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Discrimination of Norwegian farmed, ranched and wild-origin Atlantic salmon, *Salmo salar* L., by image processing

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Abstract A method of distinguishing between farmed, ranched and wild-origin Atlantic salmon, *Salmo salar* L., using scale morphology is proposed. Circuli spacing and scale texture data, as expressed as a Fourier transform of transmission luminescent patterns, were extracted by image processing. Spacing patterns and texture features were most distinct for wild salmon compared with the other two groups. Three-group quadratic discriminant function models were developed using different combinations of data types. The most efficient model to separate the three groups had a classification efficiency of 74%. When models were simplified to two groups, farmed and wild, efficiency increased to 90%, thus reflecting the feature overlap between farmed and ranched groups. The method may be a useful tool for more objective and efficient classification of wild versus husbandry-origin salmon. However, it should be stressed that farmed salmon that escape at the smolt stage are still problematic.

KEYWORDS: aquaculture, Atlantic salmon, escapee, image processing, stock identification.

Introduction

As world production of farmed Atlantic salmon, *Salmo salar* L., has increased, so has the frequency of fish farm escapees in marine and freshwater habitats utilized by wild stocks (Hansen, Lund & Hindar 1987; Egidius, Hansen, Jonsson & Nævdal 1991; Gausen & Moen 1991; Jacobsen, Hansen & Lund 1992; Webb & Youngson 1992). The appearance of sexually mature fish-farm escapees in freshwater habitats raises concerns about long- and short-term effects of genetic mixing and disease on wild stocks (Hansen *et al.* 1987; Egidius *et al.* 1991; Hindar, Ryman & Utter 1991). In addition, the appearance of escaped salmon in marine grazing habitats confounds fisheries management by altering perceived harvest impacts, thus varying exploitation to achieve conservation goals of specific spawning escapement is carried out with less accuracy and precision (Phillips, Beveridge & Ross 1985; Lund, Økland & Hansen 1991). The high apparent stock biomass caused by the abundance of escapees could

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lead to overestimation of the abundance of wild stocks and, if not accounted for, the subsequent overexploitation of wild stocks. In general, the presence of fish-farm escapees amplifies the dilemma of mixed-stock salmon fisheries (Ricker 1958).

In both marine and freshwater habitats it has become increasingly important to identify and quantify farm-escapee salmon from wild fish. Techniques of scale interpretation (Antere & Ikonen 1983; Lund & Hansen 1991), analysis of the content of carotenoids (Craik & Harvey 1987; Lura & Sægrov 1991), and multivariate analysis of body shape and fin characteristics (Hansen *et al.* 1987; Potter 1987; Lund, Hansen & Järvi 1989) are generally satisfactory, but are open to criticism. For morphology-based techniques, characteristic features are subjectively identified and included in classification rules. Techniques based on chemical identification of artificial pigments are dependent on the continued use of the specific pigments in the aquaculture industry.

This paper examines the properties of an objective set of quantitative measures to distinguish salmon of wild versus fish-farm origin. Imaging techniques to measure circuli spacing and surface texture patterns of salmon scales are used (Schwartzberg & Fryer 1989; Ross & Pickard 1990; Barlow & Gregg 1991) to develop rules to classify salmon into three groups according to origin: farmed, ranched and wild.

Methods

Scale samples were taken from salmon known to have originated from fish farms, ranching projects, and wild stocks, respectively. Scale samples were assembled to approximate the type of training set needed to develop classification rules to identify origin of salmon in Norwegian fisheries. Farm-origin salmon were sampled from five fish farms in mid-Norway and collected during 1986 and 1987. Ranched-origin salmon, also collected during 1986 and 1987, were fish released in the Imsa River and recovered in the Imsa trap; their identity was confirmed by scale reading and tagging. Wild-origin salmon were captured in marine fisheries during 1970 and 1971 before the start of extensive fish farming. The samples of wild fish were obtained from an earlier period to ensure correct identification of their origin. This is difficult to do with contemporary samples due to the large number of fish-farm escapees at large today. A total of 213 fish were used in the study, 71 from each of the three groups. Farm- and ranch-origin salmon were of river age 1 and of sea ages 1 and 2 (greater than 80% of the samples were from 1-sea-winter salmon). Wild-origin salmon were, for the most part, of freshwater ages of 2 and 3, and like the other group, of sea ages 1 and 2 (greater than 75% of the sample was from 1-sea-winter salmon). The mean fork length of each group was: farm 67.1 cm (CV 12%); ranch 63.9 cm (CV 15%); and wild 65.5 cm (CV 20%).

