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[Article]

DNA-Level Variation of Sockeye Salmon in Southeast Alaska and the Nass and Skeena Rivers, British Columbia, with Applications to Stock Identification

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Abstract.—The variation at 14 microsatellite loci and 1 major histocompatibility complex locus was surveyed in 12,000 sockeye salmon *Oncorhynchus nerka* from 35 populations in the Skeena and Nass River drainages in British Columbia and 20 populations from Southeast Alaska. Genetic differentiation among populations was observed, with an overall F_{ST} of the microsatellite loci of 0.104. Evaluation of the utility of the microsatellite loci for stock identification applications indicated that the accuracy and precision of the estimated stock compositions generally increased as the number of observed alleles at the loci increased. Analysis of simulated mixtures of sockeye salmon from the Nass River, Skeena River, and Southeast Alaska indicated that DNA variation provides a practical way to estimate stock composition, yielding population and regional estimates within 1–3% of the actual values. The validity of the results from the simulated mixtures was evaluated with an analysis of a sample of known origin. Estimated stock compositions on a regional basis (derived from application of a 203-population baseline) were within 1% of the actual contributions, and for the Southeast Alaska component the individual population estimates were generally within 0.5% of the actual contributions. Analysis of fishery samples from a fishery near Tree Point in Southeast Alaska indicated that the major contributors to the fishery in 2001 were sockeye salmon of Nass River, Skeena River, and Southeast Alaska origin, with some components from the Stikine River. Analysis of fishery samples from a fishery near the entrance to Portland Inlet in northern British Columbia indicated that sockeye salmon of Nass River origin dominated the fishery in 2002. Thus, DNA variation provides an effective method for sockeye salmon stock identification.

The sockeye salmon *Oncorhynchus nerka* returning to spawn in the Skeena and Nass rivers in northern British Columbia comprise very important stocks, both economically and culturally. They are the focus of a significant amount of management action in domestic Canadian salmon fisheries, and considerable effort is made in evaluating their status. They are similar in that there are a number of distinct spawning populations within each river and there are good escapement counts for the major substock in each drainage. In the Nass River, sockeye salmon returning to Meziadin Lake comprise 50–90% of drainage escapement (Rutherford et al. 1994; Beacham and Wood 1999), escapement being thought to be reliably determined at a fishway close to Victoria Falls near the outlet of the lake. In the Skeena River, sockeye salmon returning to Babine Lake comprise 80–95% of drainage production (Larkin and McDonald 1968; West and Mason 1987; Beacham et al. 2000b), Babine Lake escapement being estimated from a counting fence near the lake outlet. In Southeast Alaska, most sockeye salmon production is derived from coastal lakes rather than large river drainages like the Nass and Skeena rivers. Sockeye salmon from both rivers are caught concurrently in fisheries in marine waters in northern British Columbia and Southeast Alaska.

Knowledge of the distribution of genetic variation in Pacific salmon *Oncorhynchus* spp. has become increasingly valuable in the assessment and management of the different species. Initial descriptions of population structure based on genetic variation centered on surveys of allozymes (Varnavskaya et al. 1994; Wood 1995; Winans et al. 1996). The advent of the ability to analyze DNA-level markers, particularly microsatellites, has dramatically increased the number of highly polymorphic loci that are potentially available to include in a survey of population genetic variation. There are large differences among microsatellites

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in terms of the number of alleles observed at a locus, the range of allele sizes, and the level of differentiation among populations. Criteria for including microsatellite loci in a survey of population variation can differ among laboratories. Theoretical studies of locus characteristics to guide selection suggested that a modest number of independent loci was best, each locus having a modest number of alleles and each allele modest in frequency (Smouse and Chevillon 1998). In Pacific salmon population structure and stock identification studies, there has been some question as to which locus characteristics to include in surveys of microsatellite variation. One could choose loci with (1) a restricted number of alleles and presumably a restricted size range; (2) a moderate number of alleles, as suggested by Smouse and Chevillon (1998); or (3) a large number of alleles. Although there are theoretical studies to guide the choice of loci, there are few practical examples of evaluating the utility of locus characteristics for regional, population, and individual identification applications.

Microsatellites are very useful genetic markers with which to survey genetic variation among salmonid populations. Microsatellites have provided the ability to determine fine-scale population structure (Nielsen et al. 1997; Wenburg et al. 1998) and obtain estimates of stock composition on either a local or regional basis (Shaklee et al. 1999; Beacham et al. 2001). Surveys of microsatellite variation have been demonstrated to be effective in determining the population structure of sockeye salmon (Beacham and Wood 1999; Withler et al. 2000) and of considerable use in estimating stock composition in mixed-stock fisheries, both on a specific-population and on a regional basis (Beacham et al. 2000a, 2000b). However, only six loci were analyzed in those studies, not enough to allow for comparisons among loci in terms of their effectiveness in estimating stock composition. Surveys incorporating additional microsatellite loci and another type of DNA-level marker, the variation at a major histocompatibility complex (MHC) locus (Miller et al. 2001), would probably be of value in improving the accuracy and precision of estimated stock compositions.

The objectives of the present study were to analyze the variation at 14 microsatellite loci and 1 MHC locus in Nass River, Skeena River, and Southeast Alaska sockeye salmon populations so as to evaluate the suite of loci for application to stock identification. These loci were then applied to the practical issue of estimating the stock compositions in two fisheries, one in Southeast Alaska (where populations from a number of areas could contribute to the fishery) and one in northern British Columbia (where, given the location at the mouth of Portland Inlet, Nass River sockeye salmon would be expected to dominate the catch).

Methods

Collection of DNA samples and laboratory analysis.—Tissue samples were collected from adult fish from sockeye salmon populations in the Nass and Skeena River drainages in northern British Columbia and from coastal lakes in Southeast Alaska (Figure 1). DNA was extracted from the samples as described by Withler et al. (2000). For the survey of baseline populations, polymerase chain reaction products at 14 microsatellite loci—*Ots2, Ots3* (Banks et al. 1999), *Ots100, Ots103, Ots107,* and *Ots108* (Beacham et al. 1998; Nelson and Beacham 1999), *Oki1a, Oki1b, Oki6, Oki10, Oki16,* and *Oki29* (Smith et al. 1998; Nelson et al. 2003), *One8* (Scribner et al. 1996), and *Omy77* (Morris et al. 1996)—were size-fractionated on denaturing polyacrylamide gels and their allele sizes determined with the ABI 377 automated DNA sequencer. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems 1998, 2000). Genetic variation at the MHC class II *DAB*-β*1* locus (Miller et al. 2001) was surveyed by denaturing gradient gel electrophoresis (DGGE). The β*1* alleles were separated by DGGE with the Bio-Rad (Hercules, California) D Gene or D Code electrophoresis systems under conditions determined by the methods of Miller et al. (1999). Fluorescently multiplexed DGGE (Miller et al. 2000) was used in the population survey and analysis of fishery samples.

