RACIAL INVESTIGATION OF THE STRIPED BASS USING CRITICAL SCALE ANALYSIS

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SUMMARY

1. A new scale analysis system has been developed. It uses the Dektak surface profile analyzer to automatically detect scale surface microstructure. The surface profiles are digitized and stored on magnetic tape. These digital profiles may be analyzed by computer to yield objective values for circulus height and spacing.

2. A spectral analysis algorithm, "POWERFUL" has been used to process recorded scale profiles. Automatic measurement of circulus spacing is performed and a graphical display of dominant circulus spatial frequencies present within the profile is produced. Since most profiles have a single sharply peaked dominant frequency, any regional difference in circulus spacing may be detected simply and accurately if it is present.

3. Intercirculus spacing does not appear to be generally useful in discriminating between populations of striped bass derived from the Chesapeake and Hudson regions. Only fish from the Potomac area are distinguishable on the basis of scale growth anomalies in circulus spacing. No explanation for this anomaly has been found to date.

4. HBAR, or average circulus height was found to vary

significantly between the Chesapeake and Hudson groups. Mean values of 13.66 microns were recorded for the Hudson training set. A mean value of 11.08 microns was obtained for the Chesapeake training set.

5. Some portion of the observed difference in HBAR was ascribable to the non- homogeneous size composition of the two groups. Mean fork- length values were: Hudson, 723 mm; Chesapeake, 423 mm. HBAR was an increasing function of fork length, and varied more strongly among the Chesapeake training set (see table 6).

6. On the basis of training sets derived from known spawning areas, linear discriminant analysis permits objective reclassification accuracies of between 65 and 70 % for synthetic unknown test sets. Size inhomogeneities between the groups contribute slightly to the observed regional variability. Tests on limited numbers of size homogeneous fish indicate that accurate reclassification is possible at the 70 % level, but a new larger training sample is required in order to produce acceptably high levels of reclassification accuracy (> 80 %).

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The concept of a unit stock is implicit in most fishery management models. In certain cases (Icelandic cod, Peruvian anchovetta) the existence of a single, discrete spawning population is easily demonstrated. However in other cases, intermingling of stocks is a common occurrence. Sophisticated techniques must then be employed in an attempt to separate the overall mixed stock into its separate unit components.

The striped bass (Morone saxatilis) presents complex problems in this respect. Several generations of fisheries biologists have pursued extensive investigations into the migratory and reproductive behavior of this coastal species. Tagging and meristic studies have shown that this species is not truly homogeneous throughout its spawning range but instead appears to be composed of several "races" or breeding subpopulations (Raney & De Sylva,1953; Lewis,1957).

This paper will describe the results of these previous investigations as they relate to the population structure of this species. Development of a new method for racial analysis will then be described. The method is based on a precision automated analysis of scale growth patterns, and appears to possess significant advantages in terms of simplicity,speed,and accuracy,relative to earlier methods.

Merriman undertook important early investigations into the life history and migratory behavior of the striped bass (Merriman, 1941). For these investigations, a total of 3,937 fish were tagged and released. Of these, some 568 or 14.4 % were eventually recaptured. This represented the first large-scale tagging effort undertaken for Atlantic coast striped bass.

Fish tagged in the Niantic and Thames R., Connecticut provided returns which indicated a massive coastal migration to the north-east occurring each spring. Summer populations remained essentially static, but in late October a mass southern migration began and continued into early winter. Fish tagged in Connecticut and Long Island waters began to be recovered from the waters off of New Jersey, Delaware,in the Chesapeake Bay, and south to Albemarle Sound, North Carolina (Merriman, 1941,p.38). This general pattern of migration has been confirmed by numerous subsequent investigators (Vladykov & Wallace, 1952; Nichols & Miller, 1967). The results of an extensive tagging program undertaken to further understanding of migration of striped bass captured and released in the New York Bight region may be found in Clark's 1968 thorough review article (TAFS).

Since this fish regularly undertakes extensive coastal migrations, we have a general situation in which considerable stock overlap might be expected to occur. As evidence of this, we have Vladykov & Wallace's (1952) recapture data. Of 1,869 fish tagged and released in mid- Chesapeake waters, 1.5 % (28) were eventually recaptured in northern (N.J.,

Conn., Rhode Island, Massachusetts) waters. The actual percentage is almost certainly greater, since the results were biased by local recaptures of potentially migrant fish. Conversely, of 1,397 striped bass tagged by Merriman in Conn. waters, 5 % were retaken from N. J. waters and south to N. Carolina. There is ample evidence of extensive stock overlap over the spawning range of this species.

Given the foregoing we still lack any accurate knowledge as to the degree of genetic mixing actually occurring. Does a single, homogeneous population exist? Are there characteristic differences between individual fish which would make possible their assignment into discrete spawning groups? These are the questions to which the techniques of source stock analysis have been applied.

Raney & De Sylva (1953) approached this problem through a graphical analysis of countable (meristic) characters. Demonstration of a severe inhomogeneity among fish collected on different spawning grounds would provide strong arguments against a completely mixed population.

On the basis of meristic counts of young fish collected from the major river systems used in spawning by this anadromous species, Raney & De Sylva (1953) reported the following. " Frequency distributions of dorsal, anal, and pectoral fin soft-ray counts, indicate differences between the Hudson R. samples on the one hand, and the Chesapeake Bay samples on the other, the latter usually being higher." Subsequent investigations have tended to confirm the original conclusions arising from this early work. On the basis of

both meristic and morphometric (body-depth;various part-to-whole length ratios) characters, a separate population is felt to exist in the Hudson R. (Raney & De Sylva,1953 ; Lund,1957; Lewis,1957; Raney,1957; Murawski,1958). As evidence Raney & De Sylva used the character index (C.I.) of the two groups (a measure equal to the sum of anal, pectoral and dorsal counts) to achieve an 81 % separation. This level was achieved by dividing the groups along the C.I. = 56 axis. 77 % of the Ches. specimens were above this cut-off, while 85 % of the Hudson sample was below it (Raney & De Sylva,1953).

At least three subpopulations have been tentatively identified within the Chesapeake bay area. Most investigators agree that the James R. population appears highly differentiated from the remainder of the bay populations (Raney & De Sylva,1953; Lund ,1957). The York-Rappahannock complex seems to comprise a second subpopulation, while fish from the upper bay (north of the Rappahannock R.) seem to comprise a third differentiable subpopulation (Lewis,1957).

Thus, some progress has been made toward a preliminary understanding of the population structure for this species. Investigators agree however, that a far greater understanding of the nature of the population composition awaits the development of a more rapid method for source stock identification. Such a method would perhaps enable researchers to categorize individuals taken on the high seas into their component subpopulations. This would permit

accurate assessment of the net contributions of the individual spawning grounds to the overall fishery. Declining areas would clearly reveal themselves, and provide an early warning as to the need for protection and reclamation.

As early as 1941, Merriman proposed using regional growth rate variations (as reflected in differential scale growth) as a rapid means of source stock separation. Reasoning that "...conditions that affect growth rate, such as temperature and food,may differ in different localities, Merriman theorized that the scales, which reflect the growth rate, might show differences at least in the first two years, before striped bass undertake long migrations" (Raney & De Sylva,1953). Merriman produced evidence in support of this hypothesis, which will be discussed in greater detail below. His studies represent an early application of scale analysis to studies of the racial origin of the striped bass.

Background of Scale Analysis in Racial Studies.

Scales have long been used in age and growth studies of fish. Scofield (1931) was one of the first investigators to validate this technique for use on Californian striped bass. He demonstrated a satisfactory linear relationship between scale growth and fish length. In addition, he used

length-frequency data and otolith analysis to cross-check the accuracy of ages as determined from scale annuli. All results tended to confirm the validity of scale-derived age determinations for fish up to the age of eight years (Scofield,1931). Merriman (1941) examined scales from Atlantic coast striped bass, using a similar procedure, and determined scale analysis to be a valid tool for use in age and growth studies.

In addition to the important role which scales have played in the ageing of fish, numerous studies have demonstrated that from a detailed study of scale surface growth structure one may successfully elucidate the original geographic origins of migratory, mixed stock populations. Anas and Murai (1968) classified sockeye salmon (Onchorhynchus nerka) caught on the high seas into Asian and North American subgroups. For the study, three manually measured scale characters were employed. These consisted of:

1. Number of circuli within the first half of the first ocean zone.
2. Distance between circuli 1 and 6 of the first ocean growth zone.
3. Distance between circuli 13 and 18 of the first ocean growth zone

(Anas & Murai, 1968).

