EXPERIMENTS IN IDENTIFYING HATCHERY AND NATURALLY SPAWNING STOCKS OF COLUMBIA BASIN SPRING CHINOOK SALMON USING SCALE PATTERN ANALYSES

Technical Report 90-3

Jeffrey Fryer
Matthew Schwartzberg

April 2, 1990

COLUMBIA RIVER INTER-TRIBAL FISH COMMISSION
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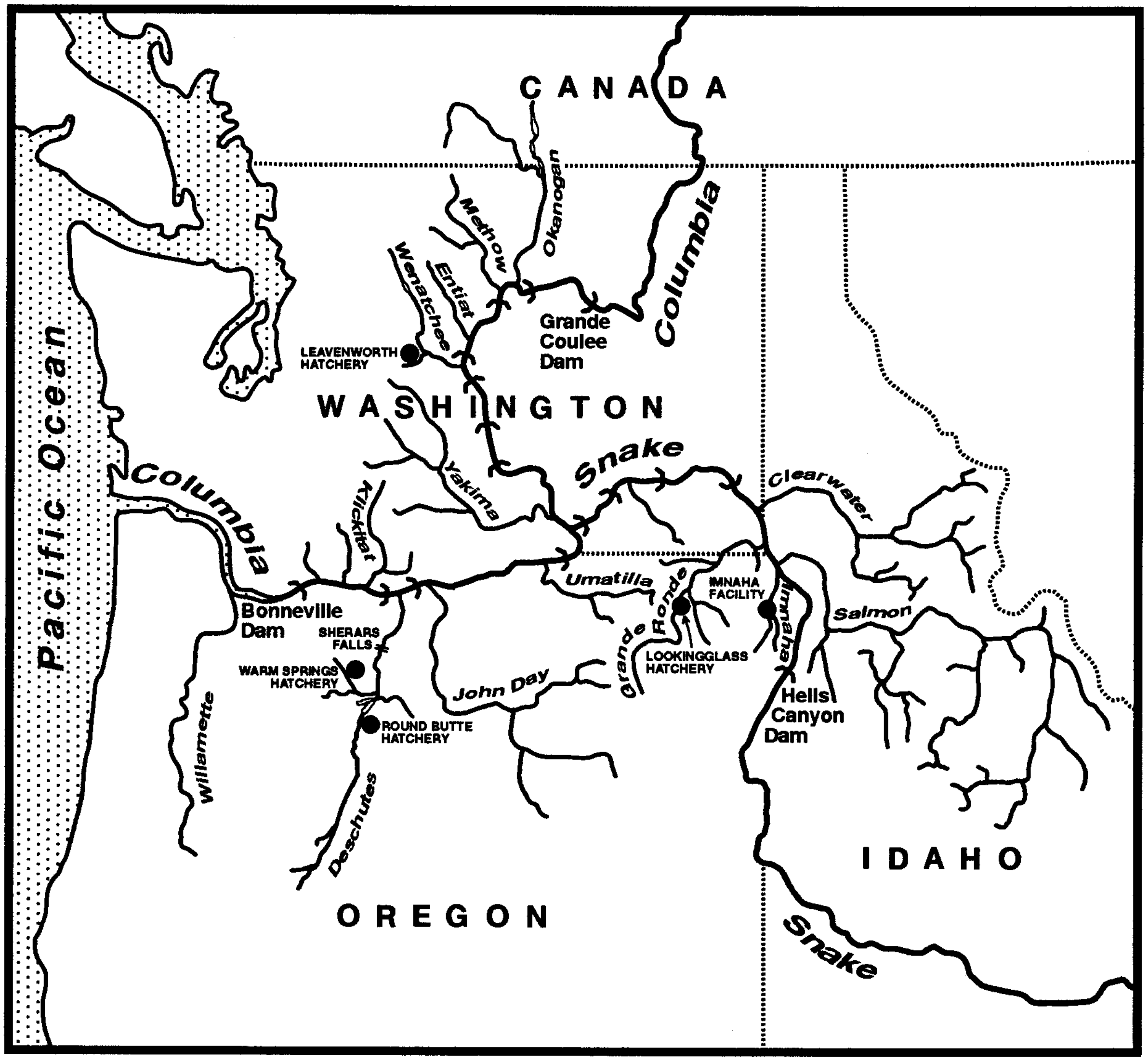
INTRODUCTION

The Columbia River Basin (Figure 1) once supported the largest population of chinook salmon *Oncorhynchus tshawytscha* (Walbaum) in the world (Fulton 1968). Based on early harvest records, estimates have been made of annual runs averaging over nine million fish (Northwest Power Planning Council [NPPC] 1986). In recent years (1979-1988), runs have averaged only about 835,000 fish (Oregon Department of Fish and Wildlife [ODFW] and Washington Department of Fisheries [WDF] 1989), with a majority of these being hatchery produced. Biologists recognize three races (spring, summer, and fall) and two major geographic groupings of chinook salmon in the Columbia Basin. These geographic groups are known as *upriver,* composed of fish originating upstream of Bonneville Dam (river km 225), and *lower river,* composed of fish originating downstream of Bonneville Dam. Historically one of the most important Columbia Basin salmon to Indian tribes and early white settlers of the region, upriver spring chinook salmon runs once may have averaged nearly two million fish per year (NPPC 1986). Annual runs over the ten year period from 1979 to 1988 have averaged only about 81,000 fish per year (Columbia River Inter-Tribal Fish Commission [CRITFC] 1989).

Because salmon return to their natal streams (or hatcheries) to spawn, different discrete groups or *stocks* have evolved, generally based on area of origin (Scheer 1939, Rich 1939). The Pacific Salmon Treaty, ratified by the United States and Canada in 1985 (Pacific Salmon Treaty 1985), requires that certain Pacific salmon stocks be monitored to determine the influence of Treaty-imposed ocean harvest regulations. A monitoring program was established containing provisions for estimating the escapement and status of key Columbia Basin salmon stocks. Columbia Basin upriver spring chinook salmon are one of these indicator stocks and the work described in this report forms a part of the Pacific Salmon Treaty monitoring program.

