PACIFIC SALMON COMMISSION
NORTHERN BOUNDARY TECHNICAL COMMITTEE

REPORT TCNB (05)-2

STOCK COMPOSITION ESTIMATES AND INDIVIDUAL STOCK ASSIGNMENTS BASED ON GENETIC MICROSATELLITESAND SCALE PATTERNS FOR TEST MIXTURES OF ALASKAN AND CANADIAN SOCKEYE SALMON

June 2005

ACRONYMS

ADFG	Alaska Department of Fish and Game
CWT	Coded-wire tag
DFO	Department of Fish and Oceans, Canada
DGGE	Denaturing Gradient Gel Electrophoresis
GSI	Genetic Stock Identification
HWLE	Hardy-Weinberg and linkage equilibrium
LDF	Linear Discriminant Functions
MAP	Maximum *a posteriori*
MCMC	Markov chain Monte Carlo
MEF	Mid-eye to Fork-of-tail Length
MSA	Mixed Stock Analysis
NBA	Northern Boundary Area
POH	Post-orbit to Hypural plate Length
SF	Tip-of-snout to Fork-of-Tail Length
SPA	Scale Pattern Analysis
SPAM	Statistical Package for the Analysis of Mixtures
SSRAA	Southern Southeast Regional Aquaculture Association
UCONLDA	Unconditional Linear Discriminant Analysis

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

This study compares the merits and compatibility of two methods currently used to estimate US and Canadian contributions of sockeye salmon to commercial fishery catches in the Pacific Salmon Treaty Northern Boundary Area (NBA). Scale pattern analysis (SPA) has been used since 1982 to estimate contributions in SE Alaskan fishery catches, and provided the run reconstruction baseline information to establish certain sharing agreements of the Pacific Salmon Treaty. Genetic stock identification (GSI) techniques based on DNA microsatellites have been used in recent years to estimate stock contributions in Canadian fisheries. Use of GSI is anticipated to increase in the future for estimating contributions to other Pacific Salmon Treaty fisheries, and several recently approved projects are actively working toward this.

A blind test mixture composed of results from matched samples collected in 2002 from sockeye salmon stocks known to be important contributors to NBA fisheries (including the Alaskan fishery at Tree Point), was used to compare accuracy of the two methods in this study. The stock composition of the blind test mixture was withheld from the scale pattern and the genetic analysts until their determinations were received.

GSI and SPA techniques displayed similar levels of accuracy in correctly estimating stock composition proportions of test mixtures, as well as in correctly classifying stock of origin for individual samples, although GSI generally performed better in this regard. For the short term, collection of scales for age and SPA should be continued until genetic baselines are adequately comprehensive, stable long term funding is sufficient to provide representative genetic sampling in mixed stock fisheries, and genetic sample processing capacity is adequate to handle the necessary sample sizes. In addition, paired sampling of genetic samples and scales in mixed stock fisheries should continue until comparative performance between the two techniques is adequately known to evaluate historic stock identification results based on SPA against future results based on genetic variation.

ABSTRACT

Matched genetic tissue and scale samples, from sockeye salmon *Oncorhynchus nerka* of known origin, were analyzed in a blind test to compare the accuracy of DNA and scale pattern stock identification analyses. Test mixtures of samples taken in 2002 from sockeye salmon of three geographic stock groups—Nass River, Skeena River, and southern Southeast Alaska—of the Northern Boundary Area between Canada and Alaska were classified to origin using scale patterns and DNA microsatellites. Estimated stock group proportions and probabilities of stock group membership for each individual fish of the test mixtures from scales and microsatellites were compared to evaluate effectiveness and compatibility of the two methodologies. Estimated stock group proportions from the two characters were quite similar for all age groups, despite differences caused by a wider range of stocks allowed by the genetic baseline than allowed by the scales baseline. Individual assignments to stock group by scales and microsatellites agreed and were correct for over 70% of test mixture samples, and only 3% were incorrectly assigned by both characters. When assignments disagreed, those from microsatellites were more often correct.

KEY WORDS: sockeye salmon, *Oncorhynchus nerka*, stock composition, linear discriminant function, scale pattern analysis, microsatellite DNA, Southeast Alaska, Canada, Northern Boundary Area

INTRODUCTION

Commercial net fisheries in the US/Canada Pacific Salmon Treaty Northern Boundary Area (NBA) (Figure 1) harvest mixed stocks of sockeye salmon *Oncorhynchus nerka* that originate from lakes, rivers, and streams in both Southeast Alaska and northern British Columbia (Figure 2) (English et al. 1984; Gazey et al. 1983; Rich and Morton 1930; Verhoeven 1952; Norenberg 1959; Logan 1967; Simpson 1968; Hoffman et al. 1983; Pella et al. 1993). The substantial total numbers of Alaska sockeye salmon contributed to NBA fisheries originate in numerous, comparatively small, individual populations of relatively low or moderately productive systems in the immediate vicinity (Figure 3). The greater total numbers of Canadian sockeye salmon in NBA fisheries originate principally from a few larger populations of the Nass River and Skeena River, which run entirely within British Columbia and flow into Portland Inlet and Chatham Sound, respectively, just south of the Alaska border (Figure 4). In both these Canadian river systems, a number of distinct spawning populations are present, and reliable escapement counts for the major population of each system have been available since at least 1950 for the Skeena River and 1990 for the Nass River. In the Nass River, sockeye salmon returning to Meziadin Lake comprise 50% to 90% of drainage escapement (Rutherford et al. 1994; Beacham and Wood 1999), with escapement thought to be reliably determined at a fishway at Victoria Falls near the outlet of the lake. In the Skeena River, sockeye salmon returning to Babine Lake comprise 80% to 95% of drainage production (Larkin and McDonald 1968; West and Mason 1987; Beacham et al. 2000), with Babine Lake escapement estimated from a counting fence near the lake outlet.

Under the Pacific Salmon Treaty of 1985 and its later annexes, catches by fishermen of either country of their neighbor's stocks are restricted in selected fisheries. In particular, the catch of Nass and Skeena sockeye salmon in Alaska's District 101 gillnet and District 104 purse seine fisheries are limited, over a ten year period, to a percentage of the total return of these stocks. Annual stock-specific run reconstructions (catch plus escapement) are required in order to accurately estimate the percentage of each stock caught in subject fisheries.

If data-based stock composition estimates from catch sampling for distinguishing characters of individual fish are not available, model-based apportionments, which are necessarily less certain, are derived from assumptions about stock-specific migratory timing, geographic entry patterns, and exploitation rates (Gazey and English 2000, English et al. 2004). In addition to their use for postseason run reconstructions, reliable data-based stock composition estimates obtained during the season could be used for up-to-date run reconstructions and revised forecasts to enable fishery managers to modify harvests to more effectively achieve catch share agreements.

This study directly compares two data-based methods currently used to estimate sockeye salmon stock composition in the NBA. Alaska Department of Fish and Game (ADFG) has used classical discriminant analysis of scale patterns (Bloomquist et al. 2002, Pella and Masuda 2004), or scale pattern analysis (SPA), to estimate the stock composition of sockeye salmon taken in southern Southeast Alaska fisheries since 1982 (Marshall et al. 1984). Recently, Canada Department of Fisheries and Oceans (DFO) has used genetic stock identification (GSI) markers, called DNA

microsatellites, to estimate stock composition in commercial Canadian fisheries of the NBA (Beacham et al. In press). The measurements of the two methods are believed to be essentially independent: SPA is based on persistent differences in growth history among stocks as manifested in scale features (Bloomquist et al. 2002), whereas GSI is based on differences in the relative frequencies of multilocus genotypes among stocks (Fournier et al. 1984, Pella and Masuda 2001, Beacham et al. in prep.). The scale features are visible by simple magnification of an imprint of the scale whereas the genotypes become visible through special biochemical techniques applied to tissues. The scale patterns of individuals are determined more by life history and geographic differences among stocks than by their microsatellite genotypes. GSI is under consideration for use in US fisheries in the NBA to supplement or replace SPA, and the present study is the first to compare the two methods for their merits and compatibility. The comparison is limited to a particular large (N = 483) blind test mixture composed of fish collected in 2002 from stocks that are known to be important contributors to NBA fisheries including the Alaskan fishery at Tree Point. The stock composition of the blind test mixture was withheld from the scale pattern and genetic analysts until their determinations were received.

The primary objective of this study was to provide a direct comparison of SPA and GSI in identifying stock of origin for the test mixture composed of sockeye salmon from southern Southeast Alaska, the Nass River, and the Skeena River. Paired scale and genetic measurements taken from the same individual fish were used. The outcome of this experiment will be used to evaluate efficacy of the two methods, as well as to provide information useful to managers in deciding appropriate techniques for inseason assessments. A secondary objective was to use the resulting information to adjust sample collection designs for either technique to most appropriately, accurately, and precisely characterize sampled fisheries. Extrapolation of comparison results to actual sampling of NBA fisheries needs also to consider the cost and sample coverage possible under each method.

METHODS

Biological Data Collection and Processing

ADFG Commercial Fisheries Division personnel collected matched samples of scales and tissues from up to 40 sockeye salmon at each of 20 escapement locations in southern Southeast Alaska. Samples were obtained at or near terminal spawning grounds at each location during one-time annual trips to collect SPA baseline samples. In northern British Columbia, DFO personnel collected matched scales and tissues from daily gillnet catches in test fisheries operating near or in the lower reaches of the Skeena River. LGL Ltd. personnel, under contract to the Nisga'a First Nation in British Columbia, collected matched scales and tissues from daily fishwheel catches in a test fishery in the lower Nass River. All samples were collected during the summer and fall of 2002.

Scales were sampled from the preferred area above the lateral line on the left side of the fish on a diagonal downward from the posterior insertion of the dorsal fin to the anterior insertion of the anal fin (INPFC 1963). Scales were mounted on gum cards and impressions made in cellulose acetate (Clutter and Whitesel 1956). For Southeast Alaska, up to 4 scales per fish were routinely collected to improve the likelihood of collecting readable scales. Canadian samples were generally collected at 2 scales per fish. Age determinations were based on examinations of scales under moderate (70x) magnification. Criteria used to assign ages were similar to those of Mosher (1968), and ages were reported in European notation (Koo 1962).

In addition, sex and mid-eye to fork-of-tail (MEF) length were recorded for each fish sampled in Southeast Alaska. Sex and post-orbit to hypural plate (POH) length were recorded for fish sampled from Skeena River test fisheries. Tip-of-snout to fork-of-tail (SF) length was recorded for Nass River fishwheel samples, but sex could not be determined due to their bright condition.

Tissues for genetic analysis consisted of two opercle punches preserved in 100% ethanol or reagent alcohol in individually numbered vials.