Baseline populations.—The baseline survey consisted of analysis of approximately 12,000 sockeye salmon derived from 11 sampling sites or populations in the Nass River drainage, 24 sites or populations in the Skeena River drainage, and 20 populations from coastal lakes or tributaries in Southeast Alaska (Table 1). Overall F_{ST} estimates for each locus and pairwise F_{ST} estimates between populations were calculated with FSTAT (Goudet 1995); the standard deviations of the estimates for individual loci were determined by jackknifing over populations and that for all loci combined by bootstrapping over loci. All of the annual samples available for a location were combined to estimate population allele frequencies, as recommended by Waples (1990). Allele frequencies for all of the baseline location samples surveyed in this study

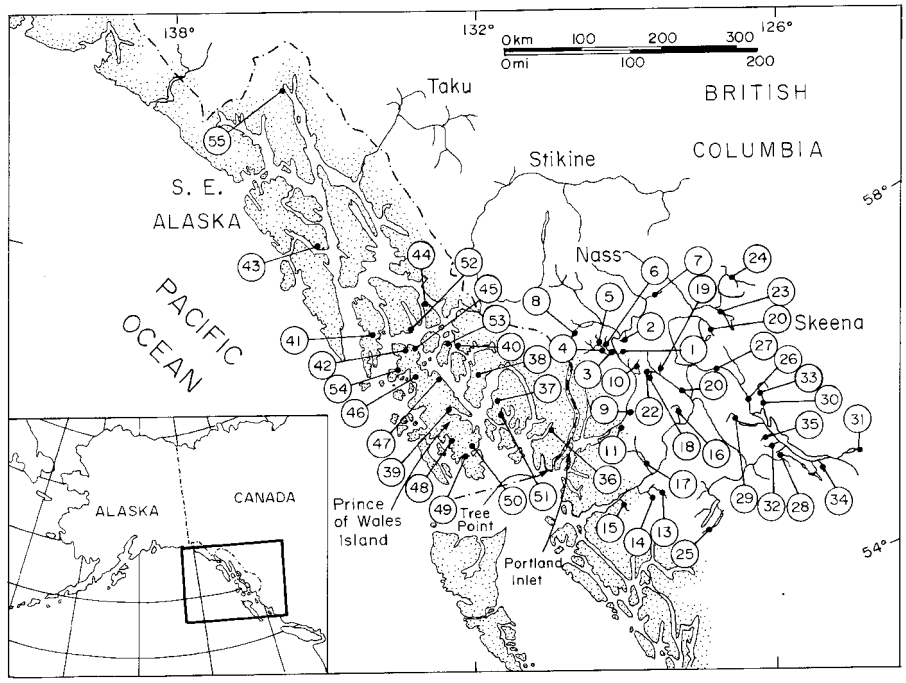

FIGURE 1.—Map showing the locations of sockeye salmon populations in the Nass River, the Skeena River, and Southeast Alaska, as well as at two fishery locations, Tree Point and Portland Inlet. The populations are identified by number in Table 1.

are available at the Web site of the Molecular Genetics Laboratory of the Department of Fisheries and Oceans, http://www-sci.pac.dfo-mpo.gc.ca/mgl/Default_e.htm.

Collection of the known sample.—Simulations provide one measure of the accuracy of estimated stock compositions, but a fundamental assumption of such simulations is that the baseline used is representative of the stocks or populations that will be encountered in fishery samples. We evaluated the accuracy of estimated stock compositions by using a sample of known origin that was completely independent of the baseline used in the estimations. The known-origin sample was composed of sockeye salmon from the Nass River, the Skeena River, and various lakes from Southeast Alaska. As the Nass and Skeena River components were derived from test fisheries in the lower portion of each drainage, the specific populations of origin of individual fish were unknown. However, as the specific lakes of origin were known for the Southeast Alaska sockeye salmon, it was possible to evaluate the level of accuracy for the individual lake components. We then compared the estimated stock composition of this sample with the known spawning locations.

Collection of mixed-stock samples.—All mixed-stock samples were collected from commercial fisheries near Tree Point in Southeast Alaska (in 2001) or from commercial or test fisheries in Management Area 3-7 at the entrance to Portland Inlet in northern British Columbia (in 2002) (Figure 1). Samples of DNA from the fisheries were obtained from either operculum punches or fin clips preserved in 95% solutions of ethanol. The mixed-stock samples were screened at all 14 microsatellite loci and the 1 MHC locus.

Comparisons among loci.—The 14 microsatellite loci surveyed were divided into three classes based on the number of alleles observed at each locus, the first set containing five loci displaying 7–21 alleles per locus, the second set containing five loci displaying 23–28 alleles per locus, and the third set containing four loci with more than 30 alleles per locus. Single-population mixtures composed of 21 representative populations from the Nass River, Skeena River, and Southeast Alaska were evaluated to determine the power of the classes to provide accurate and precise estimates of their composition using a wide-ranging, 203-population coastal baseline. In addition to the 55 populations surveyed in the present study, the

TABLE 1.—Population, nursery lake, sample collection years, number of fish sampled (*N*) per year, and total number of fish sampled for 55 populations of Nass River, Skeena River, and Southeast Alaska sockeye salmon. (The Bulkley River and Babine Lake are components of the Skeena River drainage.) Population numbers refer to the locations in Figure 1.