These three quantitative scale parameters were input to a quadratic discriminant function. This function mathematically maximizes the intergroup separation obtainable from a given set of characters. Using this method, between

80 and 90 % of those fish caught on the high seas were correctly reclassified into their respective continental subgroups. The accuracy of this technique was cross-checked using the results of tagging programs, and morphometric racial analysis studies. All techniques were in good agreement with scale deduced classifications, when applied to identical samples of fish. This successful study provides dramatic evidence of the significant effect of environmental and genetic differences on the patterns of scale growth and development.

Returning to Merriman's earlier study of the striped bass, manually measured widths of the annual growth zones present on the scales were plotted for fish from Cape Cod Bay, Harkness Pt., Conn., Montauk, L.I., Chesapeake Bay, and Currituck Sound, N.C. Striking similarities in annual growth rates were observed among fish from Mass., Conn., L.I., and the Chesapeake area. In sharp contrast to the foregoing areas, the fish from Currituck Sound, N.C. demonstrated second annual growth zone widths two to three times greater than those observed in samples of fish taken from more northerly spawning areas. Merriman strongly indicated, and confirmed in several subsequent year classes, the existence of a significant difference in scale pattern between N.C. fish, and other, more northern samples. Further, he suggested that this observed difference might form a sort of natural "tag", whereby N.C. fish might be easily distinguished from fish from other spawning areas. Unfortunately, his original study (using optical measurement

of projected scale growth patterns) failed to distinguish between fish derived from the northern spawning areas, which have been shown to differ on the basis of meristic, and other analytic techniques. Recent work performed by Texas Instruments Ecological Services Group has successfully detected regional differences in focus-to-annulus distances for the first two years of growth. This represents an additional means for eventual separation of striped bass spawning stocks.

Through the use of a high resolution electronic surface profile analyzer a new level of accuracy and resolution in scale growth pattern analysis has been achieved. Profiles of striped bass scales from the major river spawning areas have been recorded. Digital computer analysis of these profiles has provided quantitative measures of scale growth. These parameters (circulus height and spacing for the innermost two growth zones (years) of scale development) have been input to automatic classification routines, in an effort to determine whether significant differences in scale growth occur between the various major spawning areas. This method represents a natural extension of the research undertaken by Merriman, as described above.

MATERIALS AND METHODS

The primary goal of this study is an analysis of variation in striped bass scale growth patterns between fish derived from several different geographic regions. Figure 1 illustrates the morphology for a typical ctenoid fish scale. A central focus (f) is surrounded by circular ridges (circuli) which are laid down successively, as the scale grows. The fish's entire growth history is permanently recorded on the surface of each scale. (No new scales are created as the fish grows unless one of the original scale complement is damaged or destroyed. The resultant replacement scales are termed regenerate, and lack all pre-regenerate growth information). In addition to the circulus ridges, there is a natural division of each scale into separate lateral, rostral and caudal fields. Only the lateral and rostral fields possess complete and well-formed circuli.

Previous investigations into the surface structure of fish scales have been forced to rely upon manual measurement of scale growth parameters. These measurements have frequently been performed on optical projections of plastic scale growth impressions (Anas and Murai, 1969; Mosher, 1963; Messinger & Bilton, 1974). At the heart of our new technique (outlined schematically in Figure 2) is the Dektak surface

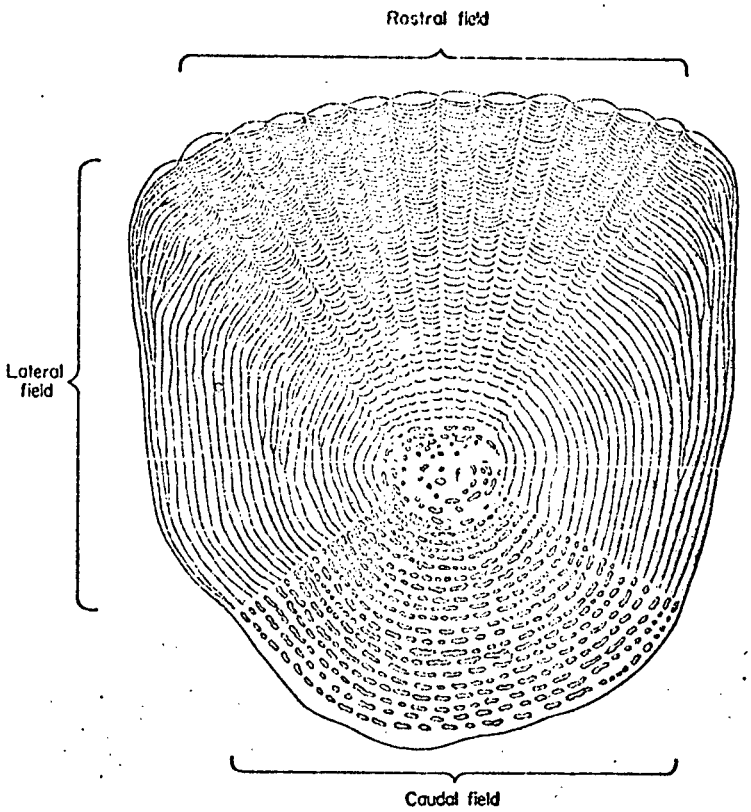

Figure 1. Morphology of a typical ctenoid fish scale (Lanzing, 1974).

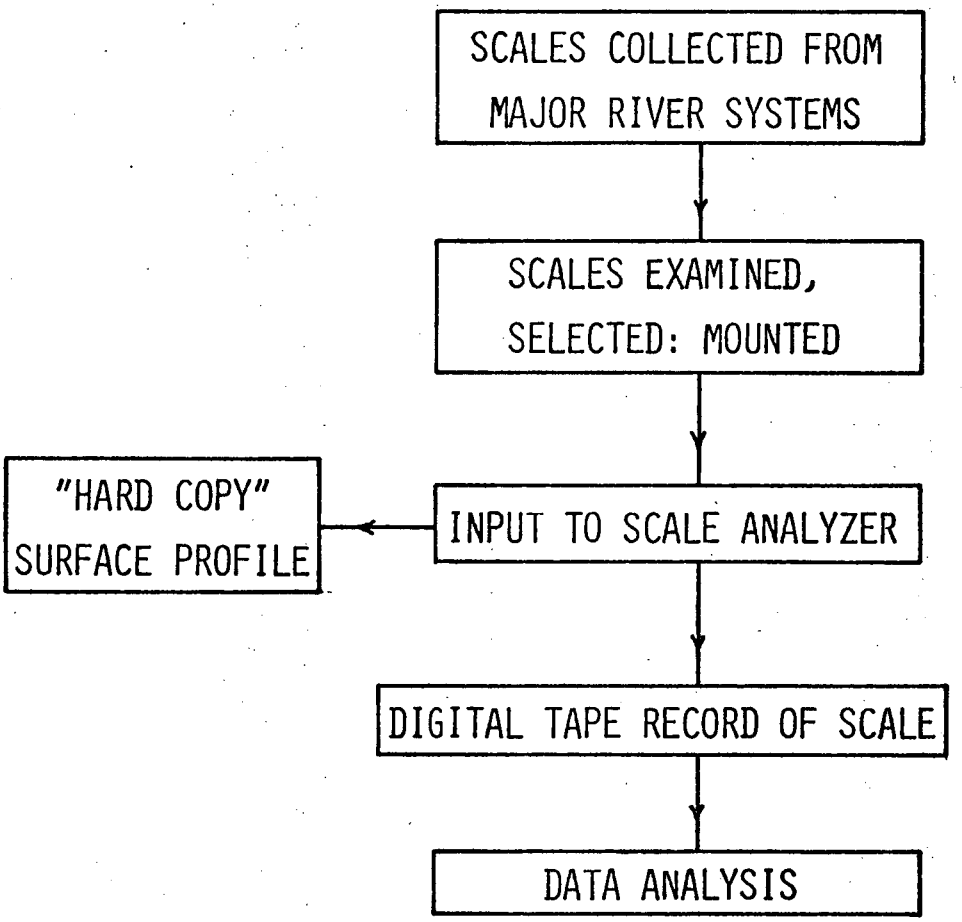

Figure 2. Scale analysis system.

profile analyzer. This device consists of a diamond-tipped sensor stylus(lightly mounted on gimbals) which provides input to an electro- mechanical transducer. The sample is transported at a constant rate underneath the fixed sensor. The internal electronic circuitry of the device automatically converts the minute sample height variations, detected by the stylus, into corresponding electrical signals. The transducer consists of a moveable core connected to the sensor stylus. Motion of the core within a transformer produces a variation in output voltage which is linearly proportional to displacement of the stylus. Motion of the sample is in a straight one-dimensional track under the sensor stylus.