Test Mixture and Baseline Data Set Construction

A total of 2,834 matched genetic tissue and scale samples were collected in 2002 from the Nass River (n=940), Skeena River (n=1,507), and from 20 distinct spawning locations in Southeast Alaska (n=387). Gum cards with scales from all sampling locations were submitted to the ADFG Douglas scale laboratory for acetate pressing and age determination. During age determination, scale readers identified specific scales that were suitable for digitizing and use in a test mixture from among the total available for each fish. Only fish with scales suitable for digitizing from among the four main age classes used in SPA studies (1.2, 1.3, 2.2, and 2.3) were considered for inclusion in the test mixture. After assigning ages, gum cards and biological data were forwarded to Ketchikan for assembly of the test mixture and SPA baseline.

Age summaries for each stock group were examined in Ketchikan to determine sample sizes for the test mixture and SPA baseline. The SPA baseline was composed of Nass River and Skeena River fish of the matched samples not assigned to the test mixture, and of Southeast Alaska fish from regular annual SPA baseline sampling. Assignment of Nass River and Skeena River fish to the test mixture or baseline was random, and no samples were used in both. Because the number of matched samples for the Nass River and Skeena River stocks was relatively large, samples from these two locations for the test mixture and baseline were selected from among all samples available for the year. For each of these two stocks, assignment criteria were as follows: 1) if an age group had more than 280 usable fish, a maximum of 80 was used for the test mixture and a maximum of 200 used for the baseline; 2) if an age group had between 160 and 280 usable fish, a maximum of 80 fish was used for the test mixture and all remaining samples were reserved for the baseline; and 3) if an age group contained less than 160 usable fish, half were used for the test mixture and half were reserved for the baseline. The genetic baseline was collected separately from the matched samples and is described later.

Matched samples from Southeast Alaska locations were collected separately from regular SPA baseline samples. Samples for the test mixture were selected from among all available matched samples without regard to specific location of collection. For the Southeast Alaska stock group, if an age group had more than 80 usable fish, a maximum of 80 fish was used for the test mixture. If an age group had less than 80 usable fish, all available matched samples were used for the test mixture. Unknown to scale and genetics laboratory personnel, the test mixture included 32 sockeye salmon from 4 Southeast Alaska locations that were not included in the SPA or genetic baselines.

Each fish assigned to the test mixture was given a new unique random specimen ID number to hide possible information of its source. Scales of each specimen were assigned a new sequential scale gum card number and scale mounting positions on the card that disregarded stock group or age. Because most Canadian scale collections included only two scales per fish, but Alaska collections included up to 4 scales per fish, the test mixture was limited to two scales per fish to hide information about fish origins from scale readers. Biological and sample identification data were recorded in a hidden master spreadsheet containing the originating stock group, length, sex, tissue sample vial numbers, original scale card and scale specimen numbers, and remounted scale card and scale specimen numbers. MEF length measurements for all Southeast Alaska samples were left unchanged. Length measurements for Nass River (SF) and Skeena River (POH) were converted to MEF length using standard linear regression relationships (Pahlke 1988) to hide information about stock origin from the scale readers.

Scales were remounted in the newly assigned order and sent back to the Douglas scale laboratory for SPA analysis. A corresponding summary spreadsheet including only the remounted scale card and scale position numbers, GSI sample number, estimated or actual MEF length, and age was forwarded to the Douglas scale laboratory. The scale laboratory supervisor was informed which samples from the original Nass River and Skeena River acetate impressions may have been included in the test mixture (not all were) to exclude them from regular baseline analysis. Because scale readers were unaware of recorded sample sequence or true number of excluded samples actually present in the test mixture, this knowledge was presumed not to be helpful to identify stock of origin in the test mixture.

All genetic tissue samples were sent to Ketchikan for selection of the test mixture. Because dissimilar tissue vials were used at various US and Canadian sampling locations, all tissues used for the test mixture were transferred to new vials of the same type to eliminate possible information about stock group from vial type. Test mixture vials were renumbered and sent to Nanaimo for genetic analysis, and the remaining Nass River and Skeena River tissue samples were returned to Prince Rupert. A copy of summary spreadsheet sent to the Douglas scale laboratory was also forwarded to the genetics laboratory at the DFO Pacific Biological Station in Nanaimo, B.C.

Statistical Estimation of Stock Composition of Test Mixtures and Source Assignment of Individuals

Although the measurements of scale patterns and DNA microsatellites are quite different in kind, the basic statistical mixture model describing the probability of measurements from individuals of test mixtures is the same. If *c* baseline stock groups (scale patterns) or stocks (DNA microsatellites) are considered possible in the test mixture, the probability that the *M* individuals of the test mixture have measurements, $\mathbf{X}_1,\ldots,\mathbf{X}_M$ is

$$p(\mathbf{X}_1,\ldots,\mathbf{X}_M) \propto \prod_{j=1}^{M}\left(\sum_{i=1}^{c} p_i f(\mathbf{X}_j \mid i)\right).$$

The measurements of the j-th individual, $\mathbf{X}_j$, are either those from its scales or else counts of its alleles at the microsatellite loci. The unknown stock group proportions composing the test mixture are denoted by $p_i,\ \ i = 1,...,c$, and the density or relative frequency of the *j*-th individual's measurements in the *i*-th stock group, or stock, is denoted by $f(\mathbf{X}_j \mid i)$. The function, $f(\mathbf{X}_j \mid i)$, differs between scale patterns and genetic measurements: the scale measurements are here assumed to have the multivariate normal distribution, whereas the genetic measurements define discrete genotypes whose relative frequencies are described by a probability model that assumes Hardy-Weinberg equilibrium at each locus and linkage equilibrium among loci (Pella and Masuda 2001). The number of baseline stock groups for scale patterns is $c = 3$, whereas the number of baseline stocks for DNA microsatellites is $c = 203$.

The so-called posterior source probabilities for the mixture individuals (Pella and Masuda 2004) are fundamental to classifying or assigning the individuals to their source stock groups (scales) or stocks (DNA microsatellites). The posterior source probabilities for the *j*-th individual are

$$p(i \mid \mathbf{X}_j) = \frac{p_i f(\mathbf{X}_j \mid i)}{\sum_{s=1}^{c} p_s f(\mathbf{X}_j \mid s)}, \qquad i = 1,...,c.$$

The test mixture individuals are viewed as drawn from a large underlying mixture, and the meaning of these posterior source probabilities is that they are the proportions of individuals in the underlying mixture with the same measurement as the *j*-th individual, $\mathbf{X}_j$, that come from each of the *c* stocks or stock groups. Given the estimated posterior source probabilities, the maximum *a posteriori* or MAP rule is used later in which individuals are assigned to the source for which their posterior source probability is greatest. Of course, these posterior source probabilities are unknown and must be estimated using methods specific to the two kinds of measurements. The posterior source probabilities of the test mixture individuals that are computed for scale pattern and genetic measurements are useful for understanding estimation and assignment discrepancies between the measurements.

In comparing stock composition and individual assignments by scale patterns and DNA microsatellites, the 203 baseline stocks of DNA microsatellites are grouped into the 3 stock groups of the scale patterns and an extra stock group composed of stocks outside the scale baseline. Composition estimates and individual assignments from DNA microsatellites are summed for

stocks within these stock groups. The two kinds of measurements and their unique modeling are described next.

Scale Pattern Analysis

Digitizing of Scales

Scale circuli were counted and incremental distances between circuli measured according to zones that represent distinct salmon life history stages (Figure 5). Scale impressions were projected onto a digitizing tablet at 100x magnification using equipment similar to that described by Ryan and Christie (1976). Counts and measurements were made on a selected radius along or near the longest axis of the scale (Anas and Murai 1969). This longest axis is roughly perpendicular to the dorsal transition zone between anterior and posterior portions of the scale and/or at 20 degrees from the dorsal line of circuli breakage in the anterior portion. Measurements of distances between circuli and growth zone information for each scale were transformed to a set of 33 standardized measurement and count characteristics (Bloomquist et al. 2002).

Data Analysis

ADFG has used linear discriminant analysis (LDA) and classification-based conditional stock composition estimation (Pella and Masuda 2004) in NBA fisheries since sampling began in 1982 (Bloomquist et al. 2002). Underlying LDA is the assumption that the scale pattern measurements have the multivariate normal distribution with different means among stock groups but a shared covariance matrix. The computer programs by which the ADFG analyses are performed are described elsewhere (Bloomquist et al. in prep). The ADFG computer program that computes the posterior source probabilities uses equal values for the unknown stock group proportions of the mixture, i.e., $p_1 = p_2 = p_3 = 1/3$. In addition, estimated proportions can also be used in place of the assumed equal values. Therefore, one of the methods of estimation for SPA described by Pella and Masuda (2004), called the direct unconditional Bayesian method for the multivariate normal distribution, is also applied to the test mixtures using their computer program UCONLDA. The ADFG programs include subset selection of variables for effective discrimination, but UCONLDA does not include this feature. Therefore, UCONLDA was applied with the same variables found effective by the ADFG programs. We will refer to the estimation methods by their acronyms: ADFG and UCONLDA methods. Age-specific models are used to estimate the stock group proportions and the posterior source probabilities of the test individuals in order to assign them to their stock group. Therefore, the test mixture is effectively composed of 4 age-specific test mixtures analyzed using SPA.

ADFG Method

Models were assembled using scales of the baseline samples from the portions of Nass River and Skeena River collections not selected for the test mixture, and scales from Southeast Alaska collected during regular annual baseline sampling. These LDF models, which are based on the

multivariate normal distribution for the scale measurements, **X**, are used to estimate the relative frequencies of measurements in the source stock groups, $f(\mathbf{X} \mid i), \quad i = 1,2,3$. These were the same models used to estimate stock contributions to commercial catch in standard annual studies (Bloomquist et al. in prep). Development of age-specific LDF baseline models involved several steps. Stepwise discriminant analysis of the baseline samples was used to select discrete scale variables with the greatest potential for discriminating among stock groups. Discriminant analysis of the pooled baseline samples was then performed iteratively by variable. Up to 14 of the initially selected variables were entered sequentially into the LDF model, or until the partial F-statistic of a variable available for entry into the model was less than 4.0. Successive classification accuracies were plotted against the respective variables. Variables were included in the model until accuracy peaked or became asymptotic for up to a total of twelve variables.

Although MEF length in age-specific LDF models in prior years has often improved discrimination between Nass River and Skeena River stock groups, MEF length was excluded from final models used to classify scales in the test mixture for several reasons. Nass River and Skeena River samples were missing MEF lengths, so approximations from other available length measurements would have been needed. However, length of Pacific salmon is known to be sexually dimorphic, and the length difference increases with advancing sexual maturity. Therefore, the missing gender information for Nass River samples would have introduced greater uncertainty in their estimated MEF lengths than occurred for the other stock groups. Finally, baseline samples from the Southeast Alaskan escapements were collected at or near the spawning grounds, and accuracy of MEF length measurements could be affected by erosion of caudal fin margins during spawning. On the other hand, samples from Canadian test fisheries, collected during upstream migration, were presumably less affected.