Population	Nursery lake	Years	*N*	
			Individual years	Total
		Nass River		
1. Bonney	Fred Wright	1987, 1994, 1996, 1998, 1999, 2001	76, 81, 93, 100, 82, 107	539
2. Kwinageese	Fred Wright	1987, 2000, 2001	81, 48, 65	194
Meziadin				
3. Fishway	Meziadin	1987, 1996, 2001	100, 111, 264	475
4. Beach spawning	Meziadin	2001	188	188
5. Tintina Creek	Meziadin	2001, 2002	51, 50	101
6. Hanna Creek	Meziadin	2001, 2002	49, 100	149
7. Damdochax	Damdochax	1987, 1994, 1998, 1999, 2000, 2001	100, 81, 100, 89, 50, 140	560
8. Bowser	Bowser	1986, 1987, 1994, 1998, 1999, 2000, 2001	80, 72, 81, 100, 160, 82, 222	797
9. Gingit	None	1987, 1988, 1997	73, 93, 169	335
10. Brown Bear	None	1997	40	40
11. Zolzap	None	1996, 1997	36, 24	60
		Skeena River		
12. McDonell Lake	McDonell	1987, 1988, 1994, 2002	81, 75, 60, 71	287
13. Williams Creek	Lakelse	1987, 1988, 1994	83, 98, 100	281
14. Schulbuckhand Creek	Lakelse	1988	77	77
15. Alastair Lake	Alastair	1987, 1988, 1994, 1998	75, 21, 100, 83	279
16. Kitwanga River	Kitwanga	1998	98	98
17. Kitsumkalum River	Kitsumkalum	1994	77	77
18. Stephens Creek	Stephens	2001	200	200
19. Nangeese River	None	2002	33	33
20. Kispiox River	None	2002	56	56
21. Motase Lake	Motase	1987	49	49
22. Swan Lake	Swan	1988, 1994	100, 81	181
23. Bear	Bear	1987, 1988	45, 71	116
24. Sustut	Sustut	1993, 2000, 2001	93, 47, 100	240
		Bulkley River		
25. Nanika River	Morice	1988, 1994	75, 63	138
		Babine Lake		
26. Lower Babine River	Babine	1987, 1994	50, 100	150
27. Upper Babine River	Babine	1987, 1994	81, 99	180
28. Pinkut Creek	Babine	1985, 1987, 1990, 1994	200, 99, 100, 100	499
29. Fulton River	Babine	1985, 1987, 1990, 1994	95, 193, 100, 100	488
30. Morrison River	Babine	1988, 1994	76, 100	176
31. Shass Creek	Babine	1987	78	78
32. Twain Creek	Babine	1987, 1990	100, 54	154
33. Tahlo Creek	Babine	1987, 1988, 1994	78, 85, 90	253
34. Four Mile Creek	Babine	1987, 1988	90, 75	165
35. Pierro Creek	Babine	1987, 1988	84, 79	163
		Southeast Alaska		
36. Hugh Smith	Hugh Smith	1992, 2000	95, 200	295
37. Heckman	Heckman	1992, 2000	100, 200	300
38. McDonald	McDonald	1992, 2000	100, 187	287
39. Karta	Karta	1992, 2000	100, 175	275
40. Thoms	Thoms	2000	212	212
41. Kutlaku	Kutlaku	2000	203	203
42. Red Bay	Red Bay	2000	201	201
43. Sitkoh	Sitkoh	2000, 2001	343, 40	383
44. Petersburg	Petersburg	2000	193	193
45. Salmon Bay	Salmon Bay	2000	197	197
46. Sarkar	Sarkar	2000	45	45
47. Luck	Luck	2000	200	200
48. Hetta	Hetta	2000, 2002	206, 108	314
49. Klakas	Klakas	2000	200	200
50. Kegan	Kegan	2000	196	196
51. Mahoney	Mahoney	2002	71	71
52. Kah Sheets	Kah Sheets	2002	105	105
53. Kunk	Kunk	2002	107	107
54. Shipley	Shipley	2002	105	105
55. Chilkat	Chilkat	1981	49	49

TABLE 2.—Number of alleles observed and F_{ST} values among 55 sockeye salmon samples (SDs in parentheses) for 14 microsatellite loci.

Locus	Number of alleles	F_{ST}
Oki1a	7	0.120 (0.029)
Oki1b	7	0.090 (0.020)
Oki6	32	0.168 (0.017)
Oki10	83	0.044 (0.004)
Oki16	25	0.127 (0.019)
Oki29	32	0.073 (0.006)
Omy77	17	0.115 (0.014)
One8	28	0.083 (0.010)
Ots2	21	0.119 (0.008)
Ots3	23	0.178 (0.019)
Ots100	30	0.131 (0.017)
Ots103	27	0.076 (0.010)
Ots107	15	0.107 (0.014)
Ots108	24	0.101 (0.011)
All loci		0.104 (0.017)

baseline consisted of (from north to south) 13 populations from the Alsek River drainage, 10 populations from the Taku River, 17 populations from the Stikine River (Beacham et al. 2004a), 1 population from the Unuk River, 5 populations from the Queen Charlotte Islands, 27 populations from the central coast of British Columbia, 17 populations from Vancouver Island (Beacham et al. 2002, in press), 3 populations from southern British Columbia, 50 populations from the Fraser River (Beacham et al. 2004b), 3 populations from coastal Washington, and 2 populations from the Columbia River. This baseline was also used in the evaluation of simulated mixtures and estimation of the known sample and was applied to the mixed-stock fishery samples.

Estimation of stock composition.—Genotypic frequencies were determined at each locus in each population, and the Statistical Package for the Analysis of Mixtures (Debevec et al. 2000) was used to estimate the stock composition of each mixture. All loci were considered to be in Hardy–Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies. We examined whether the genetic differentiation observed among sockeye salmon in the Nass River, Skeena River, and Southeast Alaska was sufficient for mixed-stock analysis, with the objective of obtaining accurate stock compositions by lake, river drainage, and region. Five simulated fishery mixture samples were evaluated for mixtures of populations from these three regions; a final simulated mixture also included populations from the Stikine River, a major river that enters the Pacific Ocean north of the Nass River through Southeast Alaska and whose populations can contribute to regional fisheries. The reported stock composition for the simulated mixtures is the bootstrap mean, which is presented along with its standard deviation. The reported stock compositions for actual fishery samples are the point estimates of each mixture analyzed, presented with variance estimates derived from 100 bootstrap simulations. Each baseline population and fishery sample was sampled with replacement in order to simulate the random variation involved in the collection of the baseline and fishery samples.