Scale samples were collected from the left side of the fish, three to six scale rows above the lateral line, and on a line defined by the posterior edge of the dorsal fin and the anterior edge of the anal fin (Reddin, Stansbury & Short 1988). Before examination, scales were cleaned manually with a mild detergent and mounted between glass slides. A single extraction transect (approximately 1.4 mm in length) was drawn from the focus towards the anterior edge of the scale along the 360° axis (Fig. 1). From this line, a luminescence profile of transmitted light was extracted which was further processed

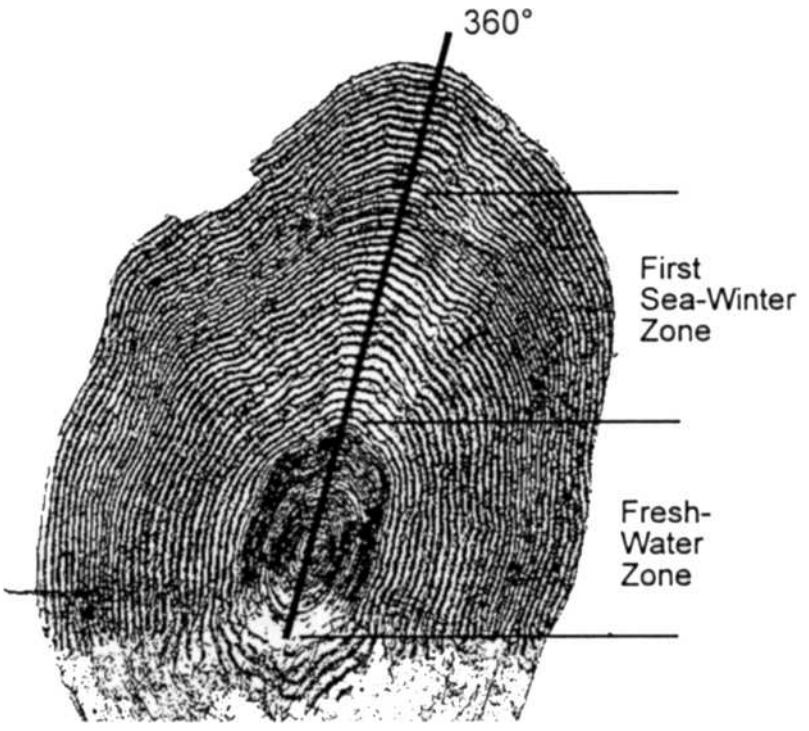

Figure 1. Illustration of salmon scale with 360° transect marked.

to determine circuli spacing and the Fourier transform of the luminescence pattern with Optimas* image-processing software (Bioscan 1989). This processing involved pattern comparison of the profile with a weighted average function to identify the location of circuli. Circuli spacing features were presented as the distances (mm) between adjacent circuli. Only the first 28 circuli pairs were used to avoid incorporation of missing values in the database. Textural features were expressed as the magnitudes of a Fourier transform of the luminescence profile of the transect sampled over 256 intervals. The magnitude was calculated as the square root of the sum of the squared coefficients for each harmonic. The first 50 harmonics were used in the analysis. Distributions of all variables were tested for normality with the Kolmogorov–Smirnov one-sample goodness-of-fit test. None of the variables was transformed.

