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The Performance of Quantitative Scale Pattern Analysis in the Identification of Hatchery and Wild Steelhead

by

Rebecca L. Bernard[1] and Katherine W. Myers
Fisheries Research Institute WH-10
School of Fisheries
University of Washington
Seattle, WA 98195

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[1] Present address: California Cooperative Fisheries Research Unit, Humboldt State University, Arcata, CA 95521.

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Abstract

The performance of quantitative scale pattern analysis in distinguishing between adult hatchery and wild origin steelhead (*Oncorhynchus mykiss*) was evaluated with a multipurpose analysis-bootstrap-simulation computer program. The freshwater and early marine portions of scales from known-origin steelhead (identified by an adipose fin mark), collected at Priest Rapids Dam, Columbia River, Washington, in 1990 and 1991, were measured using an optical digitizing system. Two-factor (origin and year) analysis of variance of 14 scale variables was used to select a subset of variables that were significantly different between origin but not between years. Hatchery steelhead had significantly more freshwater circuli and larger freshwater zones than wild steelhead. The spacing of circuli immediately following the focus of the scale was similar in both hatchery and wild fish, but hatchery steelhead generally had significantly wider spacing of freshwater circuli than wild fish. Wild fish had significantly wider spacing of ocean circuli than hatchery fish. Model performance varied depending on the estimator and variable set that was used. The best overall performance was achieved using a direct maximum likelihood estimator and the selected subset of six variables. The 1990 baseline data were tested with mixtures composed of 100% hatchery or wild fish collected in 1991, and estimated compositions and bootstrapped 95% confidence intervals were 91.5% (68.1-99.9%) hatchery and 97.2% (71.5-99.9%) wild steelhead. Similarly, the 1991 baseline data were tested with fish collected in 1990, and estimated compositions were 91.8% (65.2-99.9%) hatchery and 94.3% (77.4-99.3%) wild steelhead. In simulations using pooled-year (1990-1991) data with a range of specified mixtures (500 runs for each simulated mixture), errors of the maximum likelihood estimates were lowest when mixtures were composed of 100% hatchery (standard error (SE): .272) or wild (SE: .266) fish and highest with 0.50:0.50 mixtures (SE: .338). In simulations where the true composition was randomly generated, doubling the size of the mixed sample from 25 to 50 fish decreased the error in the maximum likelihood estimates by 43.8% (means of the squared differences between estimated and true compositions decreased from .0368 to .0207). Additional doubling of the size of the mixture decreased the error by 55.6% (50 to 100 fish), 35.9% (100 to 200 fish), and 37.3% (200 to 400 fish). In general, the models were somewhat better at identifying wild fish than hatchery fish, and it is possible that some of the fish included in our baselines were incorrectly identified by the adipose fin mark. The inclusion of additional variables that provide information on growth at or near the end of the freshwater zone might further improve the performance of the models. For general application of this technique to mixtures collected in offshore waters, development and testing of models with samples of known-origin fish from other Pacific Rim stocks are necessary.

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Introduction

Freshwater age, as determined by qualitative visual examination of scales, has been used to provide rough estimates of the proportions of hatchery and wild steelhead (*Oncorhynchus mykiss*) in mixed samples of unknown origin fish on the high seas (for example, Burgner et al. 1992, Myers and Bernard 1993). The basis of this technique is that almost all hatchery steelhead are released as age 1. smolts (one freshwater annulus on the scale) and most wild steelhead smolts are age 2. or older (two or more freshwater annuli on the scale; Burgner et al. 1992). However, accurate visual identification of freshwater annuli on the scales of hatchery steelhead is complicated by factors such as: (1) partial or complete regeneration of the freshwater portion of the scale due to scale loss, (2) resorption of the margin of the scale when the fish is released into the natural environment, (3) uniform seasonal growth in the hatchery environment, which makes it difficult to differentiate summer and winter growth, and (4) formation of checks or false annuli that occur in the same portions of the scale as true freshwater annuli in wild fish (Davis and Light 1985).

Information from high seas recovery of coded-wire tagged fish shows that hatchery steelhead are widely distributed in the North Pacific Ocean (for example, Myers et al. 1990, 1993; Burgner et al. 1992; Myers and Bernard 1993). However, Burgner et al. (1992) reported a low observed occurrence of age 1. steelhead (approximately 25% of the total) in recent (1980-85) high seas research vessel samples compared to the estimated abundance of hatchery origin fish (approximately 50%) in the population. Burgner et al. concluded that they could not be certain of the actual freshwater age composition of the offshore population because of the high percentage of regenerated scales in their samples.

Davis and Light (1985) found relatively low agreement between Fisheries Research Institute (FRI) and Fisheries Agency of Japan (FAJ) biologists on visual determinations of freshwater age of steelhead in Japanese research vessel samples (average of only 54% agreement in 1976-1981 samples). The estimated proportion of freshwater age 1. steelhead in 1990 port samples of the Japanese traditional landbased driftnet salmon fishery based on FRI age determinations was large (53%, Myers and Campbell 1991) compared to the estimated proportion of freshwater age 1. steelhead in 1982-1990 Japanese research vessel samples from the same area based on FAJ age determinations (10% in commercial gillnet and 9% in non-selective gillnet, Myers et al. 1993). Myers et al. (1993) suggested that the low occurrence of freshwater age 1. steelhead in offshore samples may result from errors in freshwater age determination from scales.

