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## **Estimation of stock composition of sockeye salmon in the North Pacific Ocean**

by

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## Abstract

Variation at 14 microsatellite and one major histocompatibility complex (MHC) loci was surveyed in approximately 29,000 sockeye salmon (*Oncorhynchus nerka*) sampled from 188 localities ranging from the Columbia River to Japan, with the majority of the sites in British Columbia. The observed regional population structure enabled an evaluation of the utility of using microsatellite and MHC variation for estimation of stock composition of sockeye salmon in mixed-stock fisheries. Stock compositions were estimated based upon 19 regional groups for a series of simulated and actual fishery samples, with the 188-population baseline used to estimate stock compositions. Application of microsatellite and MHC variation clearly has the potential to provide reliable estimates of stock composition for a local group of sockeye salmon even when there is a potential of a Pacific Rim distribution of populations contributing to the fishery sample.

## Introduction

Reliable, accurate, effective, and practical methods of stock identification are a key requirement in the assessment and management of Pacific salmon fisheries and populations. Stock identification of sockeye salmon (*Oncorhynchus nerka*) is of particular concern to many management agencies, given the economic value of sockeye salmon fisheries. Several methods of stock identification currently exist for sockeye salmon, with scale pattern analysis (Cook and Guthrie 1987), parasites (Margolis 1963), allozymes (Seeb et al. 2000), minisatellites (Beacham et al. 1995), microsatellites (Beacham and Wood 1999), and MHC variation (Miller et al. 2001) all potentially available for applications to specific problems. Genetic methods of stock identification have several advantages, among them the level of differentiation among populations and the stability of the genetic characters surveyed. For example, in Fraser River sockeye salmon, 25% of the variation at DAB- $\beta$ 1 major histocompatibility (MHC) locus was partitioned among populations, compared with 5% at neutral microsatellite loci. Differences among populations were about 24 times greater than annual variation within populations (Miller et al. 2001). As annual variation in allele frequencies in salmonid microsatellite and MHC loci is substantially less than differentiation among populations (Beacham and Wood 1999; Tessier and Bernatchez 1999; Beacham et al. 2000a,b; Miller et al. 2001), there is no requirement for annual updating of baseline populations once sufficient surveys have been conducted to characterize adequately the genetic differentiation among populations.

The requirement for increased population discrimination relative to that of other techniques that led us to evaluate minisatellite (Beacham et al. 1995), microsatellite (Beacham and Wood 1999; Beacham et al. 2000a,b), and MHC variation (Miller et al. 2001). Population-specific stock composition estimates of sockeye salmon have been available with microsatellite analysis in a local area (Beacham et al. 1998), within a river drainage (Beacham and Wood 1999), or between river drainages (Beacham et al. 2000b). Allozyme-based applications generally provide regional estimates of stock composition in a species, provided that there is a regional basis in population structure (Shaklee et al. 1999). Population-specific estimates of sockeye stock composition can sometimes be required in management decisions, as are regional-specific estimates in more wide ranging applications, and these were unlikely to be available using allozymes for stock composition analysis (Wood et al. 1994). Microsatellites can provide regional estimates as well, but may possibly provide population-specific estimates in some applications if the survey of baseline populations has been adequate (Beacham et al. 2001, Beacham et al. 2002). This greater population discrimination ability is a consequence of the higher heterozygosity and the larger number of alleles at microsatellite loci compared with allozyme loci. With respect to sockeye salmon, no relatively local regional population structure is apparent when population structure is examined with allozymes (Wood et al. 1994; Gustafson and Winans 1999), and applications in stock composition estimation in marine fisheries have been limited. Indeed, in a 14-locus allozyme-based analysis of stock composition of sockeye salmon caught in a high seas fishery, Wilmot et al. (2000) reported that only three regional reporting areas were statistically valid for the entire Pacific-rim distribution of sockeye salmon (Russia, Alaska and northern British Columbia, and southern British Columbia and Washington).

There are clearly limitations in the effectiveness of allozymes in providing accurate estimates of stock composition in complex mixed-stock fishery samples.