Results

Population Structure

Genetic differentiation was observed among the 55 sockeye salmon populations sampled in our study. The overall F_{ST} for the 14 microsatellite loci surveyed was 0.104, the values for individual loci ranging from 0.044 at *Oki10* to 0.178 at *Ots3*; all values were significantly greater than zero ($P < 0.05$; Table 2). In pairwise comparisons over all 55 populations, those in Southeast Alaska were shown to be distinct from those in the Nass and Skeena rivers, with a mean F_{ST} value exceeding 0.09 in the regional comparisons; however, there was also a considerable degree of differentiation among the Southeast Alaska populations (Table 3). Much lower differentiation was observed among the populations in Meziadin Lake in the Nass River drainage and Babine Lake in the Skeena River drainage, but there was considerable differentia-

TABLE 3.—Mean pairwise F_{ST} values (SDs in parentheses) averaged over 14 microsatellite loci for 55 populations of sockeye salmon from five regional groups in the Southeast Alaska, Nass River, and Skeena River drainages. Comparisons were conducted between individual populations in each region; populations are listed in Table 1.

Region[a]	Southeast Alaska	Meziadin	Other Nass	Babine	Other Skeena
Southeast Alaska (20)	0.100 (0.057)	0.139 (0.047)	0.100 (0.045)	0.094 (0.045)	0.132 (0.052)
Meziadin (4)		0.007 (0.006)	0.082 (0.023)	0.118 (0.024)	0.113 (0.053)
Other Nass (7)			0.049 (0.024)	0.056 (0.019)	0.085 (0.031)
Babine (10)				0.005 (0.003)	0.094 (0.031)
Other Skeena (14)					0.121 (0.045)

[a] The number of populations from each region is shown in parentheses.

TABLE 4.—Mean estimated percentage stock compositions of single-population mixtures (correct = 100%) for 21 representative populations of sockeye salmon from the Nass River, Skeena River, and Southeast Alaska drainages calculated with three classes of microsatellite (MS) loci, all MS loci combined, and all MS loci plus the major histocompatibility complex (MHC) locus. Class 1 contained five loci with 7–21 alleles each (*Oki1a*, *Oki1b*, *Ots107*, *Omy77*, and *Ots2*); class 2 contained five loci with 23–28 alleles each (*Ots3*, *Ots108*, *Oki16*, *Ots103*, and *One8*); and class 3 contained four loci with more than 30 alleles each (*Ots100*, *Oki6*, *Oki10*, and *Oki29*). Simulations were conducted using a 203-population baseline, 150 fish in the mixture sample, and 100 resamplings in the mixture sample and baseline samples. Standard deviations are given in parentheses.

Population	Class 1	Class 2	Class 3	All MS loci	MS loci + MHC
Bonney	91.9 (3.8)	95.7 (2.9)	97.1 (2.3)	98.6 (1.2)	98.6 (1.2)
Kwinageese	87.5 (5.9)	88.4 (5.0)	92.8 (4.3)	95.3 (2.4)	95.7 (2.3)
Meziadin[a]	92.9 (3.5)	92.6 (4.3)	93.1 (3.9)	94.8 (2.7)	94.9 (2.6)
Damdochax	89.0 (3.8)	92.1 (3.6)	95.8 (2.0)	98.6 (1.0)	99.1 (0.7)
Bowser	94.0 (2.8)	97.6 (1.4)	97.2 (1.4)	99.5 (0.6)	99.6 (0.4)
McDonell	97.2 (1.7)	98.8 (1.0)	98.1 (1.1)	98.5 (1.1)	98.7 (0.9)
Alastair	95.1 (2.4)	95.7 (1.7)	96.6 (1.5)	98.5 (1.0)	98.5 (1.0)
Kitsumkalum	82.2 (5.8)	92.5 (2.5)	85.7 (4.6)	92.0 (2.1)	92.5 (2.1)
Kitwanga	95.8 (2.0)	95.9 (1.8)	95.1 (1.9)	94.6 (2.1)	94.7 (1.9)
Sustut	97.1 (1.8)	98.0 (1.3)	98.5 (1.0)	98.4 (1.1)	98.3 (1.0)
Fulton	73.2 (12.0)	80.6 (9.0)	77.8 (7.3)	86.5 (5.2)	86.5 (5.2)
Hugh Smith	85.9 (5.1)	89.5 (3.9)	91.9 (2.5)	96.7 (1.8)	97.1 (1.8)
Heckman	89.7 (3.4)	94.2 (2.4)	95.2 (1.9)	97.5 (1.4)	97.8 (1.3)
McDonald	76.6 (8.2)	88.8 (4.1)	90.0 (2.9)	95.9 (1.9)	96.5 (1.8)
Karta	90.3 (4.4)	96.2 (2.1)	95.9 (1.8)	97.2 (1.3)	97.5 (1.3)
Thoms	91.2 (3.6)	95.8 (1.8)	96.6 (1.6)	97.5 (1.2)	97.8 (1.2)
Kutlaku	95.2 (2.3)	97.1 (1.5)	97.8 (1.2)	97.7 (1.2)	97.4 (1.4)
Red Bay	93.9 (2.9)	94.8 (1.9)	94.6 (2.2)	97.3 (1.4)	97.5 (1.3)
Sitkoh	98.7 (0.9)	99.0 (0.8)	98.3 (1.1)	98.9 (0.9)	98.9 (0.8)
Petersburg	97.3 (1.5)	98.6 (1.0)	97.9 (1.2)	97.8 (1.3)	97.8 (1.3)
Salmon Bay	92.8 (2.9)	95.8 (1.7)	95.4 (2.1)	97.4 (1.4)	97.3 (1.3)
Mean	90.8 (3.8)	94.2 (2.7)	94.4 (2.4)	96.6 (1.6)	96.8 (1.6)

[a] Beach-spawning fish.

tion between the populations in these lakes and all other populations. Greater differentiation was observed among non-Babine Lake populations in the Skeena River drainage than among non-Meziadin Lake populations in the Nass River drainage. Apart from the within-lake comparisons, the mean regional pairwise F_{ST} values ranged from 0.06 to 0.14, which is indicative of moderate to substantial differentiation among regions.

