IDENTIFICATION OF COLUMBIA BASIN SOCKEYE SALMON STOCKS BASED ON SCALE PATTERN ANALYSES, 1989

Technical Report 90-2

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ACKNOWLEDGEMENTS

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INTRODUCTION

Sockeye salmon *Oncorhynchus nerka* (Walbaum) is one of the five species of Pacific salmon native to the Columbia River Basin. Before white settlers developed the region, it is estimated the Columbia Basin supported an annual sockeye salmon run averaging over four million fish (Northwest Power Planning Council 1986). Since the mid-1800s, however, this sockeye population has severely declined. Annual runs over the ten year period from 1978 through 1987 averaged only about 90,000 fish (Bohn and McIsaac 1988). Run sizes have further declined over the past two years. The 1989 return at Bonneville Dam totaled only 41,900 sockeye, the lowest number since 1978.

The Columbia Basin sockeye salmon run was once composed of at least eight principal stocks (Fulton 1970). Today, only two major stocks remain (Figure 1). Both stocks are naturally sustaining, originating in the Wenatchee River-Lake Wenatchee system (Wenatchee stock) and in the Okanogan River-Osoyoos Lake system (Okanogan stock).

Until now, reliable methods for individual Columbia Basin sockeye salmon stock identification have not been developed to permit estimation of the overall run composition and the migratory characteristics of each component stock. Numerous potential research and management uses exist for such information. These include: run-reconstruction studies to permit accurate population size forecasts, escapement monitoring, establishment of spawner-recruit relationships, and in discrete stock approaches to Columbia River mainstem harvest management. The Pacific Salmon Treaty, ratified by the United States and Canada in 1985 (Pacific Salmon Treaty 1985), requires that certain Pacific salmon populations be monitored to determine the influence of Treaty-imposed ocean harvest regulations on *transboundary* stocks. Some Okanogan stock sockeye salmon originating in Canadian waters but migrating through, and harvested in, the United States portion of the Columbia River might constitute such a stock. Stock identification research would aid in estimation of the proportion and absolute number of Canadian origin sockeye caught within the United States.

The Columbia River Inter-Tribal Fish Commission's Stock Identification Project is designed to develop and apply techniques for identification of individual or aggregate stocks of Columbia Basin salmon originating above Bonneville Dam. The collection and

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Figure 1. Map of the Columbia Basin showing Bonneville, McNary, Rock Island, Rocky Reach, Wells, and Tumwater dams and the two present sockeye salmon production areas.
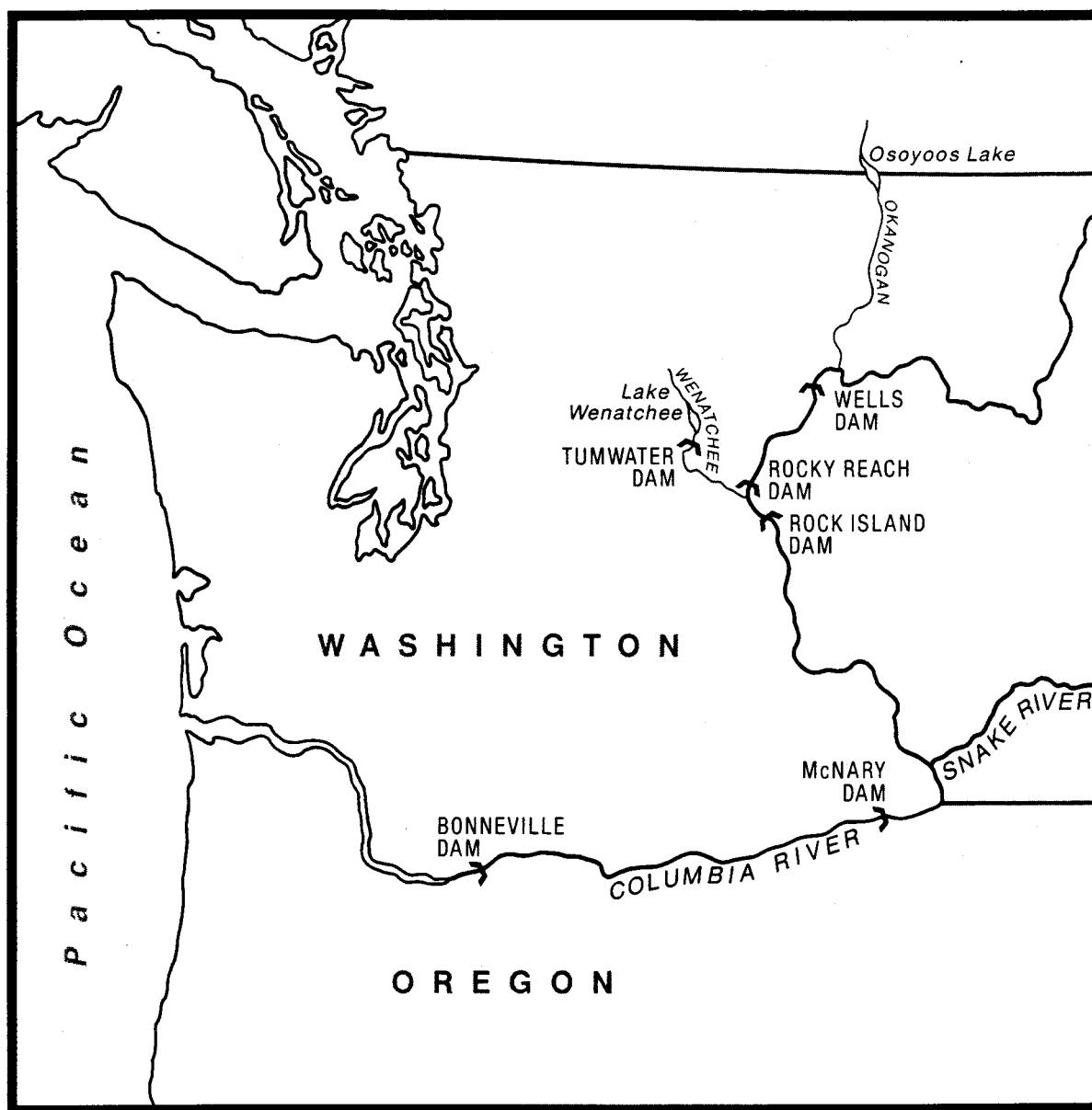
dissemination of basic stock-specific biological information is also an important project goal. Analysis of scale pattern characteristics is the method of study we are currently using for stock identification. Research on sockeye salmon was begun in 1986, and reports are available describing 1987 and 1988 results (Schwartzberg and Fryer 1988, 1989b). We have expanded the 1988 research to include age composition estimates at Wells and Tumwater Dams stratified by migratory timing. A summary of stock composition estimates at Bonneville Dam stratified by migratory timing was also added this year. Additional changes were made in statistical procedures to minimize potential biases in calculating overall stock age and length-at-age composition estimates.

METHODS

Scale pattern analysis (SPA) is a well established stock identification and class-ification technique (Clutter and Whitesel 1956, Henry 1961, Mosher 1963, Anas and Murai 1969). In many species of fish including Pacific salmon, the use of SPA as a tool for stock identification depends on a high correlation between individual fish growth and scale growth (Koo 1955, Clutter and Whitesel 1956). Fish growth and scale growth are influenced by genetic factors and by such environmental conditions as water tempera-ture, length of growing season, and food availability. Stock identification based on SPA assumes that genetically or environmentally influenced growth patterns will differ throughout a species' range and that these differences will be exhibited in the scales of entire groups or *stocks* of fish.

Markedly different conditions exist in the two remaining Columbia Basin sockeye salmon spawning and rearing areas. Lake Wenatchee is oligotrophic, with relatively deep, cold, and biologically unproductive waters. Conversely, Osoyoos Lake has the shallow, warm, and agriculturally enriched waters characteristic of eutrophic lake hab-itats (Allen and Meekin 1980, Mullan 1986). We believed it probable that scales from the two stocks would reflect these differences in freshwater spawning and rearing condi-tions.