Discriminant function models were constructed using circuli spacing and periodogram magnitude data. Three discriminant functions were tested: (A) a three-group model using farmed, ranched and wild salmon; (B) a two-group model using all husbandry salmon, farmed and ranched combined, and wild salmon; and (C) a two-group model using farmed and wild salmon only. Models were constructed from circuli spacing data, magnitude data, and a combination of both types of data. Stepwise linear discriminant

* Reference to trade name does not imply endorsement.

analysis, using forward and backward stepping, was used to guide selection of variable sets used in each function (SAS 1990). The number of variables used was constrained at 20, which conservatively stays below the limits suggested by Saila & Martin (1987). Homogeneity of the within-group covariance matrices was tested for all models. In all cases the matrices were not homogeneous and the individual within-group matrices were used, thus a quadratic discriminant function was computed (SAS 1990). All prior probabilities of classification were proportional to sample sizes in the respective analysis (i.e. priors equal 0.33 in model A, 0.66 and 0.33 in model B, and 0.5 in model C). Classification efficiencies (per cent correctly classified) were cross-validated according to the methods of Lachenbruch & Mickey (1968). Cohen's kappa (κ) statistic, and its 95% confidence interval, were computed for each discriminant function (Titus, Mosher & Williams 1984). This statistic estimates the improvement over chance of the per cent corrected classification rates.

Stepwise linear discriminant analyses were performed based on two sets of composite variables representing circuli spacings and magnitudes of the Fourier functions averaged over adjacent pairs and quadruplets of variables, respectively. None of the data were excluded with the pairwise averaging because there was an even number of input variables. The same data were also averaged by groups of four, or quads, again beginning with variable one of each data set. Because the magnitude data were not divisible by four, some of the higher-order magnitudes were dropped from the analysis. Because there were no tests of the homogeneity of the within-group covariance matrices for these functions, the results are presented for comparative purposes on variable sampling and selection. No meaning was attached to the classification efficiencies associated with each function.

Results

The mean patterns of circuli spacing for farmed, ranched and wild-origin salmon show differences between groups (Fig. 2). Wild-origin salmon have wider-spaced circuli than the other groups, particularly for circuli pairs near the end of the collection transect (pairs 20–28). Although not greatly different, circuli spacings for the farm-origin group appear slightly wider than observed for the ranch-origin group. Likewise, the mean patterns of periodogram magnitudes of surface texture for farmed, ranched and wild-origin salmon show differences between groups (Fig. 3). The most striking difference between groups is the peak in spectral density seen for wild-origin fish between harmonics 19 and 22. Spectral density between groups is also different for the region of the periodogram between harmonics 30 and 45.

Results of the three-group discriminate function analysis to allocate fish of farm, ranch or wild origin were mixed depending on data type used in the function. Models constructed from only one data type, either spacing or magnitude data, had classification efficiencies below 65% (Table 1A). Mixed models (using both spacing and magnitude data) produced better results and were optimized to a classification efficiency of 74% with group classification biases of −1%, 6% and −6%, for farmed, ranched and wild fish, respectively. Examination of the group biases and a canonical variate plot shows