Understanding the stock composition of fish populations enables fisheries resource managers to better utilize fisheries resources by treating individual stocks as discrete management units (Ricker 1954, MacLean and Evans 1981, McDonald 1981). One fundamental strategy of this management technique is the proportional distribution of harvest relative to individual stock health and abundance. To be applied effectively, this form of fisheries management requires dependable methods for stock recognition

Figure 1. **Map of the Columbia Basin showing Bonneville Dam; the principal (upriver) spring chinook salmon natural production areas; Sherars Falls; Round Butte and Warm Springs hatcheries on the Deschutes River; Leavenworth Hatchery on the Wenatchee River; Lookingglass Hatchery on the Grande Ronde River; and the Imnaha Facility on the Imnaha River.**



and mixed stock run composition estimation, as well as an understanding of the biological and migratory characteristics of each stock or stock group. Such information does not now exist for Columbia Basin upriver spring chinook salmon stocks. The proportional composition of hatchery and naturally produced fish in the mixed stock population is also not known.

It is expected that reliable stock identification procedures would be specifically useful in a variety of Columbia Basin upriver spring chinook salmon management applications including run-reconstruction studies to more accurately forecast population sizes, escapement monitoring, and for establishment of spawner-recruit relationships. Although the mainstem Columbia River commercial fishery for these stocks has been suspended since 1977, future in-river harvest management will require an established database that includes such information as stock composition and stock-specific escapement.

The purpose of this study, a part of the Columbia River Inter-Tribal Fish Commission Stock Assessment Project, is to explore the feasibility of Columbia Basin upriver spring chinook salmon stock identification through the use of scale pattern analyses. This research began in 1988 with a study designed to differentiate hatchery and naturally spawning stocks in the Deschutes and Wenatchee subbasins of the Columbia River (Schwartzberg and Fryer 1989a). A long term goal of the Stock Assessment Project has been to develop stock composition estimation techniques applicable in mixed stock areas to differentiate hatchery and naturally spawning stocks. Data from the two study areas was therefore pooled in 1988 to test the subsequent effects on scale-variable dispersion and on group classification accuracies.

In 1989, the study was expanded to include spring chinook stocks from the Snake River Basin. In addition, mixed stock scale samples of unknown origin collected from Bonneville Dam were used in the 1989 analysis to estimate the hatchery and naturally spawning stock composition of the entire Columbia Basin adult upriver spring chinook salmon population.

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 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 an entire group or *stock* of fish.

Fish reared in hatcheries generally grow faster than those produced in natural habitat. In addition, hatchery growth is generally more uniform with less pronounced growth retardation during winter and early spring periods (Peck 1970). Throughout this study our hypothesis has been that these differences in growth would be reflected in scales of hatchery and naturally produced fish and that the differences could be detected and classified by SPA.

Columbia Basin upriver spring chinook salmon are produced naturally in many subbasins and at over 15 hatcheries (United States, et. al. 1988). A subset of eight of these stocks, of both hatchery and naturally spawning origin, were selected for use in this study. Stocks selected originated in three geographically distinct subbasins—the Deschutes, Wenatchee, and Snake. Two spring chinook hatcheries located in the Deschutes subbasin provided samples used in this study. These hatcheries are the Round Butte Hatchery, operated by Portland General Electric Corporation (at river km 175 on the Deschutes River), and Warm Springs National Fish Hatchery, operated by the U.S. Fish and Wildlife Service (at river km 11 on the Warm Springs River). One spring chinook hatchery located in the Wenatchee subbasin provided samples used in this study. This hatchery is the Leavenworth National Fish Hatchery, operated by the U.S. Fish and Wildlife Service and located at river km 4 on Icicle Creek. Deschutes and Wenatchee subbasin naturally spawning spring chinook salmon samples were also collected.

Snake River subbasin samples were obtained from hatchery fish reared at the Lookingglass Hatchery, operated by the Oregon Department of Fish and Wildlife (at river km 3 of Lookingglass Creek on the Grande Ronde River), and the Imnaha Satellite Facility of the Lookingglass Hatchery (at river km 73 on the Imnaha River), hereafter referred to as the Imnaha Facility. All Snake subbasin naturally spawning spring chinook samples were collected from the Imnaha River.

Within all of the above-mentioned hatcheries, a variety of rearing and release strategies are typically employed. For this study, however, all release groups from a particular hatchery were treated as a single stock. All natural production from within a particular subbasin was also treated as a single stock. A representative sample of unknown origin spring chinook salmon fish was collected at Bonneville Dam on the mainstem Columbia River (river km 225). This work was done in conjunction with a separate age and length-at-age composition study (Schwartzberg and Fryer 1990a).

In total, five hatchery and three natural stocks were sampled along with a single mixed stock sample. In various combinations, these samples provided the data used in the following stock identification analyses:

Test A. A three-stock identification test of the two hatchery and one natural Deschutes stocks.

Test B. A two-stock identification test of hatchery and natural stock separability in the Deschutes subbasin. Both Round Butte and Warm Springs hatchery stock samples were combined and compared to the Deschutes natural stock sample.

Test C. A two-stock identification test of hatchery and natural stock separability in the Wenatchee subbasin. The Leavenworth Hatchery stock sample was compared to the Wenatchee natural stock sample.

Test D. A three-stock identification test of the two Snake River hatchery and one natural Snake River stocks.

Test E. A two-stock identification test of hatchery and natural stock separability in the Snake subbasin. Both Lookingglass and Imnaha hatchery stock samples were combined and compared to the Imnaha natural stock sample.

Test F. A two-stock identification test in which the five groups of hatchery stock samples from the Deschutes, Wenatchee, and Snake subbasins were pooled and compared to the three groups of pooled natural stock samples.

The classification function calculated in Test F was used to classify unknown origin spring chinook samples (from Bonneville Dam) as either hatchery or natural origin.

Sampling

The eight hatchery and natural stocks sampled in terminal areas represented *known stock* scale sample groups. Deschutes subbasin stocks were sampled at the Sherars Falls sport and the tribal dipnet fisheries as well as at a trap at the Warm Springs Hatchery (Figure 1). All adult returns from both of the Deschutes hatchery stocks were uniquely marked with fin clips (for other research projects) while natural stock fish carried no artificial external marks. Identity of stock origin was, therefore, easily determined. In the Wenatchee subbasin, hatchery fish were sampled at the Leavenworth National Fish Hatchery, and natural stock samples were collected from carcasses on Wenatchee River tributary spawning grounds (Chiwawa, White, and Little Wenatchee rivers and Nason Creek). Snake subbasin hatchery stocks were represented by samples of broodstock returns to Lookingglass Hatchery and the Imnaha Facility. Imnaha Facility stock fish were reared at Lookingglass Hatchery. However, all adult returns to Lookingglass Hatchery were uniquely marked with fin clips (for other research projects) and, therefore, stock origin was identifiable. Natural stock fish from the Imnaha River were sampled at a weir located adjacent to the Imnaha Facility. Scales from mixed spring chinook stocks (*unknown stocks*) were obtained from fish sampled at the Bonneville Dam Fisheries Engineering and Research Laboratory located beside the Second Powerhouse of Bonneville Dam.