This machine, originally designed for quality control inspection of integrated circuits, is easily adaptable to scale surface profile analysis. Scales, collected from major spawning areas (Rappahannock R., Elk, Choptank, Potomac, and Hudson Rivers) have been provided by the Texas Instrument Ecological Services Group, Buchanan, N.Y. Each scale envelope provides data on date and location of capture, sex and identification number for each fish. Scales are examined initially under a 40x light microscope for evidences of damage or regeneration. If found suitable, scales are moistened with water, and rubbed dry on paper towelling, to remove adhering mucus. Scales are then dried and glued, textured surface uppermost, to an acrylic scale slide (of dimensions 2 in. x 2 in. x .125 in.). Eastman 910 adhesive has been used, as it rapidly and securely bonds scale to

slide. This step is vital to the operation of the technique, since unmounted scales, when dry, tend to have considerable curl. This curl must be eliminated, since the maximum linear region of the Dektak surface profile sensor is limited to a total relief of .1 mm. The method described above has served to maintain scales well within acceptable deformation tolerances.

Reference to fig. 1 indicates the various regions (lateral, rostral, caudal) of a typical ctenoid fish scale. Initially, the rostral field was investigated, using the surface analyzer. Severe difficulty was encountered in this region due to interference from radial grooves in the rostral region. Performance improved dramatically when the site of investigation was transferred to the lateral field, and this became the standard area for analysis.

Scales are oriented using the 40x eyepiece integrally mounted on the surface analyzer. Tracking of each scale begins near the center of the focus area, and extends outward approximately 3 mm into the lateral field. As an aid in orientation, the left edge of the rostral field is aligned with a small fixed mark in the microscope eyepiece. Use of this mark serves to standardize the scale region over which the analyzer passes. Variability in repositioning the scales is also significantly reduced (see App. 1).

A potentiometric pen recorder (±50 mv full scale deflection) responds to the analog height signals generated by the surface analyzer. The recorder has a linear response, to within 0.5 % over its normal range, and provides an

immediate, permanent "hard copy" output of the overall surface profile transmitted by the analyzer.

A typical surface profile is illustrated in fig. 3. Beginning at the left, we observe that the area near the focus (f) displays several irregular low ridges. Within one mm of the origin, however we begin to note the regular pattern of circuli ridges characteristic of the lateral field. This record represents surface structure taken along a 3 mm track outward from the focus. Prior investigations have determined the innermost 3 mm of scale growth to correspond to the first two years of development, a period during which the fish is normally resident within its native river (Merriman, 1941).

The most important feature of our new method is its interface capabilities, which permit computer assisted collection of surface profile data. Upon receipt of an operator-actuated 6 VDC trigger signal, the computer (a Data General Nova 1200 mini-computer) begins sampling the data. Data sampling is controlled at 381.4 samples/mm by an external pulse generator (General Radio GR-1217-c unit pulse generator). Sampling at this rate permits us to detect spatial variation rates of up to 190 cycle/mm without exceeding the Nyquist criterion (FSAMPLE > 2 x FMAX). In this case, FMAX is well above the actual circulus frequencies observed. This prevents occurrence of aliasing (spurious low frequency indications). Sampling continues until the computer has accumulated 1,024 data points. With a fixed transport speed of .1 cm/min. this results in an overall

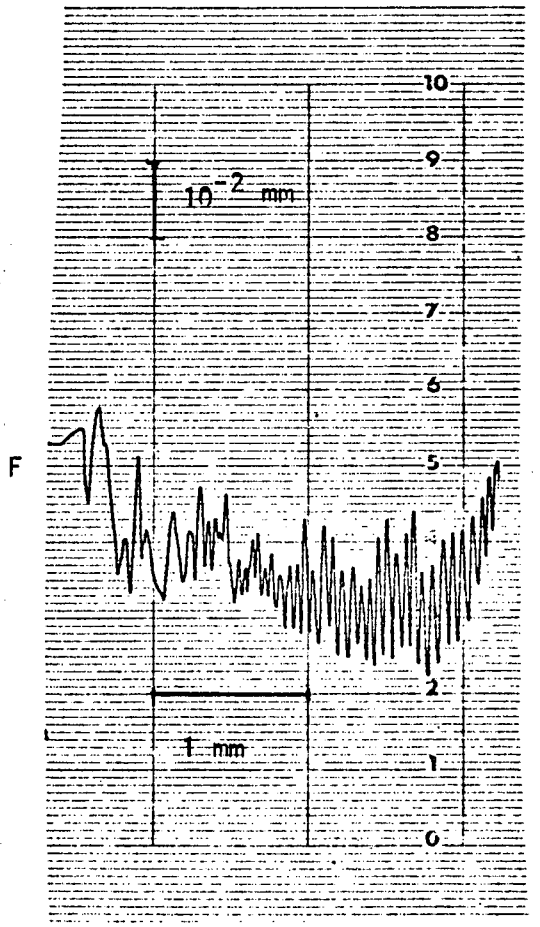

Figure 3. Dektak surface profile of Hudson River No. 1407 scale.

transcription of 2.685 mm of scale growth, subsequent to receipt of the initiating trigger signal.

The analog signal generated by the profile analyzer is first amplified 20 d B and then fed to an analog- to-digital converter. Each sampled voltage level is transformed to a corresponding digital value, and stored in successive core memory locations. Upon reception and storage of the entire series of data points, the entire (digital) surface profile is transcribed onto a magnetic tape.

This digital signal thus represents a permanent recording of the original analog signal. It may be plotted for visual inspection, as shown in fig. 4. In addition to this feature, the data in this form are well suited to automatic analysis by digital computer. Several sophisticated routines have been developed in the area of digital analysis of complex waveforms (Blackman & Tukey,1958; Colbert,1974). In the following sections, these routines will be described more fully, and their application to the automatic analysis of scale profiles demonstrated.

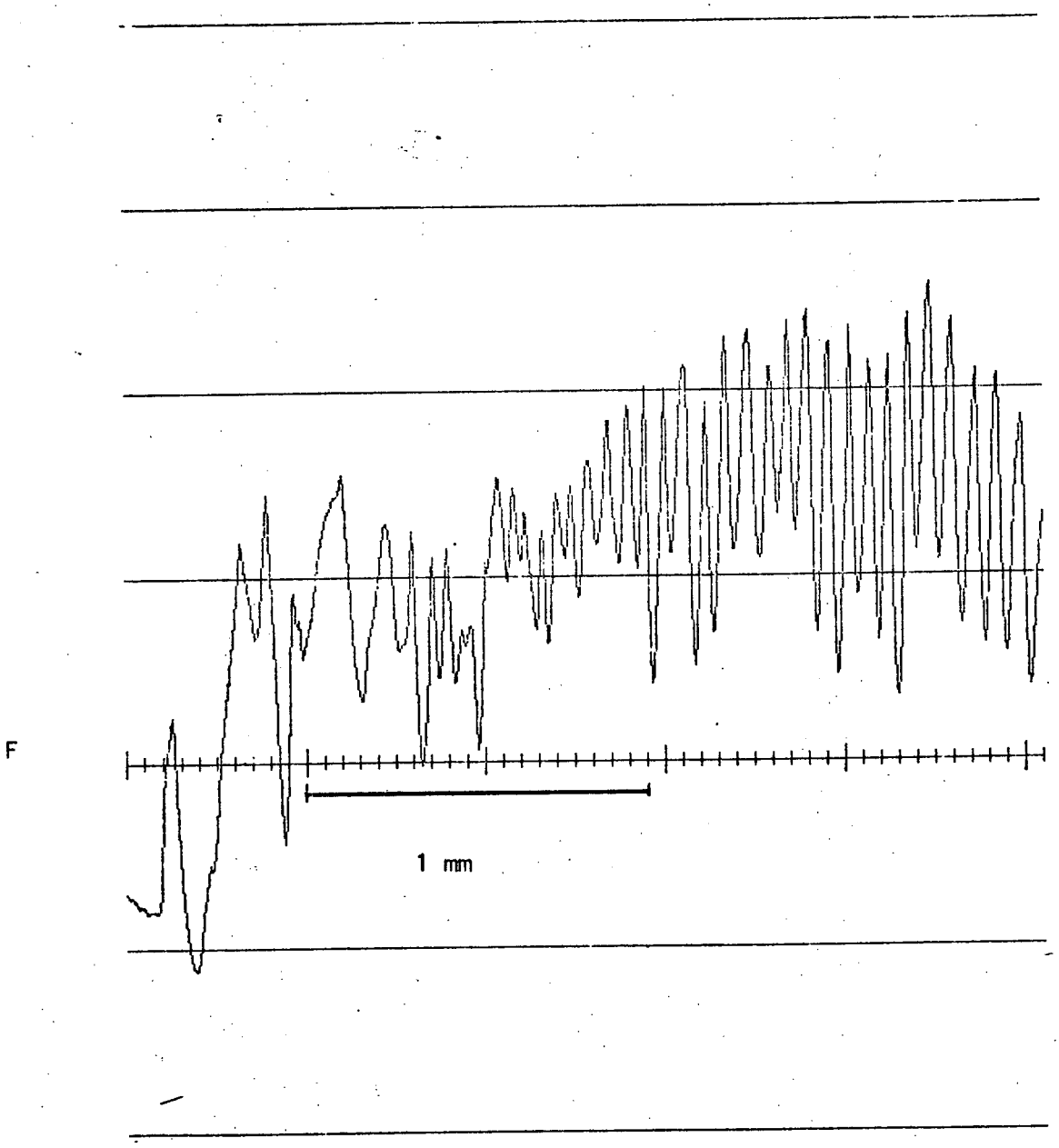

Figure 4. Digital profile of Hudson River No. 1407 scale.