Scales in the age-specific test mixtures were classified to stock group of origin by the MAP rule using LDF models. An almost unbiased estimate of classification accuracy for each LDF model was determined with a cross validation procedure similar to leaving-one-out applied to the baseline samples (Lachenbruch 1967). Estimates of proportions of each stock group in an age-specific test mixture were computed by classification-based conditional maximum likelihood estimation assuming multivariate normality (see pp. 532-536 of Pella and Masuda 2004). Age-specific sets of scale variables were submitted to LDF procedures of the statistical program SAS® to compute the posterior source probabilities for individuals in the age-specific test mixtures.

UCONLDA Method

The UCONLDA method is Bayesian and generates a probability distribution of the age-specific model unknowns. The model unknowns are the stock group proportions and the means and covariance matrix of the measurement distributions in the sources. The distribution is called the Bayesian posterior distribution of the unknowns. Some care is needed in order to not confuse the name with that of the posterior source probabilities of mixture individuals: the posterior source probabilities are functions of the unknowns whereas the Bayesian posterior distribution describes the uncertainty in the unknowns. The algorithm is a Markov chain Monte Carlo (MCMC) method in which a sequence of draws is made from distributions for the unknowns. At each full cycle of draws, each mixture individual is randomly assigned to one of the sources with probabilities equal to the posterior source probabilities given the current draw of the unknowns. After the mixture

individuals are assigned to the sources, the distributions of the unknowns are updated with their measurements added to the appropriate original baseline sample and draws of the unknowns are repeated. A total of 50,000 cycles were computed with the initial 25,000 discarded as burn-in. In contrast to the ADFG method that uses only the baseline samples to estimate the measurement distributions in the sources, the UCONLDA method includes any available information in the mixture sample as well. The algorithm is provided in complete detail in section 2 of the appendix in Pella and Masuda (2004).

Genetic Stock Identification and Mixture Analysis

DNA was extracted from preserved tissue 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* (Beacham et al. 1998; Nelson and Beacham 1999), *Oki1a, Oki1b, Oki6, Oki10, Oki16*, and *Oki29* (Smith et al. 1998 and unpub.), *One8* (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. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA). Genetic variation at the MHC class II *DAB-β1* locus (Miller et al. 2001) was surveyed by denaturing gradient gel electrophoresis (DGGE). β1 alleles were separated by DGGE with the Bio-Rad (Hercules, CA) D Gene™ or D Code™ 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 and analysis of fishery samples.

The baseline used for analysis of the test mixture consisted of 203 populations ranging from southeast Alaska to the Columbia River, and was surveyed for variation at 14 microsatellite loci and one MHC locus (Beacham et al. In press). This extensive baseline included many populations outside the 55 populations generally believed likely to contribute to NBA mixed stock fisheries, but did not include four Southeast Alaska populations included in the test mixture.

Genotypic frequencies were determined at each locus in each population and the statistical package for the analysis of mixtures software program (SPAM version 3.7) (Debevec et al. 2000) was used to estimate stock composition of the test mixture. All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies.

Posterior distribution for individual unknowns from allele relative frequencies of each locus in the baseline stocks was performed by Bayesian methods with the computer program BAYES (Pella and Masuda 2001). At each cycle of MCMC sampling, the posterior source probabilities of each mixture individual are computed from the draws of the unknowns and the individual is randomly assigned to one of the baseline stocks with assignment probabilities equal to the posterior source probabilities. Four MCMC chains of 20,000 samples were drawn and an initial burn-in of 15,000 samples from each chain was discarded. The long run proportions of each individual's assignments to the various baseline stocks equals their average posterior source probabilities generated under

the uncertainty in allele relative frequencies and test mixture stock proportions. Just as for scales, the MAP principle of assignment was applied to the long run assignment proportions from microsatellites to decide the stock group sources of the test mixture individuals.

RESULTS

A total of 626 fish was originally selected for mixture analysis (Table 1). Of the 626 matched samples, 79 were excluded from classification, because tissue samples were either unsuitable for lab analysis, or because vial numbers could not be unambiguously determined due to leakage of preservative during shipment of one sample batch. Another 55 samples were excluded from mixture classification, because scale impressions were not suitable for measurement, or were originally assigned improper ages. Nine samples were unsuitable for classification using either method (Table 2). SPA baseline samples included Nass River and Skeena River samples not assigned to the test mixture, and Southeast Alaska samples from regular annual SPA baseline sampling (Table 3).

Table 1. Total sample size of the test mixture by stock and age group.

Stock Group	Age Group				Grand Total
	1.2	1.3	2.2	2.3	
Nass River	80	50	80	16	226
Skeena River	80	80	20	15	195
SE Alaska	80	70	31	24	205
Grand Total	240	200	131	55	626

Table 2. Effective sample size of the test mixture by stock and age group for which both GSI and SPA results were available.

Stock Group	Age Group				Grand Total
	1.2	1.3	2.2	2.3	
Nass River	66	43	69	11	189
Skeena River	63	63	18	12	156
SE Alaska	43	60	24	11	138
Grand Total	172	166	111	34	483

Table 3. Total baseline sample size by stock and age group of scale pattern analysis used to classify the test mixture.

Stock Group	Age Group				Grand Total
	1.2	1.3	2.2	2.3	
Nass River	199	94	200	14	507
Skeena River	199	200	32	15	446
SE Alaska	200	200	148	122	670
Grand Total	598	494	380	151	1,623

Scale Pattern Analysis

The stock group composition estimates by the ADFG and UCONLDA methods are provided (Tables 4-5). Point estimates for the ADFG method (Table 4) were reasonably accurate for age 1.2, 1.3, and 2.2, differing from actual proportions by a maximum of 7.7% for age 1.3 from the Skeena River. The UCONLDA posterior means for stock group proportions (Table 5) were remarkably accurate for these age groups. The greatest absolute discrepancies between actual and posterior mean of estimated test mixture stock group percentages by age group were 0.4%, 2.7%, and 2.6% for age group 1.2, 1.3, and 2.2, respectively.

The ADFG method underestimated stock group proportions for age 2.3 from the Nass River by 10.7%, and UCONLDA underestimated the same stock by 17.9%. Stock proportions were overestimated for Southeast Alaska by more than 22% for both methods. The small sample size of 34 fish for the test mixture and total baseline of only 151 fish (14 Nass River, 15 Skeena River, and 122 Southeast Alaska individuals) for age 2.3 (Table 3) resulted in high uncertainty (SE in stock group composition between 11.2% and 17.6% for ADFG method, and SD in stock group composition between 11.2% and 14.9% for UCONLDA) in the test mixture composition. In every case, the 95% probability interval covered the true composition of the test mixtures for both methods.

Table 4. Point estimates, standard errors, and lower and upper 95% confidence bounds for the unknown source proportions (%) in the test mixtures for the 4 age groups. Point estimates were computed by the ADFG method and confidence intervals by bootstrap resampling.

Age Group	Stock Group	True	Point Estimate	SE	Confidence Interval Lower 2.5%	Confidence Interval Upper 97.5%
1.2	Nass River	38.4	37.3	5.4	24.3	50.2
	Skeena River	36.6	37.8	4.7	26.4	49.3
	SE Alaska	25.0	24.9	4.6	13.8	36.0
1.3	Nass River	25.9	30.5	7.3	13.0	48.1
	Skeena River	38.0	30.3	6.6	14.5	46.0
	SE Alaska	36.1	39.2	5.2	26.8	51.5
2.2	Nass River	62.2	59.4	8.3	39.7	79.2
	Skeena River	16.2	18.6	8.5	0.0	39.0
	SE Alaska	21.6	22.0	4.8	10.4	33.5
2.3	Nass River	32.4	21.7	17.6	0.0	63.7
	Skeena River	35.3	23.6	11.2	0.0	50.4
	SE Alaska	32.4	54.7	15.4	17.9	91.6

Table 5. Means, standard deviations, and quantiles of the marginal posterior density for the unknown source proportions (%) in the test mixtures for the 4 age groups. Parameters were computed by UCONLDA from 25,000 MCMC samples after 25,000 samples were discarded as burn-in.

Age Group	Stock Group	True	Mean	SD	Posterior quantiles 2.5%	Posterior quantiles Median	Posterior quantiles 97.5%
1.2	Nass River	38.4	38.6	6.0	27.1	38.5	50.4
	Skeena River	36.6	36.2	5.0	26.7	36.1	46.2
	SE Alaska	25.0	25.3	4.8	16.2	25.1	35.2
1.3	Nass River	25.9	23.4	9.3	4.3	23.5	41.2
	Skeena River	38.0	37.8	7.0	24.8	37.5	52.0
	SE Alaska	36.1	38.8	5.9	27.5	38.7	50.8
2.2	Nass River	62.2	63.5	5.9	51.3	63.7	74.5
	Skeena River	16.2	13.6	5.1	5.0	13.2	24.9
	SE Alaska	21.6	22.9	4.5	14.7	22.7	32.0
2.3	Nass River	32.4	14.5	14.9	0.0	9.9	51.5
	Skeena River	35.3	30.9	11.2	10.8	30.3	54.5
	SE Alaska	32.4	54.6	14.4	24.2	55.6	79.7

The source identification of individuals in the test mixture is more challenging and of interest here primarily to explain errors in composition estimation and discrepancies between methods. The outcomes for the individuals in the test mixtures by the ADFG and UCONLDA methods are summarized in Tables 6 and 7. Corresponding numbers of fish of combined ages correctly classified by the UCONLDA method were within a few percentage points of the ADFG method (Table 6).

Table 6. Number of Southeast Alaskan, Nass River, and Skeena River sockeye salmon scales of the test mixtures classified to stock group of origin using linear discriminant models, 2002.

