

**IDENTIFICATION OF COLUMBIA BASIN
SOCKEYE SALMON STOCKS BASED
ON SCALE PATTERN ANALYSES, 1990**

Technical Report 91-2

**Jeffrey K. Fryer
Matthew Schwartzberg**

March 25, 1991



**COLUMBIA RIVER INTER-TRIBAL FISH COMMISSION
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INTRODUCTION

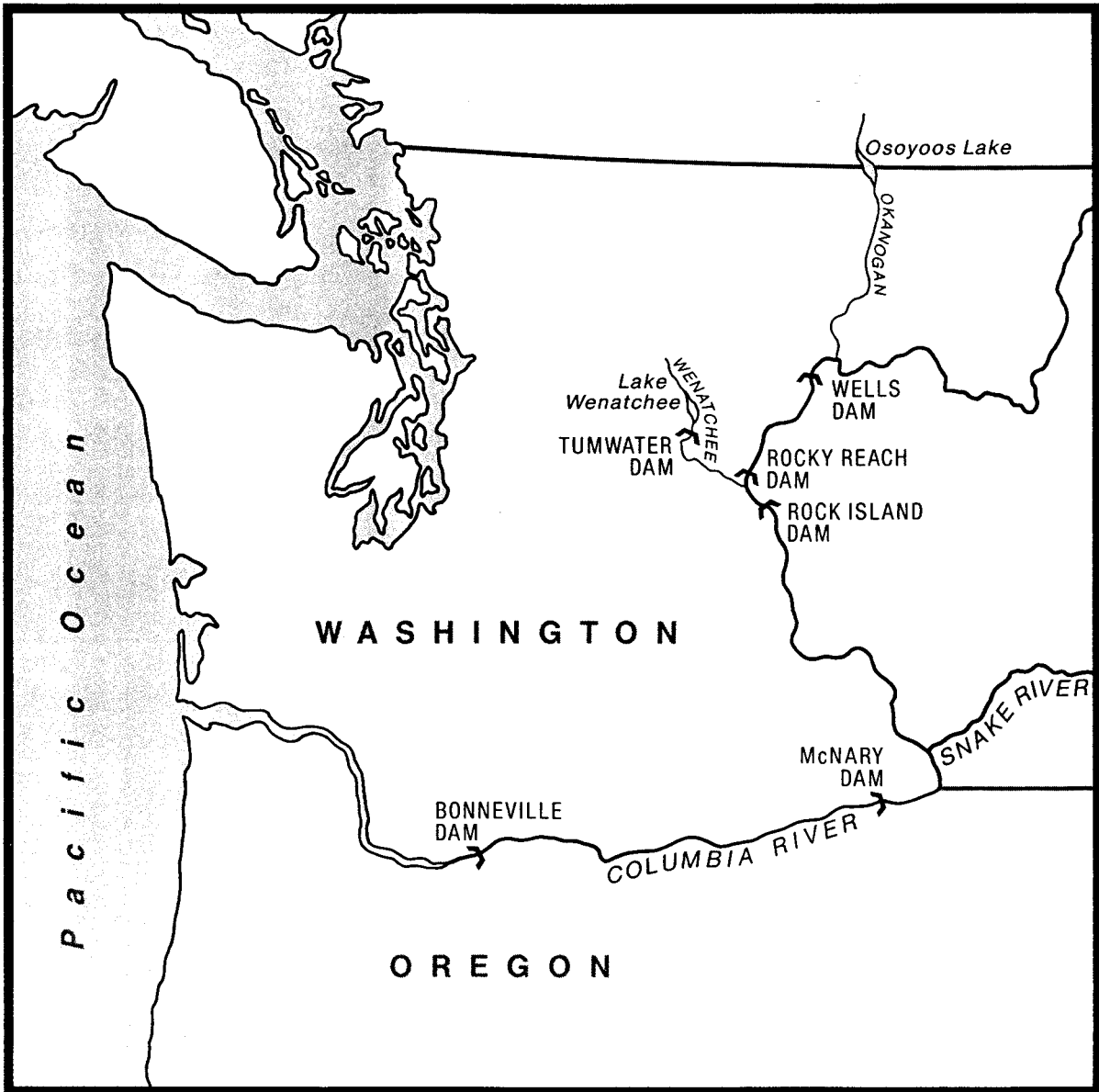
Sockeye salmon *Oncorhynchus nerka* (Walbaum) is one of the five species of Pacific salmon native to the Columbia River Basin. Before white settlers developed the region, it is estimated the Columbia Basin supported an annual sockeye salmon run averaging over four million fish (Northwest Power Planning Council 1986). Since the mid-1800s, however, this sockeye salmon population has severely declined. The estimated number of sockeye salmon entering the Columbia River over the ten year period from 1980 through 1989 has averaged only about 98,000 fish (King and McIsaac 1990). In both 1989 and 1990, fewer than 50,000 sockeye salmon returned to the Columbia Basin (CRITFC 1990).

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 development of discrete-stock approaches to Columbia River mainstem harvest management. The Pacific Salmon Treaty, ratified by the United States and Canada in 1985 (PST 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 salmon 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

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.



stocks of Columbia Basin salmon originating above Bonneville Dam. The collection and 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 began in 1985, and reports are available describing 1987, 1988, and 1989 results (Schwartzberg and Fryer 1988, 1989, 1990). In 1990, the study was expanded to include an additional age class in scale pattern analysis procedures. Changes in methods used to compute sample sizes were also made in 1990.

METHODS

Scale pattern analysis (SPA) is a well established stock identification and classification 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 temperature, 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 habitats (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 conditions.

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 km 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 salmon 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 length measurements, allowed to recover, and released. 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. Fork 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

Predetermined composite sample sizes for estimation of mixed and known stock age compositions were computed based on preferred levels of precision and accuracy ($d=0.05$, 90% c.i.). These estimates (Tables 1 and 2) were obtained using a method based on previous years' escapement estimates (Fryer 1991). Escapement estimates are made from fish ladder counts at hydroelectric dams in the Columbia Basin that, for a particular location, generally include visual observations of all upstream migrating salmon¹ (CRITFC 1990). Predetermined weekly sample sizes for Bonneville and Wells dam samples were derived from estimates of the average weekly proportion of the total annual migration during the previous ten years. The predetermined weekly sample size for the Tumwater Dam sample was derived using a similar methodology but based solely on 1989 escapement data (Hatch and Schwartzberg 1989) because a time series of escapement estimates was not available. All weekly age and length-at-age estimates were weighted post season using a stratified sampling method to account for deviations between estimated and actual 1990 migration. To improve the accuracy of weekly age and length-at-age composition estimates, a minimum weekly sample size of 50 was collected, when possible. The actual mixed stock (Bonneville Dam) composite sample size used for age composition estimation was 744 fish. The actual known stock composite sample sizes used for age composition estimation were 500 fish from Tumwater Dam and 406 from Wells Dam. Differences in desired and actual sample sizes used reflected over- or under-sampling in certain weeks and the rejection of poor quality scale samples.

1. An unknown and presumably small number of fish use navigation locks for upstream passage. Others that migrate during certain nighttime hours are not counted.

Table 1. Sample sizes for age composition estimates of Columbia Basin sockeye salmon sampled at Bonneville Dam in 1990.

Date	Statistical Week^a	Desired Number^b	Actual Number
6/14/90	24	50 ^c	4
6/18,21/90	25	158	139
6/25,28/90	26	198	173
7/03,05/90	27	158	184
7/09,12/90	28	73	123
7/16,18/90	29	50 ^c	94
7/23,25/90	30	50 ^c	27
Composite Sample		737^d	744^e

- a. 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 1990, for example, Statistical Week 24 began on June 10 and Statistical Week 30 began on July 22.
- b. Desired weekly sample proportions and numbers were based on the proportional weekly migration rate for previous years' sockeye salmon fish ladder counts at Bonneville Dam (ten year average, 1980-1989).
- c. A minimum weekly sample size of 50 per week was set to allow for more accurate weekly age composition estimates.
- d. The composite sample size was based on the number of samples necessary to obtain a population composition estimate ($d = 0.05$, 90% c.i.). Simulation techniques were used to calculate the composite sample size taking into account errors in run timing prediction (Fryer 1991). A 15% rate of unusable samples was assumed.
- e. Differences between the number of samples desired and the number of samples collected and analyzed reflect over- or under-sampling during certain weeks and the rejection of unusable scale samples.