Clearly, the development of quantitative techniques for identifying hatchery and wild steelhead in high-seas scale samples would improve the accuracy of freshwater age composition estimates and estimates of the proportions of hatchery and wild-origin steelhead (*Oncorhynchus mykiss*) migrating in the Convention area. In this study, scale measurement data from known-origin fish are used to evaluate the potential use of quantitative scale pattern analysis in estimating proportions of hatchery and wild steelhead in mixed fishery samples.

Methods

Known Origin Scale Samples

Scales were collected by the Washington Department of Wildlife (WDW) from adult summer-run steelhead at Priest Rapids Dam, Columbia River, in 1990 and 1991. All Columbia River Basin hatchery steelhead are marked with an adipose fin clip prior to release, and WDW biologists used this mark to identify adult steelhead collected at Priest Rapids Dam as either hatchery origin (adipose fin absent) or wild origin (adipose fin present). Use of this mark can result in some errors in identification because of regeneration of clipped fins in hatchery fish and natural loss of fins in wild fish (Blankenship 1990). The 1990 scale samples were examined by a scale expert at WDW, and in a few cases he noted that the scale patterns contradicted the original designation of origin based on the adipose fin mark (Robert Leland, WDW, Olympia). These scales were not used in our study. The scales collected in 1991 were not examined by WDW scale experts, and consequently a few of the 1991 scales used in our study may have been incorrectly designated to origin.

Scale Selection and Qualitative Visual Examination

Acetate impressions, made from the original scale cards provided by WDW, were viewed under a microfiche reader, and the highest quality (clean, not regenerated) and most representative scale was selected from each fish. While examining the scales under the microfiche reader, qualitative differences in hatchery and wild steelhead scale patterns were observed in both freshwater and early ocean growth on the scales. Hatchery fish appeared to have larger freshwater zones and more freshwater circuli than wild fish (for example, see Fig. 1). Freshwater circuli appeared to be more even and regularly spaced in hatchery steelhead than in wild steelhead. In hatchery fish, the spacing between circuli at the end of the freshwater zone was similar to spacing at the beginning of the first ocean zone, and spacing between circuli at the beginning of the first ocean zone was narrower than in wild fish. Because of these observed differences and qualitative differences reported in previous studies (for example, Davis and Light 1985), we decided to limit our measurements in this study to the freshwater and early marine portions of the scale.

Scale Measurement

Acetate impressions of scales were all measured by one person using an image analysis system (OPRS, BioSonics, Inc., Seattle, WA). To maximize the length of the measurement axis that could be viewed on the OPRS monitor and to insure consistency of the measurement data, each scale was first magnified (60X) and positioned on the screen in an identical manner. The focus of each scale was placed at the same location in the lower left hand corner of the OPRS monitor with the long axis of the scale running diagonally across the screen. At a higher power (170X), counts and measurements were made along the longest axis of the scale from the center of the focus to the outer edge of each circulus through the last ocean circulus that could be viewed on the OPRS monitor.

The transition between freshwater and ocean growth was identified by locating the last freshwater circulus (for example, see Fig. 1), and the outer edge of this circulus was marked with two very closely placed measurements (one or two OPRS sampling units apart) that could later be detected by a computer program used to reformat the measurement data. The freshwater-ocean transition was usually associated with the first widely spaced, thicker ocean circulus, although some variation occurred. In several cases, resorption at the margin of the freshwater zone of hatchery steelhead enabled easy detection of the freshwater-ocean transition. Some of the wild origin steelhead scales displayed considerable freshwater plus growth after their last freshwater annulus, and the last freshwater circulus was determined by circulus spacing, circulus thickness, and the appearance of any resorption around the freshwater zone.

A preliminary analysis was used to assess consistency in method and technique of measuring steelhead scales on the OPRS. A set of hatchery origin and wild origin steelhead scales was selected and measured at one sitting. The following day these same scales were measured again. The data were analyzed using a paired sample t-test on 25 measurement variables. No significant differences were found between the measurements taken on separate days, which indicated that our scale measuring technique was consistent.

Scale Variables

The original OPRS measurement data consisted of the distances from the center of the focus to the outer edge of each circulus that was marked on the OPRS monitor. These data were reformatted into an initial set of 25 variables: (1) size of focus, (2) size of freshwater (fw) zone, (3) fw circuli count, (4-18) 15 fw triplets, (19) size of the ocean zone that could fit on the OPRS monitor at 170X, (20) ocean circuli count that could be marked on the OPRS monitor at 170X, and (21-25) 5 ocean triplets. Triplet measurements, calculated by combining individual circulus measurements into groups of threes for a maximum of 15 freshwater triplets and 5 ocean triplets, are intended to 'smooth' the increments of individual circuli on a particular fish scale in order to better characterize the stock (Davis 1987).