ADFG Method

True Stock	Classified Stock						
	Number of Samples				Percent		
	Nass River	Skeena River	SE Alaska	Total	Nass River	Skeena River	SE Alaska
Nass River	**139**	23	27	189	73.5%	12.2%	14.3%
Skeena River	36	**112**	8	156	23.1%	71.8%	5.1%
SE Alaska	14	12	**112**	138	10.1%	8.7%	81.2%
Total	189	147	147	483			

UCONLDA Method

True Stock	Classified Stock						
	Number of Samples				Percent		
	Nass River	Skeena River	SE Alaska	Total	Nass River	Skeena River	SE Alaska
Nass River	**133**	25	31	189	70.4%	13.2%	16.4%
Skeena River	28	**117**	11	156	17.9%	75.0%	7.1%
SE Alaska	11	16	**111**	138	8.0%	11.6%	80.4%
Total	172	158	153	483			

Although individual assignment accuracy of ADFG age-specific models (Table 7) was somewhat variable (ranging from 50.0% correctly classified for Skeena River age 2.2 to 100.0% for Southeast Alaska age 2.3), the average accuracy over stock groups was similar for all age groups (range 71.1-77.8%). Except for age 1.2 (69.8% correctly classified), Southeast Alaska scale samples were correctly classified to group of origin at a higher rate than the Nass River of Skeena stocks (total correct over ages: Southeast Alaska 81.2%, Nass River 72.8%, and Skeena River 72.0%). Corresponding individual assignment accuracy of UCONLDA age-specific models (Table 7) was more variable (ranging from 9.1% to 100%) and average accuracy over stock groups was lower for each age group (range 61.4 – 72.9%). Despite the method differences

in accuracy for age groups, the overall accuracies for stock groups by summing over age groups were similar (Table 7, "Total" column) and their averages were identical at 75.3%. The greater variation of UCONLDA compared to ADFG is mainly due to the use of estimated (UCONLDA) versus assumed (ADFG) stock group proportions in computing the posterior source probabilities of individuals: the equal stock group proportions for the age-specific mixtures assumed by the ADFG method anchored the assignments better than the estimates used by the UCONLDA method. Had the actual stock group proportions of the age-specific mixtures differed more from equal proportions, the UCONLDA method would be expected to perform better than the ADFG method.

Table 7. Classification success for age groups comprising a test mixture of Southeast Alaskan, Nass River, and Skeena River sockeye salmon scales using the ADFG and UCONLDA method, 2002.

ADFG Method

	Percent of Age Group Correctly Classified				
Stock Group	1.2	1.3	2.2	2.3	Total
Nass River	72.7%	65.1%	80.0%	58.3%	72.8%
Skeena River	79.4%	70.3%	50.0%	75.0%	72.0%
SE Alaska	69.8%	85.0%	83.3%	100.0%	81.2%
Average	74.0%	73.5%	71.1%	77.8%	75.3%

UCONLDA Method

	Percent of Age Group Correctly Classified				
Stock Group	1.2	1.3	2.2	2.3	Total
Nass River	74.2%	41.9%	94.2%	9.1%	70.4%
Skeena River	79.4%	82.5%	33.3%	75.0%	75.0%
SE Alaska	65.1%	88.3%	79.2%	100.0%	80.4%
Average	72.9%	70.9%	68.9%	61.4%	75.3%

Genetic Stock Identification and Mixture Analysis

Estimated stock composition of 526 sockeye salmon in the test mixture, all samples for which GSI results were available, were within 1% of actual contributions of Skeena River and Nass River components, but the Southeast Alaska component was underestimated by about 4% (Figure 6). The Stikine River component was overestimated by about 4%, as there were no Stikine River sockeye salmon in the sample. Some portion of the Southeast Alaska component was allocated to the Stikine River (Figure 6A). 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 32 fish removed (Beacham et al. In press). Estimated stock compositions on a regional basis were within 1% of actual contributions, and for the southeast Alaska component, individual population estimates were generally within 0.5% of actual contributions (Figure 6B). These four 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 accuracy levels outlined in Figure 6B would be obtained.

Individual assignment accuracy of 483 sockeye salmon from the test mixture for which both GSI and SPA results were available, was generally higher for DNA microsatellites (Tables 8 and 9) than for SPA (Tables 6 and 7). The percent correctly classified ranged from 82.6% for Southeast Alaska to 97.4% for Nass River. Similar to the GSI stock composition estimate for all samples in the text mixture, 24 samples were incorrectly assigned to the Stikine River, which was not represented in the test mixture. Of these 24 samples, 16 were actually collected from the 4 locations not included in the genetic baseline. Misclassification of other individual samples was generally very low, although 10.3% of Skeena River fish were incorrectly classified to the Nass River.

Table 8. Classification matrices from DNA microsatellites for the subset of test mixture individuals previously analyzed using scale patterns: numbers and percentages of Southeast Alaskan, Nass River, and Skeena River sockeye salmon classified to stock groups of origin, 2002.

True Stock	Classified Stock								
	Number of Samples					Percent			
	Nass River	Skeena River	SE Alaska	Stikine River	Total	Nass River	Skeena River	SE Alaska	Stikine River
Nass River	**184**	4	1	0	189	97.4%	2.1%	0.5%	0.0%
Skeena River	16	**136**	2	2	156	10.3%	87.2%	1.3%	1.3%
SE Alaska	1	1	**114**	22	138	0.7%	0.7%	82.6%	15.9%
Total	201	141	117	24	483				

Interestingly, the age-specific stock groups with the lowest classification accuracy for SPA, were also least frequently classified correctly using microsatellites (50.0% Skeena River age 2.2, and 74.4% SE Alaska age 1.2). Proportions correctly classified for all other age and stock groups exceeded 80% (range 83.3% to 98.6%).

Table 9. Classification success from DNA microsatellites for the subset of test mixture individuals previously analyzed using scale patterns: percentages of Southeast Alaskan, Nass River, and Skeena River sockeye salmon correctly classified to their stock groups of origin, 2002.

	Percent of Age Group Correctly Classified				
Stock Group	1.2	1.3	2.2	2.3	Total
Nass River	97.0%	97.7%	98.6%	91.7%	97.4%
Skeena River	92.1%	92.2%	50.0%	83.3%	86.6%
SE Alaska	74.4%	86.7%	83.3%	90.9%	82.6%
Average	89.5%	91.6%	87.5%	88.6%	89.7%

Comparison of Classification Methods

Classification of individual fish from populations included the test mixture, but not represented in scale pattern or genetic baselines, demonstrates some potential error that may occur when baselines do not adequately represent populations potentially present in mixed-stock fishery samples. Although direct comparison between the two stock identification techniques are not strictly valid due to fundamental differences in how baselines were applied in the two analyses, the comparison is of interest, because it mimics possible conditions in some real-world mixed-stock fishery situations. Of the 26 fish present in this test mixture from four populations unrepresented in either baseline (Table 10), more were classified correctly to the Southeast Alaska stock group by SPA (61.5%) than microsatellites (23.1%). However, of the 20 fish incorrectly assigned to source stock by microsatellites, 18 were assigned to the Stikine River, a disallowed stock classification for SPA.

Table 10. Number and percent of sockeye salmon extra-baseline samples in the test mixture correctly and incorrectly assigned to stock group of origin by the maximum posterior probability from scale patterns (ADFG method) and genetic microsatellites (program BAYES).

	SPA (ADFG)					
	Number of Samples			Percent		
GSI	Incorrect	Correct	Total	Incorrect	Correct	Total
Incorrect	5	15	20	19.2%	57.7%	76.9%
Correct	5	1	6	19.2%	3.8%	23.1%
Total	10	16	26	38.5%	61.5%	100.0%

Comparison between individual posterior source probabilities computed from scale patterns and microsatellites (Figures 7 – 10) demonstrates the essential independence in information provided by the two kinds of measurements, i.e., no evidence of a relationship between the posterior

source probabilities occurs. Generally speaking, as the posterior source probability increases for either scale patterns or microsatellites, the proportion correctly assigned to stock group increases as expected. For example, for age group 1.2, the percentages correctly assigned by scales ranged from 47.8%, to 62.7%, to 88.8% for the posterior source probability intervals, 0.4 to 0.6, 0.6 to 0.8, and 0.8 to 1.0, respectively. Corresponding percentages for microsatellites are much higher and range from 83.3% to 98.4%.

Although the majority of individual assignments from microsatellites and scale patterns agreed and were correct (333 of 460 or 72%), microsatellites more frequently assigned the individuals to their correct stock group than scale patterns did (99 assignments correct only by microsatellites vs. 14 correct only by scales (Table 11) when the two methods disagreed.

Table 11. Numbers of individuals by age assigned to stock group of origin by the maximum of the average posterior probability from scale patterns (program UCONLDA) and genetic microsatellites (program BAYES).

Age	Both incorrect	Correct		
		Both	Microsatellites only	Scale patterns only
1.2	3	117	38	5
1.3	3	113	38	5
2.2	7	85	11	2
2.3	1	18	12	2
Total	14	333	99	14

DISCUSSION

Stock identification analysis of mixed-stock fisheries requires both representative samples from the fishery and an adequate baseline. The baseline used in estimating stock composition is critical, and must be comprehensive for the estimated stock compositions to be accurate and practically useful. It must encompass all stocks in the fishery samples, and there must be sufficient resolution among stocks or populations. If a wide range of stocks or populations is potentially present in fishery samples, the baseline must, of necessity, be wide-ranging and complex.

Genetic characters such as microsatellites offer some relief in this concern for complete baseline sampling. If the genetic characters are sufficiently informative, they present the potential to detect and estimate contributions of extra-baseline stocks in a mixture. If such contributions appear sufficiently large as to be practically important, a search for the missing baseline populations is necessary in order to sample and include them in the baseline. If the contributions are not important, further baseline sampling is not needed. Smouse et al. (1990) first attempted to accommodate extra-baseline stocks by noting, as had Makela and Richardson (1977) in a related problem, that a mixture of large panmictic populations results in an excess of homozygotes and a deficiency of heterozygotes at any genetic locus. On the other hand, individuals from a single large panmictic population can be expected to meet Hardy-Weinberg and linkage equilibrium (HWLE) conditions. Pritchard et al. (2000) extended this approach by devising a Gibbs sampler called STRUCTURE that partitions a genetic mixture sample into subsets that meet the HWLE conditions. These subsets can be considered as derived from separate populations. The program STRUCTURE can perform this analysis with or without a baseline. If the baseline is incomplete, STRUCTURE may succeed in determining that extra-baseline stocks are present and possibly even allow estimation of their contributions as well. This capacity of genetic data for detection and estimation of extra-baseline contributions is less generally available to scale pattern data.

Accuracy of estimated stock compositions is a key question in application of genetic or scale variation to estimate stock composition of mixed-stock fisheries. One approach to evaluate accuracy and precision of estimated stock compositions is analysis of simulated mixtures and comparison of estimated results with the known composition. While this is an important first step, a key assumption in this method is that results obtained are representative of results when the baseline is applied to estimate the stock composition of a sample of unknown origin. Results will be comparable only if the baseline used to estimate stock composition includes adequate representation from stocks or populations present in the sample. For example, in microsatellite analysis of simulated mixture samples (Beacham et al. In press), the error of the Southeast Alaska component ranged from 1-2%. However, microsatellite analysis of the test mixture of known origin sockeye salmon that included samples from four populations not in the baseline, underestimated the Southeast Alaska component by 4%. Removing fish from these four populations from the sample so that the genetic baseline was completely representative of the southeast Alaska component reduced the error to about 1% for this component, very similar to the simulated mixtures. Although scale patterns correctly classified more of the unrepresented

samples in the test mixture than did microsatellites, stock contributions for Southeast Alaska were overestimated similarly, ranging from 2-4%. It is clear that baselines applied to mixed-stock fishery samples must be comprehensive and representative if management applications require highly accurate stock composition estimates.