Automatic Analysis and Classification Scheme

Having recorded scale surface profiles in convenient digital form, we are now in a position to submit them for automatic profile analysis. The flow diagram for the analytic technique is illustrated in fig. 5.

Initially, signals recorded on the 9-track magnetic tape, consisting of the 1,024 serial data points previously described, are input to the NOVA "POWERFUL" Fourier Transform Routine (Stutheit, 1971). This routine takes a finite-length, time- sampled complex waveform, and computes its normalized Power Spectral Density (P.S.D.). This method uses the Fast Fourier Transform (FFT), a digital method for obtaining rapid solutions to the Discrete Fourier Transform (DFT):

$$X(j) = \frac{1}{N} \sum_{k=0}^{N-1} x(k) \cdot e^{-i2\pi jk/N}$$

for $j = 0, 1, \ldots, N-1$; $k = 0, 1, \ldots, N-1$

where: N = Number of sampled points

$X(j)$ = j'th coefficient of DFT

$x(k)$ = k'th sample point of original waveform

$i = \sqrt{-1}$ (Bergland, 1969).

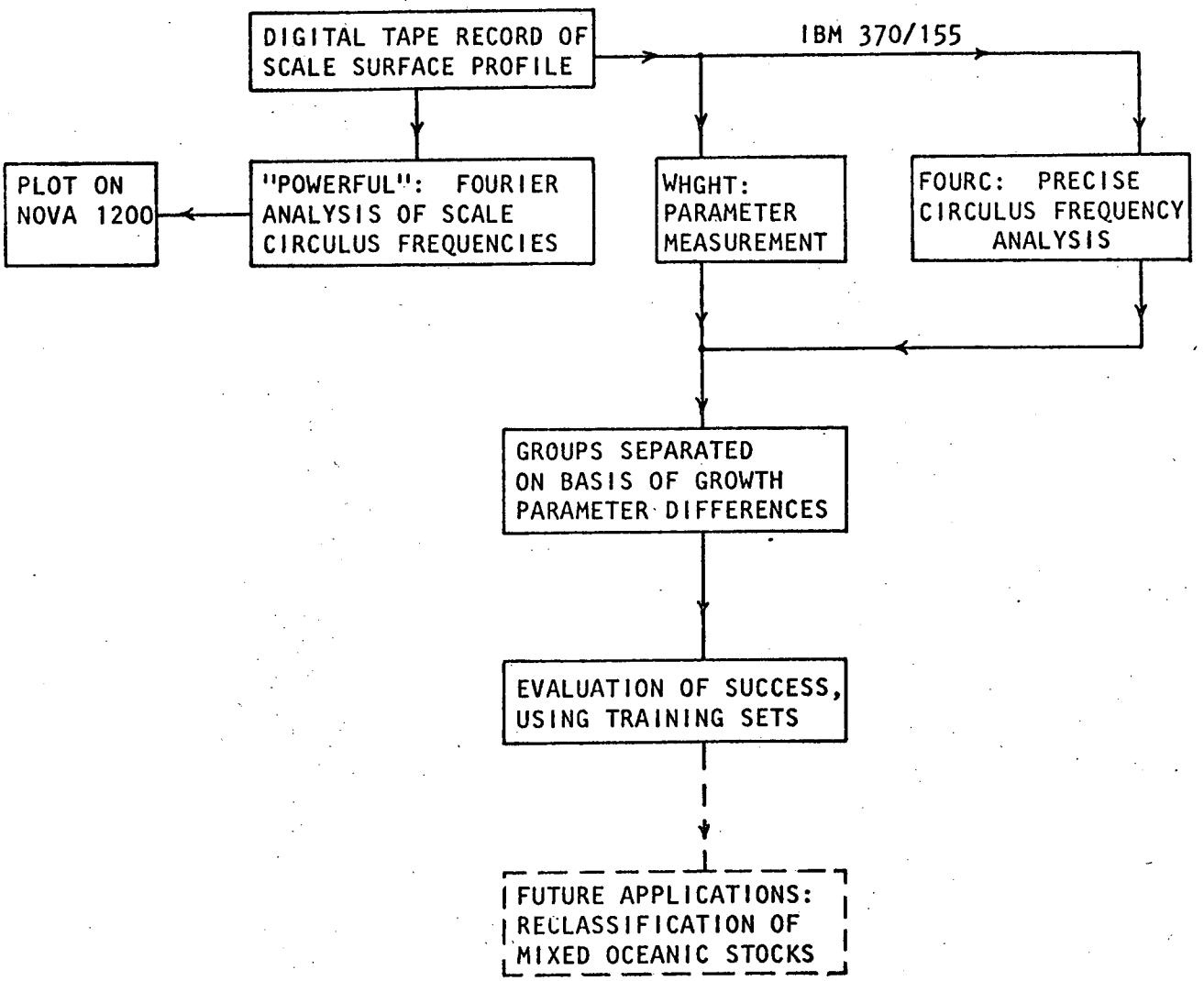

Figure 5. Scale pattern analysis method.

Expressed in simpler terms, in this step the overall scale waveform, representing a trace of circulus ridge height structure, is analyzed into its component circulus frequencies. Thus, if the circuli occur with a regular spacing (i.e. possess a dominant spatial frequency) a single peak, representing that particular frequency, will be plotted on the Fourier spectrum. Should the scale possess a polymodal distribution of circulus spatial frequencies (i.e. some circuli closer together, some further apart) then a characteristic polymodal P.S.D. frequency spectrum would be output.

A sample "POWERFUL" Fourier transform is illustrated in fig. 6. The scale waveform from which this spectrum is derived has been presented visually in figs. 3 and 4. On the basis of visual inspection of the rightmost 2 mm of scale profile we have already observed the dominant regularity present within the profile. We are not surprised then to find the Power Spectrum for this scale displaying a characteristic single sharp peak, at a point corresponding to the observed dominant spatial frequency. The exact location of the peak maximum permits us to accurately quantify the dominant circulus frequency. In this case, this corresponds to a value of 4.5 units x 3.74 circ/mm/unit = 16.8 circuli/mm. This represents then an initial technique for quantifying scale parameters, on the basis of circulus spacing. Results of this technique will be described below.

Figure 6. Power spectrum of dominant circulus frequencies of Hudson River No. 1407 scale.

Histogram Analysis

The above technique (POWERFUL) although extremely useful in qualitative investigations of regional shifts in dominant circulus frequency patterns, possesses severe shortcomings. The coefficients are available only in the form of graphic output. No numerical values for the coefficients are normally obtainable. The results are thus of limited usefulness, in terms of accurate quantitative parameter description. For this reason, it was decided to transfer the original data from its 9-track format (which was Nova compatible only) to a 7-track tape, which would be compatible with the main frame of the IBM 370/155, a much faster machine. To implement this transfer, a new program (SCALE) was written (S. Milligan,prog.) This served to transfer the serial records from the original tape to a new tape, which would be permanently resident on the IBM 370 system. Records were successfully transferred using this routine. In addition to mere transcription, a linear regression technique (Bendat and Piersol,1971) was used in detrending the original data. This was done to remove the residual curl or non-level component in the profiles, which had been previously obscuring the more important higher frequency circulus information. In other words, if the surface profile of the entire scale sloped gradually upward, succesively larger values (computed from the linear regression coefficients) were subtracted from each serial data point, in order to

remove all residual slope (detrend the series).

Once the scale profiles were in a form suitable for analysis by the IBM 370, a new program for the histogram analysis of circulus height and spacing was implemented. This program (WHGHT) was derived from a computer program originally designed to quantify and characterize sea state, based on data derived from a free floating wave height sensor buoy (Colbert, 1974). Five significant parameters are obtained when the program is used to analyze a typical scale profile. These are:

1. HBAR: Average height of circuli, microns (10^{-4} cm)
2. TBAR: Average spacing of circuli, microns
3. HTHRD: Average height of highest one-third of circuli, microns
4. TTHRD: Average period of highest one-third of circuli, microns
5. TNW: Total number of circuli.