Sampling

Sampling Methods

Scales from mixed sockeye salmon stocks *(unknowns)* were obtained from fish sampled at the Bonneville Dam Fisheries Engineering and Research Laboratory, located at river kilometer 225 on the mainstem Columbia River. Each stock was also sampled in terminal areas to obtain representative scale groups for each of the two Columbia Basin sockeye groups *(knowns)*. Wenatchee stock scales were collected at Tumwater Dam on the Wenatchee River (river km 31), while Okanogan stock scales were obtained at Wells Dam on the mainstem Columbia River (river km 829).

Fish were trapped, anesthetized, quickly sampled for scales and biological data and allowed to recover. Scales were collected and mounted according to methods described in Clutter and Whitesel (1956) and the International North Pacific Fisheries

Commission (1963). Four scales per fish were collected to minimize the sample rejection rate. At Bonneville Dam, fish were then returned to the exit fishway leading to a main fish ladder. At Tumwater Dam, fish were returned to the project forebay while fish at Wells Dam were returned to the project tailrace. Length measurements were recorded to the nearest 0.5 cm. Observed mark and/or tag information were also recorded. No live fish were sacrificed in this study. Consequently, sex of specimens collected at Bonneville Dam, all in the earliest stages of sexual maturation, could not be determined. The sex of most specimens collected at Tumwater and Wells dams could be determined, and was recorded. Field sampling procedures followed guidelines established for this project (Schwartzberg 1987).

Sample Design

The mixed stock Bonneville Dam sample size was 561 fish. The known stock sample sizes were 728 fish from Tumwater Dam, and 469 from Wells Dam. At Bonneville and Wells dams, sample sizes were based on desired levels of precision and accuracy (p=0.05, c.i.=0.90; Bernard 1982). Weekly sample sizes were proportional to sockeye counts at those dams averaged over the previous ten years (1979-1988). To improve precision in weekly age-composition as well as weekly stock-composition estimates, the minimum weekly sample sizes was set to 50. At Tumwater Dam, sockeye samples were collected during the three-week period during which large numbers of fish were observed moving through the fish ladder at the dam. Due to unreadable scales caused by scale regeneration, usable samples collected for estimating age and length-at-age were 543 from Bonneville Dam, 721 from Tumwater Dam, and 451 from Wells Dam.

For SPA studies, subsamples of age 1.2 scale samples were selected. Target subsample sizes were 200 from each known group and 100 from the mixed group (Conrad 1985). Actual subsamples consisted of 257 systematically selected Wenatchee stock and 195 randomly selected Okanogan stock age 1.2 scales. The latter sample was selected using a stratified (by statistical week) random sampling technique. Instead of using the target sample size of 100, all 426 useable mixed stock (age 1.2) samples were used in the analysis. This was done to permit more precise weekly stock composition estimates.

In both 1987 and 1988, no corrections were made to account for any deviations in actual run timing from the ten year average. Corrections were made for the 1989 weekly sample sizes by determining actual run timing from post-season analysis of 1989

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Figure 2. Age 1.2 Okanogan-stock sockeye salmon scale showing growth and measurement zones.
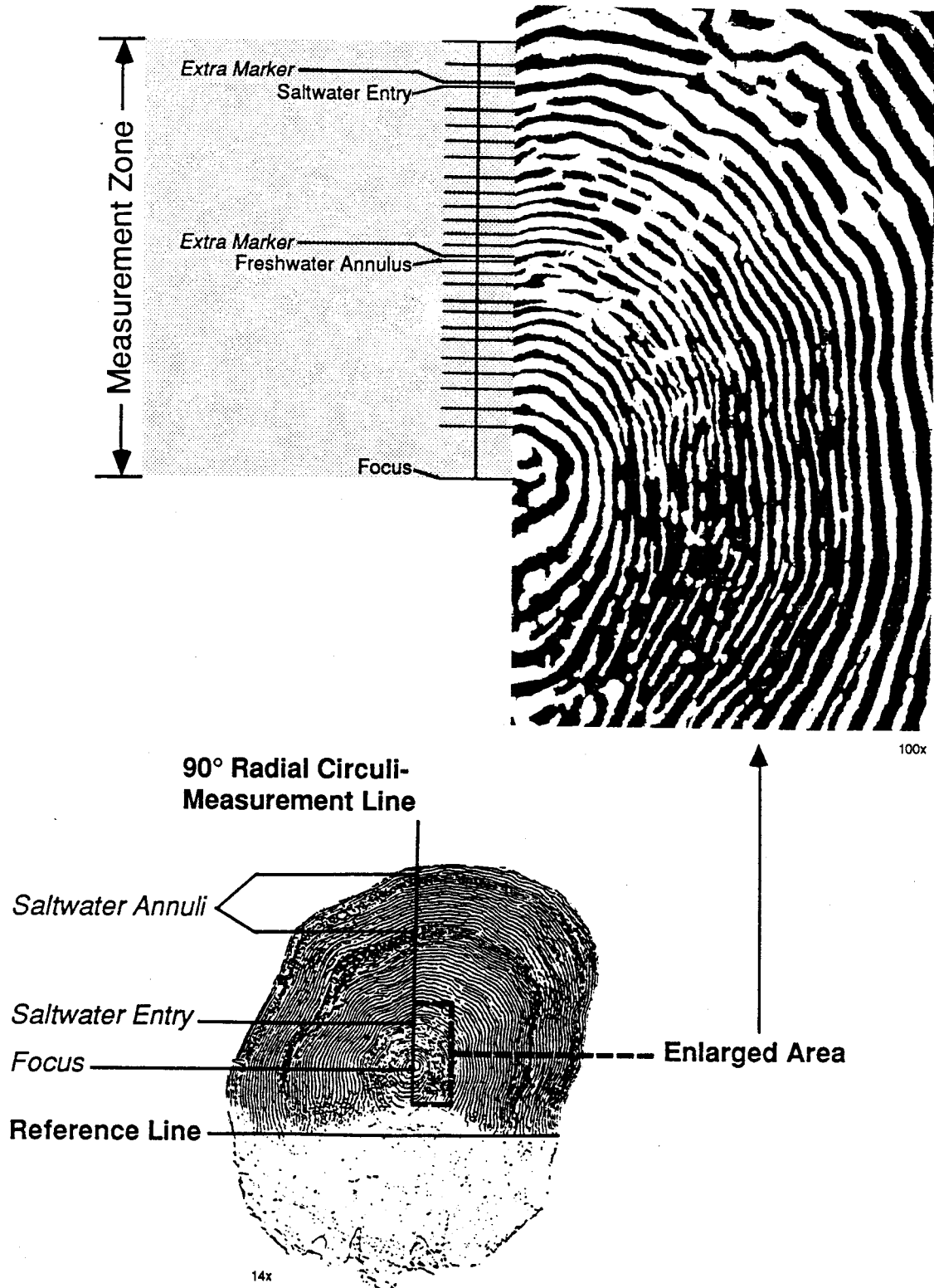
visual fish counts at Bonneville and Wells dams. If actual run timing differed significantly from that predicted by the ten year average, the uncorrected total age composition and length estimates were considered biased. An approach was used to correct for this potential bias that was based on a stratified sampling method (Cochran 1977). A corrected and unbiased estimate of the total proportion of fish in a given age class, p_{st}, was calculated as:

$$p_{st} = \frac{\sum_h (N_h p_h)}{N}$$

where h represents the statistical week, N_h the total number of sockeye migrating over Bonneville or Wells dam during that week, p_h the proportion of fish belonging to the given age class in the sample for week h, and N the total number of fish counted at the given dam over the entire year. The quantity in the parentheses, the estimated number of fish of a given age class in week h, is summed over all weeks. The average length by age class was computed as:

$$\overline{y}_{st} = \frac{\sum_h (N_h \overline{y}_h)}{N}$$

where y_h represents the average length within a given age class for the particular week. The quantity in the parentheses, the average length weighted by the weekly population size for the same age class, is summed over all weeks. The variance for this estimate was calculated as:

$$V(\overline{y}_{st}) = \sum_h \left(\frac{N_h}{N}\right)^2 V(\overline{y}_h)$$

with the quantity in the parentheses, a weighting factor which weights the variance of the length for a given week by the size of the run for that week, again being summed over all weeks. This stratified variance estimate cannot be calculated for age classes where only one sample was observed in a given week. In such cases, the standard unstratified variance estimate was presented.