DNA variation has provided the opportunity for accurate estimation of stock composition for a range of sockeye salmon fisheries in British Columbia (Beacham et al. 2002, 2004b). Rapid processing of samples during the fishing season could allow fishery managers the flexibility to structure fisheries to achieve the twin management objectives of restricting exploitation on populations of conservation concern while enabling the harvest of abundant populations (Beacham et al. 2004c).

In the current study, DNA and SPA techniques displayed similar levels of accuracy in correctly estimating stock composition proportions of test mixtures, as well as in correctly classifying stock of origin for individual samples, although DNA generally performed better in this regard. Because genetic stock identification techniques do not require annual sampling and analysis of baselines, and the number of discrete stocks that they are capable of resolving is relatively large, DNA based techniques are expected to eventually become the dominant form of stock identification used for Pacific salmon species. Indeed, several projects funded and proposed from a variety of sources since conduct of this study, are specifically aimed at improving accuracy and comparability of genetic stock composition results between laboratories, and genetic sample processing capacity at labs involved in Pacific Salmon Treaty research. For the short term, collection of scales for age and SPA should be continued until genetic baselines are adequately comprehensive, stable long term funding is sufficient to provide representative genetic sampling in mixed stock fisheries, and genetic sample processing capacity is adequate to handle the necessary sample sizes. In addition, paired sampling of genetic samples and scales in mixed stock fisheries should continue until comparative performance between the two techniques is adequately known to evaluate historic stock identification results based on SPA against future results based on DNA variation.

LITERATURE CITED

Anas, R. E., and S. Murai. 1969. Use of scale characters as a disrcriminant function for classifying sockeye salmon (*Oncorhynchus nerka*) by continent of origin. International North Pacific Fisheries Commission, Bulletin 26:157-192.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (Oncorhynchus tshawytscha). Journal of Heredity. 90: 281-288.

Beacham, T. D., and C. C. Wood. 1999. Application of microsatellite DNA variation to estimation of stock composition and escapement of Nass River sockeye salmon (Oncorhynchus nerka). Canadian Journal of Fisheries and Aquatic Sciences 56: 1-14.

Beacham, T. D., L. Margolis, and R. J. Nelson. 1998. A comparison of methods of stock identification for sockeye salmon (Oncorhynchus nerka) in Barkley Sound, British Columbia. North Pacific Anadromous Fish Commission Bulletin 1: 227-239.

Beacham, T. D., and C. E. Withler, and K. M. Miller. 2000. Application of microsatellite DNA variation to estimation of stock composition and escapement of Skeena River sockeye salmon (Oncorhynchus nerka). . North Pacific Anadromous Fish Commission Bulletin 2: 263-276.

Beacham, T. D., J. R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. M. Miller, and R. E. Withler. 2005. DNA-level variation of sockeye salmon (*Oncorhynchus nerka*) in southeast Alaska and the Nass and Skeena rivers, British Columbia, with applications to stock identification. N. Amer. J. Fish. Manage. In press.

Bloomquist, Richard, Anne Reynolds, and Iris S. Frank. 2002. Contribution of Alaskan, Canadian, and transboundary sockeye salmon stocks to catches in Southeast Alaska purse seine and gillnet fisheries, districts 101-108, based on analysis of scale patterns, 1998. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 1J02-40, Juneau.

Clutter R. and L. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Bulletin International Pacific Salmon Fisheries Commission, No. 9.

Cook, R. and G. Lord. 1978. Identification of stocks of Bristol Bay sockeye salmon by evaluating scale patterns with a polynomial discriminant method. U.S. Fish and Wildlife Service, Fisheries Bulletin 76:415-423.

English, K.K., W.J. Gazey, and J.A. Taylor. 1984. Part C. The 1983 North Coast tagging study. In Gazey, W.J., and D.A. Birdsall (editors), Design and execution of a stock interception study. Draft of Unpubl. Rep. by LGL Limited and ESSA Environmental Social Systems Analysts Ltd., for Fisheries and Oceans Canada, 115p., LGL Limited, No. 333, 2453 Beacon Ave., Sidney, B.C., Canada.

English, K.K., W.J. Gazey, D. Peacock and G. Oliver. 2004. Assessment of the Canadian and Alaskan sockeye stocks harvested in the Northern Boundary Fisheries using Run Reconstruction Techniques, 1982-2001. PSC Tech. Rep. No. 13, December 2004.

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400-408.

LITERATURE CITED (continued)

Gazey, W.J., J. Taylor, K.K. English, T. Webb, and D.A. Birdsall. 1983. Part B. 1982 North Coast tagging study. In Gazey, W.J., and D.A. Birdsall (editors), Design and execution of a stock interception study. Unpubl. Rep. by LGL Limited and ESSA Environmental Social Systems Analysts Ltd., for Fisheries and Oceans Canada, 107 p., LGL Limited, No. 333, 2453 Beacon Ave., Sidney, B.C., Canada.

Gazey, W.J., and K.K. English. 2000. Assessment of sockeye and pink salmon stocks in the northern boundary area using run reconstruction techniques, 1982-95. Can. Tech. Rep. Fish. Aquat. Sci. 2320: 132p.

Hoffman, S., L. Tally, and M. C. Seibel. 1983. 1983 chum and sockeye tagging, interception rates, migration patterns, run timing, and stock intermingling research in southern Southeast Alaska and northern British Columbia. Alaska Department of Fish and Game, Technical Data Report. Juneau.

International North Pacific Fisheries Commission (INPFC). 1963. Annual Report 1961. Vancouver, British Columbia.

Koo, T. S. Y. 1962. Age designation in salmon. Pages 37–48 in T. S. Y. Koo, editor, Studies of Alaska red salmon. University of Washington Press, Seattle, Washington.

Lachenbruch, P. A. 1967. An almost unbiased method of obtaining confidence intervals for the probability of misclassification in discriminant analysis. Biometrics 23(4):639-645.

Larkin, P.A., and J.G. McDonald. 1968. Factors in the population biology of the sockeye salmon of the Skeena River. Journal of Animal Ecology 37: 229-258.

Logan, R. 1967. The Noyes Island salmon fishing conflict. British Columbia Geographic Series, Occasional Paper 8:18-26.

Makela, M. E., and R. H. Richardson. 1977. The detection of sympatric sibling species using genetic correlation analysis. I. Two loci, two gamodemes. Genetics 86: 665-678.

Marshall, S. L., G. T. Oliver, D. R. Bernard, and S. A. McPherson. 1984. Accuracy of scale pattern analysis in separating major stocks of sockeye salmon (*Oncorhynchus nerka*) from southern Southeastern Alaska and northern British Columbia. Alaska Department of Fish and Game, Informational Leaflet 230, Juneau.

Miller, K. M., T. J. Ming, A. D. Schulze, and R. E. Withler. 1999. Denaturing gradient gel electrophoresis (DGGE): A rapid and sensitive technique to screen nucleotide sequence variation in populations. Biotechniques 27: 1016-1030.

Miller, K. M., K. H. Kaukinen, T. D. Beacham, and R. E. Withler. 2001. Geographic heterogeneity in natural selection of an MHC locus in sockeye salmon. Genetica 111: 237-251.

Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (Oncorhynchus mykiss) and their use for genetic study of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53: 120-126.

Mosher, K. H. 1968. Photographic atlas of sockeye salmon scales. Fishery Bulletin 67(2):243-279.

Nelson, R. J., and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics. 30: 228-229.

LITERATURE CITED (continued)

Norenberg, W. 1959. Salmon migrations in Southeastern Alaska. U.S. Fish and Wildlife Service, Circular No. 59.

Pahlke, K. 1988. Length conversion equations for sockeye, chinook and coho salmon in Southeast Alaska. Alaska Department of Fish and Game, Division of Commercial Fisheries, Fishery Research Bulletin 89-02, Juneau.Pella, J. and T. Robertson. 1979. Assessment of composition of stock mixtures. Fishery Bulletin 77:387-389.

Pella, J., M. Hoffman, M. Hoffman, S. Hoffman, M. Masuda, S. Nelson and L. Talley. 1993. Adult sockeye and pink salmon tagging experiments for separating stocks in Northern British Columbia and Southeast Alaska, 1982-1985. NOAA Tech. Memo. NMFS-AFSC-18.

Pella, J. and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fish. Bull. 99: 151-167.

Pella, J., and M. Masuda. 2004. Classical discriminant analysis, classification of individuals, and source population composition of mixtures, in S. Cadrin, K. Friedland, and J. Waldman (Eds.) Stock Identification Methods: applications in fishery science. Academic Press.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Rich, W. and F. Morton. 1930. Salmon-tagging experiments in Alaska, 1927–1928. U. S. Bureau of Fisheries Bulletin 45:1-23.

Rutherford, D.T., C.C. Wood, A.L. Jantz, and D.R. Southgate. 1994. Biological characteristics of Nass River sockeye salmon (Oncorhynchus nerka) and their utility for stock composition analysis of test fishery samples. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1988.

Ryan, P. and M. Christie. 1976. Scale reading equipment. Fisheries and Marine Service, Canada Technical Report no. PAC/T - 758.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53: 833-841.

Simpson, L. 1968. Sockeye salmon migratory behavior and biological statistics collection, Southeastern Alaska. Annual Progress Report, 1 July, 1967 to 30 June, 1968. Anadromous Fish Act (P. L. 89-304), Project No. AFC-2-2.

Smith, C. T., B. F. Koop, and R. J. Nelson. 1998. Isolation and characterization of coho salmon (Oncorhynchus kisutch) microsatellites and their use in other salmonids. Molecular Ecology 7: 1613-1621.

Smouse, P. E., R. S. Waples, and J. A. Tworek. 1990. A genetic mixture analysis for use with incomplete data. Can J. Fish. Aquat. Sci. 47: 620-634.

LITERATURE CITED (continued)

Verhoven, L. 1952. A report to the salmon fishing industry of Alaska on the results of the 1947 tagging experiments. Unpublished manuscript. Fisheries Research Institute, University of Washington, Seattle.

West, C. J., and J. C. Mason. 1987. Evaluation of sockeye salmon (Oncorhynchus nerka) production from the Babine Lake development project. In Sockeye salmon (Oncorhynchus nerka) population biology and future management. Edited by H.D. Smith, L. Margolis, and C.C. Wood. Canadian Special Publication of Fisheries and Aquatic Sciences 96: 176-190.