Table 2. Sample sizes for age composition estimates of Wenatchee and Okanogan sockeye salmon stocks in 1990.

Wenatchee Stock			
Date	Statistical Week^a	Desired Number^b	Actual Number
7/26/90	30	270	159
8/01,02/90	31	289	274
8/09/90	32	101	45
8/15/90	33	59	22
Composite Sample		719^c	500^d

Okanogan Stock			
Date	Statistical Week^a	Desired Number^e	Actual Number
7/18/90	29	458	23
7/25/90	30	139	113
8/01/90	31	70	140
8/08/90	32	50 ^f	87
8/14/90	33	50 ^f	43
Composite Sample		757^c	406^d

- a. 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 1990, for example, Statistical Week 29 began on July 15 and Statistical Week 33 began on August 12.
- b. Desired weekly numbers were based on the proportional weekly migration rate for 1989 sockeye salmon fish ladder counts at Tumwater Dam.
- c. The composite sample size was based on the number of samples necessary to obtain a population composition estimate ($d = 0.05$, 90% c.i.). Simulation techniques were used to calculate the composite sample size taking into account errors in run timing prediction (Fryer 1991). A 15% rate of unusable samples was assumed.
- d. Differences between the number of samples desired and the number of samples collected and analyzed reflect over- or under-sampling during certain weeks and the rejection of unusable scale samples.
- e. Desired weekly numbers were based on the proportional weekly migration rate for previous years' sockeye salmon fish ladder counts at Wells Dam (ten year average, 1980-1989).
- f. A minimum weekly sample size of 50 per week was set to allow for more accurate weekly age composition estimates.

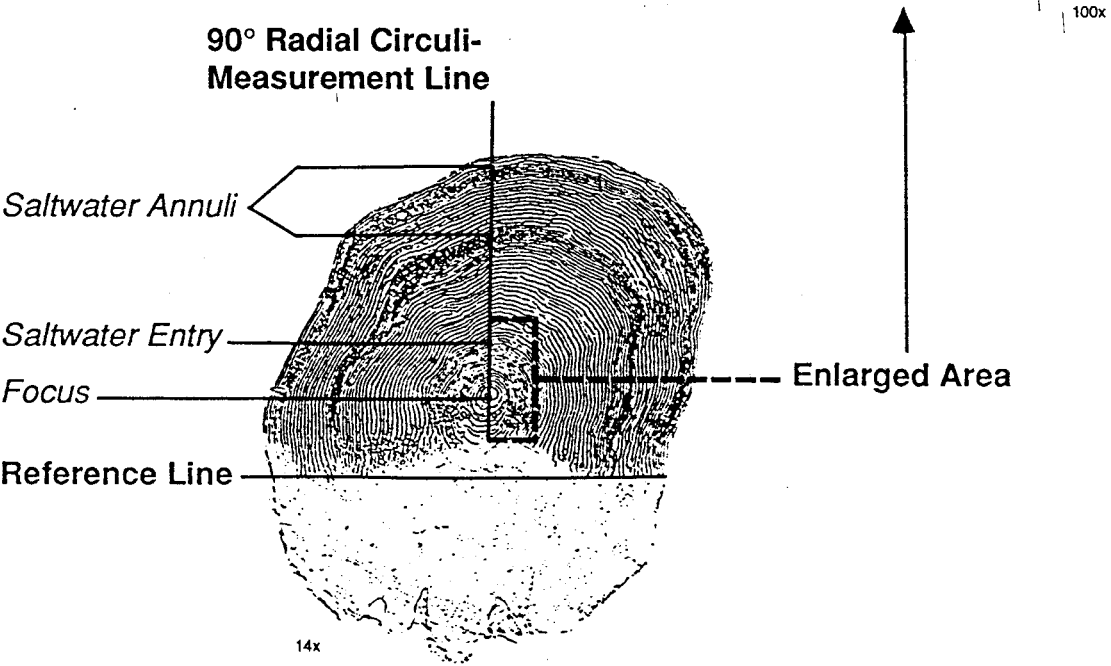
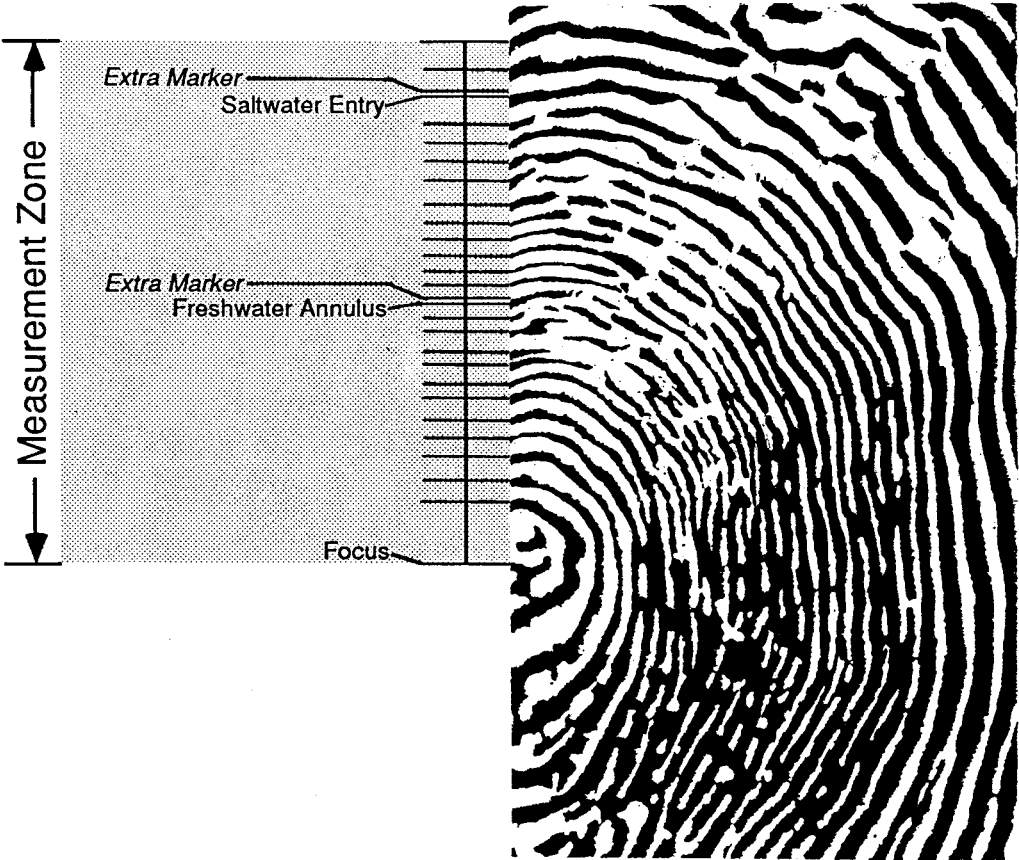
Desired sample sizes used for SPA studies were somewhat lower than those necessary for age composition estimation. For SPA studies, the desired sample size for each age group being examined was 200 from each known stock group (Conrad 1985). We therefore expected to subsample the larger known stock sample groups that were collected for age composition estimates. The Wenatchee stock subsample consisted of 219 systematically selected age 1.2 specimens (see the following section entitled 'Age Determination and Scale Measurements' for a description of the fish age notation used herein). However, because only 90 Okanogan stock age 1.2 scale specimens were collected, this group was not subsampled and all specimens collected were used in SPA tests. Although a minimum desired sample size of 100 is recommended for unknown groups in SPA studies, all 404 of the mixed stock (age 1.2) samples were used in the analysis to permit more precise weekly stock composition estimates. Minimum sample sizes were not obtained in any stock for age 1.3 scale sample classification. Nevertheless, age 1.3 specimens were used in the analysis and included 43 Okanogan stock, 46 Wenatchee stock, and 68 unknown stock samples. The use of sub-minimal sample sizes for age 1.3 fish would be expected to reduce precision in sample classification for this age group.

Age Determination and Scale Measurements

Salmon scales, under magnification, display numerous concentric rings (*circuli*) radiating 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 one 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.

Figure 2. Age 1.2 Okanogan stock sockeye salmon scale showing growth and measurement zones.