The 1989 results for the mixed and Okanogan stocks were corrected using this stratified sampling method.

Age Determination and Scale Measurements

Salmon scales, under magnification, display numerous concentric rings *(circuli)* radiating outwards from a central focal area. A freshwater-growth zone of narrowly spaced circuli (Figure 2) is clearly distinguishable from a zone of more widely spaced saltwater-growth circuli in scales of all Columbia Basin sockeye salmon, which typically spend one or two complete years in freshwater before migrating to the ocean. Fish age can be determined by counting *annuli*, which are zones of closely spaced circuli formed yearly during winter periods of slow growth.

All scales were examined visually and categorized by age using previously studied techniques (Johnston 1905, Gilbert 1913, Borodin 1924, Van Oosten 1929). The method used for fish age description is that recommended by Koo (1955) which is sometimes referred to as the *European* method. The number of winters a fish spent in freshwater (not including the winter of egg incubation) is described by an Arabic numeral followed by a period. The numeral following the period indicates the number of winters a fish spent in the ocean. Total age, therefore, is equal to one plus the sum of both numerals.

Scales were used to estimate the age composition of Bonneville Dam mixed stock as well as the Okanogan and Wenatchee known stock samples. Length-at-age relationships were also established for each stock. Otoliths from each known stock were used to support scale age estimation techniques and to corroborate scale age estimates. Validation of ages (Beamish and McFarlane 1983) was not possible as there were no known-age fish in any samples.

SPA of circuli in freshwater and early saltwater-growth zones was used to identify each known stock sample and to also classify mixed stock samples. The methodology was applied to like-age (age 1.2) samples from all stocks. SPA was not used to classify fish of age classes other than 1.2 in the mixed stock sample. Certain age classes were found to be overwhelmingly present in only one of the two known stock samples. We assumed that those age classes were attributable to a single stock (at least in this sample year) and used this relationship to categorize mixed stock samples of given age-groups.

A computer and video camera were used to measure, or *digitize*, scale features

(BioSonics 1985). The system employed consisted of a microscope (2x, 4x, 6.3x, and 10x objectives; a 1.0x, 1.25x, and 1.5x magnification changer; and a 2.5x photocompensation adapter), a monitor (13 inch) and a digitizing tablet connected to a personal computer (AT) with a video frame-grabber board. Acetate impressions of scales were placed in the microscope and projected onto the monitor. Using a keyboard and digitizing tablet, distances were measured along a radial line drawn through the scale. These measurements were then stored in a computer file.

One scale from each fish was selected, oriented diagonally with the clear (posterior) part of the scale in the lower left corner of the screen, and a reference line was drawn along its base (5x final microscope magnification and 65x projection magnification). The reference line was placed in the posterior field of the scale so that it bridged the end points of circuli in the first saltwater annulus (Figure 2). A radial line was then drawn perpendicular to the reference line, and circuli positions were measured at their points of intersection with the radial line (25x final microscope magnification and 325x projection magnification). All measurements were made to the outermost marginal edges of circuli. Additional circuli markers were placed to permit measurement of other key scale-features, specifically, freshwater annulus and saltwater-entry point. These features were respectively indicated by two sets of closely spaced circuli markers. The 'extra markers' were placed immediately after and adjacent to the original circuli position markers and were interpreted and removed by data analysis programs used in subsequent procedures (Appendix A). The freshwater annulus-position marker was placed beside the last circulus in the freshwater annulus and the saltwater-entry marker was placed immediately after the first circulus in the ocean zone.

Variable Selection

Variables, composed of selected scale-measurements from within an area from scale focus to approximately circulus 27, were tested to find those that most effectively characterized differences in growth between the two stocks. Test variables were selected and used in discriminant analyses, and classification accuracies were noted. Variables tested were grouped into four categories. These included distances measured between adjacent circuli, or *doublets*, between three adjacent circuli, or *triplets,* between four adjacent circuli, or *quadruplets,* as well as measurements between five adjacent circuli or *quintuplets.* (Note that these definitions have been changed from

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those presented in our previous reports to be consistent with those presented by Davis (1987). What were described as triplets in 1987 and 1988 are herein referred to as quadruplets.) Among the variables tested were distances and number of circuli from scale focus to saltwater-entry and from scale focus to freshwater annulus margin (anterior).

Statistical Analyses

A linear discriminant analysis technique developed by Fisher (1936) was used to differentiate stocks and classify unknown mixed stock samples. Linear discriminant analysis permits the simultaneous use of many variables to form classification functions that typify and identify groups. This methodology has proven useful for determining the origins of individual fish stocks from mixed stock samples (Bethe and Krasnowski 1977, Bethe et al. 1980, Major et al. 1978).

A linear classification function of a set of different scale measurements was computed that maximized the variance between stocks against that within each stock. This discriminant function y may be written as:

$$y = m_1 x_1 + m_2 x_2 + \ldots + m_n x_n$$

for the case where there are n different scale measurements being used in the analysis (Worlund and Fredin 1960). The ith measurement is represented by x_i while m_i represents its coefficient.

The frequency distribution of y was determined, and mean values of y computed for the two populations. The critical value for separating two populations was a function of the average of the two means $\overline{y_a}$ and $\overline{y_b}$, expressed as:

$$Y = \frac{\overline{y_a} + \overline{y_b}}{2}$$

The discriminant function was applied to each sample, producing a y value. Samples were categorized to one of the two populations depending on whether their own y values were greater or less than Y.

Accuracy of the discriminant analysis was determined by classifying the pooled known stock samples and comparing results to actual known stock identities. A jack-knife procedure was used to correct for systematically biased results created by using the same set of observations for both calculating the discriminant function and determining its accuracy.

The discriminant function was then used to classify unknown mixed stock samples. To correct for the additional bias occurring when the expected classification accuracy determined from known stock tests was not 1.00, we used the method developed by Cook and Lord (1978). Variances on estimates were also computed (Pella and Robertson 1979).

A stepwise procedure was applied in our analyses, allowing variables to be entered and/or removed from a discriminant function at each stage of function development. The steps taken by the procedure were similar to those of a stepwise regression.

Relationship of Migratory Timing to Stock and Age Composition

Tests were conducted to examine the relationship between migratory timing and stock composition as well as migratory timing and age composition in the mixed stock unknown sample. The relationship between migratory timing and age composition was also examined in known stock Wenatchee and Okanogan samples. Mean weekly stock and age composition estimates were regressed against the statistical week weighted by the inverse of the variance for each estimate (Neter, Wasserman, and Kutner 1985). In the test of the relationship between migratory timing and age composition in the Wenatchee known stock sample, a limited number of data points (three) reduced the sensitivity of the test. All other tests included at least five data points, providing sufficient degrees of freedom for more acceptable test sensitivity.

Visual Scale Classification

Visual interpretation of freshwater scale-circuli patterns was studied based on the assumption that differences between Okanogan and Wenatchee sockeye salmon

scales were large enough to permit classification through visually observable characteristics alone. A blind experiment similar to one described by McPherson and Jones (1987) was used to test this hypothesis.

Testing for Consistency in Scale-Feature Measurement

The identification of such scale features as annulus position and point of saltwater entry may be a highly subjective process. In the discriminant analysis procedures, we therefore did not use variables dependent on the location of such features in the discriminant analysis procedures. Freshwater annulus and saltwater-entry locations were, however, estimated and marked in the digitizing process and a test was later conducted to determine whether an operator could consistently locate these two scale features. A subsample of 13 mixed stock scales were selected, measured, and compared to those same previously digitized scales. A paired t-test was made testing among scale differences in the distance and number of circuli from scale focus to freshwater annulus and from scale focus to saltwater entry. The test was designed to detect a difference in freshwater annulus position of two circuli and saltwater entry of three circuli at a significance level of 0.10 and a power of 0.90 (Snedecor and Cochran 1980).

RESULTS

Age and Length-at-Age Composition

Mixed stocks of Columbia Basin sockeye salmon sampled at Bonneville Dam in 1989 were estimated to include fish of ages 1.1, 2.1, 1.2, 2.2, and 1.3 (ages were not validated, therefore all sample age and stock compositions and length-at-age relationships reported must be considered estimates). Age 1.2 fish composed 0.81 of the sample (Table 1). Age 1.3 and 2.2 fish were found in significant proportions, 0.09 and 0.07, respectively. Age 2.1 and 1.1 fish each comprised 0.01 of the sample while age 2.3 fish made up less than 0.01 of the sample.