Comparisons among Loci

As noted above, the 14 microsatellite loci surveyed were divided into three classes based on the number of observed alleles at each locus, and 21 single-population simulated mixtures were evaluated with each of the three classes and estimated with a 203-population baseline. Some populations, such as the Sustut River population in the upper Skeena River drainage, were readily identified with a high degree of accuracy and precision using any of the three classes of loci (Table 4). For other populations, such as the Fulton River population from Babine Lake, it was more difficult to obtain reliable estimates of stock composition. The accuracy and precision of the estimated stock compositions generally increased as the number of observed alleles at the loci increased. Although the third locus set only contained four loci while the other sets each contained five loci, the accuracy and precision of the estimates derived from the third set were generally higher than those of estimates derived from the other two sets. Relative to locus set 1, there was on average a reduction in error of 37% for set 2, 39% for set 3, 63% for all microsatellites, and 65% for all loci. In comparison with the results from the 21 single-population evaluations, the results with locus set 2 were significantly more accurate than those with locus set 1 (sign test analysis; $P < 0.01$), as were the results with locus set 3 (sign test analysis; $P < 0.01$). The corresponding reductions in standard deviations were 29% for set 2, 37% for set 3, and 58% for all microsatellites and all loci. The standard deviations obtained with locus set 2 were significantly less than those obtained with locus set 1 (sign test analysis; $P < 0.01$), as were the standard deviations for locus set 3 ($P < 0.01$). Combining all loci for stock identification applications

provided the most accurate and precise estimates of stock composition, and there was little evidence to indicate that adding loci to the set used for stock composition estimation resulted in any degradation in the accuracy and precision of the estimates.

Analysis of Simulated Mixtures

With a baseline comprised of 203 coastal populations ranging from the Columbia River in the south to Alaska in the north, mixtures composed solely of Southeast Alaska populations (mixture 1) were estimated with a high degree of accuracy, the error of estimation of specific populations being less than 1% and the error of the regional estimate being about 1% (Table 5). Meziadin Lake is the dominant sockeye salmon production lake in the Nass River drainage, and mixtures comprising only this single stock (mixture 2) were estimated with less than 3% error. Similarly, Babine Lake is the dominant production lake in the Skeena River drainage, and mixtures comprised solely of this stock (mixture 3) were estimated with less than 1% error. Mixtures comprised of a range of populations from both the Nass and Skeena drainages (mixture 4) were well estimated, with drainage estimates within 2% of actual contributions and similar levels of accuracy for the individual population components (with the exception of the Babine Lake populations). Mixtures containing populations from the three regional components—the Nass River, the Skeena River, and Southeast Alaska (mixture 5)—were again well estimated, the error of regional components being about 1% and a similar level of accuracy for the individual populations. The final simulated mixture evaluated included the three previous regional components and some populations from the Stikine River (mixture 6). As in the previous mixtures, regional and population components were estimated with an error of 1–2%. Analysis of simulated mixtures of sockeye salmon from the Nass River, Skeena River, and Southeast Alaska indicated that DNA variation provides a practical way to estimate stock composition with a high degree of both population and regional accuracy.

Analysis of a Known-Origin Mixture

The estimated stock compositions of a sample of 526 sockeye salmon of known origin were within 1% of the actual contributions for the Skeena River and Nass River components, but the Southeast Alaska component was underestimated by about 4%. The Stikine River component was overestimated by about 4%, as there were no Stikine

TABLE 5.—Estimated percentage composition of six simulated mixtures of sockeye salmon from the Nass River, Skeena River, Stikine River, and Southeast Alaska using the variation at 14 microsatellite loci and a 203-population baseline. Each mixture of 150 fish was generated 100 times with replacement, and the stock compositions of the mixtures were estimated by resampling each baseline population with replacement. Standard deviations are shown in parentheses.

Population	Actual	Estimated
Mixture 1: Southeast Alaska		
Hugh Smith	20	19.6 (3.6)
Kegan	20	19.9 (3.5)
Kutlaku	20	19.4 (3.1)
Petersburg	20	19.3 (3.2)
Salmon Bay	20	19.2 (3.2)
All Southeast Alaska	100	98.8 (0.9)
Mixture 2: Meziadin Lake		
Meziadin (beach)	20	17.4 (4.1)
Meziadin (fishway)	20	29.2 (5.4)
Hanna	20	20.6 (4.9)
Tintina	40	29.8 (5.1)
All Nass River	100	97.6 (1.6)
Mixture 3: Babine Lake		
Fulton	30	34.9 (6.1)
Lower Babine	20	14.7 (3.0)
Morrison	20	14.2 (4.4)
Pinkut	20	19.5 (5.5)
Tahlo	10	10.0 (4.2)
All Babine Lake	100	99.4 (0.6)
Mixture 4: Skeena and Nass rivers		
Morrison	20	13.7 (3.5)
Upper Babine	20	15.5 (3.4)
All Babine Lake	40	39.4 (4.2)
Kalum	10	8.9 (3.2)
Sustut	10	10.0 (2.6)
All Skeena River	60	58.6 (3.8)
Meziadin (beach)	20	18.0 (3.6)
Bonney	10	9.6 (2.5)
Bowser	10	10.1 (2.6)
All Nass River	40	40.1 (3.9)
Mixture 5: Southeast Alaska, Nass River, and Skeena River		
Hetta	20	19.6 (3.2)
Luck	10	9.5 (2.1)
Sarkar	10	8.9 (2.4)
All Southeast Alaska	40	38.8 (4.1)
Meziadin (fishway)	15	14.7 (2.1)
Tintina	5	3.5 (2.1)
Damdochax	10	9.8 (2.4)
All Nass River	30	29.9 (3.8)
Pinkut	20	16.3 (3.7)
Alastair	5	5.0 (1.8)
Swan	5	4.8 (1.2)
All Skeena River	30	30.0 (3.2)
Mixture 6: Southeast Alaska and Stikine, Nass, and Skeena rivers		
Verrett	10	8.8 (2.8)
Tahltan	10	8.2 (2.7)
All Stikine River	20	20.7 (3.4)
Sarkar	20	17.6 (3.1)
Sitkoh	20	19.7 (3.3)
All Southeast Alaska	40	38.3 (4.1)
Meziadin (fishway)	20	18.3 (3.4)
Bowser	10	9.8 (2.4)
All Nass River	30	29.8 (3.8)
Alastair	10	9.9 (2.4)
All Skeena River	10	10.0 (2.4)

River sockeye salmon in the sample (Figure 2A). Some portion of the Southeast Alaska component was allocated to the Stikine River. However, there were 32 fish from four Alaskan lakes not in the baseline (Andrews, Falls, Gene's, and Warmchuck), and the known sample was reanalyzed with these fish removed. The estimated stock compositions on a regional basis were then within 1% of the actual contributions, and for the Southeast Alaska component the individual population estimates were generally within 0.5% of the actual contributions (Figure 2B). These four unsampled populations were apparently more similar to Stikine River populations than they were to other populations in Southeast Alaska. However, were they to be included in the baseline used to resolve the original 526-fish sample, it is expected that the accuracy levels outlined in Figure 2B would be obtained.