Sample sizes obtained (after adjustment for unusable specimens) were 194

Deschutes natural stock, 225 Round Butte Hatchery stock, 341 Warm Springs Hatchery stock, 180 Wenatchee natural stock, 457 Leavenworth Hatchery stock, 105 Imnaha natural stock, 216 Lookingglass Hatchery stock, and 187 Imnaha Facility stock. A total of 511 fish were sampled from the Bonneville Dam mixed stock population.

For SPA studies, subsamples were selected from the predominant mixed stock age class with target sizes of 200 from each known stock group and 100 from the mixed stock group (Conrad 1985). Actual subsamples consisted of 113 Deschutes natural stock, 148 Round Butte Hatchery stock, 165 Warm Springs Hatchery stock, 117 Wenatchee natural stock, 244 Leavenworth Hatchery stock, 46 Imnaha natural stock, 70 Lookingglass Hatchery stock, and 35 Imnaha Facility stock. A subsample of 107 fish was selected from the Bonneville Dam mixed stock sample.

Scales were collected and mounted according to methods described in Clutter and Whitesel (1956) and the International North Pacific Fisheries Commission (1963). Six scales were selected from each fish sampled, except in the Leavenworth Hatchery stock sample where three scales per fish were collected.

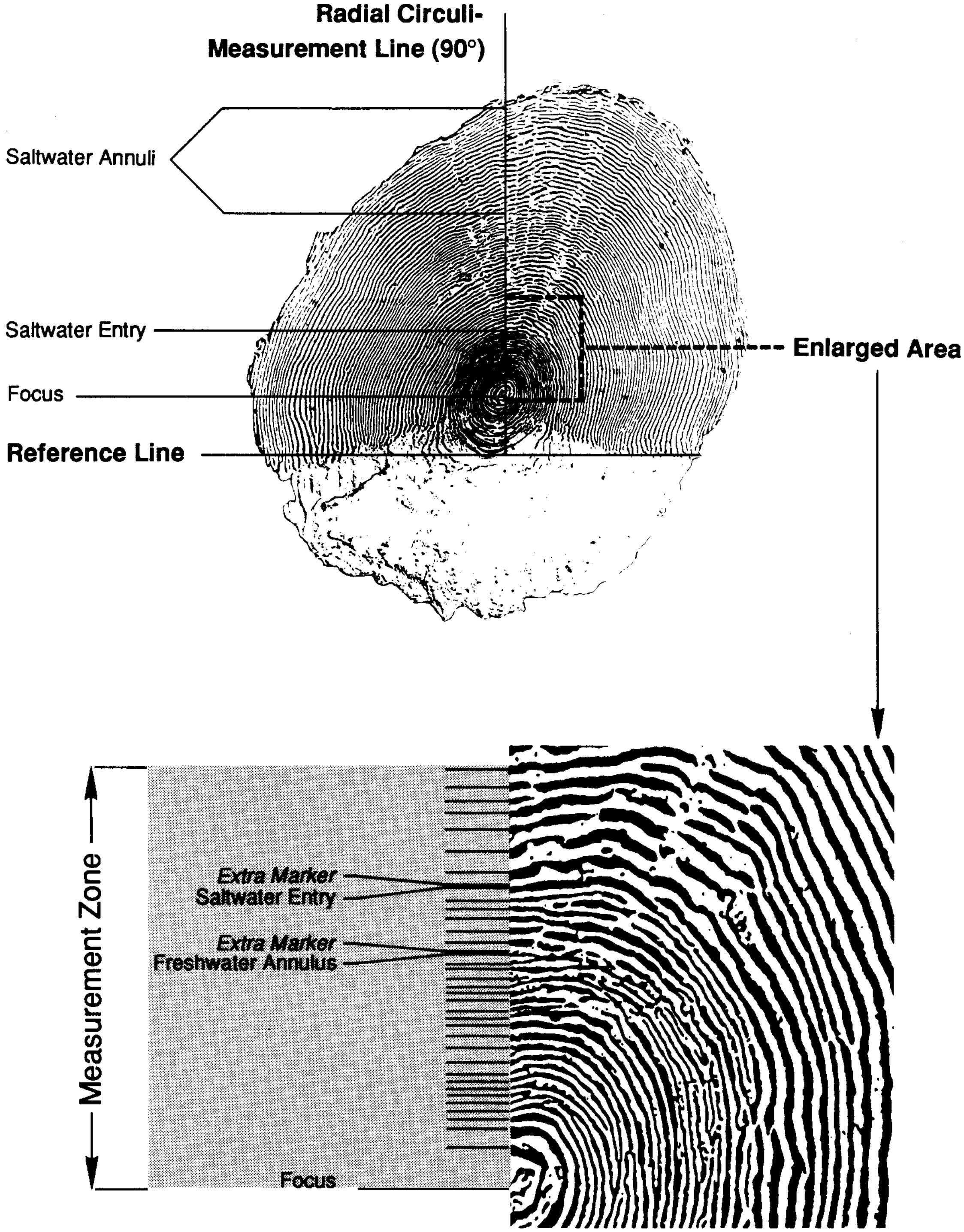
Fork lengths, measured to the nearest 0.5 cm, were recorded as were observed mark or tag information and sex of fish (sex of fish was not possible to determine for the Bonneville Dam mixed stock group). Field sampling procedures for this project followed previously established guidelines (Schwartzberg 1987).

Age Determination and Scale Measurements

Under magnification, salmon scales display numerous concentric rings (*circuli*) radiating outward 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 spring chinook salmon, which typically spend one complete year 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 were categorized by age using previously studied techniques (Johnston 1905, Gilbert 1913, Borodin 1924, Van Oosten 1929).

Figure 2. Age 1.2 Round Butte stock spring chinook salmon scale showing growth and measurement zones.