The Wenatchee stock sample consisted predominantly (0.71) of age 1.2 fish (Table 2). Age 2.2 and 1.3 fish were found in significant numbers (0.17 and 0.11 respectively). Age 2.3 fish made up an additional 0.01 of the sample. No fish in the Wenatchee-stock sample were of ages 1.1 or 2.1.

The Okanogan stock sample was primarily composed of age 1.2 fish (0.93). Small proportions of age 1.1, 1.3, 2.1, and 2.2 fish were present (0.03, 0.03, 0.01, and 0.01, respectively).

Mean fork-lengths of Bonneville Dam mixed stock samples for the three principal age classes present in that sample (1.2, 2.2, and 1.3) were 49.6, 50.2, and 58.4 cm, respectively. The 0.90 confidence intervals for these lengths were 46.0, 53.1; 47.2, 53.2; and 54.4, 62.3; respectively (Figure 3).

Mean fork-lengths of Wenatchee stock samples for the three principal age classes present in that sample (1.2, 2.2, and 1.3) were 50.4, 50.9, and 57.7, respectively. The 0.90 confidence intervals for these lengths were 47.2, 53.5; 47.2, 54.6; and 53.5, 62.0, respectively (Figure 4).

Mean fork-lengths of Okanogan stock samples for the three principal age classes present in that sample (1.1, 1.2, and 1.3) were 37.3, 49.2, and 57.9, respectively. The 0.90 confidence intervals for these lengths were 34.9, 39.7; 45.6, 52.8; and 56.3, 59.5; respectively.

Table 1. Age composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1989.

Brood Year and Age Class[1]

Statistical Week[2]	Sample Size	1986 1.1	1985 1.2	1985 2.1	1984 1.3	1984 2.2	1983 2.3
24	47	0.00	0.79	0.00	0.13	0.09	0.00
25	115	0.00	0.80	0.00	0.14	0.04	0.02
26	189	0.01	0.79	0.01	0.11	0.07	0.01
27	130	0.02	0.80	0.04	0.05	0.08	0.00
28	47	0.00	0.92	0.00	0.02	0.06	0.00
29	15	0.00	0.87	0.00	0.00	0.13	0.00
Total Sample	**543**	**0.01**	**0.81**	**0.01**	**0.09**	**0.07**	**0.00[3]**

1. Rounding errors caused sample proportions to occasionally not total 1.0.
2. Statistical weeks are sequentially numbered calendar-year weeks. Excepting the first and last week of most years, weeks are seven days long, beginning on Sunday and ending on Saturday. In 1989, for example, Statistical Week 24 began on June 11 and Statistical Week 29 began on July 16.
3. Estimated proportion was 0.004.

Table 2. Age composition estimates of Wenatchee and Okanogan sockeye salmon stocks sampled in 1989.

Wenatchee stock sampled at Tumwater Dam

Brood Year and Age Class[1]

Statistical Week[2]	Sample Size	1986 1.1	1985 1.2	2.1	1984 1.3	2.2	1983 2.3
29	473	0.00	0.68	0.00	0.12	0.18	0.02
30	149	0.00	0.74	0.00	0.13	0.11	0.01
31	99	0.00	0.82	0.00	0.06	0.12	0.00
Total Sample	721	0.00	0.71	0.00	0.11	0.17	0.01

Okanogan stock sampled at Wells Dam

Brood Year and Age Class[1]

Statistical Week[2]	Sample Size	1986 1.1	1985 1.2	2.1	1984 1.3	2.2	1983 2.3
27	63	0.02	0.95	0.00	0.03	0.00	0.00
28	76	0.00	0.96	0.00	0.01	0.01	0.00
29	144	0.03	0.95	0.01	0.01	0.01	0.00
30	111	0.04	0.92	0.01	0.03	0.00	0.00
31	57	0.09	0.82	0.02	0.07	0.00	0.00
Total Sample	451	0.03	0.93	0.01	0.03	0.01	0.00

1. Rounding errors caused sample proportions to occasionally not total 1.0.
2. Statistical weeks are sequentially numbered calendar-year weeks. Excepting the first and last week of most years, weeks are seven days long, beginning on Sunday and ending on Saturday. In 1989, for example, Statistical Week 27 began on July 2 and Statistical Week 31 began on July 30.

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Figure 3. Length-at-age composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1989.

Mean Fork-Lengths ± the 0.90 Confidence Interval for Each Age Class
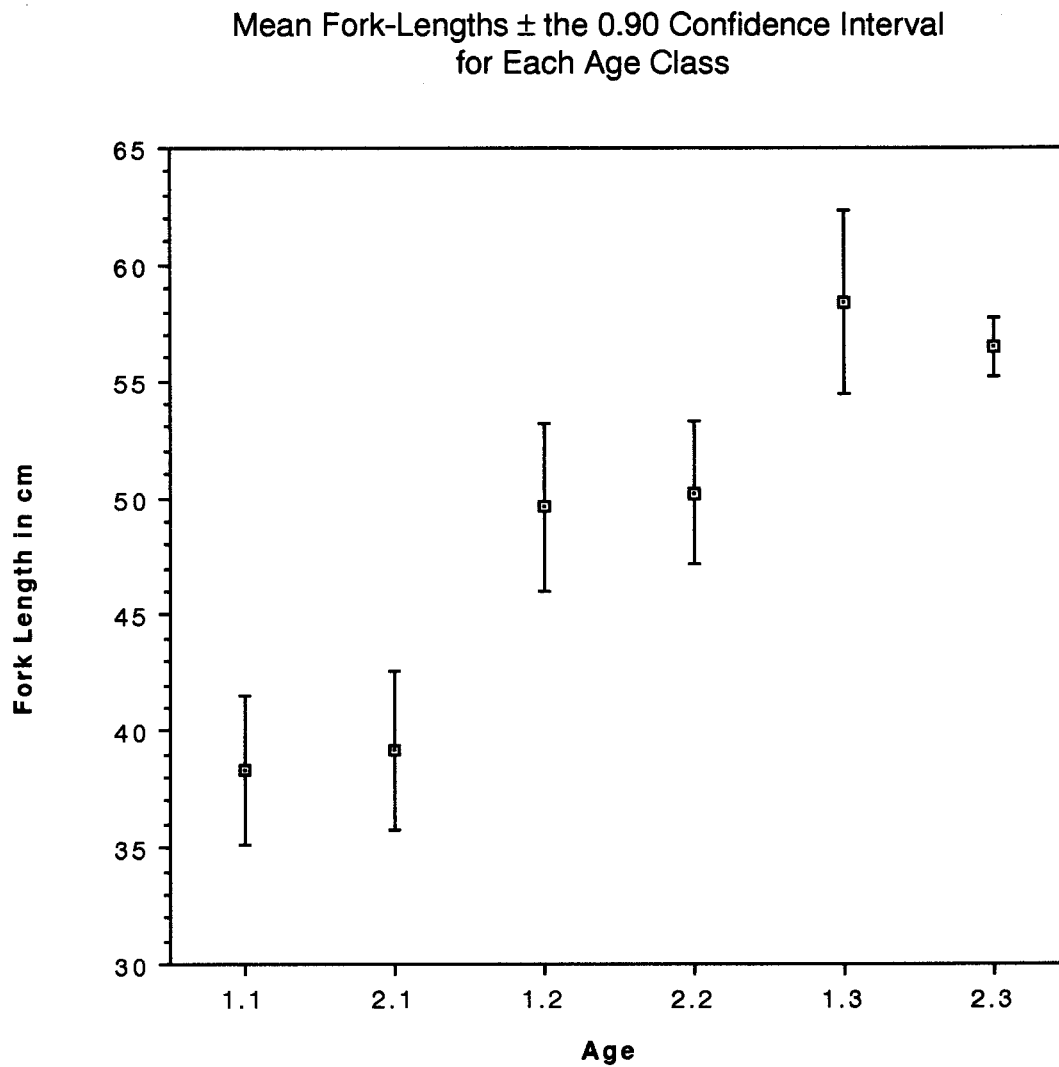
Figure 4. Length-at-age composition estimates of Wenatchee and Okanogan sockeye salmon stocks sampled in 1989.