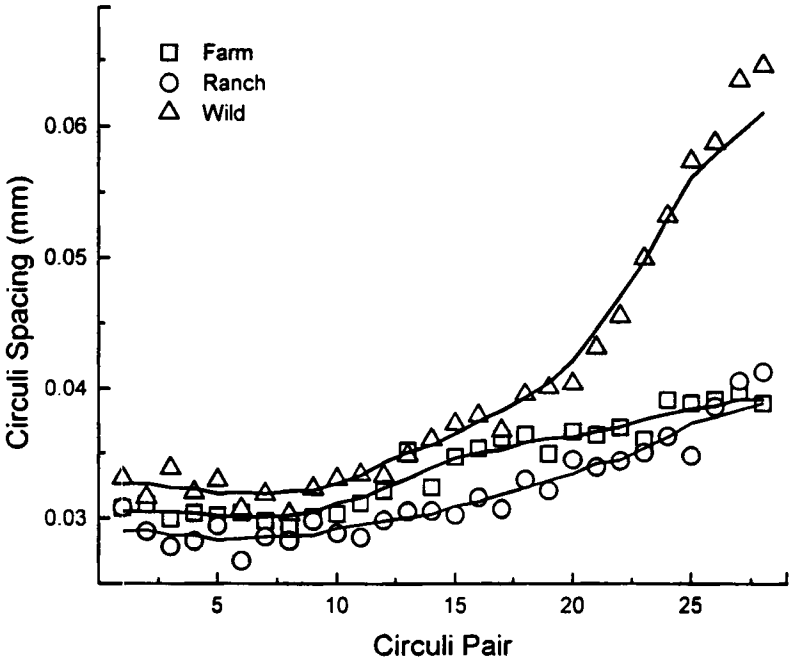

Figure 2. Patterns of mean circuli spacing versus circuli pair for farmed, ranched and wild-origin Atlantic salmon. Smooth line is simple adjacent averaging.

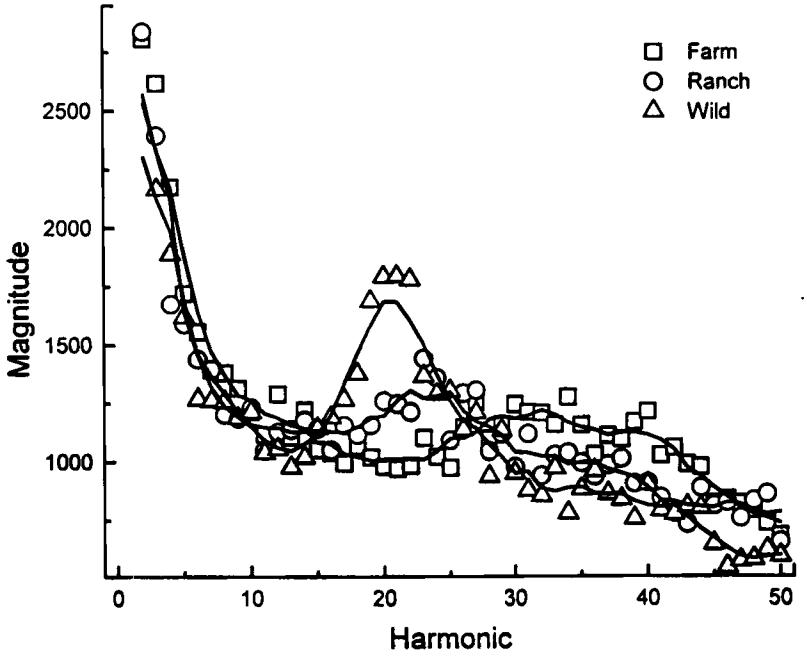

Figure 3. Patterns of mean magnitude of the Fourier transform of luminescence pattern versus harmonic for farmed, ranched and wild-origin Atlantic salmon. Smooth line is simple adjacent averaging.

Table 1. Quadratic discriminant function models to classify farmed, ranched and wild Atlantic salmon. Part A of the table presents results of three-group discriminations between farmed, ranched and wild-origin salmon. Part B gives results for two-group discriminations between husbandry salmon (farmed and ranched) versus wild salmon. Part C presents results for two-group discriminations between farmed and wild salmon.