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 each stock on a return year basis. Because of a large amount of scale *resorbtion* (the eroding of a salmon scale as the fish approaches sexual maturity), age composition of the Wenatchee natural stock sample, the Snake hatchery stocks, and some of the Deschutes samples were somewhat more difficult to reliably estimate. The only spring chinook stocks for which age validation (Beamish and McFarlane 1983) was possible were Snake and Deschutes-Round Butte hatchery stocks. Many Snake-Lookingglass and Snake-Imnaha hatchery spring chinook were, for other research programs, marked as juveniles with coded wire tags. By reading the tag, the true age of a fish could be used for comparison to estimates derived from scales. Such validation was possible for 0.18 of the Lookingglass Hatchery and 0.87 of the Imnaha Facility samples. Some Deschutes-Warm Springs and Deschutes-Round Butte hatchery spring chinook were similarly marked before release with fin clips. Fin clip patterns for Round Butte fish varied from year to year, allowing scale-age estimates to be validated for 0.48 of the samples. Length-at-age relationships were established for each stock using estimated scale ages and corresponding fork length measurements for each specimen.

In 1989, only age 1.2 scale samples were categorized by stock. This age group was predominant in all but one of the stocks examined. Sample sizes were insufficient to examine other age groups. This differed from the approach used in 1988 when it was necessary to pool age 1.2 and 1.3 samples to achieve sufficient sample sizes.

A computer and video camera were used to measure, or *digitize*, scale features (BioSonics 1985). The system 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 (33 cm), 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.

An acetate impression of 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 (6.25x final microscope magnification and 82x 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 (20x final microscope magnification and 256x projection magnification). All measurements were made to the marginal (outermost) 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 the 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 a single zone (from scale focus to approximately circulus 33, usually located in the saltwater area), were tested to find those that most effectively characterized differences in growth among the stocks. A total of 14 different variables were tested to determine those most effective in classifying different known origin samples. Test variables were selected and used in discriminant analyses and classification accuracies were noted. Variables tested included those made up of measurements among groups of four non-adjacent circuli. These four-circuli groups are herein referred to as *quadruplets* (in our earlier work, such variables were referred to as *triplets,* but the term was changed for this report to be consistent with definitions presented by Davis [1987]). Quadruplets contain three distinct intervals and had been found to give the highest classification accuracies in previous studies (Schwartzberg and Fryer 1988, 1989a, 1989c, 1990b). Distances and number of circuli from scale focus to saltwater entry point were also used in variable development. Distances and number of circuli from scale focus to freshwater-annulus margin (anterior) were not used as variables because it was felt that operator subjectivity played too great a part in the identification of these scale features.

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 been applied and proven useful in determining the origin of individual fish stocks from mixed stock samples (Bethe and Krasnowski 1977, Bethe et. al. 1980, Major et. al. 1978).

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

$$y = m_1x_1 + m_2x_2 + \ldots + m_nx_n$$

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

The frequency distribution of *y* (in the case of a two-stock test) was determined, and mean values of *y* computed for the two populations. The critical value for separating the populations was a function of the average of the two means, $\bar{y}_a$ and $\bar{y}_b$, expressed as:

$$Y = \frac{\bar{y}_a + \bar{y}_b}{2}$$

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 *y* values were greater or less than *Y*.

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 occurring when the expected classification accuracy determined from known stock tests was not 100%, 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.

RESULTS

Age and Length-at-Age Composition

Age compositions, determined on a return year basis, varied among the different stocks as well as among the different subbasins (when natural and hatchery stocks were pooled). Six of the eight Columbia Basin upriver spring chinook stocks sampled were estimated (ages were not validated and, therefore, all sample age composition and length-at-age relationships in this section must be considered estimates) to include fish of ages 1.1, 1.2, and 1.3, while only the Leavenworth Hatchery and Imnaha natural stock samples contained fish of age 1.4 (Table 1, Figure 3). The Lookingglass Hatchery stock sample was the only sample containing no age 1.3 fish. The Wenatchee natural stock sample was the only sample containing no age 1.1 fish. Fish from the Imnaha natural stock and both Wenatchee subbasin stocks were generally older and larger than fish from the three Deschutes subbasin stocks and the Snake subbasin hatchery stocks.

The Deschutes natural stock sample age composition included 0.86 age 1.2 fish and 0.12 age 1.3 fish. Age 1.1 fish represented 0.02 of the sample. No age 1.4 fish were sampled. Mean fork-lengths for age 1.1, 1.2, and 1.3 fish were, respectively, 50.8, 70.9, and 84.5 cm (Figure 4). The 0.90 confidence intervals for these lengths were, respectively, 43.8, 57.7; 62.3, 79.4; and 71.0, 98.0 cm.

The Deschutes-Round Butte Hatchery stock sample age composition included 0.80 age 1.2 fish and 0.16 age 1.3 fish. Age 1.1 fish represented 0.04 of the sample. No age 1.4 fish were sampled. Mean fork-lengths for age 1.1, 1.2, and 1.3 fish were 56.8, 72.2, and 88.8 cm, respectively. The 0.90 confidence intervals for these lengths were, respectively, 47.2, 66.4; 63.7, 80.7; and 80.8, 96.8 cm.

The Deschutes-Warm Springs Hatchery stock sample age composition included 0.96 age 1.2 fish and 0.03 age 1.3 fish. Age 1.1 fish represented 0.01 of the sample. No age 1.4 fish were sampled. Mean fork-lengths for age 1.1, 1.2, and 1.3 fish were, respectively, 50.1, 69.9, and 81.7 cm. The 0.90 confidence intervals for these lengths were, respectively, 45.5, 54.7; 62.7, 77.1; and 74.8, 88.6 cm.

Table 1. Age composition estimates of Columbia Basin spring chinook salmon stocks sampled in 1989.

		Brood Year And Age Class[1]			
Stock	Sample Size	1986 1.1	1985 1.2	1984 1.3	1983 1.4
Deschutes					
Natural	194	0.02	0.86	0.12	0.00
Round Butte Hatchery	225	0.04	0.80	0.16	0.00
Warm Springs Hatchery	341	0.01	0.96	0.03	0.00
Wenatchee					
Natural	180	0.05	0.76	0.19	0.00
Leavenworth Hatchery	457	0.00[2]	0.73	0.26	0.00[2]
Snake					
Natural (Imnaha River)	105	0.04	0.55	0.40	0.01
Lookingglass Hatchery	216	0.20	0.80	0.00	0.00
Imnaha Facility	187	0.62	0.33	0.05	0.00
Mixed Stock					
Bonneville Dam[3]	511	0.11	0.69	0.18	0.00[4]

1. Rounding errors caused sample proportions to occasionally not total 1.0.
2. Estimated proportion is 0.004.
3. The mixed stock sample is included for comparative purposes and does not include all age groups observed at Bonneville Dam. Fish of ages 0.2, 0.3, and 0.4 were also observed (Schwartzberg and Fryer 1990b).
4. Estimated proportion is 0.002.