Wenatchee Stock
Mean Fork-Lengths ± the 0.90 Confidence Interval
for Each Age Class

Okanogan Stock
Mean Fork-Lengths ± the 0.90 Confidence Interval
for Each Age Class
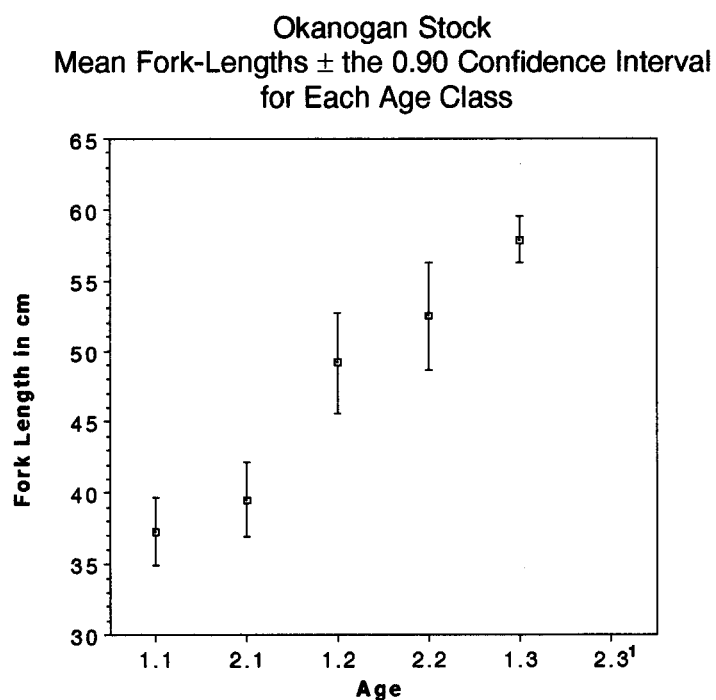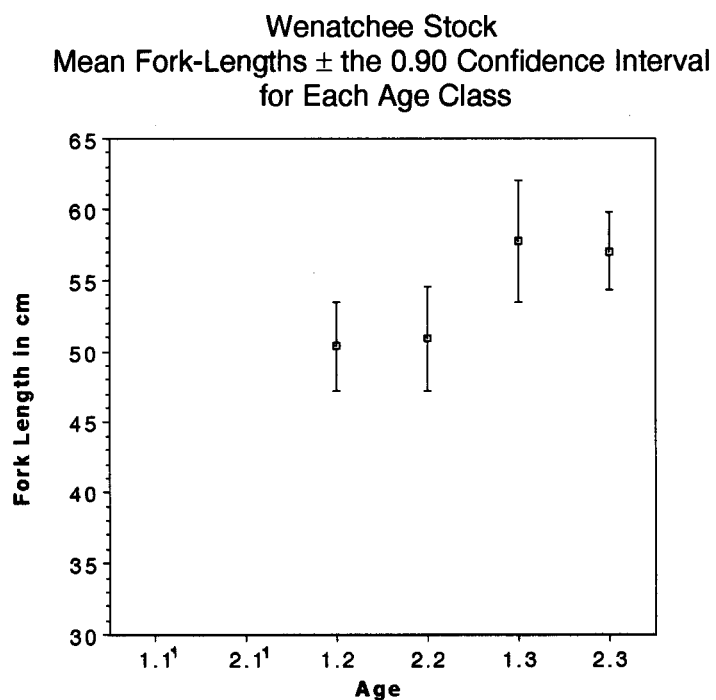
1. No fish estimated to be of these age classes were sampled.

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Classification of Known Stock Samples

Among the variables tested, (Table 3), those resulting in the highest classification accuracies were quadruplets (Group D) combined with the number of circuli and distance measurements from scale focus to freshwater annulus and to saltwater entry. A known stock classification accuracy of 0.86 was achieved using these variables. However, an attempt was made in this study to limit the degree of subjectivity in the data acquisition process. Because determining the locations of saltwater entry and freshwater annulus is highly dependent on individual operator judgement, variables directly related to those positions were rejected from the final subset.

A final subset was selected consisting of seven quadruplets. Similar to our 1987 results, it was found that the relatively larger quadruplet spacings tended to smooth random variations between samples of the same stock, emphasizing actual between-stock scale differences. (In 1988, the use of quadruplets and triplets as variables gave approximately the same classification accuracies but quadruplets were used to provide consistency with the 1987 procedures.)

The classification accuracy achieved using the final variable subset was 0.81 (Table 4). A total of 44 Wenatchee samples were misclassified as being of Okanogan stock, while 35 Okanogan samples were misclassified as Wenatchee stock. The jack-knife procedure resulted in four additional Wenatchee, and two additional Okanogan samples being misclassified.

Classification of Unknown Mixed Stock Samples

The linear discriminant function classified 0.50 of age 1.2 unknown origin samples to each stock (Table 4). Because samples were classified equally between the two stocks, the potential bias in stock abundance proved to be relatively insignificant. After correction for bias (Cook and Lord 1978), 0.51 of the sample was classified as Okanogan stock and the remaining 0.49 as Wenatchee stock. Confidence intervals (0.90) ranged from 0.43 to 0.59 for the Okanogan group and from 0.41 to 0.57 for the Wenatchee group.

Table 3. Variables tested and used in discriminant analyses, 1989.

Test Set	Group	Set Used	Description of Variables
1	A		Number of circuli from focus to freshwater annulus
2	A		Distance from focus to freshwater annulus
3	A		Number of circuli from focus to saltwater entry
4	A		Distance from focus to saltwater entry
5	B		Distance from circulus 1 to circulus 2
6	B		Distance from circulus 2 to circulus 3
7	B		Distance from circulus 3 to circulus 4
8	B		Distance from circulus 4 to circulus 5
9	B		Distance from circulus 5 to circulus 6
10	B		Distance from circulus 6 to circulus 7
11	B		Distance from circulus 7 to circulus 8
12	B		Distance from circulus 8 to circulus 9
13	B		Distance from circulus 9 to circulus 10
14	B		Distance from circulus 10 to circulus 11
15	B		Distance from circulus 11 to circulus 12
16	B		Distance from circulus 12 to circulus 13
17	B		Distance from circulus 13 to circulus 14
18	B		Distance from circulus 14 to circulus 15
19	B		Distance from circulus 15 to circulus 16
20	B		Distance from circulus 16 to circulus 17
21	B		Distance from circulus 17 to circulus 18
22	B		Distance from circulus 18 to circulus 19
23	B		Distance from circulus 19 to circulus 20
24	B		Distance from circulus 20 to circulus 21
25	B		Distance from circulus 21 to circulus 22
26	B		Distance from circulus 22 to circulus 23
27	B		Distance from circulus 23 to circulus 24
28	B		Distance from circulus 24 to circulus 25
29	B		Distance from circulus 25 to circulus 26
30	B		Distance from circulus 26 to circulus 27
31	B		Distance from circulus 27 to circulus 28
32	C		Distance from focus to circulus 2
33	C		Distance from circulus 2 to circulus 4
34	C		Distance from circulus 4 to circulus 6
35	C		Distance from circulus 6 to circulus 8
36	C		Distance from circulus 8 to circulus 10
37	C		Distance from circulus 10 to circulus 12
38	C		Distance from circulus 12 to circulus 14
39	C		Distance from circulus 14 to circulus 16
40	C		Distance from circulus 16 to circulus 18
41	C		Distance from circulus 18 to circulus 20
42	C		Distance from circulus 20 to circulus 22
43	C		Distance from circulus 22 to circulus 24
44	C		Distance from ciruclus 24 to circulus 26
45	C		Distance from circulus 26 to circulus 28
46	D	x	Distance from focus to circulus 3
47	D	x	Distance from circulus 3 to circulus 6
48	D	x	Distance from circulus 6 to circulus 9
49	D	x	Distance from circulus 9 to circulus 12
50	D	x	Distance from circulus 12 to circulus 15
51	D		Distance from circulus 15 to circulus 18
52	D	x	Distance from circulus 18 to circulus 21
53	D	x	Distance from circulus 21 to circulus 24
54	D	x	Distance from circulus 24 to circulus 27
55	E		Distance from focus to circulus 4
56	E		Distance from circulus 4 to circulus 8
57	E		Distance from circulus 8 to circulus 12
58	E		Distance from circulus 12 to circulus 16
59	E		Distance from circulus 16 to circulus 20
60	E		Distance from circulus 20 to circulus 24
61	E		Distance from circulus 24 to circulus 28
62	F		Distance from fw. annulus to 3rd circulus after fw. annulus
63	F		Distance from 3rd to 6th circulus after fw. annulus
64	F		Distance from 6th to 9th circulus after fw. annulus

Table 4. Known stock classification test using linear discriminant analyses and age 1.2 Columbia Basin sockeye salmon stocks sampled in 1989.

