtures of Barkley Sound sockeye salmon from Table 6 and mean coefficient of variation (CV) for stocks comprising 5, 10. 20, 75, and 80% mixtures.

		Mean CV for stock at (%)				
	Mean Bias	5	10	20	75	80
Parasites 1977-84	0.4	26.0	21.5	7.0	2.2	4.8
1993-95	2.5	73.0	57.0	36.0	11.2	8.0
Electrophoresis	7.0	22.0	51.5	86.5	23.0	6.8
Minisatellite DNA	6.2	-	-	25.8*	7.7	-
Microsatellite DNA	1.5	39.0	24.2	18.4	5.9	3.6

* CV at 25% of total mixture.

the magnitude of the difference between Great Central Lake and Sproat Lake fish. Accordingly, estimation of the Henderson Lake component of the simulated mixtures was the most precise with the least amount of bias (Table 6). The mean CV of a stock comprising 20% of the mixture was about 20% using microsatellite loci variation, greater than 7% observed with parasite prevalence during 1977-84, but less than all other techniques evaluated (Table 7).

Variation at minisatellite loci was available only for Sproat Lake and Great Central Lake sockeye salmon. Separation between these stocks was sufficient to provide estimates of similar precision to those observed with microsatellite loci, but accuracy was lower (Table 7). A characterization of variation for Henderson Lake sockeye salmon is required before more definitive conclusions concerning the utility of minisatellite DNA variation can be made.

Although significant differences in allele frequencies were observed among the three stocks for three protein-coding loci, and no significant differences were observed between sampling years within stocks, the level of differentiation observed among the three stocks was insufficient to provide reliable estimates of stock composition. Stocks comprising 20% or less of the mixture could not be reliably identified as being present (Table 6). The high levels of uncertainity associated with the estimated stock compositions, coupled with the bias observed in the compositions, precluded the use of protein electrophoresis as a viable method of estimating stock compositions of Barkley Sound sockeye salmon.

DISCUSSION

Parasites have been demonstrated to be quite useful in studies of population structure in marine fish (Kabata 1963; MacKenzie 1983; Williams et al. 1992), and in their application to Pacific salmon stock identification and migration (Margolis 1963; Bailey et al. 1988; Groot et al. 1989; Wood et al. 1989). Application to stock composition of adult salmon is necessarily restricted to those parasite species that are long lived, which in the case of sockeye salmon would be up to five yr. If differences in prevalence of

parasites can be found among local stocks of sockeye salmon, Konovalov (1995) suggested that there was hardly a more efficient or inexpensive method of stock identification. However, the application in the Barkley Sound fishery indicated that parasite prevalence worked very well for about 10 yr, but a subsequent change in the parasite prevalence resulted in the application becoming less reliable. Temporal changes in parasite prevalences have been recorded in marine fish (MacKenzie, 1987), some over a period of less than five yr (Nagasawa et al., 1988). The results of our study indicated that there was statistically significant but limited annual variation in the prevalence of both parasites during 1977-84, but marked annual variation in the prevalence of *Myxobolus* in Great Central Lake sockeye salmon in the 1990s.

Management of the fishery in Barkley Sound required that an accurate and precise method of stock identification of the catch be available, and the prevalence of the parasites *M. arcticus* and *H. salminicola* provided an effective method of stock identification in the late 1970s and the 1980s (Steer et al. 1986, 1988). Unfortunately, the change in the prevalence of these two parasites in Great Central Lake in the 1990s resulted in poorer discrimination among the stocks, with the resultant uncertainity in the estimates large enough to result in the cessation of the use of parasite prevalence as a method of stock identification in this fishery. The causes of the change in parasite prevalence in Great Central Lake sockeye salmon are uncertain, but may include an increase in abundance of the oligochaete alternate hosts of the myxosporeans (*Stylodrilus heringianus* in the case of *M. arcticus*; Kent et al. 1993) as a result of long-term fertilization of the lake; an increase in spawning escapements of sockeye salmon, and possibly other salmonids, resulting in progressively increasing abundance of the myxosporean spores in the lake; or an increased infection of sockeye salmon smolts in the Somass River after emigration from Great Central Lake (see Quinn et al. 1987), resulting in returns to Great Central Lake of progressively increasing numbers of *M. arcticus*-infected adult sockeye salmon over time. It is clear that in applications where parasites have been used to estimate stock compositions in Pacific salmon, periodic sampling of the baseline stocks should be conducted to confirm the utility of the technique.