Application to Fisheries

DNA variation was applied to the practical problem of estimating the stock composition in two fisheries, one in Southeast Alaska and one in northern British Columbia. The fishery sampled in Southeast Alaska was adjacent to Tree Point, in waters close to the border between Canada and the United States (Figure 1). In 2001, samples from this fishery were collected between mid-June and mid-August and analyzed during the fishing season. In mid-June, Nass River sockeye salmon dominated catches in the fishery, comprising about 75% of the samples, Skeena River sockeye salmon comprising about 20% of the catch (Figure 3A). By late June, Nass River sockeye salmon comprised nearly 80% of the catch, Skeena River fish about 10%, Stikine River fish about 5%, and Southeast Alaska fish about 2%. By the week of 11 July, the Nass River component declined to about 50% of the catch, the Skeena River component increased to 25%, and the Southeast Alaska component increased to about 20%. Sockeye salmon from Southeast Alaska comprised the highest proportions (25–30%) of the catch during the weeks of 17 July and 24 July and were virtually absent after 7 August. By this time, the Nass River component had decreased to 20% of the catch while the Skeena River component increased to nearly 75%, the Stikine River component comprising about 4%. In this fishery in 2001, there was a general trend of decreasing proportions of Nass River sockeye salmon during the sampling period, increasing proportions of Skeena River fish, and peak Southeast Alaska proportions during the middle of the sampling period.

The Tree Point fishery is an interception fishery in which it is expected that sockeye salmon from different regions will be caught, and this was reflected in the estimated stock compositions. The fishery sampled in British Columbia (i.e., at the entrance to Portland Inlet in Management Area 3-7) was more terminal in nature, and it is this inlet into which the Nass River empties. Nass River sockeye salmon comprised over 95% of the samples between 15 June and 9 July in 2002, which would be expected given the more terminal nature of this fishery (Figure 3B). At the conclusion of sampling on 29 July, Nass River sockeye salmon still comprised 83% of the sample, but the Skeena River component was estimated at 16%, concurrent with the later timing of return of this stock relative to that of the Nass River stock. Analysis of actual fishery samples suggested that DNA variation provides a practical way to estimate stock composition with a high degree of regional accuracy.

Discussion

Genetic Differentiation

There is some potential for differentiation among the sockeye salmon spawning within a lake and its tributaries. The sockeye salmon spawning in Meziadin Lake in the Nass River drainage spawn both in the lake's tributaries (Hanna and Tintina creeks) and along the lakeshore (the latter are known as beach spawners). Genetic differentiation can exist among the sockeye salmon populations spawning in the same lake system, but it is usually based on differences in the timing of spawning among the populations within the lake (Burger et al. 1997; Beacham et al. 2004b). Some differentiation was observed between the beach-spawning population and the two tributary populations (F_{ST}, 0.010 versus Hanna Creek and 0.012 versus Tintina Creek), but no difference was observed between the two tributary populations (F_{ST}, 0.000). Sockeye salmon spawn earlier in the tributaries than along the lakeshore (Rutherford et al. 1994), and this separation in the timing of spawning reflects the differentiation observed among the three populations. In contrast, in Babine Lake in the Skeena River drainage, very modest genetic differentiation was observed among the populations spawning in lake tributaries (mean F_{ST}, 0.005).