Known stock classification test[1]

Stock	Percent Correct	Sample Classification	
		Wenatchee	*Okanogan*
Wenatchee	81	209	48
Okanogan	81	37	158
Composite Accuracy	81		

1. Results reported here were corrected by using a jackknife procedure. Uncorrected results are also reported in the text.

Although age 1.3 and 2.2 fish were found in both Okanogan and Wenatchee samples, their proportional abundance was quite different in each group. Fish of these age classes composed 0.27 of the Wenatchee stock sample size (Table 2), while they composed only 0.04 of the Okanogan-stock sample. As in 1988, we therefore classified all age 1.3 and 2.2 fish in the Bonneville Dam mixed stock sample as Wenatchee stock.

Age 1.1 and 2.1 fish were similarly treated. No specimens of either age 1.1 or 2.1 were estimated to be present in the Wenatchee stock sample, although specimens of these ages were present (0.01 and 0.01, respectively) in the Bonneville Dam sample (Table 1). Although the proportion of these fish was small in the Okanogan stock sample (0.04), previous studies and our earlier work have found one-ocean fish to be extremely rare in the Wenatchee system (Major and Craddock, 1962; Schwartzberg and Fryer, 1989). We therefore classified all age 1.1 and 2.1 fish in the Bonneville Dam mixed stock sample as Okanogan stock.

Combining the results of SPA classification of age 1.2 unknowns and classification of the remaining unknowns by age alone, we estimated the total mixed stock sample to be composed of 0.56 Wenatchee and 0.44 Okanogan stock (Table 4). Confidence intervals (0.90) for the Wenatchee and Okanogan stock estimates are 0.48, 0.64 and 0.36, 0.52; respectively (Table 5).

Mixed stock composition analysis was compared for the three study years (Figure 5). In 1989, the total mixed stock sample at Bonneville Dam was estimated to be 0.56 Wenatchee stock and 0.44 Okanogan stock. In 1988 classification was 0.69 Okanogan stock and 0.31 Wenatchee stock while in 1987 classification was 0.45 Wenatchee and 0.45 Okanogan stock, with the remaining 0.10 (age 1.3 and 2.2) considered to be of undetermined origin. The stock origin of age 1.2 sockeye in the mixed stock sample has varied dramatically over the past three years. In 1987, the discriminant analysis classified 0.64 of these fish as Wenatchee stock. In 1988 and 1989, the proportion of age 1.2 fish was 0.13 and 0.49, respectively.

Relationship of Migratory Timing to Stock and Age Composition

Weekly unknown mixed stock composition estimates (Table 5) show a significant relationship at the 0.10 significance level between migratory timing and stock

Table 5. Weekly stock composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1989.

Classification of only age 1.2 sockeye

Statistical Week[1]	Sample Size	Sample Classification		Standard Error
		Wenatchee	*Okanogan*	
24	37	0.60	0.40	0.13
25	87	0.53	0.47	0.09
26	143	0.48	0.52	0.07
27	105	0.52	0.48	0.08
28	43	0.35	0.65	0.12
29	13	0.23	0.77	0.19
Total Sample	**426**	**0.49**	**0.51**	**0.05**

Classification of all age sockeye

Statistical Week[1]	Sample Size	Sample Classification		Standard Error
		Wenatchee	*Okanogan*	
24	47	0.68	0.32	0.12
25	115	0.62	0.38	0.08
26	189	0.57	0.43	0.06
27	130	0.56	0.44	0.07
28	47	0.40	0.60	0.12
29	15	0.33	0.67	0.19
Total Sample	**543**	**0.56**	**0.44**	**0.05**

1. Statistical weeks are sequentially numbered calendar-year weeks. Excepting the first and last week of most years, weeks are seven days long, beginning on Sunday and ending on Saturday. In 1989, for example, Statistical Week 24 began on June 11 and Statistical Week 29 began on July 16.

Figure 5. Stock composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam 1987—1989.

1989

1987[1]

1988
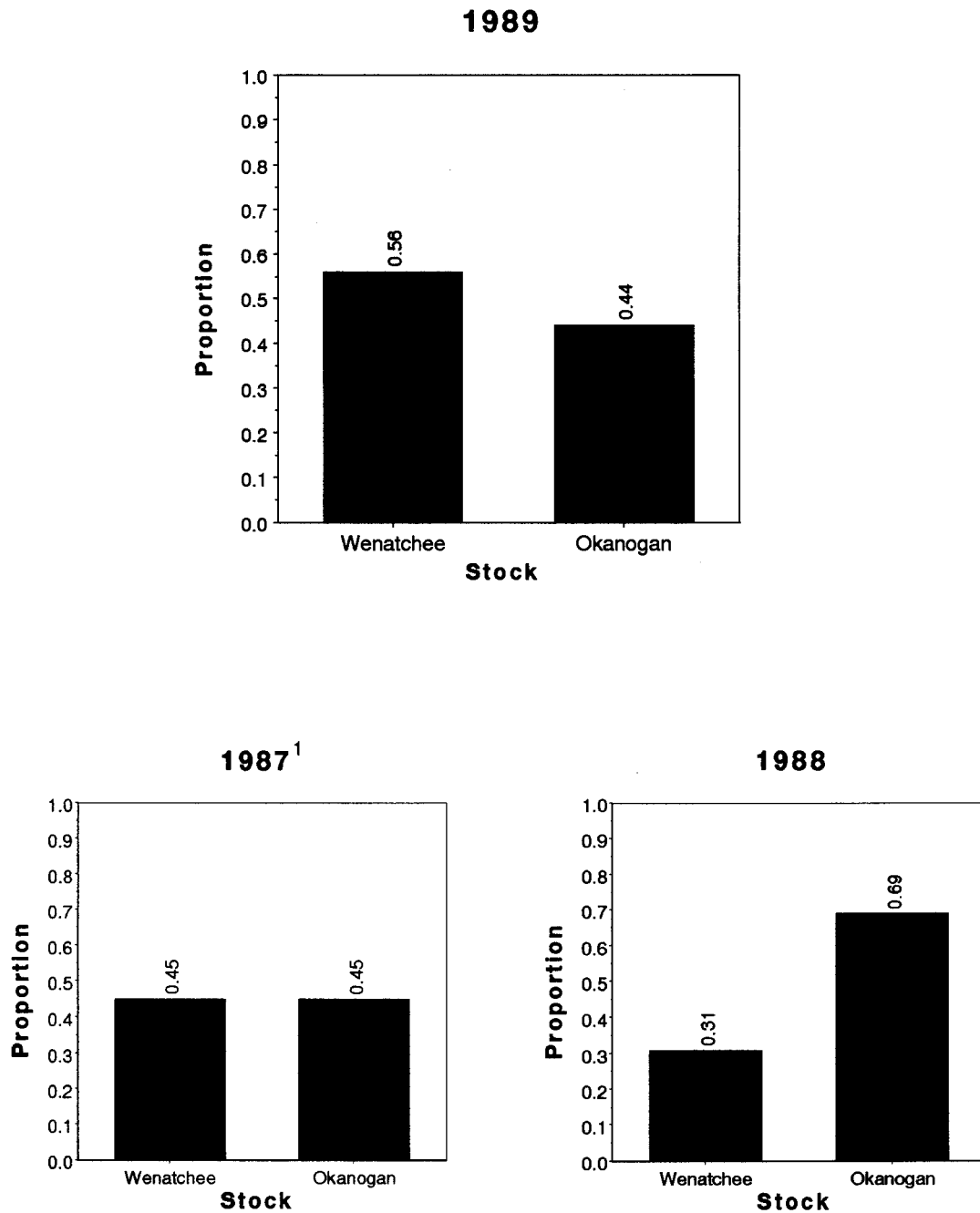
1. The origin of 0.10 of the sample could not be determined.

composition using a linear regression technique (p<0.01). These tests indicated that, in 1989, the relative proportion of Okanogan stock sockeye increased with time as the mixed stock migration progressed. Conversely, the relative proportion of Wenatchee stock sockeye decreased with time. Wenatchee stock sockeye tended to predominate early in the migratory period (Statistical weeks 24 through 27). Okanogan stock sockeye were more prevalent during the later stages of the migration (Statistical weeks 28 through 29). Comparisons to other years are not possible because 1989 was the first year that weekly stock composition was analyzed.