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 and age 1.3) samples from all stocks. SPA was not used to classify fish of age classes other than age 1.2 and age 1.3 in the mixed stock sample. Consistent with findings from previous years' research (Schwartzberg and Fryer 1988, 1989, 1990) we found that certain age classes were primarily present in only one of the two known stock samples. This relationship was used to classify mixed stock samples of certain 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, 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. Only scales with distinct foci and circuli were used in scale measurement work.

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 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 *singlets*, between three adjacent circuli, or *doublets*, between four adjacent circuli, or *triplets*, as well as measurements between five adjacent circuli, or *quadruplets* (Davis 1987). Distance measurements and number of circuli from scale focus to saltwater-entry and from scale focus to freshwater annulus margin (anterior) were among the variables tested.

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).

Accuracy of the discriminant analyses was determined by classifying the pooled known stock samples from a particular analysis and then comparing results to actual (verifiable) known stock identities. A jackknife procedure was employed to correct for systematically biased results that are created in known stock classification when the same samples are used 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 often occurring when the expected classification accuracy determined from known stock tests is not 100%, the method developed by Cook and Lord (1978) was employed. Variances on estimates were also computed (Pella and Robertson 1979). Stratified sampling techniques were used to make post-season corrections of stock composition estimates (Fryer 1991).

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. This relationship was tested by regressing mean weekly stock and age composition estimates against the statistical week weighted by the inverse of the variance for each estimate (Neter et. al 1985).

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

Although frequently included as variables in SPA research, the identification of such scale features as annulus position and point of saltwater entry may be a highly subjective process. In the final discriminant analysis models used for known and mixed stock classification, we chose not to employ variables dependent on the location of freshwater annulus and saltwater entry. However, we did test whether an operator could consistently and precisely locate these two scale features and to quantify the degree of

subjectivity inherent in their identification and measurement. The test was conducted on a subsample of 21 previously measured unknown mixed stock scales. These scales were remeasured with freshwater annulus and saltwater entry positions identified. A paired t-test was used to test within-specimen measurement differences. We also tested the effect of potential inconsistency of scale feature measurement on sample classification using SPA. The two groups of unknowns, consisting of the original and the remeasured data sets, were classified using previously developed linear discriminant analysis models and differences in classifications were noted.

RESULTS

Migratory Timing

Post-season analysis of the 1990 sample design disclosed significant time-related biases because the weekly proportions of the composite sample differed from actual 1990 migratory timing (Figure 3). The early part of the migration (Statistical weeks 25-26) at Bonneville Dam tended to be over-represented in the mixed stock sample while the later period (Statistical weeks 29-30) was under-represented. The early portion of the migration at both Tumwater and Wells dams was under-represented in the known-stock samples. This resulted in a bias in unadjusted composite sample age, length-at-age, and stock composition estimates. The effect of this bias was determined and results were subsequently adjusted to correct for it.

Age and Length-at-Age Composition

Mixed stocks of Columbia Basin sockeye salmon sampled at Bonneville Dam in 1990 were estimated to include fish of ages 1.1, 2.1, 1.2, 2.2, 1.3, and 2.3². The proportion of age 1.2 fish in the composite sample was 0.59 (Table 3). Age 1.1, 2.2, and 1.3 fish were found in significant proportions (0.15, 0.15, and 0.09, respectively). The proportion of age 2.1 fish in the sample was 0.01 while age 2.3 fish made up less than 0.01 of the sample.

The proportion of age 1.2 fish in the Wenatchee stock sample was 0.71 (Table 4). Age 2.2 and 1.3 fish were found in significant proportions (0.17 and 0.10, respectively). The proportion of age 2.3 fish in the sample was 0.02. No fish in the Wenatchee stock sample were of ages 1.1 or 2.1.

The proportion of age 1.1 fish in the Okanogan stock sample was 0.45. Age 1.2 and 1.3 fish were found in significant proportions (0.26 and 0.23, respectively). Age 2.2, 2.1, and 2.3 fish made up only small proportions of the sample (0.04, 0.02, and 0.01, respectively).

2. No known-age fish were present in the sample. Therefore, all scale age and length-at-age composition results reported could not be validated and must be considered estimates.

Figure 3. Time distribution of the composite sample with actual and predicted 1990 sockeye salmon migration timing at Bonneville, Tumwater, and Wells Dams.

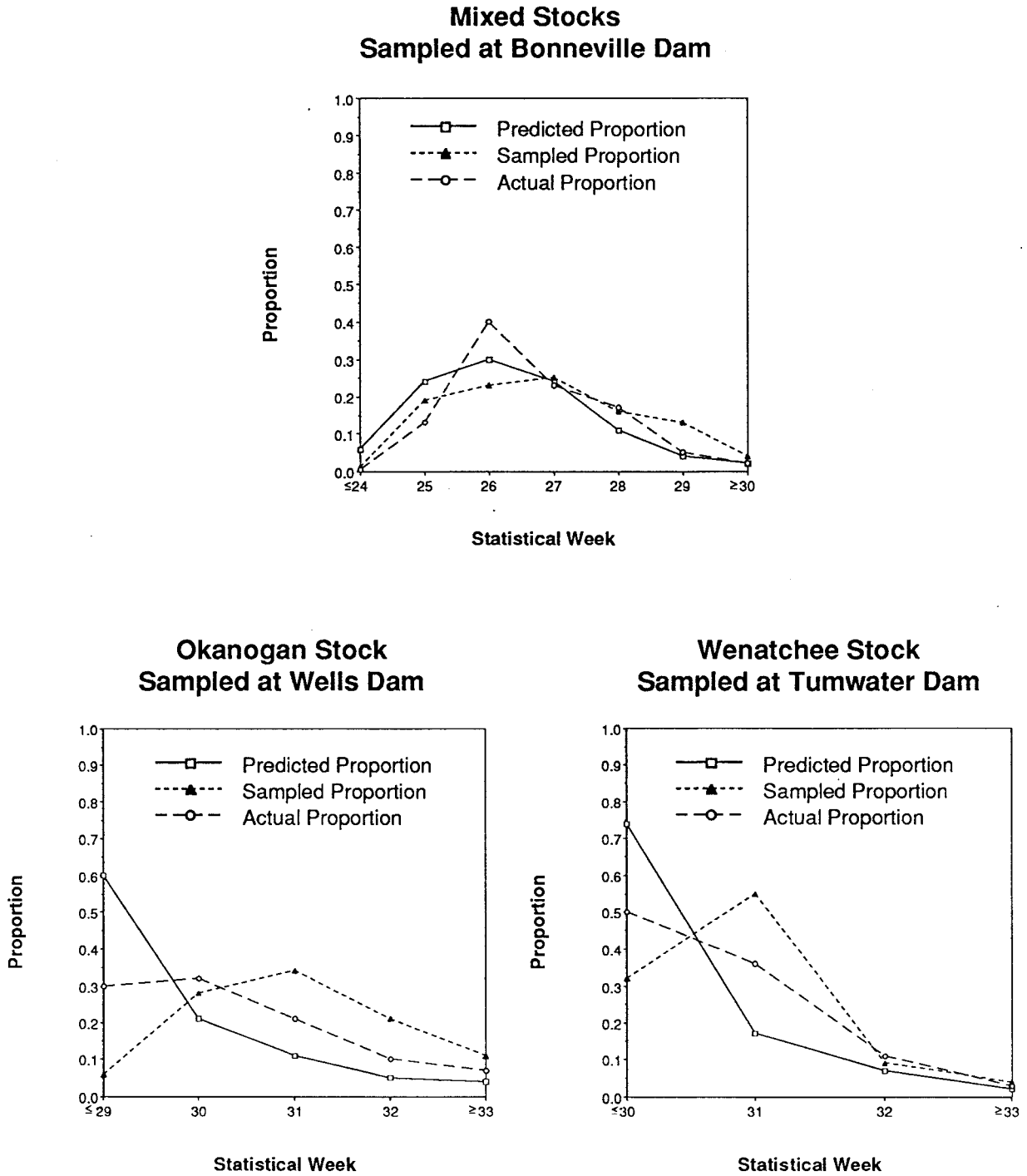


Table 3. Age composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1990.