	Model variables		Number of variables	Group	Cross-validated percent correct	Group bias (%)	Cohen's kappa	
	Circuli spacing	Magnitudes						
A	3 13 25 28		4	Farm	70.4	7.0	−95% CI	0.359
				Ranch	52.1	−2.3	κ	0.458
				Wild	69.0	−4.7	+95% CI	0.557
				Total	63.8			
		2 4 12 13	16	Farm	69.0	1.9	−95% CI	0.272
		15 19 21 22		Ranch	47.9	−2.8	κ	0.373
		23 24 30 32		Wild	57.7	0.9	+95% CI	0.475
		39 42 43 49		Total	58.2			
	3 6 10 17	12 19 22 24	13	Farm	76.1	−0.9	−95% CI	0.515
	25 27	39 43 49		Ranch	62.0	6.6	κ	0.606
				Wild	83.1	−5.6	+95% CI	0.696
				Total	73.7			
B	3 7 10 17		5	Farm-Ranch	90.1	2.3	−95% CI	0.532
	28			Wild	73.2	−2.3	κ	0.645
				Total	84.5		+95% CI	0.759
		2 3 6 13	10	Farm-Ranch	85.2	2.8	−95% CI	0.350
		17 19 21 22		Wild	62.0	−2.8	κ	0.482
		34 46		Total	77.5		+95% CI	0.614
	3 28	1 6 16 22	8	Farm-Ranch	90.1	2.3	−95% CI	0.550
		31 46		Wild	76.1	−2.3	κ	0.662
				Total	85.5		+95% CI	0.774
C	3 7 27 28		4	Farm	88.7	6.3	−95% CI	0.520
				Wild	76.1	−6.3	κ	0.648
				Total	82.4		+95% CI	0.776
		1 12 14 17	11	Farm	94.4	5.6	−95% CI	0.669
		19 20 21 22		Wild	83.1	−5.6	κ	0.775
		32 34 44		Total	88.7		+95% CI	0.881
	7 10 16 24	1 5 6 11	19	Farm	91.6	1.4	−95% CI	0.703
	27	12 16 19 21		Wild	88.7	−1.4	κ	0.803
		22 25 31 39		Total	90.1		+95% CI	0.903
		41 44						

that the greatest overlap was between ranched and either wild or farmed fish (Table 1A and Fig. 4).

Discriminant functions were also developed as simpler models by reducing the number of groups from three to two. In the first set of trials wild fish were compared

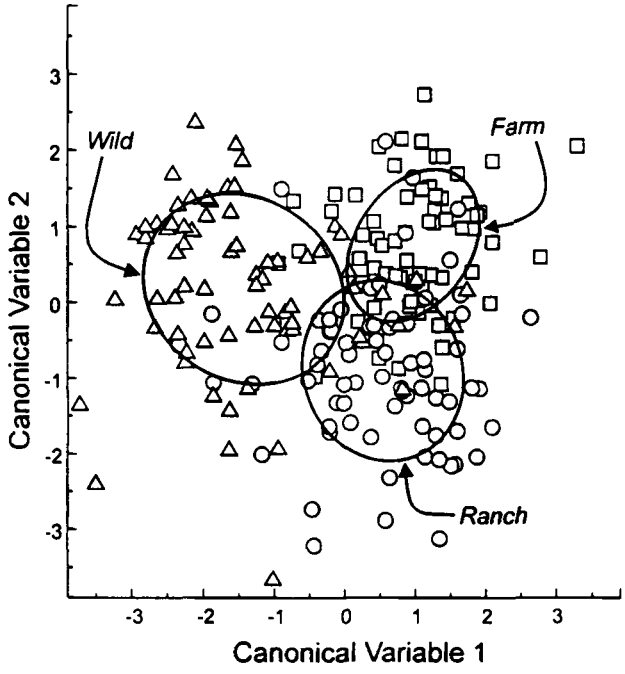

Figure 4. Canonical variate plot for three-group discriminate function using both circuli spacing and Fourier transform magnitude data. Gaussian bivariate confidence ellipses ($P = 0.5$) are drawn for each group.

with all husbandry fish (ranched and farmed combined). The models were slightly more efficient than the three-group models, with the best model being constructed with mixed data sources (Table 1B). This model had an overall classification efficiency of 86% and group classification bias of ±2.3%. Cohen's kappa (κ) was 0.662, indicating that the classification efficiency was 66% better than would have occurred by chance alone (95% confidence interval was approximately 55% to 77%). Because the expectation of encountering ranched fish in nature is significantly less than that of encountering farm- or wild-origin fish, trials were conducted with two groups excluding ranch samples. These models were relatively strong with classification efficiencies at or close to 90% (Table 1C). The best model again was constructed with mixed data sources and had an overall classification efficiency of 90% and group classification bias of ±1.4%. Cohen's kappa (κ) was 0.803, indicating that the classification efficiency was 80% better than would have occurred by chance alone (95% confidence interval was approximately 70% to 90%).