Age composition analyses using a similar linear regression technique indicated that the relative abundance of age 1.2 and 1.3 sockeye in the 1989 unknown mixed stock sample (Table 2) was related to migratory timing at the 0.10 significance level (p=0.06 and p<0.01, respectively). Age 1.3 sockeye were proportionally more numerous early in the migratory period (Statistical weeks 24 through 26). Age 1.2 sockeye were proportionally more numerous later in the migratory period (Statistical weeks 28 and 29). The relative abundance of age 1.2 sockeye in the Wenatchee known stock sample was also found to be related to migratory timing (p=0.02). The relative proportion of such fish increased significantly over the three weeks sampling was conducted. No other relationships between age and migratory timing could be detected at the 0.10 level of significance in either the mixed stock or known stock samples.

Visual Scale Classification

Known stock sample identification and separation, attempted strictly on the basis of the visual appearance of scale patterns, resulted in a 0.78 classification accuracy.

Consistency in Scale Feature Measurement

Results of the test for operator consistency in scale feature location yielded no significant differences in measurements of variables 1, 2, 3, or 4 (number of circuli from scale focus to freshwater annulus, distance from scale focus to freshwater annulus, number of circuli from focus to saltwater entry, and distance to saltwater entry, respectively). P-values ranged from 0.15 for the test of variable 1 to 0.42 for variable 3.

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DISCUSSION

This paper reports results from the third year of a Columbia Basin sockeye salmon stock identification research project (Schwartzberg and Fryer 1988, 1989). As in 1987 and 1988, Wenatchee and Okanogan stock fish were identified and separated with a reasonably high degree of accuracy by digitizing scales and using a linear discriminant analysis procedure. Our study also has indicated that reasonably accurate visual scale identification is possible.

The overall classification accuracy achieved in 1989 was 0.81, 0.05 lower than in 1988 and 0.10 lower than in 1987. While reasons for this have not yet been fully explored, an examination of between-year variation in focus-to-freshwater-annulus measurements was made for each known stock. For the Wenatchee stock, measurement of focus-to-freshwater-annulus growth averaged 0.10 more in 1989 than in 1988 while in the Okanogan stock, the measurement was only 0.01 greater in 1989 than in 1988. The 1989 results actually showed focus-to-freshwater-annulus measurements estimated to be 0.02 greater in the Wenatchee stock than in the Okanogan stock. This is the first time in three years that focus-to-freshwater distances were greater in the Wenatchee stock than in the Okanogan stock. In addition, Wenatchee stock focus-to-saltwater entry distance measurements averaged 0.34 more in 1989 than in 1988. This represents only 0.83 of Okanogan stock focus-to-saltwater-entry zone distance measurement. Nevertheless, variables based on inter-circuli spacing in these growth areas are key between-stock discriminators and relative changes in Wenatchee stock scale characteristics probably are the cause of an overall reduction in 1989 classification accuracy.

Although not used in final analyses, freshwater annulus and saltwater entry locations were again marked in the 1989 data acquisition process to permit a variety of tests. A test for consistency in scale feature measurement indicated that both freshwater annulus and saltwater entry position could be accurately located. Higher classification accuracies (0.86) were attained this year, as in 1988, in tests using variables containing positions of these subjectively determined scale features. Nevertheless, we felt it prudent to limit use of these variables in stock composition estimates, awaiting the results of further study.

Post-season analysis of the 1989 sample design showed that an averaged run timing prediction at both Bonneville and Wells dams (based on the averaged previous ten year's fish counts at each dam) differed relatively little from the actual 1989 run timings. Therefore, weekly sample sizes were predicted relatively accurately. Nevertheless, we used post-season timing data to weight sample sizes to correct for the small differences detected. Slight changes in total age and length-at-age composition estimates resulted from the application of this procedure. In contrast, a study of Columbia River spring chinook salmon sampled at Bonneville Dam showed a marked variation between predicted run timing (again using the averaged previous ten year's fish counts at Bonneville Dam) and actual run timing (Schwartzberg and Fryer 1990).

Age composition estimates of the mixed and Okanogan stocks were similar to those recorded in 1988. For mixed stocks sampled at Bonneville Dam, age 1.2 fish were again predominant comprising the highest proportion recorded in three years. Age 1.3 and 1.2 sockeye were found in significant proportions while only small proportions of age 1.1 and 2.1 fish were sampled. For the first time in our studies, age 2.3 sockeye were also recorded. Similar to 1988 results, virtually all Okanogan stock sockeye sampled at Wells Dam in 1989 were age 1.2. Small numbers of age 1.1 fish were recorded, unlike in 1988 when only one age 1.1 sockeye was observed or in 1987 when the Okanogan stock was predominantly age 1.1.

The 1989 Wenatchee stock sockeye sample was the only one in which age composition estimates differed significantly from 1988. In 1988, age 1.2, 2.2, and 1.3 sockeye were found in nearly equal proportions. In 1989, the Wenatchee stock composition was similar to that in 1987, being predominantly age 1.2 with significant proportions of age 2.2 and 1.3.

Where comparisons were possible among significant age classes, sockeye of similar ages were found to be consistently smaller in mean fork-length in 1989 than in 1988. This is a continuation of a trend observed in a 1988-1987 comparison. At Bonneville Dam, the average 1989-1988 inter-annual decrease in length for age 1.2, 1.3, and 2.2 sockeye was 1.1, 1.1, and 1.6 cm, respectively ($p < 0.01$ in all cases). In the Okanogan stock sample, the average difference for age 1.2 and 1.3 sockeye was 2.3 and 1.2 cm, respectively ($p < 0.01$ in both cases). In the Wenatchee stock sample, the average difference for age 1.2 and 2.2 sockeye was 1.5 and 0.3 cm, respectively ($p < 0.01$, $p = 0.20$; respectively). The only significant group showing an increase in length

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was Wenatchee age 1.3 sockeye which increased in size by only 0.2 cm (p=0.33). Length-at-age estimates for Columbia River spring chinook salmon, by comparison, have remained relatively stable over the last three years for four- and five-year-old fish (Schwartzberg and Fryer 1990). However, the average length of three-year-old spring chinook has declined over the past three years. No explanation is readily apparent for the general decline in sockeye length-at-age over the past three years.

Adult migrating salmon are counted at most mainstem Columbia and Snake river dams. Except for a remnant Snake River stock (two sockeye were counted at Lower Granite Dam in 1989), all Columbia Basin sockeye salmon pass Rock Island Dam (river km 729) but only Okanogan stock fish pass Rocky Reach Dam (river km 761). Using the difference between Rock Island and Rocky Reach dam counts as an estimate of the Wenatchee stock escapement, and using the Rocky Reach Dam count as an estimate of Okanogan stock escapement, 0.43 (16,175) of the 1989 escapement may be attributed to the Okanogan stock and 0.57 (21,185) to the Wenatchee stock. These proportions agree very closely with the results of our 1989 mixed stock classification estimate.