Withler, R. E, K. D. Le, R. J. Nelson, K. M. Miller, and T. D. Beacham. 2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon, Oncorhynchus nerka, populations of the Fraser River, British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences 57: 1985-1998.

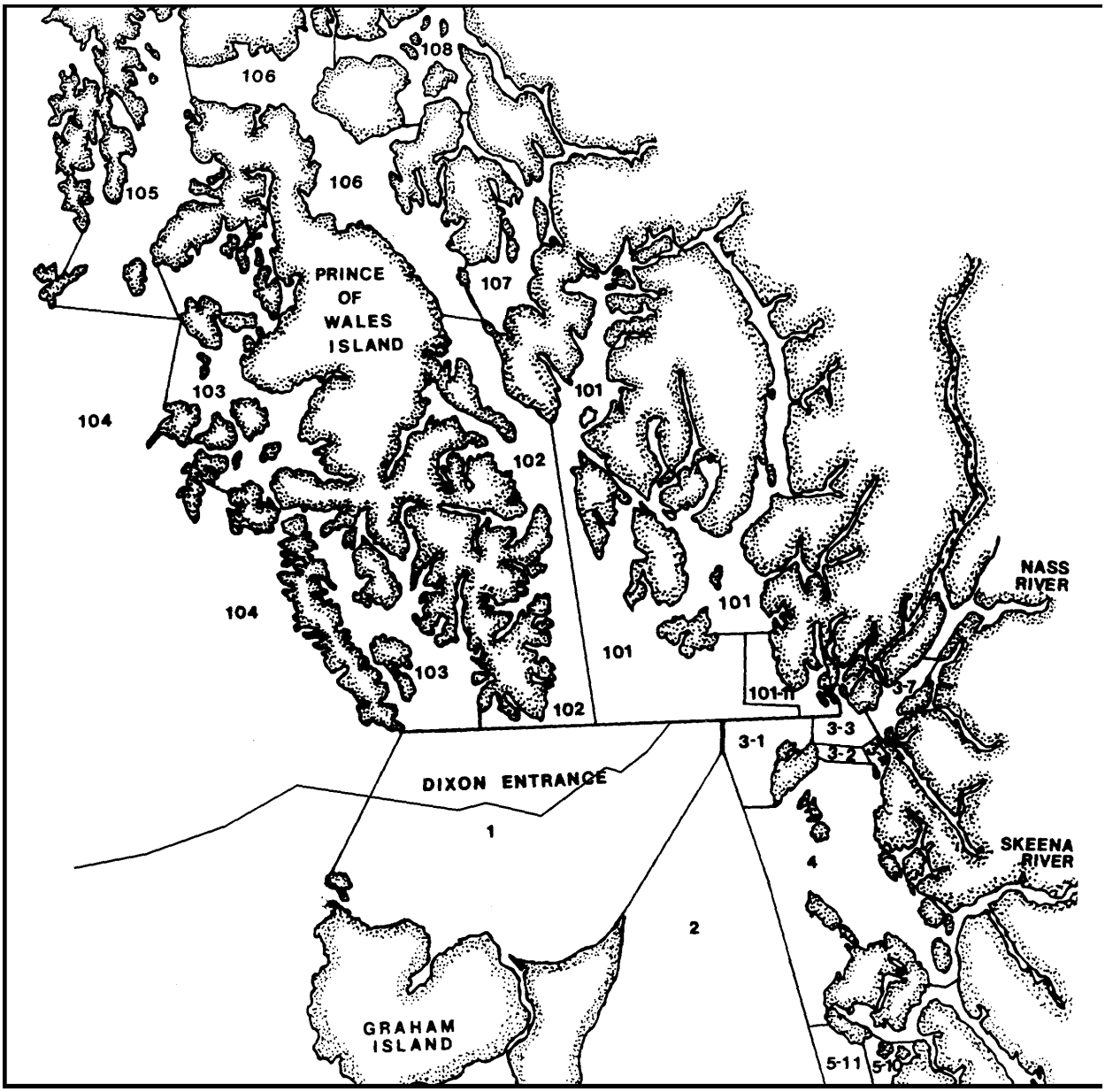

Figure 1. Fishery management districts in southern Southeast Alaska and northern British Columbia waters.

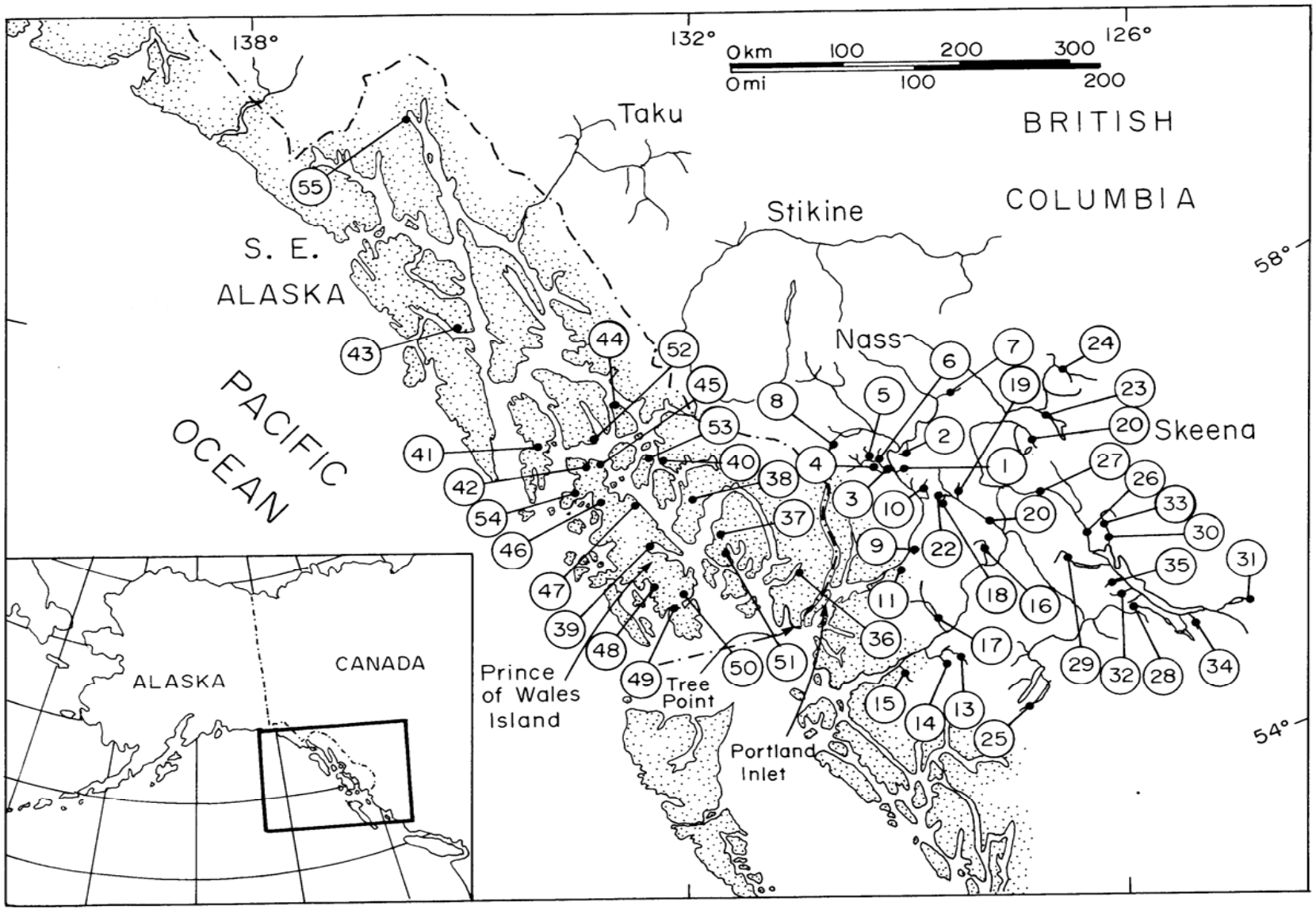

Figure 2. Locations of sockeye salmon populations in the Nass River, Skeena River, and southeast Alaska included in genetic baseline. See Beacham et al. (In press), Table 1, for explanation of numbers and locations of indicated populations.

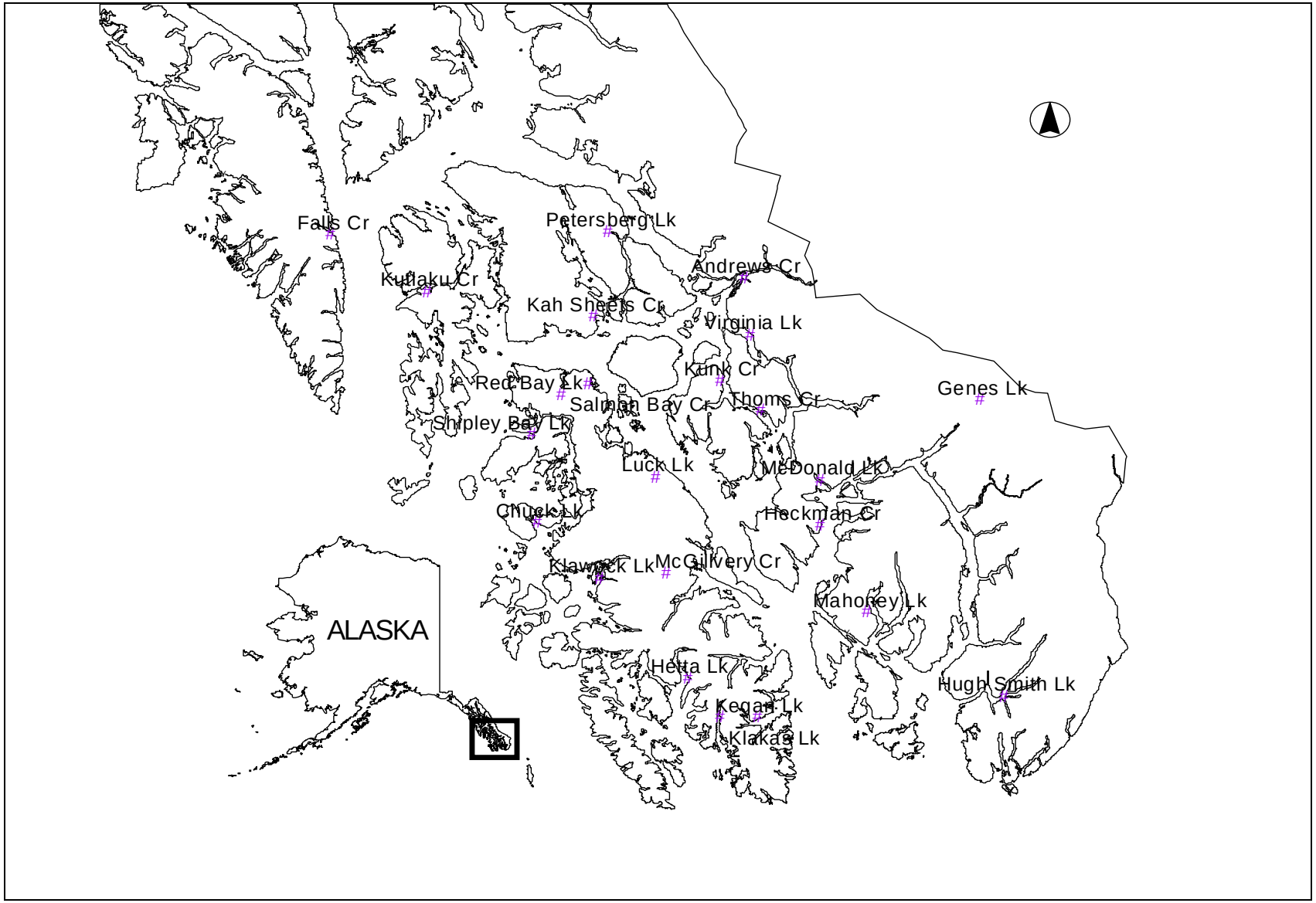

Figure 3. Major sockeye salmon systems of Southeast Alaska sampled for scales and tissues used in stock discrimination method comparison studies, 2002.