In the current study, we evaluate the utility of using variation at 14 microsatellite and one MHC loci for regional stock identification of sockeye salmon. This evaluation is conducted by examining the accuracy and precision of estimated stock compositions through analysis of simulated mixtures and samples from fisheries in coastal British Columbia, with the mixtures resolved using a 188-population baseline incorporating populations from Japan, Russia, Alaska, British Columbia, and Washington.

## Materials and Methods

### Collection of DNA samples and laboratory analysis

Tissue samples were collected from adult fish from sockeye salmon populations in the Pacific Rim and DNA extracted from the samples as described by Withler et al. (2000). For the survey of baseline populations, PCR products at 14 microsatellite loci: *Ots2*, *Ots3* (Banks et al. 1999), *Ots100*, *Ots103*, *Ots107*, and *Ots108* (Small et al. 1998; Nelson and Beacham 1999), *Oki1* (two loci), *Oki6*, *Oki10*, *Oki16*, and *Oki29* (Smith et al. 1998 and unpub.), *One8* (primers outlined by Scribner et al. 1996), and *Omy77* (Morris et al. 1996) were size fractionated on denaturing polyacrylamide gels and allele sizes determined with the ABI 377 automated DNA sequencer. Genetic variation at the MHC class II *DAB-β1* locus (Miller et al. 2001) was surveyed by denaturing gradient gel electrophoresis (DGGE).  $\beta 1$  alleles were separated by DGGE with the Bio-Rad (Hercules, CA) D Gene<sup>TM</sup> or D Code<sup>TM</sup> electrophoresis systems, with conditions determined by the methods of Miller et al. (1999). Fluorescently-multiplexed (FM)-DGGE (Miller et al. 2000) was used in the population survey.

### Baseline populations

The baseline survey consisted of analysis of approximately 29,000 sockeye salmon from 188 populations from Japan, Russia, Alaska, British Columbia, and Washington. Information on population structure derived from six microsatellites in Fraser River sockeye salmon was outlined by Withler et al. (2000) and that from the *DAB-β1* MHC locus by Miller et al (2001).

### Estimation of stock composition

Genotypic frequencies were determined at each locus in each population and the statistical package for the analysis of mixtures software program (SPAM 3.2) (Debevec et al. 2000) was used to estimate stock composition of each mixture. More alleles were present at the microsatellite loci than was practical for stock identification applications. We combined low frequency adjacent alleles to reduce the number of genotypic frequencies to be estimated with the available samples, with little loss in the ability to discriminate among populations. Summarized briefly, 24 alleles recognized at *Ots2* were condensed to 15 alleles (120 genotypes) for stock identification analysis, 24 alleles at *Ots3* were condensed to 10 alleles (55 genotypes), 33 alleles at *Ots100* were condensed to 19 alleles (190 genotypes), 30 alleles at *Ots103* were condensed to 20 alleles (210 genotypes), 14 alleles at *Ots107* were condensed to 7 alleles (28 genotypes), 28 alleles at *Ots108* were condensed to 20 alleles (210 genotypes), 8 alleles at *Oki1a* were condensed to 5 alleles (15 genotypes), 9 alleles at *Oki1b* were condensed to 4 alleles (10 genotypes), 30 alleles at *Oki6* were condensed to 11 alleles (66 genotypes), 75 alleles at *Oki10* were condensed to 29 alleles (435 genotypes), 23 alleles at *Oki16* were condensed to 17 alleles (153 genotypes), 35 alleles at *Oki29* were condensed to 20 alleles (210 genotypes), 43 alleles at *One8* were condensed to 11 alleles (66 genotypes), and 20 alleles at *Omy77* were condensed to 13 alleles (91 genotypes). For the MHC locus, no compression of 12 alleles (78 genotypes) at the *DAB-β1* locus was instituted. All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the

observed allele frequencies. Reported stock composition for simulated mixtures was the bootstrap mean, along with the standard deviation of the mean. Reported stock composition for actual fishery samples was the point estimate of each sample analyzed, with variance estimates derived from 100 bootstrap simulations.