Statistical Week ^a	Sample Size	Brood Year and Age Class					
		1987 1.1	1986 1.2 2.1		1985 1.3 2.2		1984 2.3
24	4	0.00	0.75	0.00	0.25	0.00	0.00
25	139	0.05	0.50	0.00	0.22	0.23	0.01
26	173	0.08	0.62	0.01	0.12	0.17	0.01
27	184	0.13	0.69	0.00	0.06	0.13	0.00
28	123	0.29	0.55	0.02	0.02	0.12	0.00
29	94	0.42	0.38	0.03	0.02	0.15	0.00
30	27	0.67	0.22	0.04	0.04	0.04	0.00
Composite Sample	744	0.15	0.59	0.01	0.09	0.15	<0.01

a. 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 1990, for example, Statistical Week 24 began on June 10 and Statistical Week 30 began on July 22.

Table 4. Age composition estimates of Wenatchee and Okanogan sockeye salmon stocks sampled in 1990.

Wenatchee stock

Brood Year and Age Class

Statistical Week ^a	Sample Size	1987	1986		1985		1984
		1.1	1.2	2.1	1.3	2.2	2.3
30	159	0.00	0.68	0.00	0.15	0.15	0.02
31	274	0.00	0.73	0.00	0.07	0.19	0.01
32	45	0.00	0.76	0.00	0.02	0.22	0.00
33	22	0.00	0.73	0.00	0.09	0.18	0.00
Composite Sample	500	0.00	0.71	0.00	0.10	0.17	0.02

Okanogan stock

Brood Year and Age Class

Statistical Week ^a	Sample Size	1987	1986		1985		1984
		1.1	1.2	2.1	1.3	2.2	2.3
27	23	0.13	0.30	0.00	0.52	0.04	0.00
28	113	0.51	0.25	0.03	0.16	0.04	0.01
29	140	0.66	0.22	0.04	0.04	0.02	0.01
30	87	0.63	0.29	0.01	0.03	0.03	0.00
31	43	0.65	0.23	0.02	0.09	0.00	0.00
Composite Sample	406	0.45	0.26	0.02	0.23	0.04	0.01

a. 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 1990, for example, Statistical Week 27 began on July 1 and Statistical Week 33 began on August 12.

Mean fork-lengths of the primary age component groups of the Bonneville Dam mixed stock sample were 37.6, 49.1, 50.0, and 54.9 cm for age groups 1.1, 1.2, 2.2, and 1.3, respectively. The 90% confidence intervals for these lengths were 34.7, 40.5; 45.9, 52.3; 46.4, 53.6; and 51.7, 58.0; respectively (Figure 4).

Mean fork-lengths of the primary age component groups of the Wenatchee stock sample were 49.3, 50.8, and 55.6 cm for age groups 1.2, 2.2, and 1.3, respectively. The 90% confidence intervals for these lengths were 46.0, 52.6; 47.2, 54.4; and 50.2, 60.9; respectively (Figure 5).

Mean fork-lengths of the primary age component groups of the Okanogan stock sample were 37.9, 50.1, and 57.2 for age groups 1.1, 1.2, and 1.3, respectively. The 90% confidence intervals for these lengths were 34.7, 41.1; 45.9, 54.3; and 54.2, 60.1; respectively (Figure 5).

Classification of Known Stock Samples

Among the variables tested (Table 5), those resulting in the highest classification accuracies were triplets (Group D). Combining these variables with those containing circuli number and distance measurements in focus-to-freshwater annulus and focus-to-saltwater entry (Group A) scale zones, relatively high stock classification accuracies were produced. To limit subjectivity in this study's data acquisition process we did not use those variables that were highly dependent on individual operator judgement. Therefore, variables directly related to freshwater annulus and saltwater entry positions were rejected from the final subset and classification models were exclusively based on triplet variables.

The final variable subsets selected consisted of three triplets for the classification model used for age 1.2 fish and one triplet for the classification model used for age 1.3 fish. In the variable testing process, doublets and singlets both resulted in approximately the same classification accuracies, but triplets were exclusively used to provide consistency with results from study years 1987 through 1989.

Figure 4. Length-at-age estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1990.

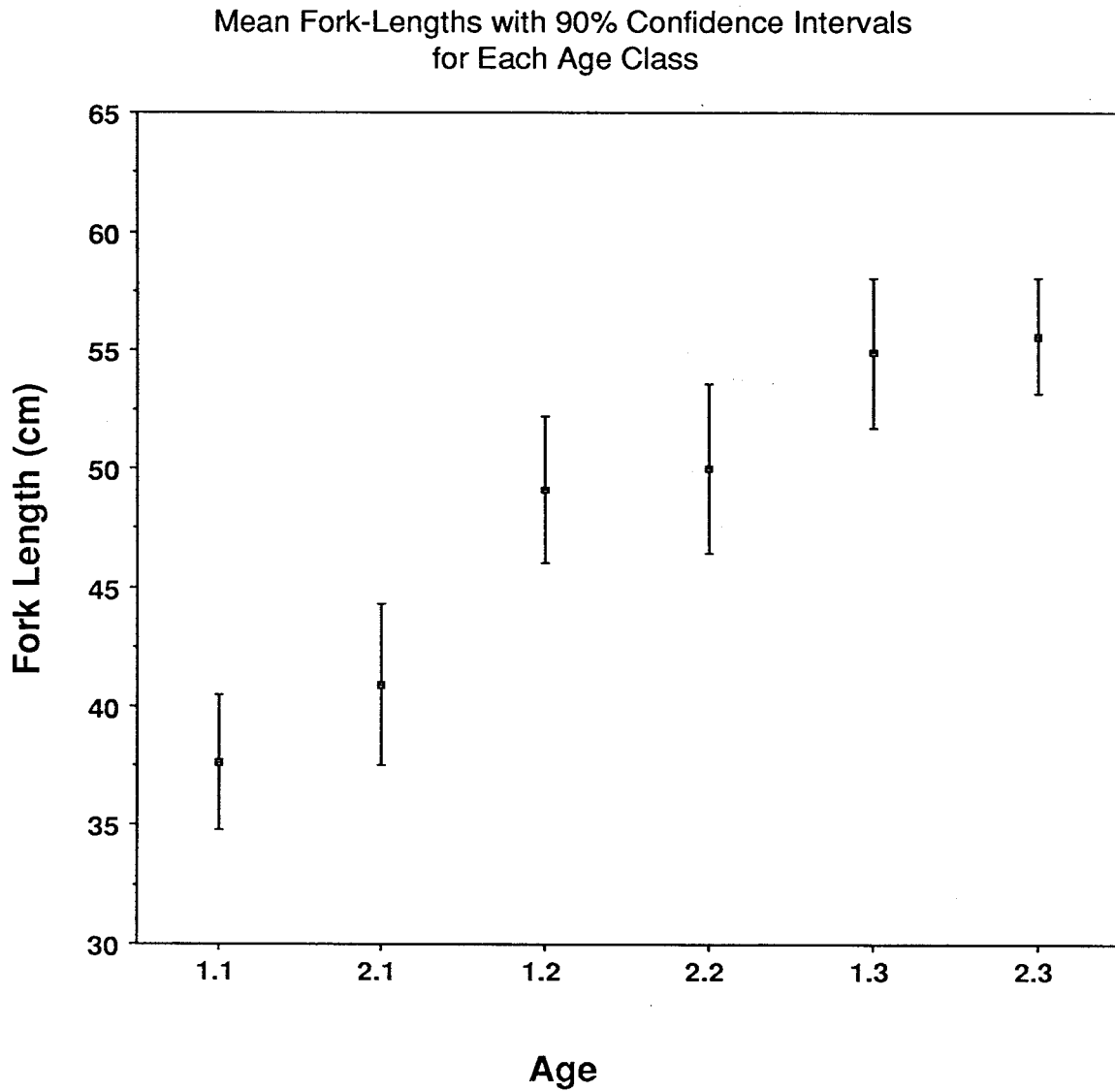
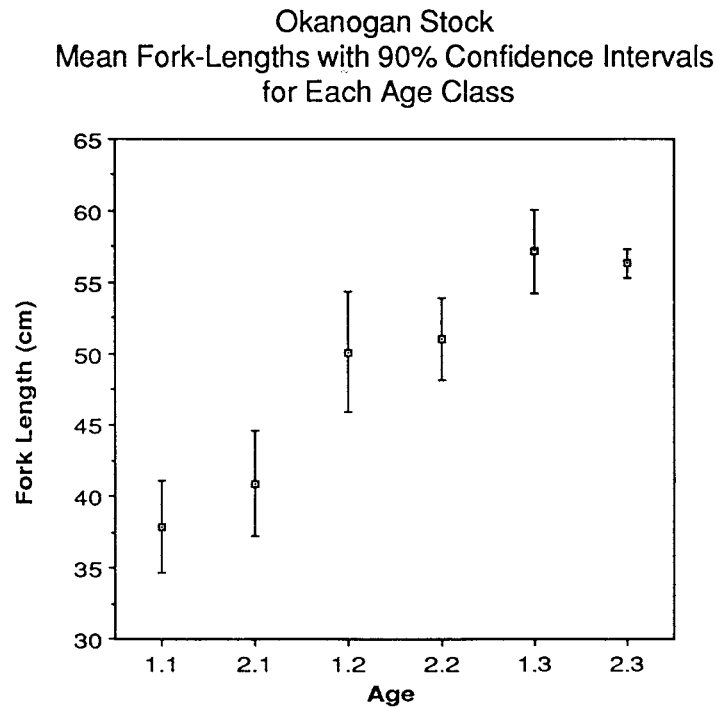
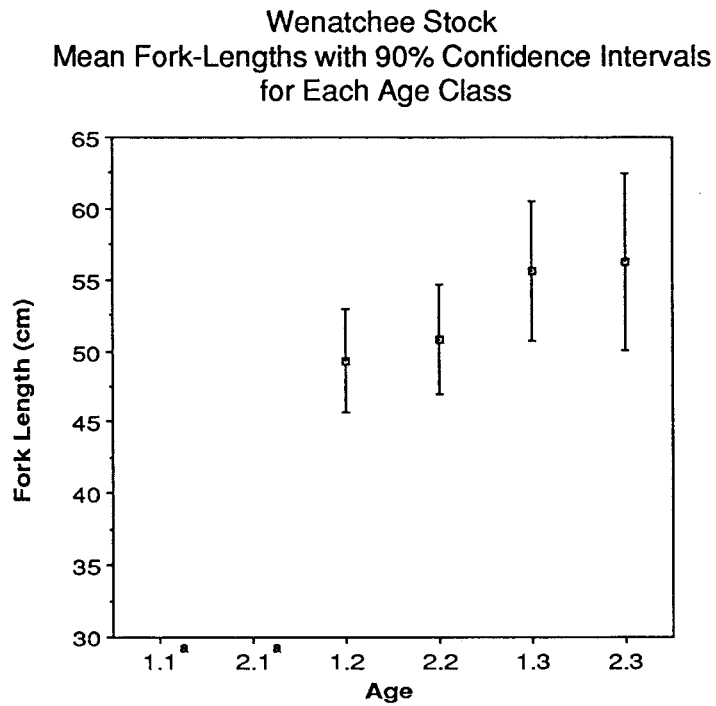