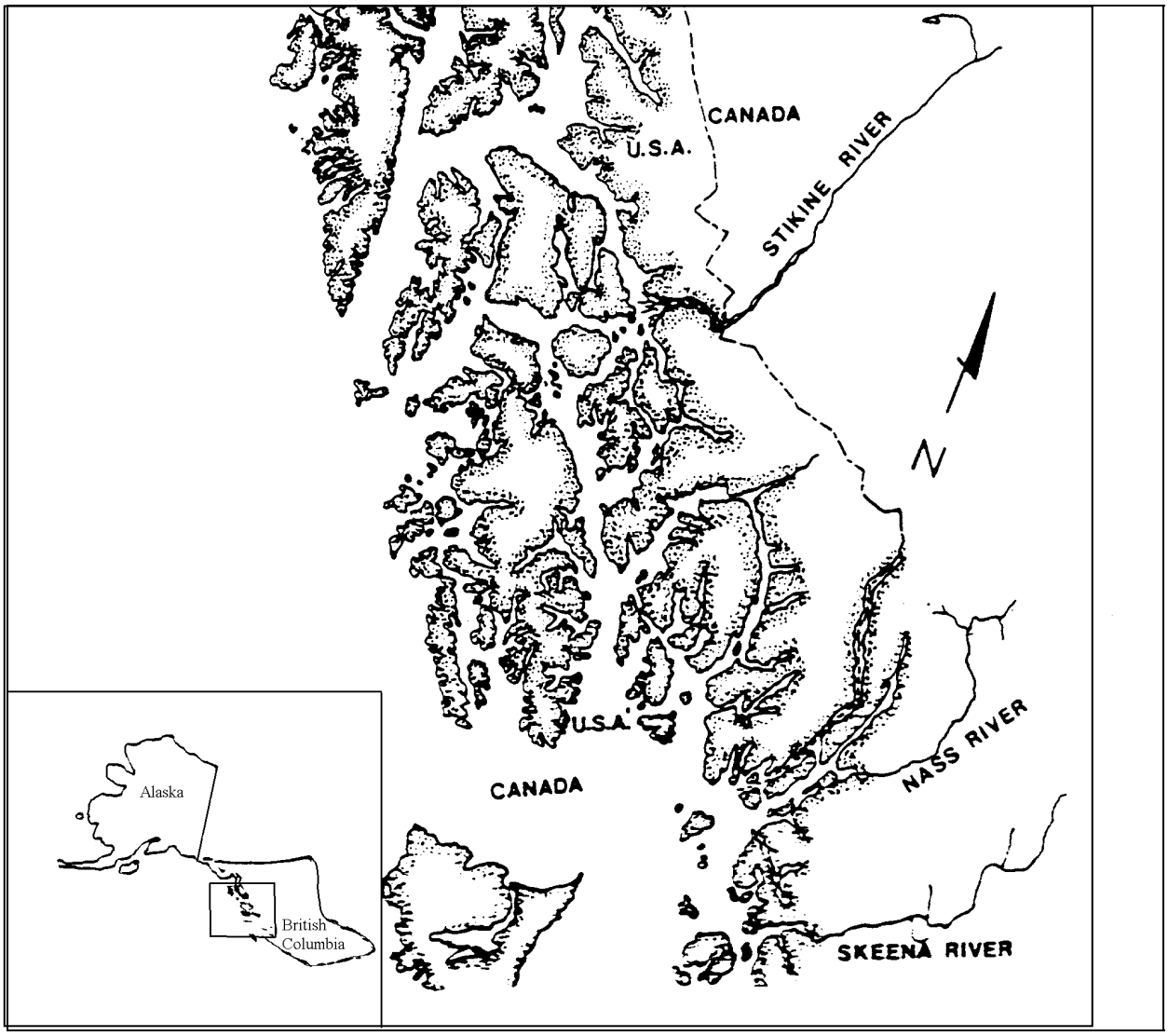

Figure 4. The Canadian Nass River and Skeena River, and the transboundary Stikine River.

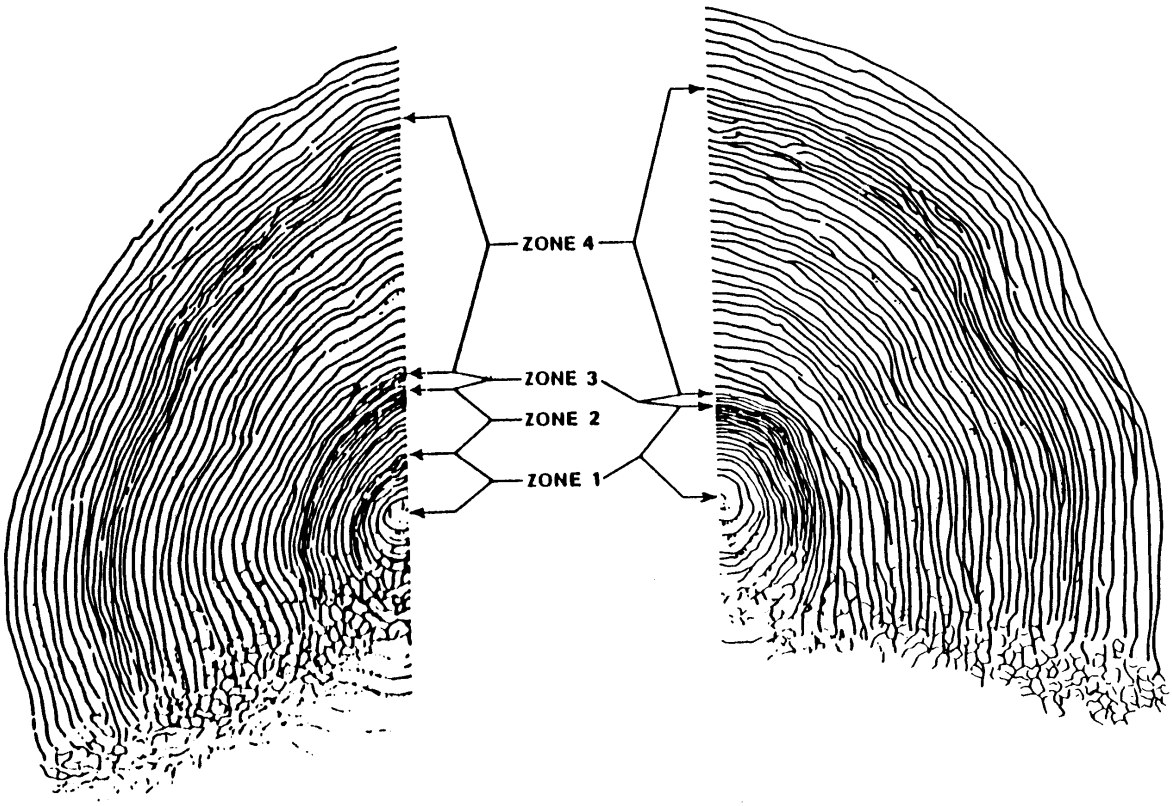

Figure 5. Typical scales with one and two freshwater growth zones showing the zones used for scale pattern analysis.