The flow chart for this program is illustrated in fig. 7. The program locates the first zero up-crossing of the digital signal. It records the exact location of this zero-crossing as TOLD and then proceeds to examine subsequent data points, storing, in the process, the maximum and minimum values lying between the initial point (TOLD) and the next zero up-crossing (TIME). This marks one complete circulus period. The process then begins again for the next circulus.

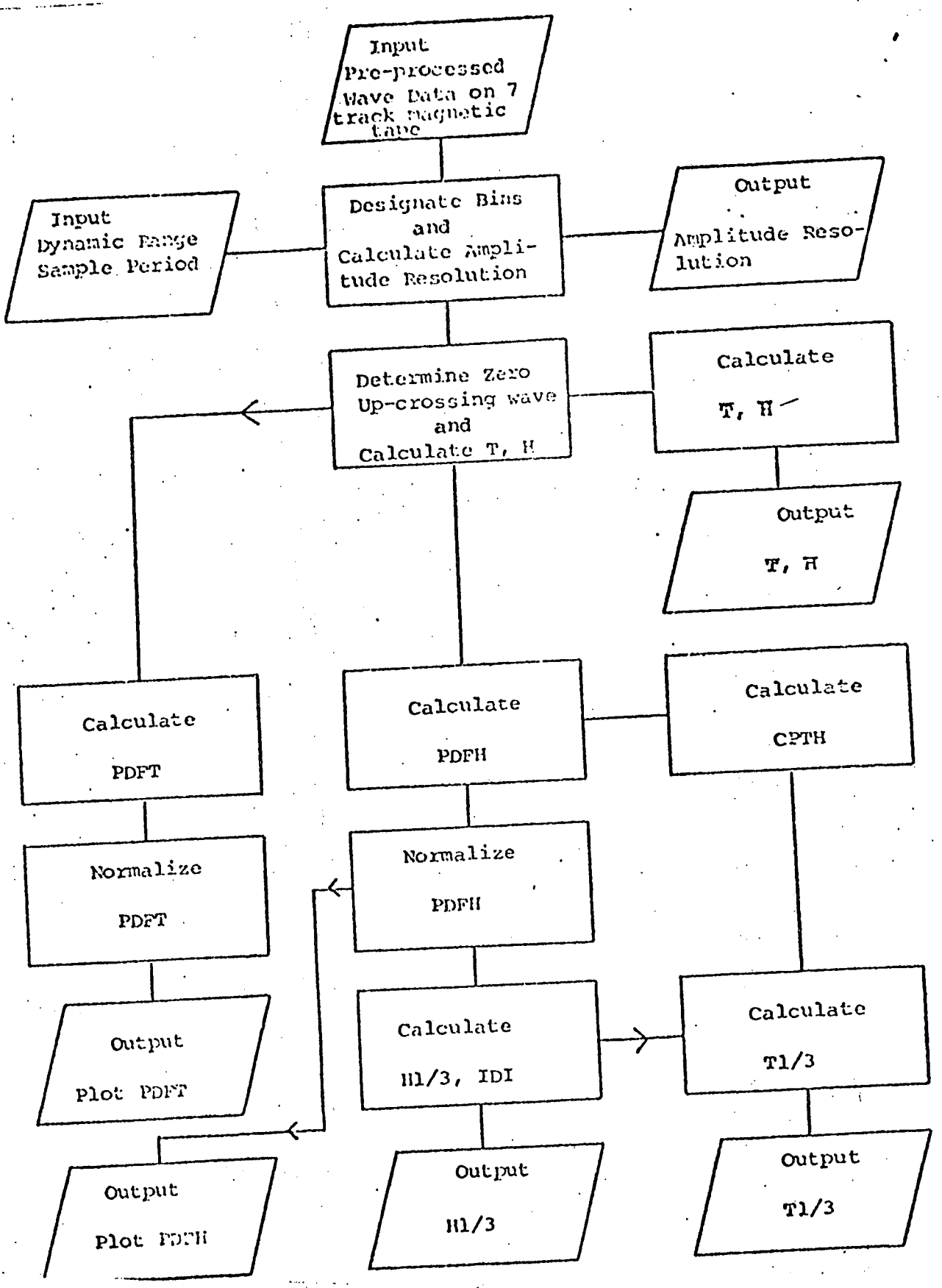

Figure 7. Histogram analysis block diagram (Colbert, 1974).

Each successive period length is recorded, and an overall average spacing (TBAR) is obtained. HBAR is obtained in much the same way, since each individual cycle's peak-to-peak (maximum-minimum) height is accumulated (as WHT) and then averaged (i.e. WHT is divided by TNW, the total number of circuli). HTHRD and TTHRD are computed by: 1. Determining the highest one-third of all the circuli; 2. Obtaining the average height of these circuli and; 3. Computing the average spacing for these circuli (Colbert,1974).

We now have five variables with which to characterize, in quantitative terms, the principal height and spacing features of the recorded scale growth patterns. This contrasts with the normalized Fourier results which only provided us with data on circulus spacing. We must now use these five new parameters in a statistical routine to optimally differentiate between our presumed racial groupings, on the basis of scale growth. For this purpose, a classical multivariate technique, Linear Discriminant analysis, has been chosen. (Dixon,1973). The assumptions, requirements, and results of this method are described below.

Classification via Linear Discriminant Analysis

The ultimate goal of this study is an ability to classify fish by region of origin on the basis of scale growth differences. In his introduction to Discriminant Analysis, Lee (1971) begins "The problem of classification arises when an investigator makes a number of measurements on an individual and wishes to classify the individual into one of several classes on the basis of these measurements." Thus, on the basis of this definition, our problem is well suited to analysis by means of the linear discriminant function. Indeed, discriminant analysis was originally developed in order to separate populations possessing multiple, overlapping character distributions (Fisher,1936; Rao,1954). In simplest terms, the discriminant function technique derives unique weighting functions for each group, which serve to maximize intergroup separability. For each presumed group i, a set of coefficients (Ai's) are derived which are then used to form weighted sums of the parameters.

$$Y_i = A_{i1}X_1 + A_{i2}X_2 + \ldots + A_{ip}X_p$$

for each member of group i, i=1,...,k.

These weighted sums in turn serve to maximize

between-to-total group variability, and permit optimal discrimination between adjacent groups. Individuals are successively evaluated by each discriminant function and are subsequently assigned to the group to which they bear greatest similarity.

Assumptions implicit in the technique are: 1. Equality of covariances of p random variables of k populations, and 2. normal distribution of random variables (Lee,1971). Validity of these assumptions as well as classification accuracies obtained through the use of this method are illustrated below.

For this study, we have selected the biomedical statistical package program, BMD07M, Stepwise linear discriminant analysis (Dixon,1973). This program, in addition to providing data concerning group means, standard deviations, and group discriminant functions, selects the measured parameters in descending order of their discriminatory power. F-ratios are output after each additional discriminatory variable has been incorporated into the discriminant function. These permit quantitative assessment of the significance of observed differences between the various group means.

RESULTS

1. "POWERFUL" Technique

For this study, a grand total of 425 scale profiles were recorded. This represents surface growth data for 172 separate fish from five major rivers.

As the scale groups were recorded, river by river, they were immediately processed via the "POWERFUL" Fourier Transform routine. As described above, this represents a rapid means of obtaining a graphical plot of principal circulus spatial frequencies present within the scale profiles. Fig. 8 illustrates the group average circulus frequency spectra for the Rappahannock R. scale sample. This spectrum represents an average over ten individual scale profiles derived from separate fish (NRAP = 10). We note that the group average possesses a single frequency maximum, at FMAXR = 18.1 circ/mm. (This correlates well with optical observations of circulus frequency in the lateral field. Using a 40x light microscope, counts were performed manually, and yielded average values of approximately 18 circ/mm.) Succeeding rivers (Rappahannock, Elk, Choptank, Potomac, and Hudson Rivers) profiles were analyzed in similar fashion.

Both the Elk and Choptank Rivers displayed peaks which closely resembled in general appearance those of the Rapp. R. previously described. Peaks occurred at FMAXE = 17.8 and FMAXC = 17.5 circ/mm respectively, with group size NC = 4 and

Figure 8. Power spectrum of Rappahannock River scales.

NE = 8. In addition, the Hudson R. sample (NH = 18) also displayed a strong, sharply peaked frequency spectrum, with an FMAXH of 18.1 circ/mm. These values are summarized in Table 1.