In all cases, averaging the data reduced the number of variables included in a linear stepwise discriminant function model (Table 2). In only the three-group models using the magnitude data was classification efficiency reduced by averaging the variables.

Discussion

The scale morphology features extracted with image-processing techniques can be used

Table 2. Performance of linear discriminant function models to classify farmed, ranched and wild Atlantic salmon tested at three levels of data averaging: direct use of variables, variables averaged as pairs, and variables averaged as quadruplets. Part A of the table includes results of three-group discriminations between farmed, ranched and wild-origin salmon. Part B includes results for two-group discriminations between husbandry salmon (farmed and ranched) versus wild salmon. Part C includes results for two-group discriminations between farmed and wild salmon.

		Circuli spacing data		Magnitude data		Circuli spacing and magnitude	
	Data sampling	Cross-validated percent correct	Number of variables	Cross-validated percent correct	Number of variables	Cross-validated percent correct	Number of variables
A	Direct	63.8	4	68.1	16	77.0	14
	Pairs	62.4	3	62.4	9	68.1	6
	Quads	64.3	2	61.5	6	67.6	8
B	Direct	83.6	5	79.8	10	86.9	10
	Pairs	85.0	2	81.2	4	85.4	6
	Quads	82.2	2	76.1	5	86.4	7
C	Direct	86.6	4	90.1	11	94.4	20
	Pairs	86.6	3	89.4	8	90.1	10
	Quads	84.5	1	87.3	8	88.7	7

to identify salmon of farm, ranch and wild origin. The approach used is quantitative and objective in that measurements are automated and without the problems associated with manual scale readings (Lund *et al.* 1989). Douglas, Minckley & Tyus (1989) suggested that qualitative characters are excellent features upon which to base group separations and presented extensive data that demonstrated that even untrained observers show a high degree of feature interpretation. Our results do not invalidate the currently used techniques to classify wild versus husbandry-origin salmon (Antere & Ikonen 1983; Lund, Hansen & Järvi 1989; Lund & Hansen 1991). However, the automated approach is an improvement because it addresses sources of procedural inaccuracy, such as those associated with reader fatigue, and removes any doubt of reader subjectivity from potentially sensitive management data. Manual scale reading and image-processing techniques use essentially the same features of the scale to form information databases to classify the samples to origin. With image processing the classification algorithm can be defined explicitly for review and there is a complete quantitative audit trail for each decision.

The robustness of the variables in the models to annual or long-term sources of variability has not been tested. Annual variations in climate and food resources affect circuli deposition in Atlantic salmon (Reddin *et al.* 1988). However, these sources of variability would not be expected to affect the general pattern of circuli deposition to the extent that these features would not be useful in the discriminant functions described here. Long-term phenomena, such as climate change or change in husbandry practices, probably have a more profound effect on the features used in the discriminant

functions. Nonetheless, as with any scale-based discrimination procedure, it is essential to maintain reference collections so that the model can be reparameterized (Reddin, Verspoor & Downton 1990). Annual or longer-term sources of variability will be irrelevant if the proper reference samples are collected and applied.

The most problematic aspect of this study and other approaches used to separate farmed, ranched and wild-origin salmon is the ability to distinguish between farm and ranch groups. The greatest overlap in morphology features and misclassification rates occurred between these two groups and these problems probably increase with decreasing age of escape for the farmed fish (Antere & Ikonen 1983; Lund *et al.* 1989; Lund & Hansen 1991). Farmed salmon that escape at the smolt stage are virtually indistinguishable from ranched salmon by conventional classification techniques, but imaging techniques could address this problem if there are systematic differences in scale characteristics between hatcheries and the correct reference samples are collected.