Figure 5. Length-at-age estimates of Wenatchee and Okanogan sockeye salmon stocks sampled in 1990.



a. No fish estimated to be of these age classes were sampled.

Table 5. Variables tested and used in discriminant analyses in 1990.

<u>Test Set</u>	<u>Group</u>	<u>Sets Used</u>		<u>Description of Variables</u>
		<u>Age 1.2</u>	<u>Age 1.3</u>	
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 circulus 24 to circulus 26
45	C			Distance from circulus 26 to circulus 28
46	D	x	x	Distance from focus to circulus 3
47	D			Distance from circulus 3 to circulus 6
48	D			Distance from circulus 6 to circulus 9
49	D			Distance from circulus 9 to circulus 12
50	D			Distance from circulus 12 to circulus 15
51	D	x		Distance from circulus 15 to circulus 18
52	D			Distance from circulus 18 to circulus 21
53	D	x		Distance from circulus 21 to circulus 24
54	D			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	E			Distance from circulus 9 to circulus 13

For classification of age 1.2 fish, the proportion of known stock samples accurately classified by the variable set containing only triplets was 0.65 (Table 6). A total of 75 Wenatchee samples were misclassified as Okanogan stock, while 30 Okanogan samples were misclassified as Wenatchee stock. Application of a jackknife procedure produced two additional Wenatchee and one additional Okanogan sample misclassifications. When Group A variables were included in the analysis, the proportion of age 1.2 known stock samples correctly classified was 0.80.

For classification of age 1.3 fish, the proportion of known stock samples accurately classified by the triplet variable set was 0.64. A total of 17 Wenatchee samples were misclassified as Okanogan stock, while 15 Okanogan samples were misclassified as Wenatchee stock. Application of a jackknife procedure produced no additional misclassifications. When Group A variables were included in the analysis, the proportion of age 1.3 known stock samples correctly classified was 0.89.

Classification of Unknown Mixed Stock Samples

Using the Bonneville Dam unknown origin mixed-stock samples, the proportion of age 1.2 fish classified by the linear discriminant function as Okanogan stock was 0.37. The remaining 0.63 of the samples were classified as Wenatchee stock (Table 6). Among age 1.3 unknown origin samples, the proportion classified as Okanogan stock was 0.41 and the remaining 0.59 was classified as Wenatchee stock. After correction for classification bias (Cook and Lord 1978) and migratory timing bias (Fryer 1991), the proportion of the age 1.2 sample classified as Wenatchee stock was 0.93 and the remaining 0.07 as Okanogan stock. Confidence intervals (90%) ranged from 0.00 to 0.23 for estimates of the Okanogan stock and from 0.77 to 1.00 for the Wenatchee stock estimates. The weekly estimated proportion of age 1.2 sockeye salmon of Wenatchee origin (Table 7) varied from 0.82 (Statistical Week 25) to 1.00 (Statistical weeks 26, 29, and 30). For classification estimates of the age 1.3 sample, classification and migratory timing biases nearly offset each other, resulting in a final classification estimate of 0.60 Wenatchee stock and 0.40 Okanogan stock. Confidence intervals (90%) ranged from 0.00 to 0.78 for the estimate of Okanogan stock and from 0.22 to 1.00 for the Wenatchee stock estimate. Weekly stock composition estimates for age 1.3 fish were not reported because mixed stock samples contained relatively small numbers of specimens in this age class.

Table 6. Known stock classification tests using linear discriminant analyses with age 1.2 and 1.3 Columbia Basin sockeye salmon stocks sampled in 1990.

Age 1.2 Known Stock Classification Test^a

Stock	Percent Correct	Sample Classification	
		<i>Wenatchee</i>	<i>Okanogan</i>
<i>Wenatchee</i>	64	139	77
<i>Okanogan</i>	65	31	59
Composite Accuracy	65		

Age 1.3 Known Stock Classification Test^a

Stock	Percent Correct	Sample Classification	
		<i>Wenatchee</i>	<i>Okanogan</i>
<i>Wenatchee</i>	61	26	17
<i>Okanogan</i>	68	15	32
Composite Accuracy	64		

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

Table 7. Stock composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam in 1990.

Classification of Only Age 1.2 Sockeye Salmon

Statistical Week ^a	Sample Size	Sample Classification		Standard Error
		<i>Wenatchee</i>	<i>Okanogan</i>	
25	66 ^b	0.82	0.18	0.22
26	107	1.00	0.00	0.19
27	125	0.88	0.12	0.18
28	65	0.85	0.15	0.22
29	35	1.00	0.00	0.29
30	6	1.00	0.00	0.31
Composite Sample	404	0.93	0.07	0.10

Classification of All Age Sockeye Salmon

Statistical Week ^a	Sample Size	Sample Classification		Standard Error
		<i>Wenatchee</i>	<i>Okanogan</i>	
25	143 ^b	0.86	0.14	0.13
26	173	0.86	0.14	0.05
27	184	0.75	0.25	0.04
28	123	0.60	0.40	0.05
29	94	0.54	0.46	0.06
30	27	0.27	0.73	0.09
Composite Sample	744	0.75	0.25	0.03

- a. 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 1990, for example, Statistical Week 25 began on June 17 and Statistical Week 30 began on July 22.
- b. Due to the very small number of fish sampled in Statistical Week 24, these fish were pooled with those sampled in Statistical Week 25 for these analyses.

Although age 2.2 fish were found in both Okanogan and Wenatchee stock samples, their proportional abundance was quite different in each group. Fish of this age class composed 0.17 of the Wenatchee stock sample size, while they composed only 0.04 of the Okanogan stock sample. Therefore, as in our 1988 and 1989 research, all age 2.2 fish in the Bonneville Dam mixed stock sample were classified as Wenatchee stock.