Figure 3. Age composition estimates of Deschutes, Wenatchee, and Snake subbasin spring chinook salmon stocks sampled in 1989.

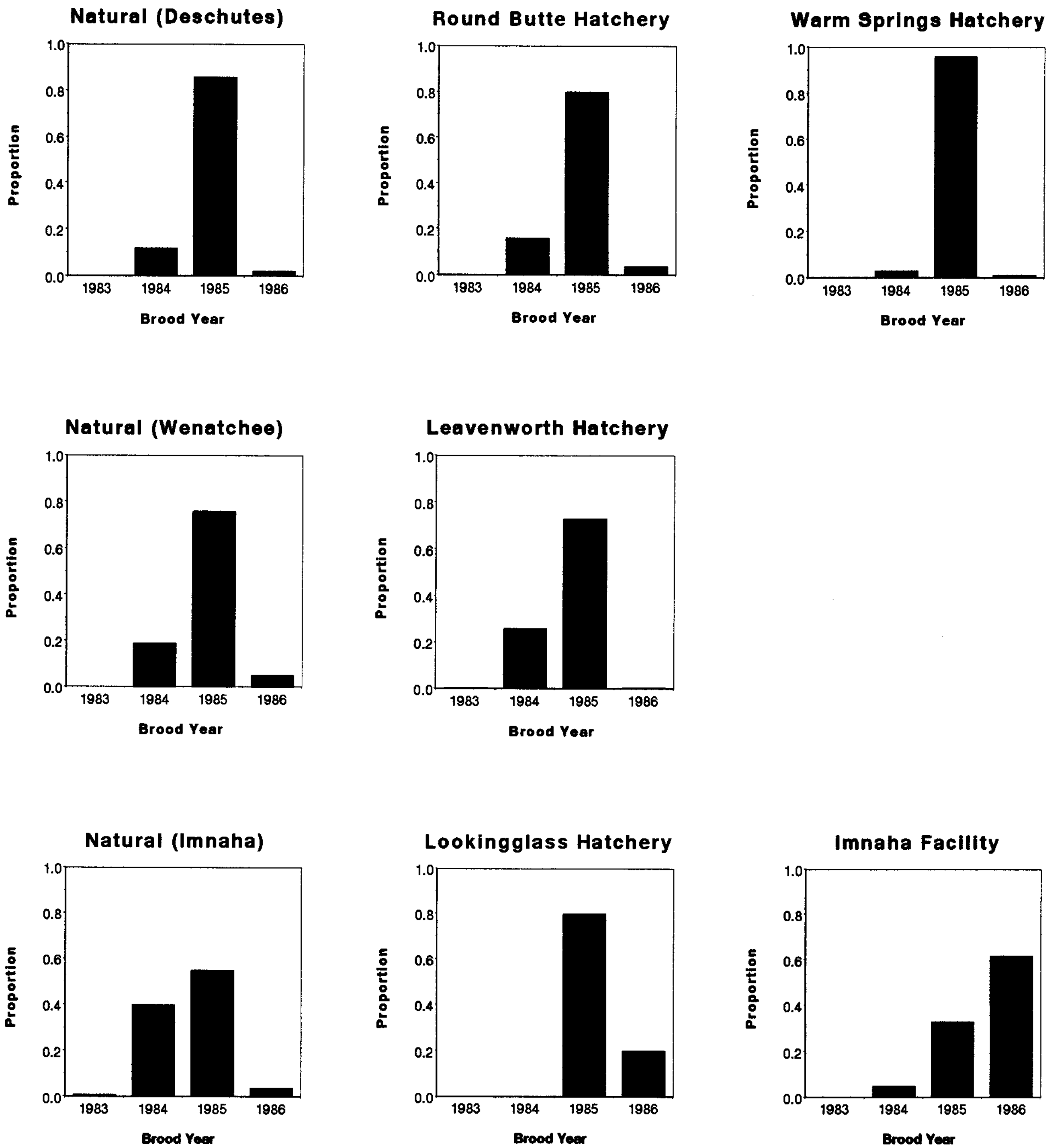
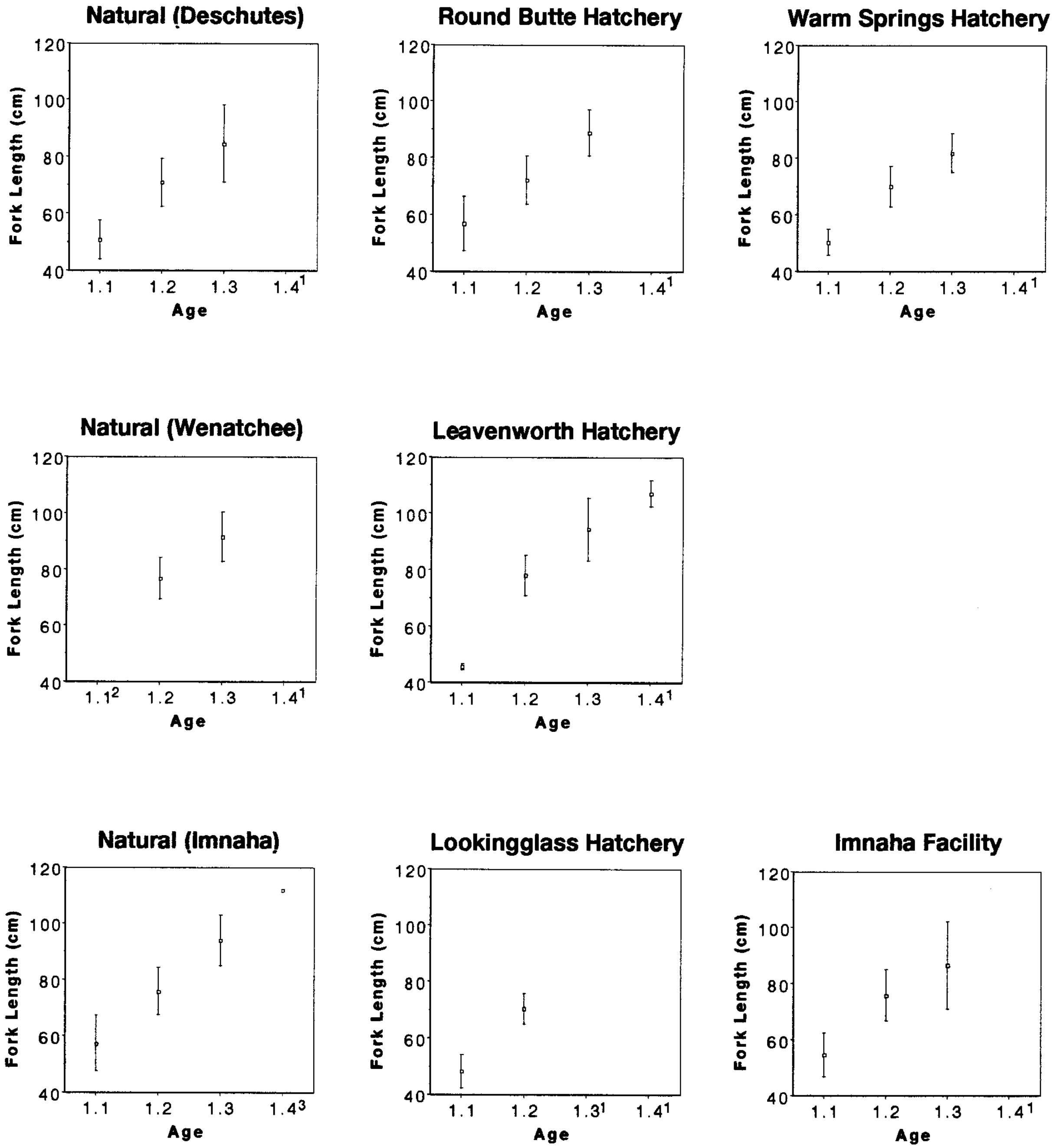