In marked contrast to the foregoing groups, all of which possess a unimodal frequency distribution, the members of the Potomac R. sample (NPOT = 7) displayed a much more diffuse, polymodal spectrum with several distinct frequency peaks (fig. 9). The three principal peaks observed were at FMAX1 = 18.4, FMAX2 = 20.5, and FMAX3 = 22.8 circuli/mm. This represents quantitative evidence of a lack of uniformity in circulus spacing. Visual inspection of a typical Potomac surface profile (fig. 10) provides clear evidence of such non-uniformity.

Male fish taken from early in the Potomac spawning run consistently displayed such non-uniform growth patterns. These types of patterns occurred much less frequently among samples of fish taken from other Chesapeake rivers, and were almost never observed in the samples of Hudson River scales examined. Possible explanations for the anomalous nature of scale growth observed among fish derived from the Potomac River will be discussed below.

In the early phases of this project, scale profiles derived from female fish were recorded. A significant difference in scale circulus frequency between the sexes would be an extremely useful tool, since at present, there are no diagnostic techniques available for the determination of sex on the basis of external characters. Internal

Table 1. Fourier Peak Circulus Frequencies

River	Sex	Frequency (circ/mm)
Rappahannock River	male	18.1
Rappahannock River	female	15.2
Elk River	male	17.8
Choptank River	male	17.5
Hudson River	male	18.1
Potomac River	male	18.4,20.5,22.78

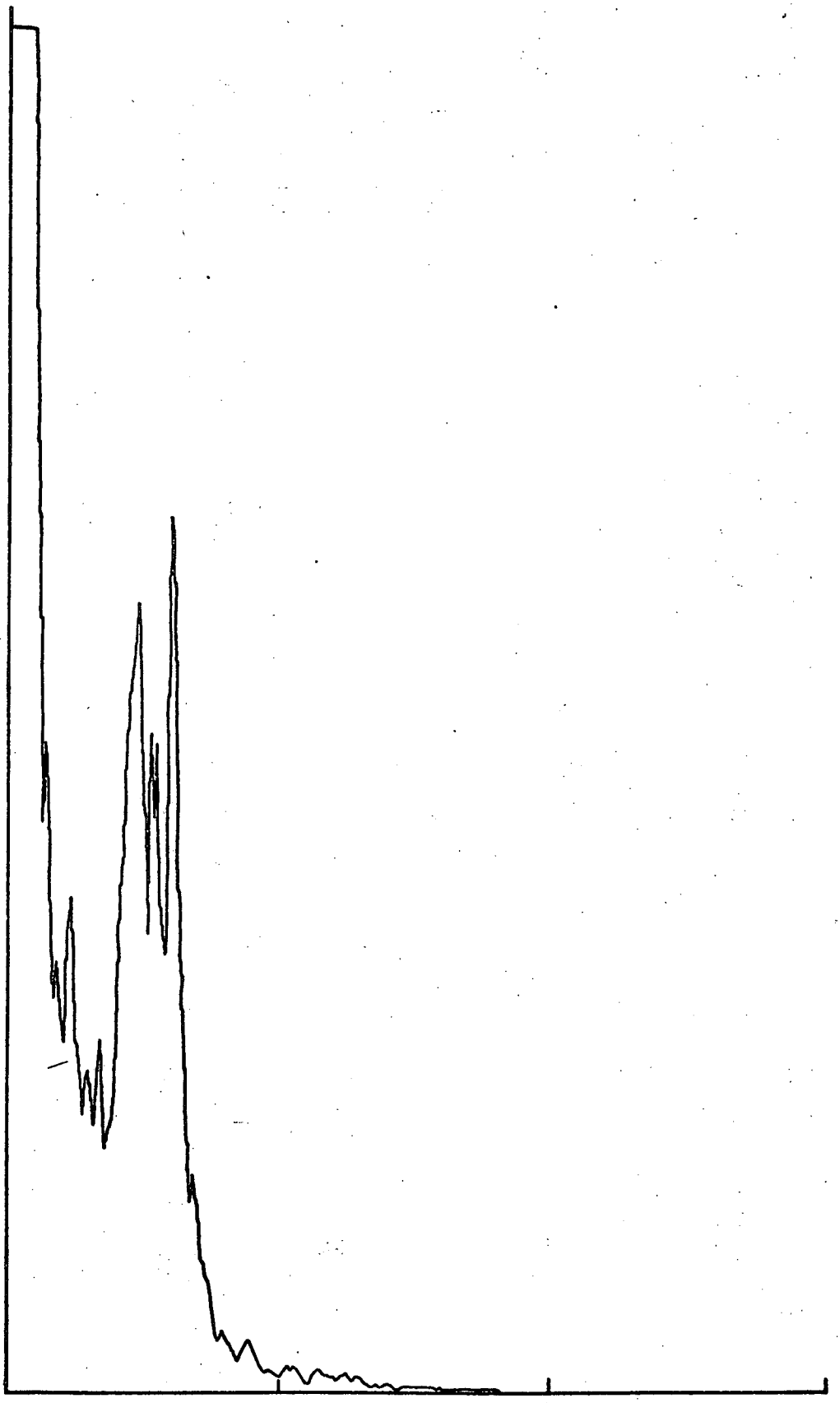

Figure 9. Anomalous power spectrum of Potomac River scales.

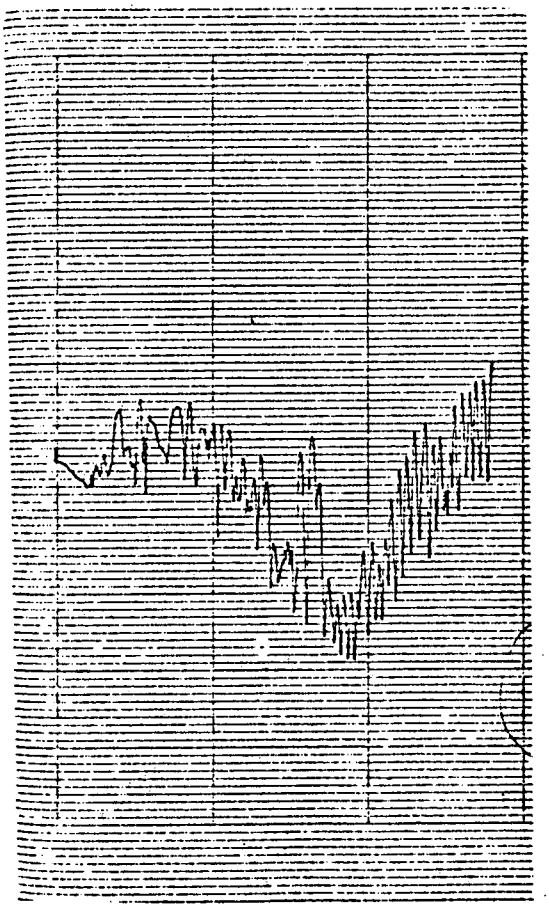

Figure 10. Typical anomalous Potomac River profile.

examination of the gonads or their products (in ripe fish) are the only methods known for sexing of striped bass (Raney, 1952). Groups of females from the Chesapeake area displayed a slightly lower group average circulus frequency peak than corresponding groups of males. Males and females from the Hudson R. displayed essentially identical circulus frequency spectra. Since even in the Chesapeake groups, individual variation between scales was generally greater than observed mean group sex differences, attempts at further differentiation by sex were discontinued. These results are not unexpected, since growth rates over the first two years of life are essentially identical for the two sexes (Scofield,1931) and this analysis only applies to growth occurring within that period.

2. "FOURC" Spectral Analysis Technique

The Nova POWERFUL program provides a rapid and graphic illustration of dominant scale circulus frequency composition. Unfortunately, the Nova "POWERFUL" program does not produce numerical output for its spectral coefficient values. To accomplish this goal a new program FOURC was written This program uses the IBM 370/155 to produce a numerical set of spectral coefficient values. Values for power spectral coefficients 20 through 68 (representing circulus frequencies from 7.46 through 25.4 circuli/mm) are output onto magnetic storage units and used

to classify fish by region of origin on the basis of circulus spacing.

On the basis of preliminary circulus frequency plots output by the POWERFUL routine, it was felt that the greatest differences in scale spatial frequency patterns were to be found between Hudson and Potomac River scale profiles. For this reason 53 Hudson and 49 Potomac profiles were processed by "FOURC" and their spectral coefficients output for classification. Classification was undertaken by means of the linear discriminant program, BMD07M, described above. Using 49 power spectral coefficients, an overall accuracy of 70 % was obtained in trials using randomly selected training subsets of Hudson and Potomac fish (NHUDS = 10; NPOT = 10). An intergroup F-ratio of 1.48 was obtained, which was significant at the $p < .10$ level. This represents a successful application of circulus frequency analysis to regional differentiation between two separate source stocks.