Missing values, correlations, and normality

The reformatted data were evaluated for missing values, correlations, and normality. Only freshwater and ocean triplet variables common to the entire scale data set were used in the final analyses. Freshwater triplets 9-15 and ocean triplet 5 had missing values for many or all of the fish in the samples, and were dropped from the analysis. The number of ocean triplets common to all scales was four, with the exception of three scales that had only three ocean triplets. These three scales were dropped from the analysis in favor of the additional information that the forth ocean triplet would provide. The size of the ocean zone was dropped as a variable because it was highly correlated with the size of each ocean triplet and was directly correlated to the size of the freshwater zone. The number of freshwater triplet measurements common to all scales was eight, yet the majority of scales had more than 24 freshwater circuli, thus the size of the freshwater zone was not highly correlated with each fw triplet, and was retained for final analysis. The size of the focus was not normally distributed, and was dropped as a variable. The elimination of variables with missing values, correlations, and non-normal distributions reduced the initial set of 25 variables to 14 variables (Fig. 2): (1) fw circuli count (fwc), (2) size of the fw zone (fwsz), (3) fw triplet 1 (fwtr1), (4) fw triplet 2 (fwtr2), (5) fw triplet 3 (fwtr3), (6) fw triplet 4 (fwtr4), (7) fw triplet 5 (fwtr5), (8) fw triplet 6 (fwtr6), (9) fw triplet 7 (fwtr7), (10) fw triplet 8 (fwtr8), (11) ocean triplet 1 (octr1), (12) ocean triplet 2 (octr2), (13) ocean triplet 3 (octr3), and (14) ocean triplet 4 (octr4).

Samples

Scale measurements from 540 fish collected in 1990 and 1991 were used in the analyses. The samples included scales from 309 hatchery steelhead (138 fish in 1990 and 171 fish in 1991) and 231 wild steelhead (73 fish in 1990 and 158 fish in 1991). The samples were pooled over all age classes and brood years.

Variable Selection

Myers (1986) examined the performance of variable subsets by comparing the classification accuracies of the same group of chinook (*O. tshawytscha*) scales measured and classified with two different sets of variables. This study clearly indicated that classification results vary depending upon the scale variables chosen, and pointed to the need for a variable selection methodology that provides consistent variable subsets and, ultimately, the most accurate classifications. We used two-factor analysis of variance (origin and year, $\alpha = .01$) of the 14 scale variables to select a subset of variables for use in our analyses. Our approach was to identify a subset of scale variables that were significantly different between hatchery and wild steelhead but not significantly different between years (1990 and 1991). A qualitative evaluation of this subset of variables in terms of the type of information that they provided to the model was made to select a final set of variables.

Tests of Model Performance

A multipurpose analysis-bootstrap-simulation computer program written by Millar (1988) was used to test scale pattern model performance. The program calculates five different estimators of mixed fishery composition: (1) raw classification proportions (Worlund and Fredin 1962); (2) Cook and Lord (1978) corrected classification estimator (see also Pella and Robertson 1979), (3) Cook (1983) constrained corrected classification estimator, (4) maximum likelihood constrained estimator (Millar 1987), and (5) the direct maximum likelihood estimator (Millar 1987, 1990). Millar's computer program uses linear discriminant analysis (LDA) as the classification rule for estimators 1-4. Estimator 1, the raw classification proportions, is not a valid estimator because it does not correct for classification errors, but we present it here because it is an intermediate step in the calculation of estimators 2-4 (Millar 1988, 1990). In addition, uncorrected classification proportions have often been reported in the scale pattern analysis literature (for example, see Myers et al. (1993) for a review of uncorrected estimates reported for stocks of salmon migrating in the western North Pacific Ocean). We present results for the raw classification estimator here to demonstrate its performance in comparison to other estimators. Estimators 2-4, which are corrected for classification errors, are identical when estimator 2 is non-negative for all groups in the mixture (Millar 1988, 1990). Applications of estimator 2 have also been reported in the scale pattern analysis literature (for example, Myers et al. 1987), where negative estimates for one or more categories are assumed to signify that these categories are not present in the mixture. In practice, when a negative estimate is calculated and there are more than two groups, the analysis is collapsed and repeated to include only those categories indicated to be present. Estimator 3 geometrically constrains the corrected estimates between 0.00-1.00, and estimator 4 uses a maximum likelihood procedure to constrain the estimates between 0.00-1.00 (Millar 1987). Estimator 5, the direct maximum likelihood estimator, uses LDA to estimate the joint probability functions of the scale variables, and an EM (expectation-maximization) algorithm (Dempster et al. 1977, Wu 1983) to calculate the maximum likelihood estimate (Millar 1987, 1990).

In the analysis mode of the program, baseline data from one year (1990 or 1991, referred to in this paper as single-year models) were used to estimate compositions of mixed samples from the other year. The actual compositions of the mixed samples used were either 100% hatchery fish or 100% wild fish. In bootstrap mode, the program was used to repeat the analyses with resampled mixed fishery data and baseline data to obtain non-parametric estimates of the variability in the estimated compositions. Simulated baselines were the same sizes as the original samples, which imitates sampling randomness in the development of the original samples (Millar 1990). Confidence intervals (CI) were derived from 500 simulations, where the CI is the actual range of 95% of the estimates. Direct maximum likelihood estimates were also calculated using the original set of 14 variables to evaluate the effect of variable selection.