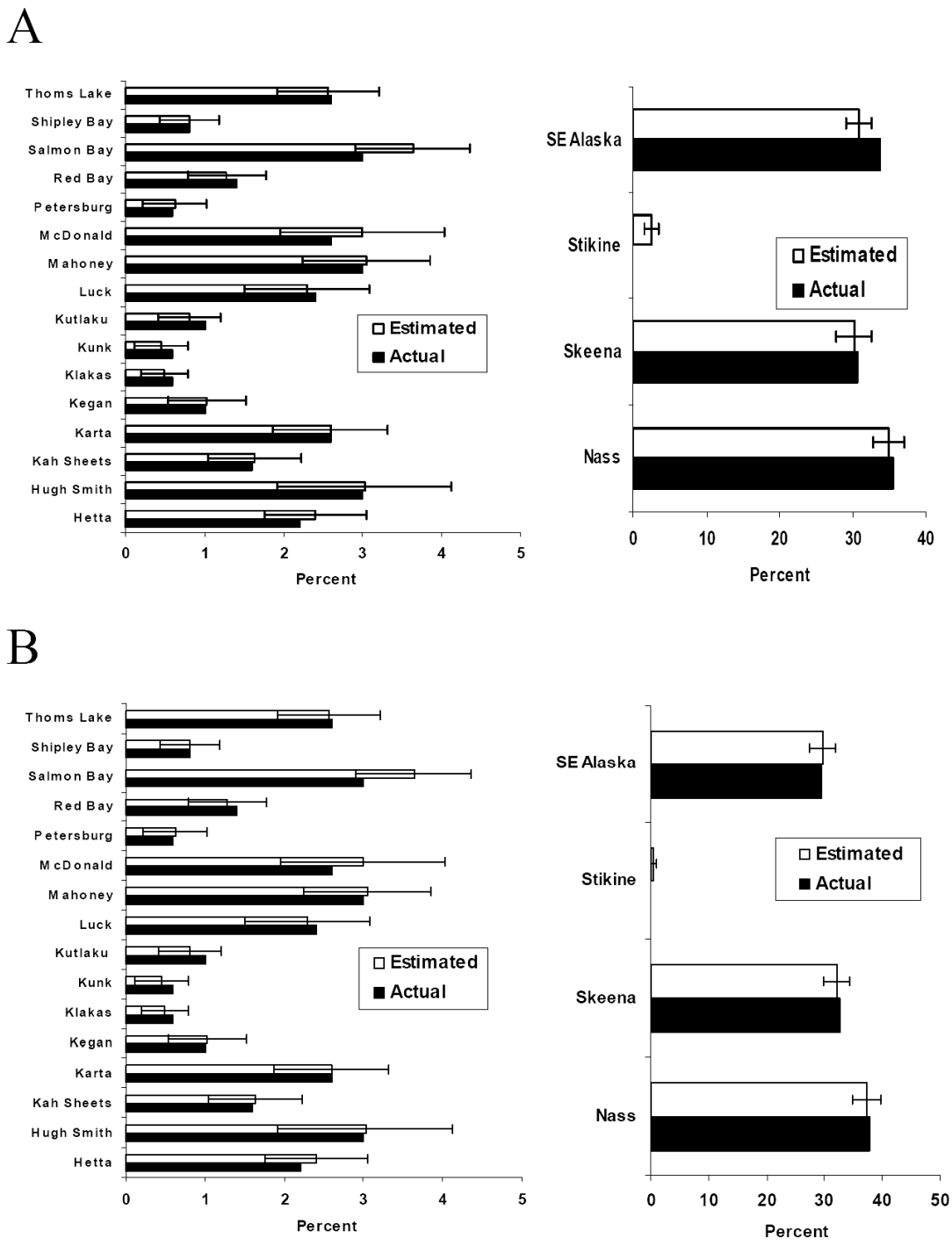

FIGURE 2.—Estimated percentage stock compositions of a sample of Nass River, Skeena River, and Southeast Alaska sockeye salmon populations of known origin collected in 2002. The baseline used for the analysis consisted of 203 populations ranging from Southeast Alaska to the Columbia River and was surveyed for variation at 14 microsatellite loci and one major histocompatibility complex locus. The known sample was constructed by sampling sockeye salmon from test fisheries in the lower Nass River and lower Skeena River and from spawning ground collections in Southeast Alaska. Panel **(A)** shows percentages estimated by population for the Southeast Alaska populations and regional estimates for a sample of 526 sockeye salmon. Panel **(B)** shows the corresponding percentages with 32 fish from the Southeast Alaska populations that were not in the baseline removed from the 526-fish sample.

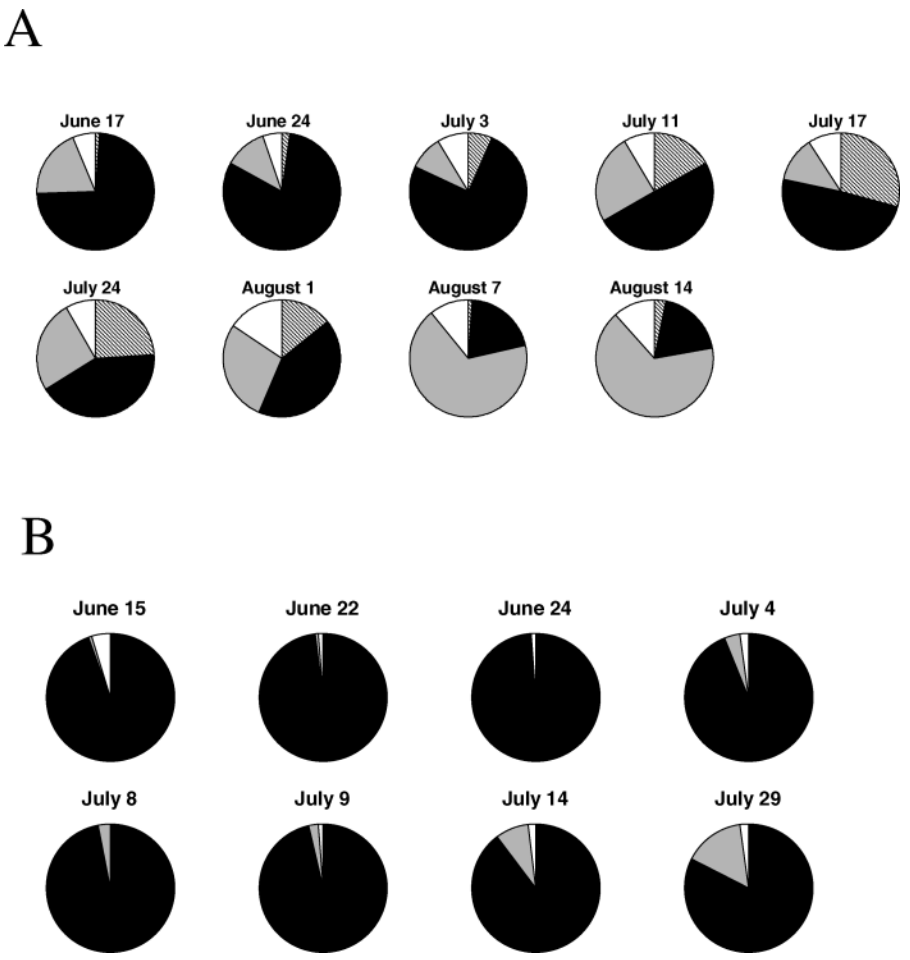

FIGURE 3.—Panel **(A)** shows the estimated stock composition of weekly samples of sockeye salmon taken from a commercial fishery during June 17–August 14, 2001, near Tree Point in Southeastern Alaska. The Nass River contribution is shown in black, the Skeena River contribution in gray, the contribution of other Canadian stocks in white, and the Southeast Alaska contribution by means of hatch marks. Weekly sample size generally ranged from 70 to 100 fish. Panel **(B)** shows the estimated stock composition of samples from a test fishery during June 15–July 29, 2002, near the mouth of Portland Inlet (Management Area 3-7). Stocks are indicated as in panel (A); sample size generally ranged from 90 to 100 fish.