As an extension to this method, parameters derived from the WHGHT program (HBAR,TBAR,HTHRD,TTHRD,TNW) were incorporated with the 49 spectral coefficients originally derived for each profile used in the analysis. Average circulus height, HBAR proved to be the single most valuable parameter for use in discrimination between the two groups. Accuracy of reclassification for new, slightly larger test subsets (NHUDS = 20; NPOT = 20) was at the 50 % level for the Hudson, and at the 70 % level for the Potomac test sets. Intergroup F-ratios were computed to be 3.67, with 51 and 112 deg. freedom. This was highly significant $p < .001$. This

demonstrates the utility of combining several types of analytical data in order to discriminate with greater assurance between selected regional populations.

3. "WHGHT" Technique

The foregoing method ("POWERFUL") proved extremely useful in providing rapid assessments of circulus frequency composition for scales taken from the principal striped bass spawning areas. However, the "POWERFUL" routine did not supply useful quantitative parameters (numbers) whose values could be used to summarize important scale growth features. For this reason, all scale profiles judged to be of acceptable quality were submitted to analysis via the "WHGHT" program. The five parameters listed earlier (HBAR,TBAR,HTHRD,TTHRD,TNW) were automatically measured for each scale profile, and written out to a permanent magnetic storage file. These fish then became our initial training set, to be used in our analysis and classification routines.

Our training groups were composed as follows: The Hudson training set consisted of 53 scale profiles (NHUDS = 53). The Chesapeake training set consisted of 63 scale profiles from the Rappahannock, York, and Choptank Rivers, and 49 profiles from the Potomac, for a total of 112 fish in the Ches. training set (NCHES = 112). Population makeup of the training sets is illustrated in Table 2. Sixty profiles were subsequently recorded from known area fish, and these

Table 2. Population Composition: Chesapeake Training Set

River	Sex	Sample Size
Rappahannock	male	42
Elk	male	17
Choptank	male	4
Potomac	male	49
		N = 112

Table 2. (continued) Hudson Training Set

River	Sex	Sample Size
Hudson	male	53
		N = 53

were used to evaluate the classification accuracy obtainable through use of the training sets.

The discriminant analysis program (described above) initially computes means and standard deviations for each of the groups noted above. Means of the parameters are listed by group in Table 3. These data are then used to derive unique discriminant functions for each group. The discriminant coefficients (Ai's) are used to form weighted sums Y of the original character values (Xi's). In the present case the Xi's represent the five parameter values derived by "WHGHT" which describe each of the profiles. The weighted sums (Y) possess the property of maximizing the between-to-within group separation capabilities. In other words, the scales are projected (in multi-dimensional space) along an axis (the discriminant axis) which maximizes the differences between the groups. Each group member is then redefined into the group it most closely resembles, on the basis of its location in the reduced discriminant space.

Separation approached 83 %, when the two groups (Ches and Huds) were classified by the stepwise linear discriminant function BMD07M, (Dixon, 1973). This was equivalent to a general misclassification error of 17 %. The most useful separator appeared to be HBAR, the average circulus height, in microns. Investigation of group means output by the discriminant program indeed showed a noticeable difference in this parameter between the groups, with HBARC = 11.08 microns, and HBARH = 13.66 microns (Table 3). Further evidence of the statistically significant nature of this

Table 3. WHGHT Parameter Summary: Circulus Height and Spacing Values

River	$\bar{H}(\mu)$	$\bar{T}(\mu)$	H1/3(μ)	T1/3(μ)	TN	Sample Size
Hudson	13.66	76.98	19.67	93.10	31.9	N = 53
Rapp.,Elk, Chop., Potomac	11.08	77.98	16.87	99.22	32.1	N = 112
Potomac	10.66	81.48	16.31	91.16	31.5	N = 49

WHGHT PARAMETERS

$\bar{H}$: AVERAGE HEIGHT OF CIRCULI, MICRONS (10^{-4} CM)

$\bar{T}$: AVERAGE SPACING OF CIRCULI, MICRONS

$H_{1/3}$: AVERAGE HEIGHT OF HIGHEST ONE-THIRD OF CIRCULI, MICRONS

$T_{1/3}$: AVERAGE SPACING OF WIDEST ONE-THIRD OF CIRCULI, MICRONS

TNW: TOTAL NUMBER OF CIRCULI COUNTED

difference was shown by an intergroup F value of 16.70, with 4 and 160 degrees of freedom. This value strongly indicated a significant difference in mean values between the groups ($p < .001$). Circulus spacing, on the other hand, appeared to be of little value in separation of the two populations. The group means for circulus spacing were extremely similar, with TBARC equal to 76.98 microns while TBARH was equal to 77.10 microns. The unsuitability of using circulus spacing for discrimination was underscored by the fact that the stepwise program chose this variable as possessing the lowest discrimination capability. It was included in calculations only after all other parameters had been exhausted.

The sixty profiles mentioned earlier were analyzed next to determine what levels of reclassification accuracy could be obtained for such synthetic unknown test sets. Of 25 new Hudson profiles, 16 were correctly reclassified, yielding a 64 % reclassification accuracy. Of 37 Chesapeake profiles, 25 were correctly assigned to their proper subgroup, for an overall accuracy approaching 67 %. This represented a direct evaluation as to the usefulness of the training sets previously established. Percentage reclassification accuracies are summarized in Table 4.

Circulus Height vs. Fork Length Regression Analysis

Table 4. Reclassification Accuracies by Region

Region	Reclassification Accuracy
Hudson Training	83%
Hudson Test	67%
Chesapeake Training	77%
Chesapeake Test	64%

Careful examination of classifications output by the linear discriminant program reveals HBAR to be the dominant classification variable. Any profile whose HBAR value exceeds 12.5 microns is classified as Hudson, while profiles with HBAR values of less than 12.5 microns are reclassified into the Chesapeake subgroup. Given the central importance of this parameter, it was considered vital to investigate further whether average scale circulus ridge height varies significantly with overall fork length of the individual fish. Since only the innermost portion of the scale was analyzed, and this portion was laid down early in the fish's life, it was not considered likely that ultimate fish length would strongly influence interior ridge structure. Nonetheless, it was considered desirable to accurately ascertain whether there was any strong relationship between HBAR and fork length of fish at capture. Since all scales had previously been selected and run without regard to ultimate fork length, this uncertainty had to be resolved in order to assure that mere size or age differences between groups might not produce differences in scale parameters which would be mistaken for true racial differences.

Accordingly, length data were requested and received from Texas Instruments Ecological Services for fish whose scales had been used for this study. 47 fish from the Chesapeake group, and 53 from the Hudson area were subjected to initial analyses. Size composition data for these fish are summarized in Table 5. The Chesapeake set gave an HBAR vs. length correlation value of .224 (not significantly

Table 5. Size Composition of Training Sets

Region	Size Range (mm)	Average Size (mm)
Hudson River	400-870 mm	723 mm
Chesapeake	270-650	423

different from zero), and a regression F-value of 2.369 (N.S.). The Hudson set yielded correlations of .319 and a regression F-value of 5.764, with 1 and 56 degrees of freedom. This was a significant value, $p < .05$. Thus some indication of a fork- length: scale height relationship appeared to be present. Further analysis yielded positive regression coefficients for each group (Table 6). This tended to confirm that HBAR was an increasing function of fork length, L. This result supports the hypothesis of continuing vertical calcification of the scale circuli during the entire period of fish growth. Previous investigators (Wallin, 1957) had assumed that calcification was limited to the period immediately following initial circulus formation.

Statistical analysis of the two training sets (Ches. and Huds.) demonstrated mean fork length among Hudson fish to be equal to 723 mm, while mean fork length for the Ches. set was only 423 mm. We see then that these samples were indeed not homogeneous as to size, and that at least part of the observed differences in HBAR between the two groups might well have been caused by the disparity in size composition among fish from the two regions. Table 5 illustrates this disparity.

It was decided to investigate new fish, selected by size class, to permit more valid intergroup comparisons. In other words, we now selected a group of shorter Hudson fish (N=5, LBAR=503 mm) and longer Chesapeakes (N=8, LBAR=748 mm). This would permit us to observe the behavior of HBAR as we extended the size limits for the two groups. Indeed, when

Table 6. Correlation Study Summary: Circulus Height vs. Fork Length

Region	Intercept (μ)	Regression Coefficient (μ/mm)	F-value for Regression	N
Hudson River	11.1 μ	.00364 μ/mm	2.897 N.S.	58
Chesapeake	7.7	.00849	19.967 $p<.001$	55

classification of the new, larger Chesapeake fish was undertaken, 7 of 8 (87 %) were found to possess HBAR values above the aforementioned 12.5 micron cutoff point and hence were (incorrectly) classified as Hudson fish. More encouraging results were obtained when the shorter Hudson fish were compared with the original Ches. training set. The shorter Hudson's maintained a high value for HBAR (14.15 microns, group average) and were correctly reclassified at the 67 % level.