Simulation mode was used to evaluate the performance of pooled-year (1990 and 1991 data sets combined) baselines by calculating estimates of hypothetical mixed fishery populations for five specified compositions: (1) 100% wild, (2) 100% hatchery, (3) 25% hatchery-75% wild, (4) 50% hatchery-50% wild, and (5) 75% hatchery-25% wild. New baseline data sets were created by resampling (with replacement) from the original baseline. Simulated baselines were the same sizes as the original samples, and 500 simulated mixed fishery samples of 100 fish each were analyzed for each specified composition.

A second simulation program written by Millar (1988) that randomly chooses the mixed fishery composition prior to the generation of each mixed fishery sample was used further to evaluate the pooled-year models for different simulated mixture sizes. Two runs of 500 simulations (500 different mixed fishery compositions) were performed for simulated mixtures of 25, 50, 100, 200, and 400 fish. As a measure of estimator performance, the program calculates the average (over the simulations) of the squared differences between estimated and true compositions. The resampled baselines were the same size as the original baselines.

Results

Mean values of circulus counts and measurements showed quantitative differences that were similar to qualitative visual observations (Table 1). Hatchery steelhead had more freshwater circuli and larger freshwater zones than wild steelhead. The spacing of circuli immediately following the focus of the scale (fwtr1) was similar in both hatchery and wild fish, but hatchery steelhead generally had wider spacing of other initial freshwater circuli (fwtr2 through fwtr5) than wild fish. Wild fish had wider spacing of initial ocean circuli (octr1 through octr4) than hatchery fish.

Two-factor analysis of variance (origin and year) of 14 scale variables showed that there were no significant differences ($p > 0.01$) between hatchery and wild steelhead in the mean size of fw triplets 1, 6, 7, and 8, and there were significant differences ($p < 0.01$) between years for ocean triplets 1 and 2 (Table 1). These variables were dropped from the analysis. Interactions between origin and year were not significant for any of the variables. Although four freshwater triplets (fwtr2-fwtr5) were significantly different between origin and not between years, they all provided similar information, that is: hatchery fish have wider spacing of circuli in the first part of the freshwater zone than wild fish (Table 1). We chose to use only two of these variables (fwtr2 and fwtr4) in our analyses. The final data set was composed of a subset of 6 variables: fwc, fwsz, fwtr2, fwtr4, octr3, and octr4 (Fig. 2).

The performance of the models changed depending on the estimator that was used (Table 2). The results show considerable biases in the raw classification proportions, which were lower (-.285 and -.288) for wild fish than for hatchery fish (-.345 and -.391). The performances of the Cook and Lord (1978) corrected, the Cook (1983) constrained corrected, and the Millar (1987) maximum likelihood constrained estimators were almost identical. The Cook and Lord (1978) estimates resulted in some values less than zero or greater than one, but in practical applications these would be assumed to be 0% and 100%, respectively. The biases in allocating wild fish were only slightly greater using the Millar (1987) estimator (approximately, -.001 and -.008) than the Cook (1983) estimator (0 and -.007). For both estimators, bias in allocating 1990 hatchery fish using the 1991 baselines (-.201) was considerable (Table 2(3)), and 95% of the bootstrap estimates for the correct (100%) origin group ranged between about .58 and 1.00. Overall, the performance of the direct maximum likelihood estimator was best, but only because of superior allocation of 1990 hatchery fish with the 1991 model. Again, biases in allocating wild fish (-.028 and -.057 in the variable-selected model) were less than for hatchery fish (-.085 and -.082 in the variable-selected model), and 95% of the bootstrap estimates for the correct (100%) origin group ranged between .65 and .999.

Variable selection affected the performance of the direct maximum likelihood estimator. Selection of a subset of six variables greatly improved the accuracy and precision of the 1990 baselines in allocating 1991 hatchery steelhead (a decrease in bias of the direct maximum likelihood estimate from -.265 in the 14-variable model to -.085 in the 6-variable model, and a change in 95% confidence intervals from .532-.884 to .681-.999; Table 2).

Performance of the 1991 baselines in allocating wild steelhead was also improved. Conversely, variable selection decreased accuracy and precision of the 1991 baseline in allocating 1990 hatchery steelhead (an increase in bias of the direct maximum likelihood estimate from 0 in the 14-variable model to -.082 in the 6-variable model, and a change in 95% confidence intervals from .745-1.00 to .652-.999. Performance of the 1990 baseline in allocating 1991 wild fish also declined.

In simulations to evaluate the performance of pooled-year baselines with different mixed fishery compositions, the mean square errors of the constrained classification and direct maximum likelihood estimates were lowest when the mixture was composed of either 100% hatchery fish or 100% wild fish and highest with 0.50:0.50 mixtures, and errors were lower when the proportion of wild fish in the mixture was higher (Table 3). Doubling the size of the mixed sample from 25 to 50 fish decreased the error in the direct maximum likelihood estimates by 43.8%, from 50 to 100 fish by 55.6%, from 100 to 200 fish by 35.9%, and from 200 to 400 fish by 37.3% (Table 4).