Figure 4. Length-at-age composition estimates of Deschutes, Wenatchee, and Snake subbasin spring chinook salmon stocks sampled in 1989 showing mean fork-lengths and the 0.90 confidence interval for each age class.



1. No fish of this age class was sampled.
2. No fork length measurements were recorded for fish of this age class.
3. Only one fish of this age class was sampled.

The Wenatchee natural stock sample age composition included 0.76 age 1.2 and 0.19 age 1.3 fish. Age 1.1 fish represented 0.05 of the sample. Mean fork-lengths for age 1.2 and 1.3 fish were 76.7 and 91.4 cm, respectively (however, for the majority of this sample, only mid-eye-to-hypural-plate lengths were available). The 0.90 confidence intervals for these (fork) lengths were 69.2, 84.3 and 82.7, 100.2 cm. Fork lengths were not recorded for any age 1.1 fish.

The Wenatchee-Leavenworth Hatchery stock sample age composition included 0.73 age 1.2 and 0.26 age 1.3 fish. Age 1.1 and 1.4 fish represented less than 0.01 of the sample. Mean fork-lengths for age 1.1, 1.2, 1.3, and 1.4 fish were, respectively, 45.5, 78.0, 94.3, and 107.0 cm. The 0.90 confidence intervals for these lengths were, respectively, 44.3, 46.7; 70.8, 85.2; 83.2, 105.4; and 102.4, 111.6 cm.

The Snake-Imnaha natural stock sample age composition included 0.55 age 1.2 and 0.40 age 1.3 fish. Age 1.1 and 1.4 fish represented 0.04 and 0.01 of the sample, respectively. Mean-fork lengths for age 1.1, 1.2, 1.3, and 1.4 fish were, respectively, 57.4, 76.0, 93.9, and 112.0 cm. The 0.90 confidence intervals for age 1.1, 1.2, and 1.3 fish lengths were, respectively, 47.5, 67.3; 67.6, 84.4; and 85.0, 102.8 cm. Only one age 1.4 fish was sampled.

The Snake-Lookingglass Hatchery stock sample age composition included 0.80 age 1.2 fish and no age 1.3 fish. Age 1.1 fish represented 0.20 of the sample. No age 1.4 fish were sampled. Mean fork-lengths for age 1.1 and 1.2 fish were 48.2 and 70.4 cm, respectively. The 0.90 confidence intervals for these lengths were, respectively, 42.2, 54.2 and 65.0, 75.8 cm.

The Snake-Imnaha Facility stock sample age composition included 0.33 age 1.2 and 0.05 age 1.3 fish. Age 1.1 fish represented 0.62 of the sample. No age 1.4 fish were sampled. Mean fork-lengths for age 1.1, 1.2, and 1.3 fish were 54.7, 75.8, and 86.4 cm, respectively. The 0.90 confidence intervals for these lengths were, respectively, 46.9, 62.5; 66.8, 84.8; and 70.8, 102 cm.

Classification Feasibility Tests

A subset of 13 variables (Table 2) was selected and used in all reported discriminant analyses. Each test, however, produced a specific function made up of different significant variables. The model used in Test C required the fewest variables (three), while the Test F model used the most variables (eight). Note that these tests were only applied to subsamples of age 1.2 fish.

Test A

The three-stock identification test of the two Deschutes hatchery stocks and one Deschutes natural stock resulted in a 0.78 composite classification accuracy (Table 3). The natural stock samples were correctly classified with 0.80 accuracy. Round Butte and Warm Springs hatchery stock samples were correctly classified with 0.75 and 0.79 accuracies, respectively.

Test B

The two-stock identification test of pooled Deschutes hatchery stocks compared with one Deschutes natural stock resulted in an 0.85 composite classification accuracy. Combined hatchery and natural stock samples were correctly classified with 0.84 and 0.85 accuracies, respectively.

Test C

The two-stock identification test of Wenatchee hatchery (Leavenworth) and Wenatchee natural stock samples resulted in a 0.93 composite classification accuracy. Natural and hatchery stock samples were correctly classified with 0.91 and 0.95 accuracies, respectively.

Test D

The three-stock identification test of two Snake hatchery stocks (Imnaha and Lookingglass) and the Snake natural stock (Imnaha) resulted in a 0.60 composite classification accuracy. Natural stock samples were correctly classified with 0.85 accuracy but the Lookingglass and Imnaha hatchery stocks were only classified with 0.44 and 0.51 accuracies, respectively.

Table 2. Variables used in discriminant analyses, 1989.