In 1989, a test was made to determine relationships between stock composition and migratory timing in the mixed stock unknown-origin sample. Wenatchee stock sockeye were estimated to predominate in the early part of the mixed stock migration (at Bonneville Dam) while Okanogan stock sockeye predominated during the latter stages of the migration. The relationship between migratory timing and stock composition was not analyzed in our previous studies but will be explored in future years. If a consistent and predictable relationship is found between stock composition and migratory timing, adjustment of future mainstem sockeye fisheries to harvest a greater proportion of a particular stock may be possible.

In 1989, sample sizes and quality improved dramatically from 1988 for the Wenatchee stock. Samples in 1988 were collected late in the run at Tumwater Dam resulting in fewer fish being sampled and scales being of poorer quality. The Okanogan stock sample from Wells Dam may have been slightly more respresentative this year than in 1988. In 1989, a screen with a small mesh size (5 cm) was added to the existing trap to prevent any smaller sockeye from avoiding the trap.

This research will be continued in 1990. Larger and more frequently collected samples of the mixed stock population at Bonneville Dam and the Wenatchee known stock at Tumwater Dam are expected in conjunction with a new summer chinook sampling program. Expanded sampling should allow for much better estimates of changes in stock and age composition over the migration period. At Tumwater Dam, results from a video counting system will be incorporated into our study. An understanding of run timing will allow more representative sampling procedures (similar in design to that employed in the Bonneville and Wells dam sampling programs). Work will continue towards the development of an accurate age, length-at-age, and stock composition data base for the Columbia Basin sockeye salmon population. This information will aid fisheries managers in predicting changes in population sizes and in the composition of stocks.

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Appendix A. Data handling and manipulation for scale pattern analysis.

During the scale data-acquisition process, information associated with each scale-data record is stored along with actual scale measurements in four separate fields of the data record *header* (Optical Pattern Recognition System, OPRS; Biosonics Inc. 1985). The sample number, denoted by the appropriate scale-card and the sample-position number (separated by a period) is recorded in the *sample id* field. Thus, sample number 3 on card number 1 would be recorded as 1.03. The estimated age of the fish from which the scale sample was taken is recorded in the *specimen id* field while the length, sex (M, F, or U), and stock (if known) are recorded in the field labeled *other*. In addition to this user-supplied information, the header includes system-supplied data including a sequential record number, the microscope magnification-conversion factor, a microscope lens-calibration factor, a record-validity indicator, the total number of circuli in the record, and the angle of the radial line used.

Once circuli measurements are made and a scale-data record is saved to the computer's hard disk, scale-measurement data cannot be further edited. The operator may, however, edit user-supplied header information. The desired record can be located and displayed (by using the OPRS *EDT* page and the *display data* command). Each record is displayed and contains header information and distance measurements from the scale focus to each circulus measured in *sampling units* (Figure A1). Measurements in sampling units can range from 1 to 700 and must be multiplied by the microscope magnification-conversion factor to determine metric distances from the scale focus to each marked circulus. Each circulus measurement is stored on a separate line.

To more effectively edit and prepare data for further statistical analysis, the *convert to ASCII* feature of the EDT page is used. The ASCII file this command creates can be manipulated by a program we have written that detects the extra markers in each record (marking freshwater annulus margin (anterior) and saltwater-entry point), converts scale-data measurements to actual metric distances, and stores this information in a more compact format (Figure A2). Using still another program, the information necessary to perform statistical analyses is extracted from this file and transferred to statistical software.

Figure A1. A sample OPRS data record for a single spring chinook salmon scale freshwater-growth-zone measurement[1].
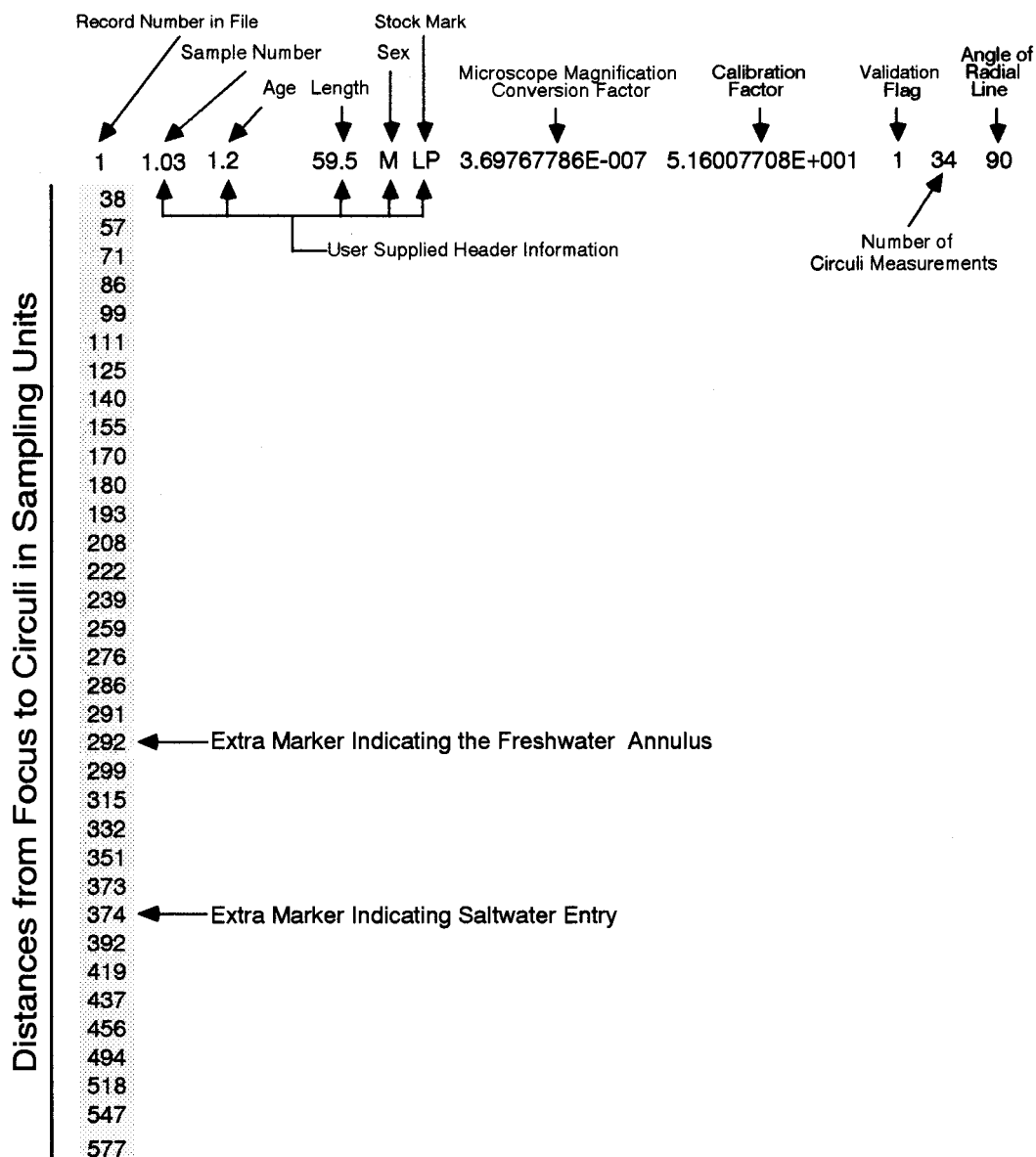
1. The data-acquisition process used for sockeye salmon scales was essentially the same as that used for spring chinook salmon scales.

Figure A2. **A sample compact-format data record for a single spring chinook salmon scale freshwater-growth-zone measurement[1].**

Sample Number
Sample Age
Sample Length (cm)
Stock Number
Number of Circuli
Distances from Focus to Circuli in Micrometers

1.03	1.2	59.5	2	32	1.40512	2.10768	2.62535	3.18000	3.66070
		4.10442	4.62210	5.17675	5.73140	6.28605	6.65582	7.13652	7.69117
		8.20884	8.83745	9.57697	10.20559	10.57536	10.76024	11.05606	11.64769
		12.27629	12.97885	13.79234	14.49490	15.49327	16.15885	16.86141	18.26653
		19.15397	20.22630	21.33560	19	24			

Number of Circuli from Focus to Freshwater Annulus

Number of Circuli from Focus to end of Freshwater Growth Zone

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1. The data-acquisition process used for sockeye salmon scales was essentially the same as that used for spring chinook salmon scales.