Discussion

Qualitative differences observed in freshwater and early marine growth on the scales of adult hatchery and wild steelhead were easily and rapidly quantified using an optical digitizing system (see Figs. 1 and 2). Scale circulus spacing, which is a function of the rates of circulus formation and scale growth, is usually positively correlated with fish growth rate, and may be negatively correlated with size or age of fish (for example, see Fisher and Pearcy 1990). Statistically significant differences in the scale patterns of hatchery and wild steelhead collected at Priest Rapids, Columbia River, indicate, not surprisingly, that freshwater growth rates of age l. hatchery steelhead are faster than freshwater growth rates of wild fish (Table 1). An unexpected result, however, was that the scales of wild steelhead showed significantly better early ocean growth (that is, wider circulus spacing) than hatchery fish (Table 1). Although not quantified in this paper, there also appeared to be more instances in hatchery steelhead of scale resorption in the freshwater-ocean transition zone than in wild steelhead. These differences in scale patterns may reflect the abrupt changes in food and feeding habits and other behaviors that hatchery steelhead smolts must undergo when they are released into the natural environment.

The performance of scale pattern models in identifying hatchery and wild steelhead varied depending on the estimator that was used (Tables 2-4). Millar (1987) advised that there may be no advantage to using direct maximum likelihood estimator when there are only two or three groups in the mixed fishery. However, in our tests, overall performance of the direct maximum likelihood estimator was substantially better than other estimators.

Experience from discriminant analysis has shown that as variables are added, classification accuracy increases and then decreases (Hand 1981; J. Pella, pers. comm.). Variable selection dramatically improved the accuracy and precision of a model using the 1990 baseline data to estimate the composition of a "mixed" sample of 1991 hatchery steelhead (Table 2(1)). Although variable selection also decreased performance in some cases, we think that overall performance was better and results were more consistent with the selected subset of six variables. In general, the models were somewhat better at identifying wild fish than hatchery fish (Tables 2 and 3), and it is possible that some of the fish included in our baselines were incorrectly identified by the adipose fin mark.

The use of additional variables that provide information on freshwater plus growth and other features at or near the end of the freshwater growth zone might further improve the models. In our analysis, freshwater triplet variables were calculated by combining groups of three circulus increments measured outward from the focus of the scale to the end of the freshwater zone (Fig. 2). There was a significant loss of information on circulus spacing in the latter portion of the freshwater zone because we dropped triplet variables with missing values. In future analyses, additional triplet variables could be calculated by combining groups of circulus increments inward from the end of the freshwater zone toward the focus.

The performance of the models improved substantially when mixed samples were large (Table 4). However, samples of steelhead in offshore waters are likely to be small because of their low population abundance.

Because the our models included only stocks from the Columbia River, we do not know whether the models would accurately identify hatchery and wild steelhead from other stocks. However, we think that our initial results are promising, and we recommend additional testing and development of scale pattern models with samples of known-origin steelhead from other Pacific Rim stocks.

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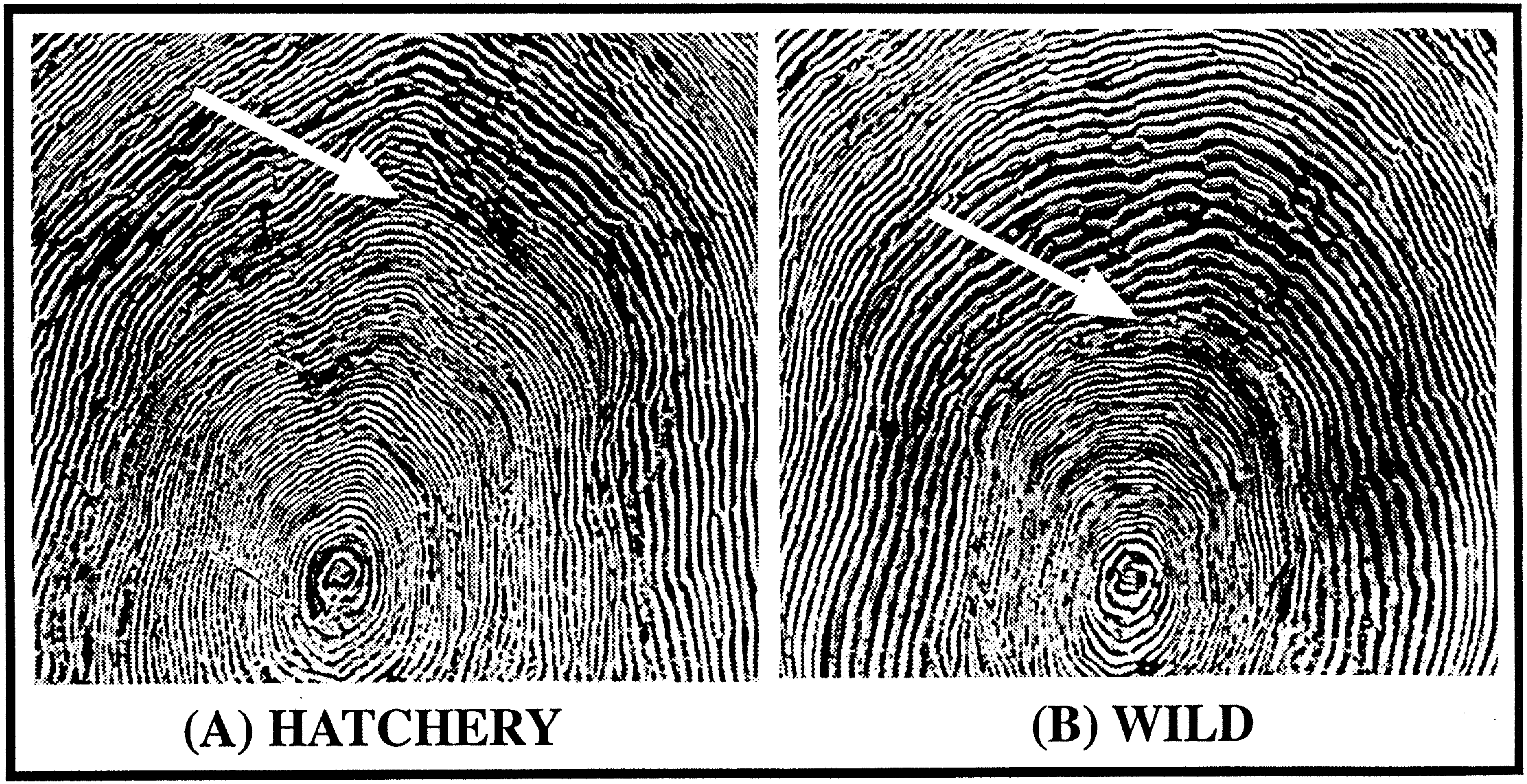