Variables	Analyses A	B	C	D	E	F	Description of Variables
1	•	•					No. of circuli from focus to saltwater entry
2			•	•	•	•	Distance from focus to saltwater entry
3						•	Distance from focus to circulus 3
4							Distance from circulus 3 to circulus 6
5	•	•				•	Distance from circulus 6 to circulus 9
6	•	•	•	•	•	•	Distance from circulus 9 to circulus 12
7	•	•					Distance from circulus 12 to circulus 15
8	•		•				Distance from circulus 15 to circulus 18
9	•	•				•	Distance from circulus 18 to circulus 21
10	•	•		•	•	•	Distance from circulus 21 to circulus 24
11				•	•	•	Distance from circulus 24 to circulus 27
12						•	Distance from circulus 27 to circulus 30
13							Distance from circulus 30 to circulus 33

Table 3. Classification accuracies achieved in linear discriminant analyses (Tests A through F) of Columbia Basin spring chinook salmon stocks sampled in 1989.

Test	Natural[1]		Hatchery[1]		Composite[1]	
	n[2]	C.A.[3]	n[2]	C.A.[3]	n[2]	C.A.[3]
A Deschutes 3-stock	113	0.80	148[4] 165[5]	0.75 0.79	426	0.78
B Deschutes 2-stock	113	0.85	313	0.84	426	0.85
C Wenatchee 2-stock	117	0.91	244	0.95	361	0.93
D Snake 3-stock	46	0.85	35[6] 70[7]	0.51 0.44	151	0.60
E Snake 2-stock	46	0.87	105	0.93	151	0.90
F Pooled Basin 2-stock	276	0.85	662	0.91	938	0.88

1. The stock or group of stocks tested. Two different hatchery stocks were tested in both the Deschutes and the Snake subbasins (see footnotes 4 through 7, below).
2. The number of samples from each stock submitted to a particular test.
3. The classification accuracy (C.A.) achieved in a particular test. Accuracy represents the proportion of samples classified to their correct stock (or stock group) of origin.
4. Round Butte Hatchery.
5. Warm Springs Hatchery.
6. Imnaha Facility.
7. Lookingglass Hatchery.

Test E

The two-stock identification test of pooled Snake hatchery stocks compared with the Snake (Imnaha) natural stock resulted in a 0.90 composite classification accuracy. Combined hatchery and natural stock samples were correctly classified with 0.93 and 0.87 accuracies, respectively.

Test F

The two-stock identification test of the pooled hatchery stock samples from the Deschutes, Wenatchee, and Snake subbasins compared with the pooled natural origin stock samples from the Deschutes, Wenatchee, and Snake subbasins resulted in an 0.88 composite classification accuracy. Hatchery and natural stock samples were correctly classified with 0.91 and 0.85 accuracies, respectively.

Classification of Unknown Mixed Stock Samples

The linear discriminant function used in Test F classified 0.69 of the unknown origin age 1.2 samples from the Bonneville Dam mixed stock group as being of hatchery stock and the remaining 0.31 as natural stock. After correction for bias (Cook and Lord 1978), 0.71 of the samples were classified as hatchery stock and the remaining 0.29 as natural stock. Confidence intervals (0.90) ranged from 0.61 to 0.81 for the hatchery stock group and 0.19 to 0.39 for the natural stock group.

DISCUSSION

This study has shown that hatchery and naturally spawning stocks of age 1.2 Wenatchee, Deschutes, and Snake subbasin spring chinook salmon can be identified with a relatively high degree of accuracy by digitizing scales and using a linear discriminant analysis procedure. Four of the six feasibility tests performed (Tests B, C, E, and F) were two-stock analyses testing classification accuracies using hatchery and naturally spawning stock samples. In these tests, scale samples were correctly identified according to their place of origin with accuracies of 0.85 or greater. A three-stock analysis (Test A) using Deschutes subbasin stocks (two hatchery and one natural) produced a classification accuracy of 0.78. The three-stock analysis (Test D) performed on Snake subbasin stock samples (two hatchery and one natural) produced a classification accuracy of only 0.60. However, both of the hatchery stocks included in Test D were reared at the same location (Lookingglass Hatchery) but were of different genetic origin (Lookingglass Hatchery samples originated from Rapid River Hatchery broodstock and Imnaha Hatchery samples originated from Imnaha River naturally spawning stocks). This suggests that, at least in this case, differences in rearing conditions are more significant than other variables such as genetic differences when using SPA for Columbia Basin upriver spring chinook stock identification.

The high classification accuracies achieved in Tests B, C, and E, each being two-stock analyses from within a single subbasin, suggest that this technique could be usefully applied in the management of Columbia River subbasins where both hatchery and naturally spawning upriver spring chinook stocks are present. In such areas, separate management strategies for each stock are generally advantageous. This methodology could most immediately be applicable in those subbasins containing mixed stock fisheries. Emphasis in these cases is usually placed on measuring and ensuring desired escapement levels for natural stock fish.

High classification accuracies were obtained in Test F which compared pooled hatchery stocks from three Columbia River subbasins with pooled natural stocks from those same subbasins. This suggests that, in hatchery and naturally spawning Columbia Basin spring chinook salmon stocks, among-stock differences in scale patterns are great enough to permit pooled stock identification despite within-stock differences.

Test F results indicate that a composite mixed stock analysis of unknown origin samples might be possible using a classification function based on a limited set of known stock samples. Such a test was conducted using the classification function (derived from test F) to obtain a composition estimate for hatchery and naturally spawning upriver spring chinook salmon from a mixed stock sample of unknown origin age 1.2 specimens collected at Bonneville Dam. After an adjustment for bias, 0.71 of age 1.2 spring chinook were estimated to be of hatchery origin. It is impossible to test the accuracy of this estimate because stock origin could only be verified for a portion of the mixed stock sample. Nevertheless, of the 107 fish in the Bonneville mixed-stock sample, all 23 chinook which were known to be of hatchery origin (ventral or pectoral fin-clipped) were correctly classified by the discriminant analysis procedure. Of the 13 adipose fin-clipped spring chinook likely to be of hatchery origin (some natural stock chinook are adipose clipped in transportation and passage studies), 12 were classified as being of hatchery origin. Thus it seems likely that, even though all known stocks of Columbia Basin upriver spring chinook were not used to create the discriminant function, the stock composition estimate obtained was reasonably accurate.