Stock Identification

There are two general types of samples required for stock identification analysis of mixed-stock fisheries, namely, samples from the fishery and an adequate baseline. The key component of the analysis is the baseline, and for the estimated stock compositions to be accurate the baseline needs to be comprehensive, encompassing all stocks in the fishery samples and providing sufficient resolution among the stocks or populations. If a wide range of stocks or populations could be present in fishery samples, the baseline must be wide ranging and complex. Given the technical effort and costs associated with assembling these baselines, the annual variation in the characters used in stock identification analysis must be small relative to the differentiation among stocks or populations, as it would not be practical or cost-effective to assemble a new baseline each year. This stability of allele frequencies of genetic characters relative to population differentiation is a key characteristic of both allozyme (Grant et al. 1980; Gharrett et al. 1987) and DNA-level variation (Beacham and Wood 1999; Tessier and Bernatchez 1999; Miller et al. 2001; Beacham et al. 2004b) and is in sharp contrast to environmentally induced variation, such as that

found in scale pattern or elemental analysis, where annual variability requires annual sampling of the baseline. Although annual estimation of baseline allele frequencies is not required for practical applications, some level of monitoring in key baseline populations would be prudent.

The loci used in stock composition estimation are assumed to be in Hardy–Weinberg equilibrium (HWE) in the baseline populations (Debevec et al. 2000). In our survey, the *Oki10* locus was not in HWE in all populations in all years (17% of tests significant at $P < 0.05$), yet it was used in the estimation of stock compositions. Given that 83 alleles were observed at *Oki10* in our survey and that a large number of fish were sampled per population, it would not be practical to use observed genotypic frequencies, as there will very likely be fish in the mixture from a population displaying *Oki10* genotypes not observed in the baseline sample. Beacham et al. (2001) illustrated that the accuracy of stock composition estimates is enhanced by assuming HWE distribution of genotypic frequencies for loci at which observed genotypic frequencies did not conform to those expected under HWE. The accuracy of the estimated stock compositions of a known sample was within 0.5% of the actual population values when estimated with a 203-population baseline, so inclusion of a locus not in HWE in all populations did not induce significant bias in the estimated population contributions.

The characteristics of the loci to include in a survey of microsatellite variation with a view to stock identification applications has been an important question. Size range and the number of alleles observed at a locus are clearly important characters. While some investigators have suggested that loci with modest numbers of alleles are preferred for population structure and individual identification (Smouse and Chevillon 1998; Bernatchez and Duchesne 2000), others have indicated that equivalent information can be obtained by examining either a few loci with many alleles or many loci with a few alleles (Kalinowski 2002). Negative relationships have been reported between locus variability and the magnitude of estimated population subdivision (O'Reilly et al. 2004). However, in an analysis of the power of individual microsatellite loci for stock identification of Fraser River sockeye salmon, Beacham et al. (2004b) reported that the number of alleles observed at a locus was significantly correlated with the power of the locus to provide accurate estimates of stock composition. Our results, which incorporate this same set of microsatellite loci, indicate that the accuracy and precision of estimated stock compositions are related to the number of alleles observed at the loci used in the analysis as well as to the number of loci used. Stock compositions derived from loci with larger numbers of observed alleles were more accurate and precise than those derived from loci with smaller numbers of alleles, a finding similar to the results outlined by Cornuet et al. (1999) and Kalinowski (2002). Loci with modest numbers of alleles (6–10) would not be the first choice in surveys of microsatellite variation for stock identification applications, but if the size range of the alleles is such that a number of loci can be analyzed on a single gel, then loci with these characteristics can be considered.

In the application of genetic variation to estimate stock composition in mixed-stock fisheries, one key question relates to the accuracy of the estimated compositions. One approach is to evaluate the accuracy and precision of the estimated stock compositions by analysis of simulated mixtures and to compare the estimated results with the known composition. While this is an important first step, a key assumption of this method is that the results obtained are representative of those that are obtained when the baseline is applied to estimate the stock composition of a sample of unknown origin. The results will be comparable only if the baseline used to estimate stock compositions includes adequate representation from the stocks or populations present in the sample. For example, in the simulated-mixture samples, the error of the Southeast Alaska component ranged from 1% to 2%, but in the actual sample of sockeye salmon of known origin (which included fish from four populations not in the baseline) the Southeast Alaska component was underestimated by 4%. Removing fish from these four populations from the sample so that the baseline was completely representative of the Southeast Alaska component reduced the error to about 1% for this component, very similar to the that of the simulated mixtures. If management applications require that estimated stock compositions be accurate to within 1–2% for stocks of interest when applied to fishery samples, it is clear that baselines must be quite comprehensive for the analysis.

In the analysis of fishery samples from Tree Point in Southeast Alaska, the major contributors to the fishery in 2001 were estimated to be sockeye salmon of Nass River, Skeena River, and Southeast Alaska origin. However, sockeye salmon of Stikine River origin were consistently estimated to be

present in the samples, comprising 5–10% of the sample from each week. In the analysis of the sample of known origin, Southeast Alaska populations present in the sample but not adequately represented in the baseline were mistakenly allocated to the Stikine River. The question arises as to whether the 5–10% allocation to the Stikine River in the weekly fishery samples is simply misallocation due to the presence of Southeast Alaska populations in the samples but not in the baseline or there are actually sockeye salmon of Stikine River origin in the samples. Tagging studies conducted about 20 years earlier in the same area indicated that Stikine River sockeye salmon were at times significant contributors to the fisheries at Tree Point (Pella et al. 1993). The estimated contributions of Stikine River sockeye salmon are consistent with their relative abundance derived from earlier tagging studies. However, if management applications require discrimination between fish of Stikine River and Southeast Alaska origin to within 1–2%, some enhancement of the Southeast Alaska baseline will be required if there is significant production from unrepresented populations.

The estimated stock compositions of the fisheries at Tree Point in Southeast Alaska and the entrance to Portland Inlet in northern British Columbia conformed to those that would be expected based on their geographic locations. The Tree Point fishery has previously been demonstrated to intercept sockeye salmon from the Nass River, Skeena River, and Southeast Alaska (Pella et al. 1993), and the relative abundance of the major stocks in the fishery samples reflected their general timing of return to spawn. The entrance to Portland Inlet is a more terminal area, and sockeye salmon of Nass River origin would be expected to be a very major contributor to this fishery. The estimated stock compositions from both fisheries reflected prior expectations.

DNA variation has provided the opportunity to accurately estimate the stock composition for a range of sockeye salmon fisheries in British Columbia (Beacham et al. 2002, 2004b), and with rapid processing of samples during the fishing season it allows fishery managers the flexibility to structure fisheries to achieve the twin objectives of restricting exploitation of populations of conservation concern while enabling the harvest of abundant populations (Beacham et al. 2004c). It is our expectation that DNA variation will become the dominant technique of stock identification used for all Pacific salmon species.

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