Fig. 1. The freshwater and early marine portions of the scales of (A) a male hatchery steelhead (510 mm fork length) and (B) a female wild steelhead (690 mm fork length) sampled at Priest Rapids Dam, Columbia River, Washington on September 18, 1990. The white arrows indicate the transition between freshwater and marine growth.

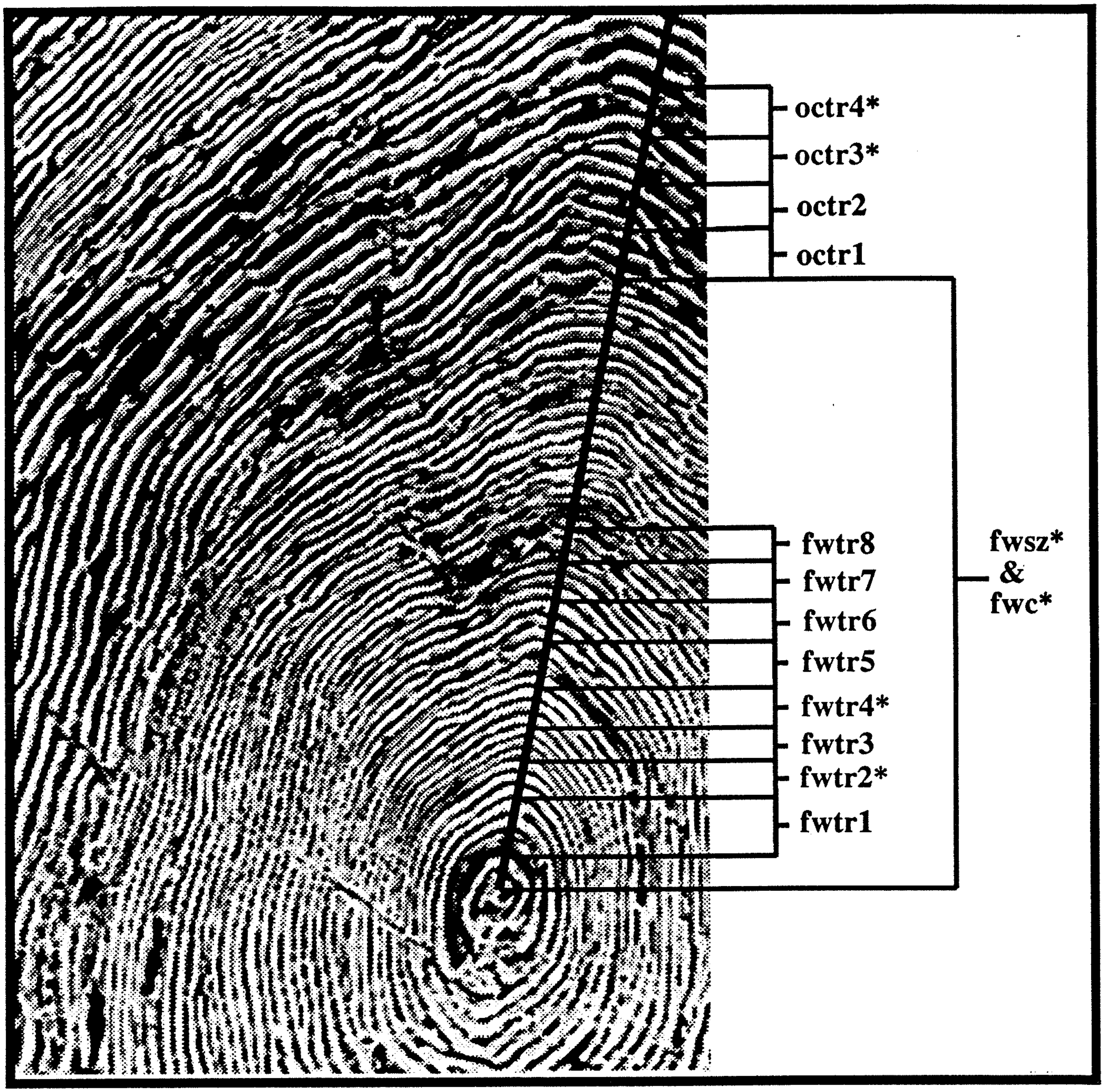

Fig. 2. Scale of a hatchery steelhead showing fourteen scale variables used in the analysis. fwc = freshwater circulus count; fwsz=size of freshwater zone; fwtr1-fwtr8 = eight freshwater triplet variables, calculated by combining individual circulus increments into groups of threes; octr1-octr4 = four ocean triplet variables. The asterisks indicate a subset of six variables that were selected for use in the scale pattern models. Magnification of scale is 115X.