Since visually detectable differences between hatchery and natural stock spring chinook scales often appeared obvious, an effort was made to classify the unknown stock scales using visual interpretation and identification alone. This procedure resulted in the classification of 0.69 of the age 1.2 mixed stock samples as hatchery origin, an estimate very similar to that produced using the discriminant analysis function. However, 0.30 of the samples visually classified as natural origin were misclassified according to the results of the mixed stock discriminant analysis estimate and 0.12 of the samples visually classified as hatchery origin were similarly misclassified. Since a greater proportion of the smaller (natural origin) sample and a smaller proportion of the larger (hatchery origin) sample were misclassified, the misclassifications for each group offset each other and produced a misleading composite result.

Salmon populations spawning in a given year produces progeny that will return as adult spawners in several different years. All progeny of a given year's spawning population are known as a *brood*. Many salmon population-size prediction techniques are based on an understanding of patterns in age compositions from successive broods. Although it is not possible at the present time to make any comparisons in age distributions by brood year, this study, if continued, should permit such analyses in future years.

For this study, samples were selected from eight major spring chinook stocks. In any comprehensive future study conducted to identify the hatchery and naturally spawning stock composition of Columbia Basin upriver spring chinook salmon, we recommend that additional stocks be included. Stocks that should be added to those represented in this study include the Rapid River and Sawtooth hatchery stocks from Idaho as well as the Carson/Little White Salmon Hatchery Complex stock from Washington. Naturally spawning stocks that should be added include those from the Salmon, Clearwater, and Yakima rivers.

Management opportunities for this research include evaluation of the degree to which stock-specific escapement goals are being met and, in mixed stock harvest areas, the assessment and regulation of fisheries. At the present time, separate escapement goals do exist for certain hatchery and naturally spawning Columbia Basin upriver spring chinook stocks, but the accuracy of the run-reconstruction methods now used for monitoring and estimation of annual stock abundance could be greatly improved using the research methods incorporated in this study.

A further potential application of the SPA methodology herein described is in evaluation of Pacific Salmon Treaty impacts (or other ocean harvest control measures) on a key indicator stock such as Columbia Basin upriver spring chinook. The ability to distinguish hatchery and naturally spawning stocks within mixed stock areas is an essential element of a comprehensive Treaty monitoring and evaluation program. Studies such as this will give an indication of the success of the Treaty in achieving the purposes for which it was established.

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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 anterior freshwater annulus margin 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.**

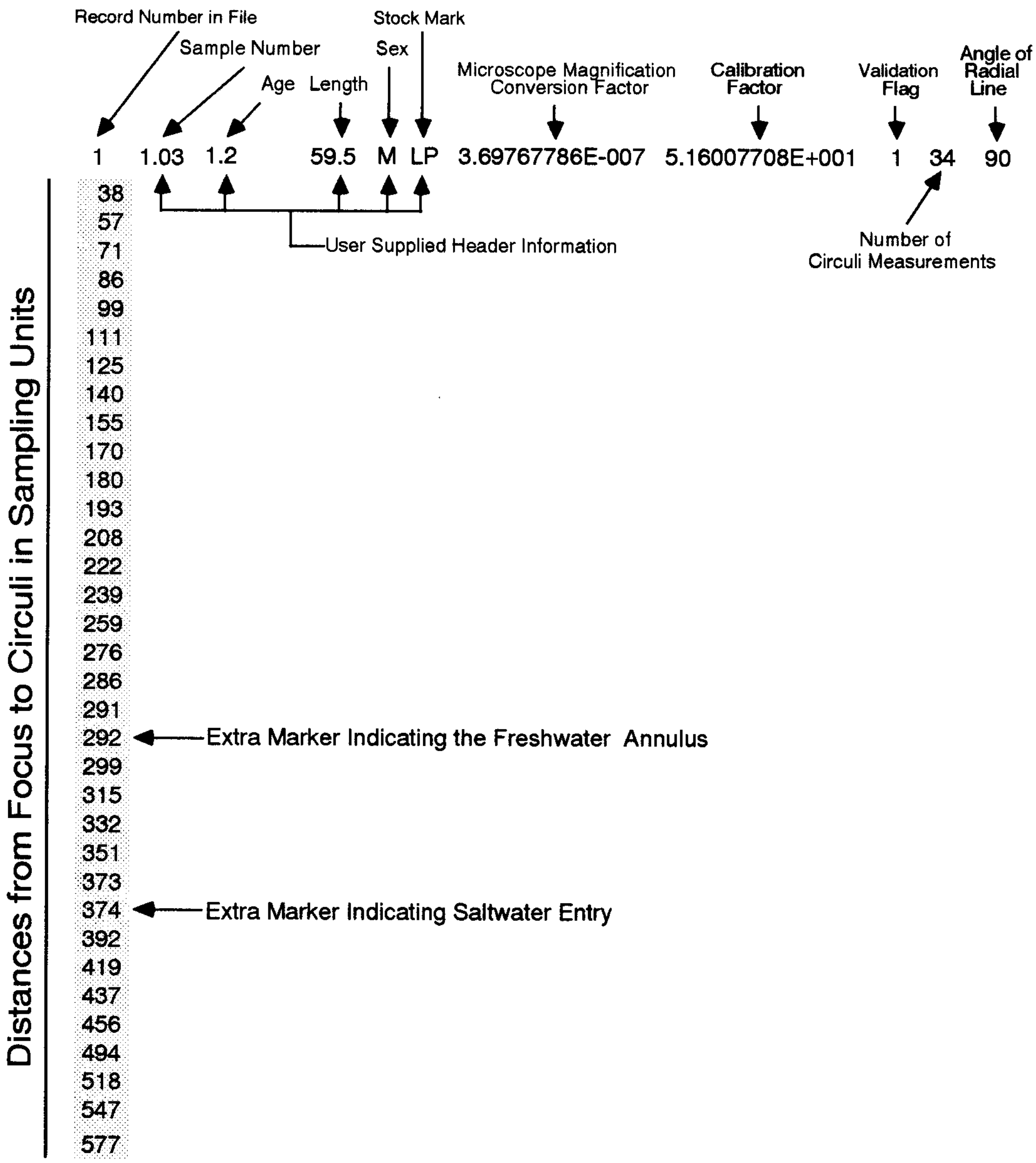

Figure A2. **A sample compact format data record for a single spring chinook salmon scale freshwater-growth-zone measurement.**

Stock Number

Sample Number | Sample Age | Sample Length (cm) | Number of Circuli in Record | 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			

19 — Number of Circuli from Focus to Freshwater Annulus

24 — Number of Circuli from Focus to end of Freshwater Growth