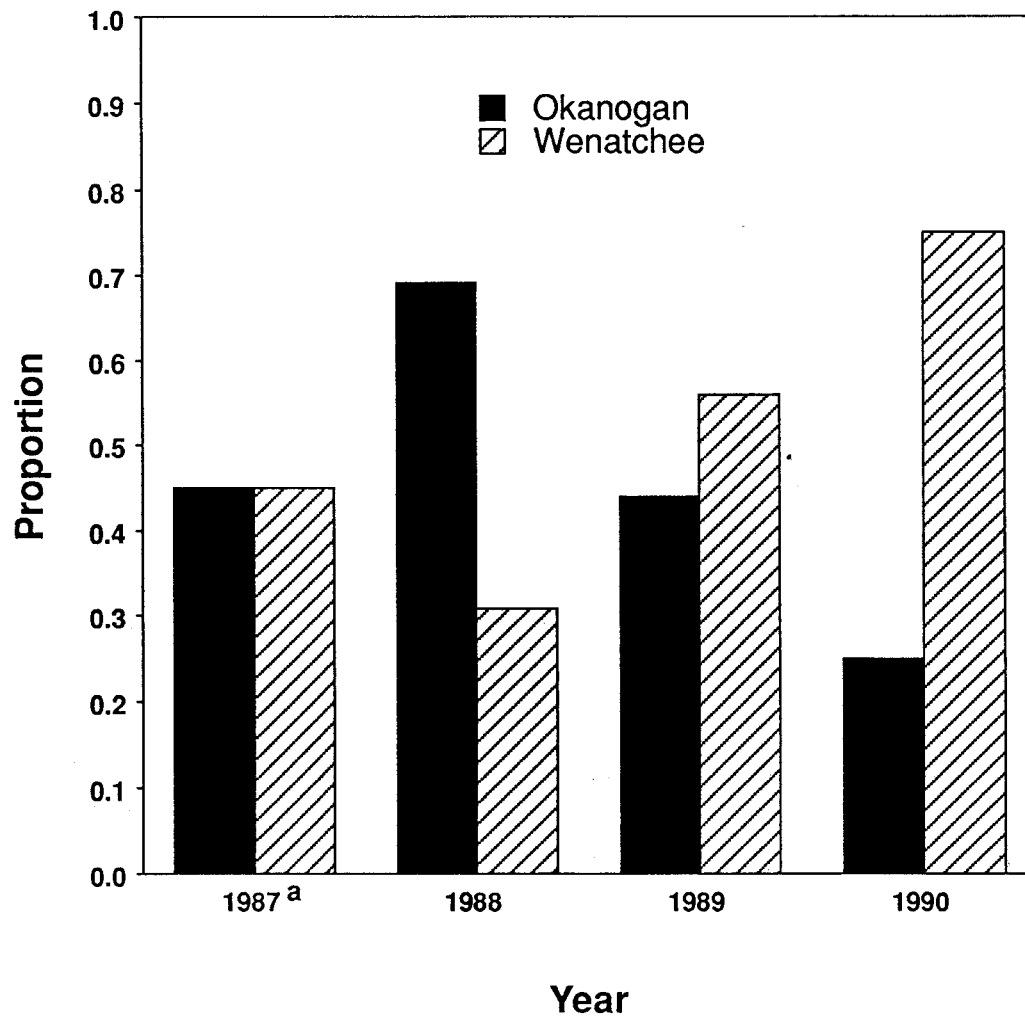
Age 1.1 and 2.1 fish were similarly treated. No specimens of either age 1.1 or 2.1 were estimated to be present in the Wenatchee stock sample, although specimens of these ages were present (0.15 and 0.01, respectively) in the Bonneville Dam sample. Fish of these age classes comprised 0.47 of the Okanogan known stock sample while none were found in the Wenatchee sample. Therefore, all age 1.1 and 2.1 fish in the Bonneville Dam mixed stock sample were classified as Okanogan stock.

Age 2.3 sockeye salmon made up a relatively insignificant proportion of each sample. In past years, age 2.3 sockeye salmon have not been found in the Okanogan sample. In 1990, only 0.006 of a relatively small Okanogan population was estimated to be of age 2.3 while 0.015 of a much larger Wenatchee population was estimated to be of age 2.3. Thus, all age 2.3 fish in the Bonneville Dam mixed stock sample (in actuality only 0.003 of that sample) were classified as Wenatchee stock.

Combining the results of SPA classification of age 1.2 and 1.3 unknowns (based on linear discriminant analyses) with the classification of the remaining unknowns (based on age alone), the proportion of the composite mixed stock sample estimated to be Wenatchee stock was 0.75 with the remaining 0.25 Okanogan stock (Table 7). Confidence intervals (90%) for the Wenatchee and Okanogan stock estimates are 0.71, 0.79 and 0.21, 0.29; respectively. Using the above stock composition estimates, individual stock escapement size estimates were made at Bonneville Dam. Of the 49,851 sockeye salmon recorded by Bonneville Dam visual fish counts, 37,186 were estimated to be Wenatchee origin and the remaining 12,463 Okanogan origin.

Mixed stock composition analysis was compared for the four study years (Figure 6). In 1989, the composite 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 of age 1.3 and 2.2

Figure 6. Stock composition estimates of Columbia Basin sockeye salmon stocks sampled at Bonneville Dam from 1987 through 1990.



a. In 1987, the origin of 0.10 of the sample could not be determined.

samples considered to be of undetermined origin). The stock origin of age 1.2 sockeye salmon in the mixed stock sample has varied dramatically over the past four years. In 1987, the proportion of fish classified by the discriminant analysis as Wenatchee stock was 0.64. In 1988 and 1989, the proportion of age 1.2 fish classified as being of Wenatchee origin was 0.13 and 0.49, respectively. In 1990, as reported above, 0.93 of the age 1.2 fish were classified as of Wenatchee origin.

Relationship of Migratory Timing to Stock and Age Composition

Weekly unknown mixed stock composition estimates (Table 7) show a significant relationship ($\alpha=0.10$) between migratory timing and stock composition using linear regression. These tests indicated that, in 1990, the relative proportion of Okanogan stock sockeye salmon increased with time as the mixed stock migration progressed. Conversely, the relative proportion of Wenatchee stock sockeye salmon decreased over the migratory period. Overall, however, Wenatchee stock fish predominated throughout the migratory period with the exception of Statistical Week 30.

Age composition analyses using a similar linear regression technique indicated that the relative abundance of age 1.1, 2.2, and 1.3 sockeye salmon in the 1990 unknown mixed stock sample (Table 3) was related to migratory timing ($\alpha=0.10$). Age 1.3 sockeye salmon were proportionally more numerous early in the migratory period. Proportions of age 1.1 fish increased as the migration progressed. No linear or quadratic relationship was found ($p>0.10$) between age 1.2 sockeye salmon abundance and mixed stock migratory timing at Bonneville Dam. Similarly, no linear relationship was found between any age class abundance and known stock migratory timing at either Tumwater or Wells Dam.

Visual Scale Classification

Sixty-nine percent of a subsample ($n=39$) of known stock origin was correctly identified strictly on the basis of the visual appearance of the scale patterns.

Consistency in Scale Feature Measurement

Using the two sets of scale measurements made from a subsample of unknown mixed stock scales, the t-test comparing the measurements of number of circuli and distance from scale focus to saltwater entry found significant differences ($\alpha=0.10$). No significant difference was found in the number of circuli and distance from scale focus to freshwater annulus ($p>0.10$). Due to the relatively large numbers of scales remeasured (21), these paired t-tests were very powerful, capable of detecting a difference of one circulus at $\alpha = 0.10$ and $\beta = 0.99$.

The subsample of 21 unknown mixed stock age 1.2 scale samples classified differently after the scales in the subsample were remeasured. Using a model consisting solely of Group D triplet variables (Table 5), 11 of 21 were originally classified as being of Wenatchee stock with the remaining 10 being classified as Okanogan stock. After this subsample was remeasured, the classification of four samples changed, resulting in 9 out of the 21 being classified as Wenatchee stock with the remaining 12 being classified as Okanogan stock. Using a model consisting of Group D plus Group A variables, 12 of the 21 samples were originally classified as Wenatchee stock while the remaining 9 were classified as Okanogan stock. After remeasuring scales in this subsample, the classification of five samples changed, resulting in 15 out of the 21 samples being classified as Wenatchee stock and the remaining 6 classified as Okanogan stock.