Microsatellite DNA consists of tandemly arrayed repeated nucleotide sequences of two, three, or four nucleotides, flanked by regions of non-repetitve DNA (Tautz 1989, Weber and May 1989). Microsatellites have a number of attributes that are useful in studies of population structure and stock identification, including their high levels of variation, alleles show codominant Mendelian inheritance, and only small amounts of tissue (such as scales) are required for analysis (O'Reilly and Wright 1995). Microsatellite loci have been shown to be quite useful in determination of population structure of salmonids (Angers et al. 1995; McConnell et al. 1995). The four microsatellite loci examined in our study showed significant differentiation among stocks, but no significant annual variation in allele frequencies, with samples spanning a range of six to eight yr. The two features of substantial variation in allele frequencies among populations and annual stability of allele frequencies resulted in the highest levels of accuracy and precision of estimated stock compositions of the three genetic methods evaluated.

Minisatellite DNA is similar to microsatellite DNA in that it consists of tandemly arrayed repeated nucleotide sequences, but the repeated sequences are longer, perhaps up to 75 nucleotides (Jarman and Wells 1989), with the alleles consequently larger in size than at microsatellite loci. Substantial differentiation at minisatellite loci has been observed among salmonid stocks, and application of variation at these loci can be an effective method of salmonid stock identification (Miller et al. 1996; Beacham et al. 1996). However, the larger allele size at minisatellite loci usually requires that variation be analyzed by Southern blotting techniques, whereas variation at microsatellite loci is analyzed via the polymerase chain reaction, which can be substantially faster and cheaper than Southern blotting. In our study, variation at minisatellite loci for Henderson Lake sockeye would have to be analyzed before the utility of applying variation at minisatellite loci for stock identification of Barkley Sound sockeye salmon could be fully evaluated.

There have been many applications of using protein electrophoresis for stock identification of Pacific salmon (eg. Utter et al. 1987), and specifically for sockeye salmon (Wood et al. 1989). Although 35 loci were screened for the Barkley Sound stocks, there was sufficient variation at only three loci to be of use in stock differentiation and identification. However, not all loci that are known to be polymorphic in sockeye salmon have been analyzed in our study, as they have not been analyzed for Barkley Sound sockeye salmon stocks. However, the low level of differentiation observed at protein-coding loci that have been analyzed for the Barkley Sound stocks indicated that protein electrophoresis was unlikely to be a viable method of stock identification for this fishery.

Two alternate methods of stock identification of Barkley Sound sockeye salmon have been explored. Scale pattern analysis has been reported to be an effective method of stock identification of some sockeye salmon stocks (Cook and Lord 1978; Marshall et al. 1987). However, the annual variability

in the characters used in stock discrimination usually requires that samples from contributing stocks be collected and analyzed annually (Wood et al. 1989), with age-specific functions usually employed (Bilton and Messinger 1975). An analysis of scale pattern variation in Barkley Sound sockeye salmon did not reveal variation that could be consistently used to provide reliable discrimination among the stocks (K. D. Hyatt, Pac. Biol. Stat., Nanaimo, pers. comm.). Another stock identification method involved an examination of variation in mitochondrial (mtDNA) DNA, as the cytochrome b sequence in mtDNA has been reported to differ among sockeye salmon populations (Bickham et al. 1995). For both Sproat Lake and Great Central Lake sockeye salmon, the cytochrome b and ND1 regions of mtDNA were amplified via the polymerase chain reaction (PCR), the amplified products restricted with ApaI, BfaI, BseEII, and RsaI, and the DNA fragments were separated electrophoretically. The observed frequency of the haplotypes did not differ for Sproat Lake and Great Central Lake sockeye salmon (C. C. Wood, Pac. Biol. Stat., Nanaimo, pers. comm.), rendering variation at these segments of mtDNA of little value in applications to stock identification of Barkley Sound sockeye salmon.

The choice of a particular stock identification method is dependent upon the stock resolution required in the application, the accuracy and precision that is required of the estimates, technical capability of laboratory procedures, and cost of the analysis. For Barkley Sound sockeye salmon, the cost of obtaining samples from the commercial fishery would be highest for the parasite and electrophoretic applications, as whole fish would be required to be purchased, compared with minimal scale, blood, or fin samples required for minisatellite or microsatellite analysis. However, the laboratory procedures for parasite and electrophoretic applications are less complex (and hence less expensive and require less technical training) than for the DNA applications, particularly the Southern blotting approach generally used to survey variation at minisatellite loci. For applications that are currently available to managers of the Barkley Sound sockeye salmon fishery, microsatellite DNA would be the preferred choice, given the minimal additional costs of obtaining samples, the reliability of the estimates, and the relative ease and cost of laboratory analysis.

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