## Results

### Population structure

If there is a regional genetic structure among populations contributing to a fishery, then it is unnecessary to survey all individual populations that contribute to the fishery. The portion of the mixed-stock sample derived from unsampled populations is allocated to sampled populations from the same region, thus reducing the cost and complexity of establishing a baseline sufficient for mixture analysis. The sampled populations constitute the baseline used to estimate stock compositions in mixed-fishery samples. Regional structure was observed in the baseline populations, with Japanese, Alek River, and Fraser River populations comprising the most distinct of 19 geographically-based groups (Table 1; Beacham et al., unpublished).

### Estimation of stock composition

This regional structure observed in sockeye salmon population structure resulted in good discrimination among the 19 regional groups of sockeye salmon. Analysis of simulated mixtures, with each mixture composition 100% from each of the 19 regions and estimated with a 188-population baseline, usually resulted in estimates above 90% for the region (Table 1). The notable exception was the non-Kuril Lake Russian component, where only about 50% of the simulated sample was estimated to have been derived from non-Kuril Lake Russian populations. Although Russian populations are more similarly genetically to each other than to populations in other regions, the lack of ability to estimate the Russian component without significant bias is due to the small population sample sizes (typically < 20 fish per population) rather than any inherent lack of ability to discriminate Russian populations. Better characterization of non-Kuril Russian populations by increased sample size would reduce the underestimation of this component.

Analysis of the simulated 100% Fraser River and Skeena River mixtures indicated that sockeye salmon from these two drainages should be well differentiated from other regional groups of sockeye salmon. We tested model performance for these two regional groups by analyzing samples of sockeye salmon derived from fishery sampling during 2001 within each of these two river drainages. The fishery samples were independent of the baseline samples. The expectation would clearly be that 100% of each sample should be allocated to either the Fraser River or Skeena River, as these were samples taken from fish caught within the drainage, and thus their origin is known. Stock composition of the Fraser River sample estimated with a 188-population baseline was 98.8% Fraser River origin, and that for the Skeena River was 96.4% (Table 2). This analysis confirmed the results of analysis of simulated samples, and indicate that Fraser River and Skeena River estimated stock compositions should have little bias.

In analysis of samples from marine fisheries, it is rare to have sockeye salmon from only a single region present in the samples. We evaluated whether the genetic differentiation observed among the 188 populations included in the baseline was sufficient for mixed-stock analysis aimed at estimating regional contributions to fishery samples. Three fishery mixture samples were simulated, and stock compositions were estimated for the 19 regions. Regional stock compositions of a simulated mixture containing fish from the Columbia River, Washington, and central and southern British Columbia were all within 2% of the actual regional contribution for all 19 regions included in the analysis (Table 3, mixture 1). Regional compositions of a simulated mixture of fish primarily from southeast Alaska and northern British Columbia were also all generally within 2% of the actual regional contribution (Table 3, mixture 2). Regional compositions of a simulated mixture of fish primarily from Bristol Bay, Kodiak Island, Russia, and Japan were within 3% of the actual regional contribution for the Bristol Bay, Kodiak Island, Kuril Lake, and Japanese components, but seriously underestimated the non-Kuril Lake Russian component (Table 3, mixture 3). This “missing” Russian

component was spread among several regions, with the main one being the Alaska Peninsula component. As previously noted, this problem would be corrected by increased sample sizes and better characterization of the Russian component.