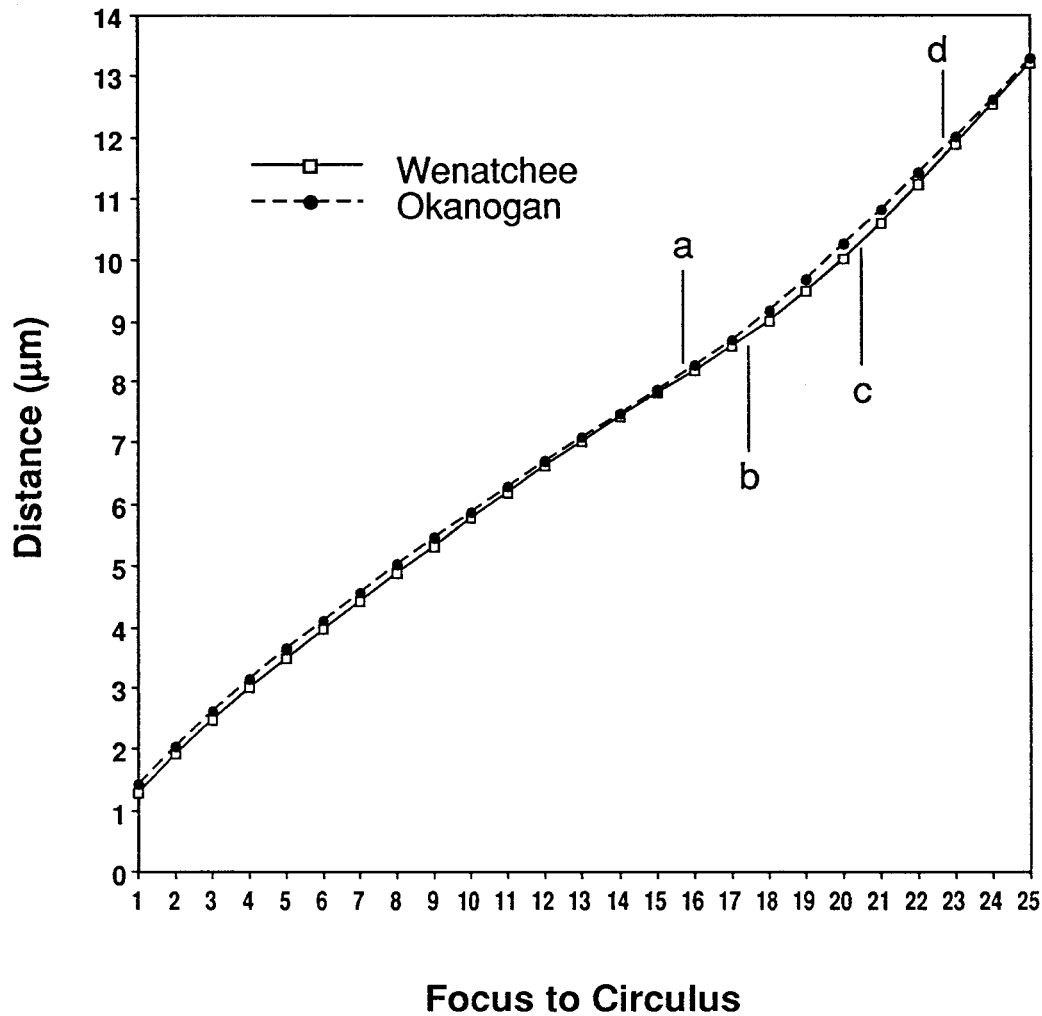
DISCUSSION

This paper reports results from the fourth year of a Columbia Basin sockeye salmon stock identification research project (Schwartzberg and Fryer 1988, 1989, 1990). Wenatchee and Okanogan stock fish were identified using a linear discriminant analysis procedure. However, classification accuracy in 1990 was significantly lower than in previous study years 1987 through 1989. The proportion accurately classified has decreased from 0.91 in 1987, to 0.86 in 1988, to 0.81 in 1989, and to 0.65 in 1990.

It is likely that the low classification accuracies achieved in 1990 were due to similar growth patterns in Okanogan and Wenatchee stock fish as reflected in scale samples. There appeared to be little between-stock difference in mean scale-circuli measurements in 1990 (Figure 7). Between-stock differences in the mean distance from scale focus to freshwater annulus were only seven percent in 1990. Since variables based on inter-circuli spacing in this growth area are key between-stock discriminators, similarities in measurements were probable reasons for low 1990 classification accuracies.

Throughout the course of this study, we have been concerned about biases resulting from excessive operator subjectivity inherent in Group A variable location (freshwater annulus and saltwater entry locations). We excluded Group A variables from final classification analyses to eliminate this potential bias but performed tests to study the presence and nature of the error. One indirect test of the accuracy of Group A variable location resulted from the observation that much higher classification accuracies were obtained with inclusion of Group A variables in classification analyses. These higher classification accuracies may be indirect evidence of the bias inherent in their use. In previous study years, mean distances from scale focus to freshwater annulus and to saltwater entry were usually smaller in Wenatchee stock scale samples than in Okanogan stock scale samples. Since the origin of the 1990 known stock scale samples was known to the operator before measurement, it is possible that location of these scale features may have been affected by predetermined assumptions. If such a bias did in fact occur, the result would be maximization of between-stock differences. Inclusion of such potentially biased variables in a variable set submitted to a stepwise discriminant analysis, because of the nature of the stepwise procedure, would likely result in a final model made up primarily, if not exclusively, of Group A variables.

Figure 7. Similarities in 1990 known stock circuli measurements as measured from scale focus to circulus 1 through 25.



- a. Estimated mean freshwater annulus position for Okanogan stock scale samples.
- b. Estimated mean freshwater annulus position for Wenatchee stock scale samples.
- c. Estimated mean saltwater entry position for Wenatchee stock scale samples.
- d. Estimated mean saltwater entry position for Okanogan stock scale samples.

Although potentially biased, such a model would be expected to classify known stock scale samples with a much higher degree of accuracy. Further compounding the potential bias, classification of mixed stock samples would also depend on the Group A variable location in those mixed stock samples.

We believe it is this bias in operator location of Group A variables that explains our observations regarding the effects of these variables on classification analyses. When Group A variables were included in the test variable set, all Group B through E variables were excluded from the final classification model by the stepwise procedure. These models had much higher known stock classification accuracies than models excluding Group A variables. We also noted that the use of Group A variables resulted in significant changes in the classification of mixed stock samples. For example, using variables exclusively from Group D, the proportion of the Bonneville Dam mixed stock sample classified as Okanogan stock was 0.07 and the remaining 0.93 as Wenatchee stock. Addition of Group A variables to the classification model (based on freshwater annulus location) resulted in the proportion of the unknown stock sample, formerly classified as Okanogan stock, changing to 0.56 with the remaining 0.44 classified as Wenatchee stock. If all Group A variables (based on both freshwater annulus and saltwater entry location) were added to the classification model, 0.30 of the unknown origin sample was classified as Okanogan and the remaining 0.70 as Wenatchee stock.

In addition to our concern about the ability of operators to accurately determine the absolute location of Group A variables, we wanted to test operator precision (or consistency) in Group A variable location. The test for consistency in scale feature measurement indicated that the freshwater annulus could be consistently located while saltwater entry position could not be consistently located. However, the power of this test was quite high. Furthermore, what effects these potential inaccuracies and biases in the location of Group A variables may have on SPA were not known. We hope to conduct further research in this area.

For the first time in the four years of this study (1987-1990), four age classes (1.1, 1.2, 1.3, and 2.2) were estimated to be present in significant numbers at Bonneville Dam. Most notable in 1990 was the large proportion of age 1.1 fish. Similar to findings in previous years, all fish of this age class were believed to be destined for the Okanogan Basin.