Table 1. Descriptive statistics, mean ± standard deviation (s) of freshwater circulus count (fwc) and measurements of freshwater (fw) and early ocean (oc) growth on scales of hatchery and wild steelhead collected at Priest Rapids Dam, Columbia River in 1990 and 1991. All measurements are in microns. sz=size, tr= triplets or groups of three circuli. n = sample size. P-values are the results of 2-factor (origin (orig) and year(yr)) analysis of variance (ANOVA, $\alpha = 0.01$). Variables are shown in Fig. 2.

	Mean ± s Hatchery origin		Mean ± s Wild origin		ANOVA P-Value		
Variable	1990 n=138	1991 n=171	1990 n=73	1991 n=158	Orig (A)	Yr (B)	AB
fwc	44 ±6	45 ±6	41 ±7	42 ±8	.0001 *	.2716	.6057
fwsz	1062 ±165	1088 ±148	976 ±187	981 ±188	.0001 *	.3148	.5002
fwtr1	110 ±18	110 ±18	108 ±21	104 ±20	.0191	.1902	.3225
fwtr2	75 ±15	77 ±15	69 ±15	69 ±13	.0001 *	.3308	.3251
fwtr3	63 ±12	65 ±11	60 ±12	58 ±11	.0001 *	.2430	.2411
fwtr4	62 ±10	62 ±11	59 ±11	57 ±10	.0001 *	.2430	.2411
fwtr5	64 ±12	64 ±12	61 ±12	60 ±12	.0007 *	.6448	.9462
fwtr6	67 ±12	68 ±12	67 ±14	64 ±13	.1129	.3475	.1411
fwtr7	68 ±13	69 ±12	68 ±12	70 ±14	.6660	.3515	.9921
fwtr8	68 ±11	67 ±11	69 ±15	70 ±14	.0808	.9382	.5178
octr1	111 ±17	120 ±19	125 ±19	128 ±21	.0001 *	.0006 *	.1075
octr2	119 ±22	128 ±24	132 ±23	139 ±23	.0001 *	.0002 *	.5553
octr3	124 ±23	129 ±25	144 ±24	142 ±22	.0001 *	.4690	.0992
octr4	133 ±23	128 ±25	141 ±22	140 ±25	.0001 *	.1455	.3969

*Significant difference ($p \leq 0.01$)

Table 2. Composition estimates for wild and hatchery adult steelhead scales collected in 1990 and 1991 at Priest Rapids Dam, Columbia River. Estimates are derived using the method of Millar (1990) with confidence intervals (CI) derived from bootstrapping (500 runs) where the CI is the actual range of 95% of the bootstrap estimates.

					Millar (1990)	
Estimated origin	6-Variable raw classification proportions	6-Variable Cook and Lord (1978) corrected	6-Variable Cook (1983) constrained corrected	6-Variable Millar (1987) maximum likelihood constrained	6-Variable direct maximum likelihood	14-Variable direct maximum likelihood
(1) Baseline = Hatchery & Wild 1990, Mixed sample = 100% Hatchery 1991 (n=171 fish)						
Hatchery	0.655	0.933	0.933	0.933	0.915	0.735
95% CI	(.555-.760)	(.595-1.164)	(.595-1.000)	(.595-.999)	(.681-.999)	(.532-.884)
Wild	0.345	0.067	0.067	0.067	0.085	.265
95% CI	(.234-.444)	(-.015-.372)	(.000-.372)	(.000-.372)	(.000-.309)	(.110-.462)
(2) Baseline = Hatchery & Wild 1990, Mixed sample = 100% Wild 1991 (n=158 fish)						
Hatchery	0.285	-0.007	0.000	0.001	0.028	0.003
95% CI	(.215-.411)	(-.019-.355)	(.000-.355)	(.000-.355)	(.000-.281)	(.000-.266)
Wild	0.715	1.007	1.000	0.999	0.972	0.997
95% CI	(.582-.785)	(.638-1.210)	(.638-1.000)	(.638-.999)	(.715-.999)	(.733-.999)
(3) Baseline = Hatchery & Wild 1991, Mixed sample = 100% Hatchery 1990 (n=138 fish)						
Hatchery	0.609	0.799	0.799	0.799	0.918	1.000
95% CI	(.529-.732)	(.582-1.168)	(.582-1.000)	(.582-.999)	(.652-.999)	(.745-1.00)
Wild	0.391	0.201	0.201	0.201	0.082	0.000
95% CI	(.261-.471)	(-.012-.401)	(.000-.401)	(.000-.401)	(.000-.341)	(.000-.251)
(4) Baseline = Hatchery & Wild 1991, Mixed sample = 100% Wild 1990 (n=73 fish)						
Hatchery	0.288	0.007	0.007	0.008	0.057	0.148
95% CI	(.178-.411)	(-.012-.346)	(.000-.346)	(.000-.346)	(.000-.206)	(.003-.350)
Wild	0.712	0.993	0.993	0.992	0.943	0.852
95% CI	(.575-.822)	(.639-1.288)	(.639-1.000)	(.639-.999)	(.774-.993)	(.637-.988)