Analysis of simulated mixtures of sockeye salmon suggested that regional estimates of stock composition should be accurate for most of the 19 regions identified, with significant bias observed only in the Russian component. We tested model performance by analyzing three samples of sockeye salmon obtained in nearshore waters in British Columbia with the 188-population baseline, and for which some prior knowledge of stock composition is believed to be available. The first sample was obtained from a test fishery conducted in early July at the south end of Vancouver Island in the Strait of Juan de Fuca. Fraser River sockeye salmon would be expected to dominate in the fishery sample at this time, although sockeye salmon from Washington may also be present. Analysis of the sample indicated that Fraser River sockeye salmon dominated the sample, but 5% of the sample was estimated to be of Washington origin (Table 4). Scale pattern analysis from the same sample also indicated that about 5% of the fish in the sample originated from Lake Washington (M. Lapointe, Pacific Salmon Commission, pers. comm.), in agreement with the genetic analysis. The second sample was obtained from a survey of juvenile distribution in northern British Columbia. As the sample was obtained from northern and central coastal British Columbia marine waters during June-July 1998, and as juvenile salmon are generally believed to migrate northward after entry into salt water, it is logical to expect that juveniles from regions south of or adjacent to the sampling sites would be present in the sample. Juveniles from the Fraser River and Skeena River were estimated to be the major contributors to the sample, likely reflective of their population abundance, but juveniles from a number of other southern regions and the adjacent southeast Alaska region were also estimated to be present (Table 4). The third sample was obtained from a fishery near Portland Inlet in northern British Columbia, near the border with Alaska. Given the location and of year, Nass River sockeye would be expected to dominate the fishery sample. Nass River sockeye salmon were estimated to have comprised 92% of the sample, with Skeena River the next most abundant contributor (Table 4). As with the previous samples, these results are in line with expectations.

## Discussion

Ideal technologies for mixed-stock analysis are those based on biological variation in characters which differ substantially among stocks, show little temporal or annual variation within stocks relative to stock differences, and can be screened in a rapid, non-lethal, and cost-effective manner for both baseline and mixed-stock samples. The PCR-based survey of single-locus allele frequencies at microsatellite DNA and MHC loci meet these criteria, and can be readily used for in-season fishery management decisions requiring stock composition analysis. During 2001, estimates of stock composition were available approximately 30 hours after the morning reception of the samples in our laboratory, illustrating the practicality of using solely microsatellite and MHC variation for in-season stock composition analysis.

In the current analysis, 19 regional groups were defined for estimation of stock compositions. However, depending upon the completeness of the baseline survey, it was possible to define additional regions if required for stock composition analysis. For example, as there has been complete baseline coverage of sockeye salmon in the Fraser River drainage, with the exception of the early Stuart complex of populations, it is possible to provide reliable estimates of stock compositions for a population complex in local geographic areas within the drainage, or indeed specific populations in the drainage. It was this ability to identify individual populations in the Fraser River drainage that led to the in-season applications in 2001 at the request of the Pacific Salmon Commission. Similarly, in the Nass River and Skeena River drainages in northern British Columbia,

estimates of the contributions of individual populations are possible (except for Babine Lake complex of populations in the Skeena River drainage), given the coverage of the baseline populations and the level of population differentiation. In the central coastal region of British Columbia, populations from Owikeno Lake in Rivers Inlet are distinct, as are sockeye salmon from Long Lake in Smith Inlet. Similarly, sockeye salmon populations from Karluk Lake and Frazer Lake on Kodiak Island are well defined from each other and from populations in other regions. In the Kvichak River in Bristol Bay, Iliamna Lake populations are well defined from those in Lake Clark. In specific circumstances, it is possible to provide estimates of stock composition for a specific population or a specific group of populations in marine fishery samples. For the purposes of the current analysis, we assumed that drainage-specific estimates of stock composition were sufficient for major river drainages.

Application of microsatellite and MHC variation clearly has the potential to provide reliable estimates of stock composition for a local group of sockeye salmon even when there is a potential of a Pacific Rim distribution of populations contributing to the fishery sample. However, there are limitations in the current 188-population baseline with respect to applications requiring a Pacific Rim distribution of sockeye populations. In Asia, non-Kuril Lake Russian populations will require better representation in the baseline in order to provide reliable estimation for this component. In Alaska, samples from major populations in the Copper River drainage, Prince William Sound, Cook Inlet, as well as additional samples from Bristol Bay, are required to account for populations originating from these areas. Regardless of the current baseline status, the results outlined in the present study are an example of the power of microsatellite and MHC variation that will likely be applied to an increasing number of species and fisheries for which the management concerns of identifying population structure and detecting specific populations or stocks in mixed-stock fisheries arise.