Where comparisons were possible among significant age classes, sockeye salmon of similar ages sampled at Bonneville Dam were found to be consistently smaller in mean fork-length in 1990 than in 1989. This is a continuation of a trend observed in comparisons made between 1988-1989 and 1987-1988. Comparing mean fork-length measurements for the 1989 and 1990 Bonneville Dam mixed stock samples, the inter-annual decrease in length for age 1.2, 1.3, and 2.2 sockeye salmon was 0.5, 3.3, and 0.3 cm, respectively. In the Wenatchee stock sample, the mean length decrease for age 1.2, 1.3, and 2.2 fish was 1.6, 1.7, and 0.7 cm, respectively. In the Okanogan stock samples, the mean length decrease of age 1.2 fish was 0.7 cm. Okanogan age 1.3 sockeye salmon was the only sample group of significant size that exhibited an inter-annual length increase. Between 1989 and 1990, this sample group increased in length by 0.7 cm. Length-at-age estimates for Columbia River spring chinook salmon, by comparison, have remained relatively stable over the past four years for four- and five-year-old fish (Fryer and Schwartzberg 1991). However, the average length of three-year-old spring chinook salmon has decreased over the past three years. No explanation is readily apparent for the general decrease in sockeye salmon length-at-age over the past three years.

Adult migrating salmon are counted visually in fish ladders at most mainstem Columbia and Snake river dams. Except for a remnant Snake River stock (no sockeye salmon were counted at Lower Granite Dam in 1990 and only two were counted in 1989), all Columbia Basin sockeye salmon pass Rock Island Dam (river km 729). 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, 0.26 (9,296) of the 1990 escapement estimate at Rocky Reach Dam may be attributed to the Okanogan stock and 0.74 (34,846) to the Wenatchee stock. These proportions agree relatively closely with the results of our 1990 mixed stock classification estimate at Bonneville Dam (0.25, 0.75; Table 7).

The number of sockeye salmon counted at Wells Dam in 1990 (7,957, including approximately 349 fish transported around Wells Dam as part of this study and not included in visual fish count records) was the second lowest total in over 40 years. This followed a relatively low escapement estimate of 15,976 for the 1989 migratory population. In addition, a large proportion of the sockeye salmon we sampled at Wells Dam in 1990 appeared to be in poor condition, exhibiting scale loss and body wounds.

The difference in 1990 sockeye salmon counts between Rocky Reach and Wells Dams (located 62 km apart) indicated that the proportion of sockeye salmon passing Rocky Reach which failed to pass Wells Dam was 0.14. This differential was the highest since 1977 and far exceeded the 24 year average of 0.05 recorded since Wells Dam was constructed (CRITFC 1990). It is likely that the major difference between the two dam counts represents inter-dam mortality, although a small number of sockeye salmon are occasionally reported in areas between Rocky Reach and Wells dams such as in the Entiat River or at the base of Chelan Falls (Mullan 1986). Comparing the Wells Dam escapement estimate with our estimate of the number of Okanogan stock fish in the Bonneville Dam migratory population (12,463), the estimated mortality rate for this stock was 0.36. By comparison, the estimated number of Wenatchee stock sockeye salmon passing Tumwater Dam on the Wenatchee River was 34,163³ (Hatch and Schwartzberg 1991). A Bonneville-to-Tumwater Dam mortality rate of only 0.08 was estimated for this stock. The number of sockeye salmon that actually survived to reach the spawning grounds in either the Okanogan or Wenatchee rivers is unknown.

In 1990, a test was made to determine relationships between stock composition and migratory timing in the mixed stock unknown-origin sample. Wenatchee stock sockeye salmon were estimated to predominate in all except the very end of the mixed stock migratory period at Bonneville Dam. The proportion of Okanogan stock sockeye salmon steadily increased through the migratory period. This is the second consecutive year that such a relationship between migratory timing and stock composition has been examined and discovered. If a consistent and predictable relationship is found between stock composition and migratory timing, adjustment of potential future mainstem Columbia River sockeye salmon fisheries may be possible to permit harvest of a greater proportion of a particular stock.

This research will be continued in 1991. It is hoped that additional work can be conducted to determine the level of accuracy required in the location of life history variables before they can be successfully included in classification models. Work will continue to develop of an accurate age, length-at-age, and stock composition database for the Columbia Basin sockeye salmon population. This information will aid fisheries managers in predicting changes in population sizes and in the stock composition of the sockeye salmon population.

3. Tumwater Dam video fish counts, unlike the Bonneville Dam fish ladder counts, include estimates of nighttime fish passage. If nighttime passage at Tumwater Dam is factored out of the escapement estimate, the estimated Bonneville-to-Tumwater Dam mortality rate is 0.15.

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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. This command creates an ASCII file that 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 sockeye salmon scale freshwater-growth-zone measurement.

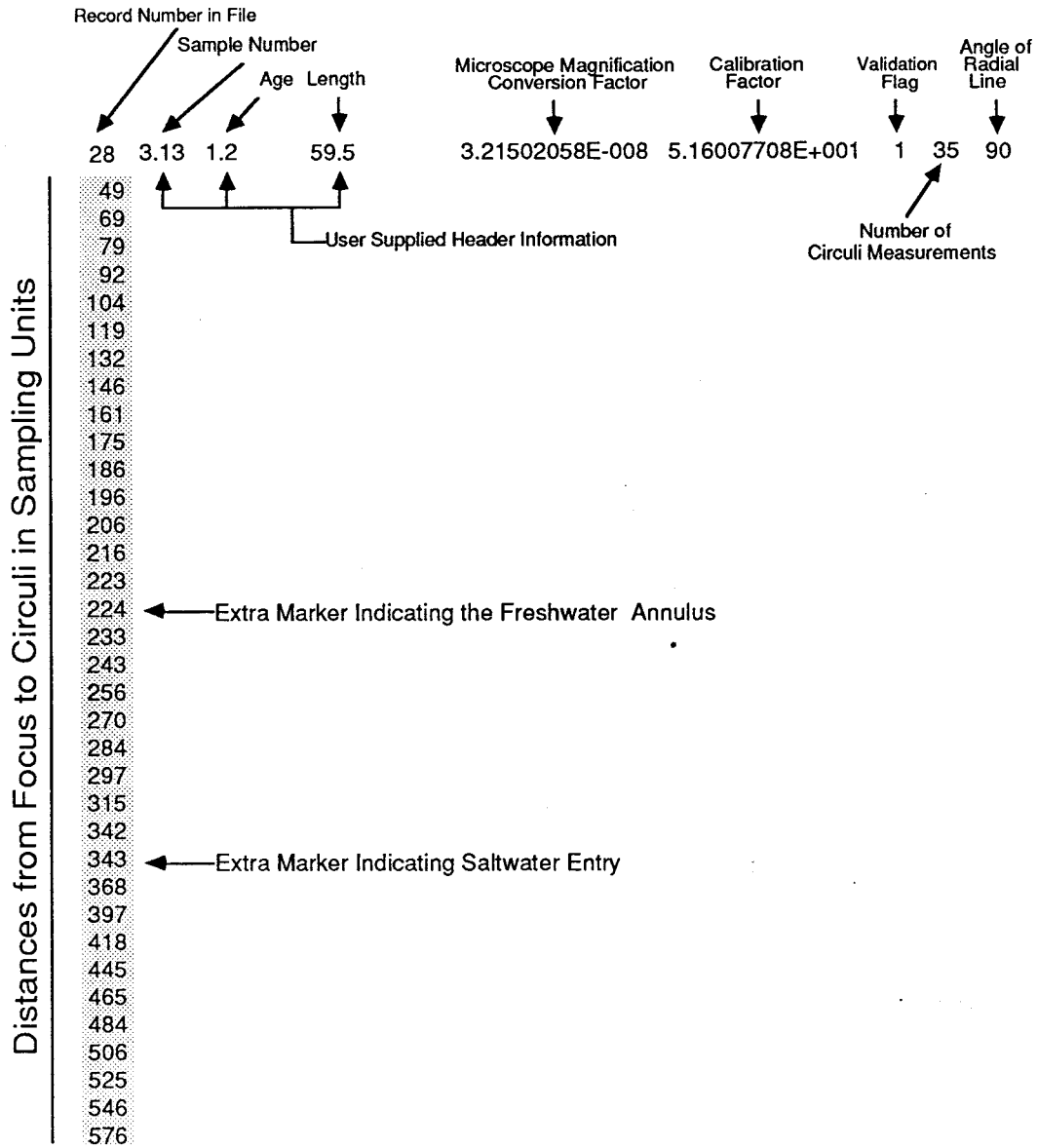


Figure A2. A sample compact-format data record for a single sockeye salmon scale freshwater-growth-zone measurement.