The responsiveness of scale morphology to environmental features makes the use of circuli spacing and scale texture more useful than other Atlantic salmon stock identification characters in the context of this study. Use of genetic characters (de Pontual & Prouzet 1987; Bermingham, Forbes, Friedland & Pla 1991; Cutler, Bartlett, Hartley & Davidson 1991) to separate wild and escapee fish is problematic, because escapee salmon are likely to be mixed with progenitor stocks in local fisheries and rivers. The obvious exception is when the cultured stock is not derived from local stocks (Garcia de Leániz, Verspoor & Hawkins 1989). Another class of approaches, yet to be evaluated with Atlantic salmon, is the microchemical analysis of easily sampled hard body parts (Coutant 1990). The immediate appeal of the microchemical approach is that wild and husbandry fish have radically different diets during juvenile growth. Whether there is sufficient divergence in hard body part microchemistry, or if it can be detected at reasonable costs, is unknown.

Variable averaging, expressed as sampling by pairs and quadruplets, had the expected effect of reducing the number of variables selected by a stepwise discriminant function procedure. In only one case did variable averaging reduce resolution to the extent that model classification efficiency went down. In similar studies using circuli spacing data, Barlow & Gregg (1991) reported that model efficiency was similar or only slightly higher for averaged data. The appeal of averaged data lies in the anticipated robustness of models to the potential problem that information content may be dispersed over a number of adjacent variables. However, from this study it appears that there is loss of information content when averaging is performed. Even with the reduced number of variables included in quadruplet trials, the actual number of data values in these trials exceeded the number used when data were applied without averaging. Thus, the pair and quadruplet averaging trials used more data with no improvement in model efficiency. This study suggests that important features can be expressed directly in the individual spacing and magnitude values, thus averaging should be applied on a case-by-case basis where it improves classification efficiency.

While several sources of model inaccuracy can be identified, such as overlap in morphological traits and poor sample quality, the only potential source of bias identified was related to the automated procedure used to mark circuli. As described above, scale

features of farmed and ranched salmon were similar because most of the scale is laid down during early life history events which are similar for the two groups. The scale features of wild fish were most dissimilar to these two groups due to wider circuli spacing in both freshwater and marine growth zones. When the image-processing system marks a circulus for measurement, it assesses a moving average function of luminescence values to determine where the circuli occurred. It is relatively infrequent that circuli would not be marked or corrected manually before the data are saved. However, circuli could be marked more than once and the double mark could then escape manual correction. This has been observed to occur with circuli that are very wide or are of complex morphology. This error will tend to add to the total number of circuli for a specimen and decrease the circuli spacing for the adjacent circuli pairs. The direction of the bias would be to classify wild salmon as hatchery-origin fish because hatchery fish tend to have narrower circuli spacings.

Precision with this technique is very high, which is in contrast to approaches dependent on a scale reader. It is well known that fatigue, pattern of prior observations, and long-term familiarity can affect the precision of scale readers (Lund *et al.* 1989). Feature extraction with the image processor is identical regardless of stage of the analysis and it is also insensitive to problems created by changes in project personnel.

As salmon aquaculture continues to expand, the rapid and accurate identification of fish-farm escapees will be useful in defining the ecological and fisheries management of Atlantic salmon. Managers can only adapt to, rather than control, some changes in salmon productivity, i.e. due to climate and environment. Loss of salmon productivity to the point of local stock extinction, however, may arise due to aquaculture through epizootic infections or genome corruption (Skaala, Dahle, Jørstad & Nævdal 1990; Egidius *et al.* 1991; Gausen & Moen 1991). Understanding when and where husbandry salmon are occurring in the environment will provide useful, but currently unavailable, feedback to managers and industry about siting practices, pen construction, and weather tolerance.

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