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Table 1. Regions and number of populations sampled within regions included in a survey of 14 microsatellite and one MHC loci in sockeye salmon, and estimated percentage composition of simulated mixtures of sockeye salmon, with each mixture composed of 100% of fish from that region and estimated with a 188-population baseline. Each mixture of 150 fish was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each baseline population with replacement to obtain a new distribution of allele frequencies. Standard deviation is in parentheses.

| Region              | Number of populations | Estimated % |
|---------------------|-----------------------|-------------|
| Columbia River      | 2                     | 92.7 (2.7)  |
| Washington          | 3                     | 90.5 (3.5)  |
| Fraser River        | 38                    | 99.8 (0.4)  |
| Vancouver Island    | 5                     | 95.8 (1.9)  |
| Central Coast       | 24                    | 82.0 (4.3)  |
| Nass River          | 8                     | 93.3 (2.3)  |
| Skeena River        | 21                    | 94.7 (2.0)  |
| Queen Charlotte Is. | 4                     | 91.2 (2.8)  |
| Unuk River          | 1                     | 76.2 (6.9)  |
| Stikine River       | 9                     | 93.5 (2.8)  |
| Taku River          | 10                    | 83.3 (3.9)  |
| Alsek River         | 3                     | 98.9 (1.1)  |
| Southeast Alaska    | 15                    | 98.0 (1.2)  |
| Kodiak Island       | 13                    | 95.1 (2.2)  |
| Bristol Bay         | 9                     | 91.5 (2.7)  |
| Alaska Peninsula    | 2                     | 87.5 (3.6)  |
| Kuril Lake          | 9                     | 91.1 (3.0)  |
| Russia              | 9                     | 49.5 (7.4)  |
| Japan               | 3                     | 91.9 (2.6)  |

Table 2. Estimated percentage stock compositions of pure samples of Fraser River and Skeena River sockeye salmon obtained from fisheries within each river system in 2001 and estimated with a 188-population baseline incorporating variation at 14 microsatellite and one MHC loci. N is the number of fish sampled in each fishery. Standard deviation is in parentheses.

| Region       | N    | Estimated %       |
|--------------|------|-------------------|
| Fraser River | 1649 | 98.8 (0.3) Fraser |
| Skeena River | 192  | 96.4 (1.9) Skeena |

Table 3. Estimated percentage stock compositions of simulated mixtures of sockeye salmon as may be encountered in samples from the high seas. Each mixture of 150 fish was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each of the 188 baseline populations with replacement to obtain a new distribution of allele frequencies. Standard deviation is in parentheses.

| Region              | Mixture 1 |            | Mixture 2 |            | Mixture 3 |            |
|---------------------|-----------|------------|-----------|------------|-----------|------------|
|                     | Actual    | Estimated  | Actual    | Estimated  | Actual    | Estimated  |
| Columbia River      | 5         | 4.4 (1.7)  |           | 0.0 (0.1)  |           | 0.0 (0.1)  |
| Washington          | 20        | 18.1 (3.1) |           | 0.0 (0.2)  |           | 0.1 (0.2)  |
| Fraser River        | 50        | 51.3 (4.2) | 10        | 10.7 (2.5) |           | 0.9 (0.9)  |
| Vancouver Island    | 10        | 9.9 (2.4)  |           | 0.1 (0.2)  |           | 0.1 (0.2)  |
| Central Coast       | 10        | 7.2 (2.3)  | 5         | 5.2 (1.8)  |           | 1.5 (1.0)  |
| Nass River          |           | 0.2 (0.3)  | 20        | 17.6 (3.4) |           | 0.1 (0.3)  |
| Skeena River        | 5         | 4.9 (1.9)  | 30        | 29.0 (3.3) |           | 0.1 (0.2)  |
| Queen Charlotte Is. |           | 0.0 (0.1)  | 10        | 8.7 (2.3)  |           | 0.0 (0.1)  |
| Unuk River          |           | 0.0 (0.1)  |           | 0.0 (0.0)  |           | 0.0 (0.1)  |
| Stikine River       |           | 1.9 (1.5)  |           | 3.1 (1.9)  |           | 0.7 (0.9)  |
| Taku River          |           | 0.2 (0.3)  |           | 0.4 (0.7)  |           | 0.2 (0.3)  |
| Alsek River         |           | 0.0 (0.1)  |           | 0.0 (0.0)  |           | 0.1 (0.3)  |
| Southeast Alaska    |           | 1.7 (1.2)  | 25        | 24.7 (3.3) |           | 1.8 (2.1)  |
| Kodiak Island       |           | 0.2 (0.4)  |           | 0.3 (0.5)  | 20        | 22.4 (3.8) |
| Bristol Bay         |           | 0.0 (0.1)  |           | 0.0 (0.0)  | 50        | 47.2 (4.1) |
| Alaska Peninsula    |           | 0.0 (0.0)  |           | 0.0 (0.0)  |           | 4.9 (2.4)  |
| Kuril Lake          |           | 0.0 (0.1)  |           | 0.0 (0.1)  | 15        | 14.9 (3.1) |
| Russia              |           | 0.0 (0.1)  |           | 0.0 (0.1)  | 10        | 1.0 (0.9)  |
| Japan               |           | 0.0 (0.0)  |           | 0.0 (0.0)  | 5         | 4.1 (1.8)  |