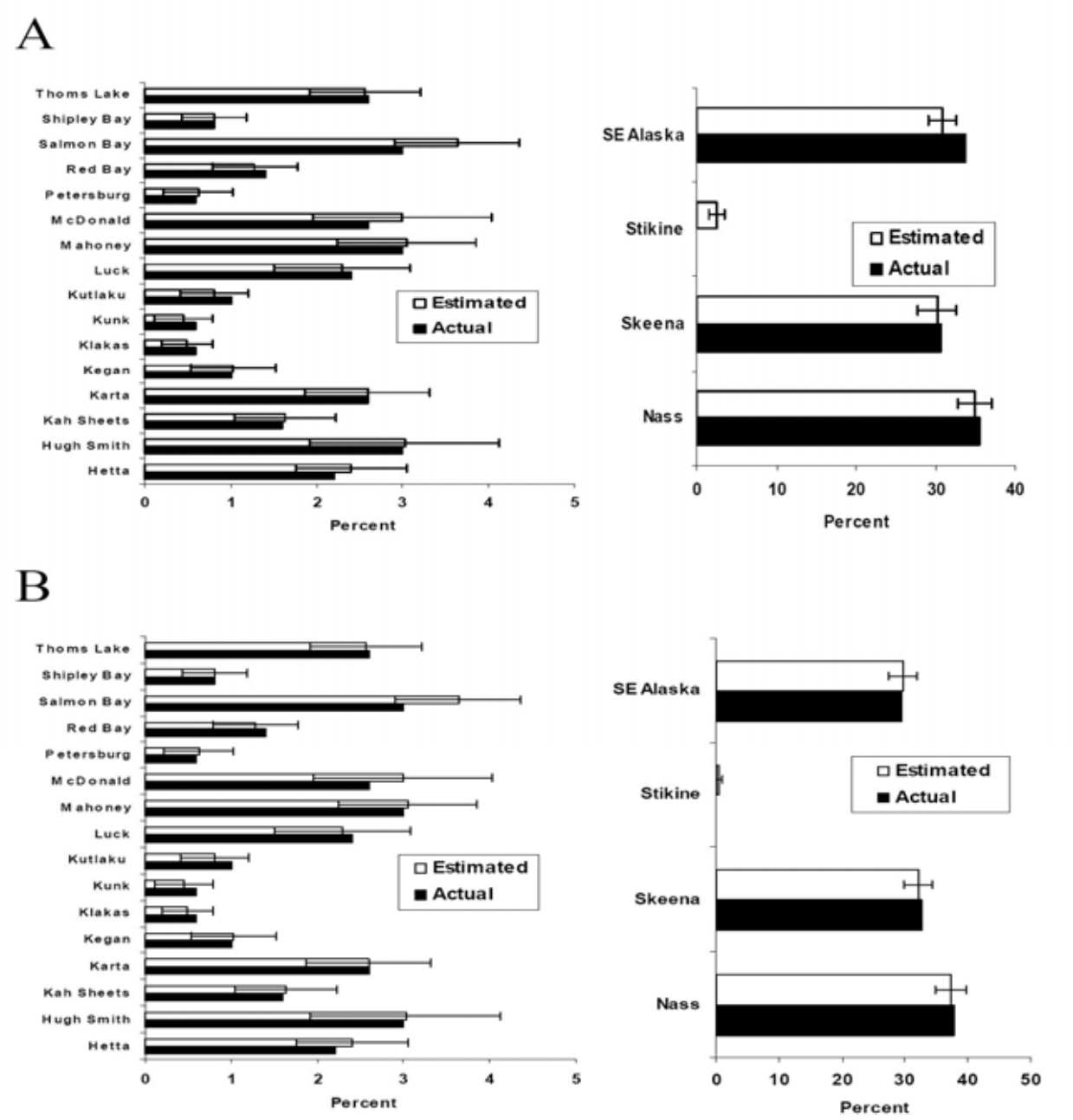

Figure 6. Estimated percentage stock compositions of a sample of of Nass River, Skeena River, and Southeast Alaska sockeye salmon populations in a test mixture of known origin collected in 2002. The baseline used for the analysis consisted of 203 populations ranging from Southeast Alaska to the Columbia River and was surveyed for variation at 14 microsatellite loci and one MHC locus. The test mixture 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. Actual percentages are in black, estimated percentages, with standard deviation, in white. A. Percentages estimated by population for Southeast Alaska populations, and regional estimates for a sample of 526 sockeye salmon, B. The 526-fish sample included 32 fish from southeast Alaska populations not in the baseline, and these fish were removed, and percentages estimated for the remaining 494 sockeye salmon.

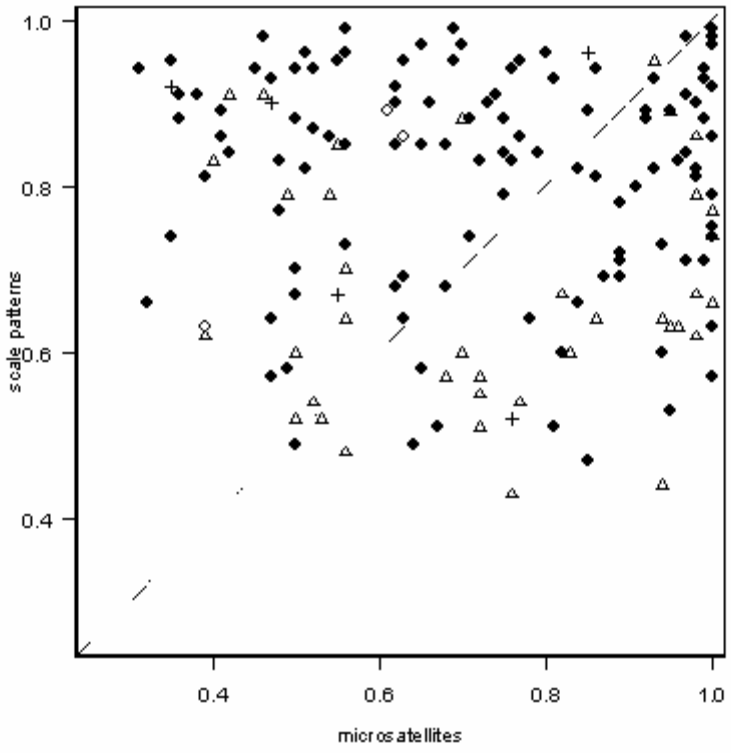

Figure 7. Comparison of maximum average posterior (MAP) source probabilities from scale patterns (program UCONLDA) and genetic microsatellites (program BAYES) for age 1.2 mixture individuals. The individuals were assigned by their MAP source probabilities for the two kinds of measurements, and either both were correct (□), both incorrect (□), only microsatellites were correct (Δ), or only scale patterns were correct (+).

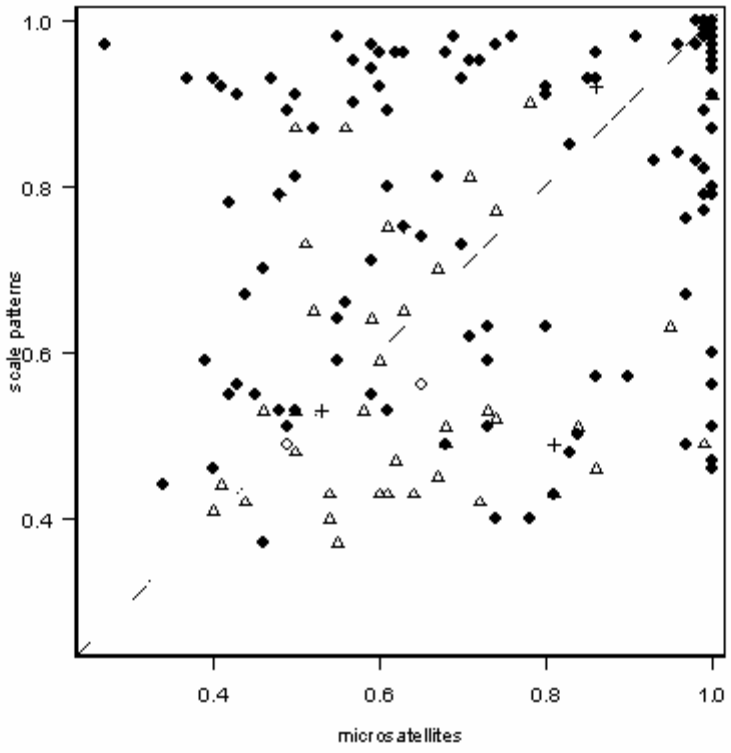

Figure 8. Comparison of maximum average posterior (MAP) source probabilities from scale patterns (program UCONLDA) and genetic microsatellites (program BAYES) for age 1.3 mixture individuals. The individuals were assigned by their MAP source probabilities for the two kinds of measurements, and either both were correct (□), both incorrect (□), only microsatellites were correct (∆), or only scale patterns were correct (+).

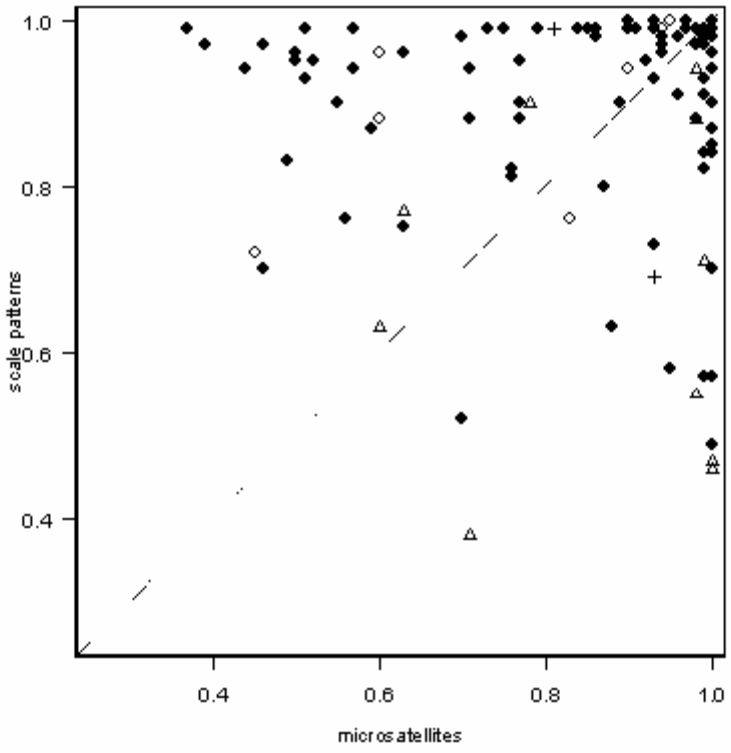

Figure 9. Comparison of maximum average posterior (MAP) source probabilities from scale patterns (program UCONLDA) and genetic microsatellites (program BAYES) for age 2.2 mixture individuals. The individuals were assigned by their MAP source probabilities for the two kinds of measurements, and either both were correct (□), both incorrect (□), only microsatellites were correct (∆), or only scale patterns were correct (+).

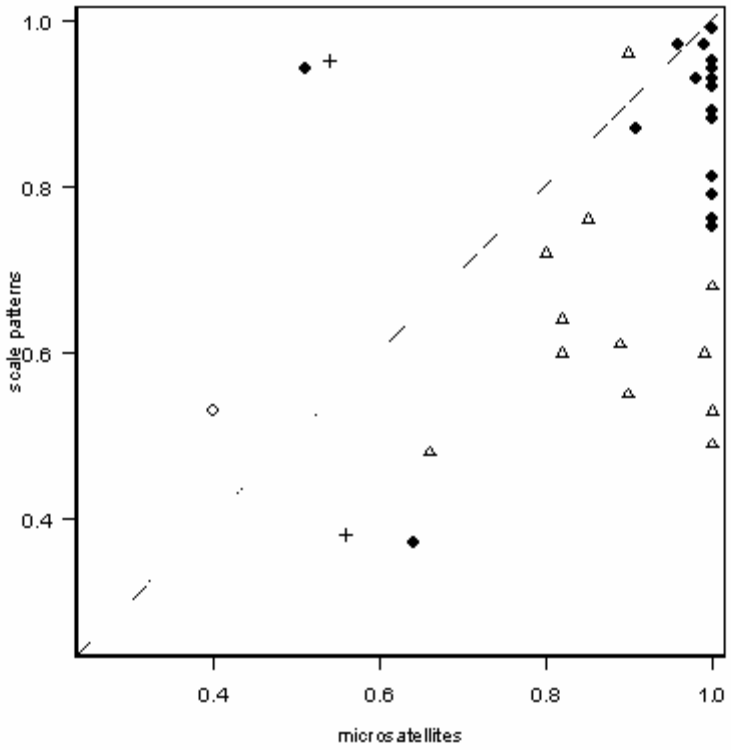

Figure 10. Comparison of maximum average posterior (MAP) source probabilities from scale patterns (program UCONLDA) and genetic microsatellites (program BAYES) for age 2.3 mixture individuals. The individuals were assigned by their MAP source probabilities for the two kinds of measurements, and either both were correct (□), both incorrect (□), only microsatellites were correct (∆), or only scale patterns were correct (+).