Table 3. The means, standard deviations (St. Dev.), and mean squared errors (Mean SE) of the composition estimators over 500 simulations using pooled-year (1990-1991) hatchery (n=309) and wild (n=231) steelhead data. In each run a 100 fish simulated mixture with a specified composition was randomly generated. The baseline data were resampled.

Origin	Raw classification proportions	Cook and Lord (1978) corrected	Cook (1983) constrained corrected	Millar (1987) maximum likelihood constrained	Millar (1990) direct maximum likelihood
(1) Specified mixed sample composition: 0.00 Hatchery, 1.00 Wild					
Means					
Hatch.	0.2834	0.0188	0.0625	0.0627	0.0405
Wild	0.7166	0.9812	0.9375	0.9373	0.9595
St. Dev.	0.0491	0.1326	0.0815	0.0813	0.0583
Mean SE	0.2876	0.1340	0.1027	0.1027	0.0710
(2) Specified mixed sample composition: 1.00 Hatchery, 0.00 Wild					
Means					
Hatch.	0.6696	0.9862	0.9376	0.9375	0.9595
Wild	0.3304	0.0138	0.0624	0.0625	0.0405
St. Dev.	0.0529	0.1371	0.0858	0.0857	0.0622
Mean SE	0.3346	0.1378	0.1061	0.1061	0.0742
(3) Specified mixed sample composition: 0.25 Hatchery, 0.75 Wild					
Means					
Hatch.	0.3857	0.2711	0.2721	0.2721	0.2578
Wild	0.6143	0.7289	0.7279	0.7279	0.7422
St. Dev.	0.0510	0.1304	0.1281	0.1280	0.1045
Mean SE	0.1450	0.1321	0.1299	0.1299	0.1048

Table 3. (cont'd)

Origin	Raw classification proportions	Cook and Lord (1978) corrected	Cook (1983) constrained corrected	Millar (1987) maximum likelihood constrained	Millar (1990) direct maximum likelihood
(4) Specified mixed sample composition: 0.50 Hatchery, 0.50 Wild					
Means					
Hatch.	0.4735	0.4892	0.4892	0.4892	0.4840
Wild	0.5265	0.5108	0.5108	0.5108	0.5160
St. Dev.	0.0533	0.1371	0.1371	0.1370	0.1135
Mean SE	0.0595	0.1375	0.1375	0.1375	0.1146
(5) Specified mixed sample composition: 0.75 Hatchery, 0.25 Wild					
Means					
Hatch.	0.5767	0.7503	0.7470	0.7470	0.7299
Wild	0.4233	0.2497	0.2430	0.2530	0.2701
St. Dev.	0.0547	0.1429	0.1358	0.1358	0.1097
Mean SE	0.1817	0.1429	0.1359	0.1358	0.1116

Table 4. The means and standard deviations of the squared differences between estimated and true compositions over 500 simulations using specified sizes of simulated mixtures of pooled-year (1990-1991) hatchery (n=309) and wild (n=231) steelhead data. Prior to each simulation the true composition was randomly generated. The baseline data were resampled.

	Raw classification proportions	Cook and Lord (1978) corrected	Cook (1983) constrained corrected	Millar (1987) maximum likelihood constrained	Millar (1990) direct maximum likelihood
(1) <u>Specified size of mixed sample = 25 fish</u>					
<u>Mean</u>	0.0424	0.0693	0.0545	0.0544	0.0368
<u>St. Dev.</u>	0.0506	0.1001	0.0848	0.0848	0.0566
(2) <u>Specified size of mixed sample = 50 fish</u>					
<u>Mean</u>	0.0360	0.0371	0.0302	0.0301	0.0207
<u>St. Dev.</u>	0.0402	0.0543	0.0455	0.0455	0.0314
(3) <u>Specified size of mixed sample = 100 fish</u>					
<u>Mean</u>	0.0344	0.0185	0.0155	0.0155	0.0092
<u>St. Dev.</u>	0.0323	0.0270	0.0244	0.0244	0.0138
(4) <u>Specified size of mixed sample = 200 fish</u>					
<u>Mean</u>	0.0358	0.0113	0.0100	0.0100	0.0059
<u>St. Dev.</u>	0.0331	0.0149	0.0138	0.0138	0.0081

Table 4. (cont'd)

	Raw classification proportions	Cook and Lord (1978) corrected	Cook (1983) constrained corrected	Millar (1987) maximum likelihood constrained	Millar (1990) direct maximum likelihood
(5) <u>Specified size of mixed sample = 400 fish</u>					
<u>Mean</u>					
	0.0325	0.0066	0.0059	0.0059	0.0037
<u>St. Dev.</u>					
	0.0315	0.0092	0.0083	0.0083	0.0053