Table 4. Estimated percentage stock compositions of sockeye salmon from a test fishery in the Strait of Juan de Fuca in southern British Columbia (Area 20, July 10, 2001), a 1998 juvenile sample from the northern coast region of British Columbia (Queen Charlotte Sound, Dixon Entrance, Southeast Alaska ), and a test fishery in the north coast region of British Columbia (Area 3, June 19, 2001). Stock compositions were estimated with a 188-population baseline. Standard deviation was estimated from 100 bootstrap resamplings of both the baseline and mixtures.

| Region              | Southern Coast | Northern Coast (juvenile) | Northern Coast (adult) |
|---------------------|----------------|---------------------------|------------------------|
| Sample size         | 190            | 337                       | 170                    |
| Columbia River      | 0.0 (0.0)      | 1.2 (0.5)                 | 0.0 (0.0)              |
| Washington          | 5.4 (1.7)      | 2.6 (1.0)                 | 0.0 (0.0)              |
| Fraser River        | 91.4 (2.1)     | 33.0 (2.9)                | 0.0 (0.0)              |
| Vancouver Island    | 0.0 (0.4)      | 8.0 (1.6)                 | 0.0 (0.1)              |
| Central Coast       | 1.7 (1.2)      | 0.8 (0.8)                 | 0.6 (0.7)              |
| Nass River          | 0.0 (0.0)      | 7.7 (1.6)                 | 91.8 (2.8)             |
| Skeena River        | 0.6 (0.6)      | 37.3 (2.7)                | 4.9 (2.0)              |
| Queen Charlotte Is. | 0.0 (0.0)      | 0.0 (0.0)                 | 0.0 (0.0)              |
| Unuk River          | 0.0 (0.0)      | 0.0 (0.0)                 | 0.0 (0.1)              |
| Stikine River       | 0.0 (0.4)      | 2.5 (1.2)                 | 1.6 (1.8)              |
| Taku River          | 0.5 (0.5)      | 0.7 (0.5)                 | 0.6 (0.7)              |
| Alek River          | 0.0 (0.0)      | 0.0 (0.3)                 | 0.0 (0.1)              |
| Southeast Alaska    | 0.4 (1.2)      | 5.9 (1.5)                 | 0.6 (0.7)              |
| Kodiak Island       | 0.0 (0.2)      | 0.0 (0.3)                 | 0.0 (0.6)              |
| Bristol Bay         | 0.0 (0.3)      | 0.0 (0.0)                 | 0.0 (0.0)              |
| Alaska Peninsula    | 0.0 (0.3)      | 0.0 (0.1)                 | 0.0 (0.3)              |
| Kuril Lake          | 0.0 (0.0)      | 0.0 (0.0)                 | 0.0 (0.0)              |
| Russia              | 0.0 (0.1)      | 0.3 (0.2)                 | 0.0 (0.0)              |
| Japan               | 0.0 (0.0)      | 0.0 (0.0)                 | 0.0 (0.0)              |