Thus while a strong length vs. HBAR dependence was demonstrated by the Chesapeake fish when the new longer fish were incorporated (F- value for regression of 19.967, $p < .001$) the Hudson fish maintained their larger HBAR values down to quite low fork lengths. The regression coefficient values are summarized in Table 6. The functional dependence of HBAR on fork length was found to be much weaker among the Hudson fish investigated (F- value for regression of 2.897, N.S.) In order to check more fully as to whether short Hudson fish retained their greater HBAR values down to quite low fork lengths, another series of runs were performed on remaining shorter Hudson scales. These fish ranged in length from a minimum of 250 mm to a maximum of 560 mm, LBAR = 499 mm. These would demonstrate more clearly whether the observed differences in Hudson scale growth structure were primarily racial, or could be actually ascribed to inhomogeneity among size classes. Results of this run, for 7 Hudson fish provided an average value of 12.96 microns, with a reclassification accuracy of 56 %.

DISCUSSION

The basic objective of this investigation has been to determine the feasibility of automatic detection and permanent transcription of scale surface profiles. The foregoing research demonstrates that these two fundamental goals have been successfully accomplished. This system may be used to rapidly and accurately produce records of scale surface growth and structure. The system is suitable for use with almost any species of scaled fish, and holds great promise in terms of providing an increased ability to process much greater quantities of scale growth information. A further objective of this study was to determine the feasibility of using the automatically measured values of scale surface morphology as diagnostic characters for use in determination of the geographic origins of the migratory striped bass populations.

With regard to this particular investigation, several conclusions may be drawn. First, intercirculus spacing does not appear to provide a useful tool for striped bass stock discrimination. This is in accord with several previous studies. Van Utrecht (1973) found that for scales of the Ide (Leuciscus idus), spacing of the individual circuli did not vary in accord with differing growth stanzas in the life of the fish. Bilton (1974), in starvation experiments performed

on several species of salmon found that alterations in feeding did not alter the spacing of circuli, although more circuli were produced among well-fed fish. On the basis of power spectral analysis of Chesapeake and Hudson scale profiles, circulus spacing appears generally constant over the range covered by this investigation. The single exception to this general statement is found among fish from the Potomac R. It is believed that either excessive pollution or some other unfavorable condition contributes to the observed abnormality in Potomac scale growth patterns. The alternate possibility, that some genetic mechanism is the cause of this anomaly cannot be dismissed, since the Potomac R. appears to contain a strongly isolated breeding subpopulation (Nichols & Miller,1967; Lewis,1957).

Speaking in broader terms, the use of power spectral analysis represents a precise, rapid technique for the evaluation of regional differences in circulus spacing. Certainly, as noted by Anas & Murai (1968) circulus spacing has been observed to vary as much as 20 % between sockeye salmon derived from either Asian or North American subgroups. This technique would be accurate enough to easily detect such a level of regional variation, and would provide a valuable tool for use in population analyses of such species.

A valuable and unique feature of this surface profile technique is its ability to produce accurate transcriptions of actual scale surface ridge height microstructure. In all previous scale growth investigation techniques, analyses have been performed on projected scale growth impressions. Never

before has quantitative data concerning ridge height values been so easily available for use as a parameter in racial separation studies. Indeed, our results tend to confirm that for striped bass, circulus height represents the best single indicator of racial origin. This is in accord with the results of the Van Utrecht study, where a correlation between scale density (thickness) and growth rate variation was found. However, results to date indicate that the effects of fork length must be considered when circulus height is used in racial analysis.

More explicitly, when HBAR values for the two populations were tabulated, a significant difference was noted. Part of this difference was attributable to the non-homogeneous size composition of the two samples. (Mature male fish captured within the Hudson during the spawning run of 1974 tended to be generally older and larger than male fish taken from the Chesapeake rivers). Nonetheless, Hudson derived scales tended to retain their greater circulus ridge height character down to fork lengths which were directly comparable to mean Ches. fork lengths. Figure 11, derived from the HBAR vs. fork length regression lines calculated for the two samples makes clear this difference in behavior of HBAR between the two groups. Both slope and intercept of the two lines are markedly different. Reasons for this observed difference are not clear, but may result from environmental influences on early scale growth, and general calcium metabolism. These differences could be due to different chemical concentrations of calcium in the local waters, since

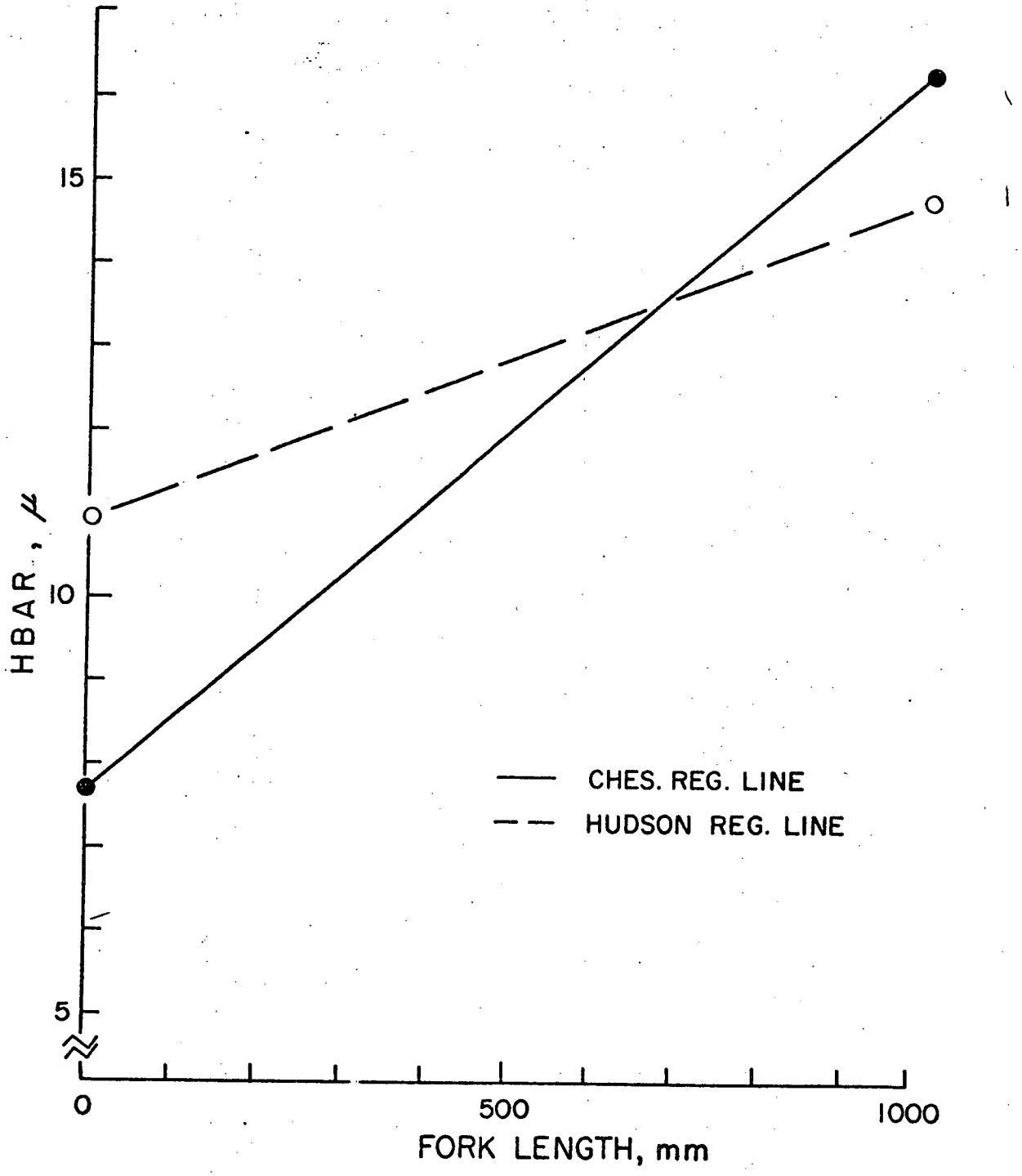

Figure 11. Regression line for HBAR (average circulus height, microns) vs. fork length. Chesapeake vs. Hudson groups.

it is known that fish actively exchange calcium between their bodies and the surrounding medium (Bogoiavlenskaia, 1960). Other environmental factors affecting early scale growth might include: altered thermal regimes between the two areas; differential growth rates between the two regions (which has been found to be true for these fish) (pers. comm., T. Berggren); variations in food availability and/or preference between the two groups. Finally, the possibility of a true genetic difference, rather than mere clinal variation must be considered. Previous studies (Raney & De Sylva, 1953; Lewis, 1957) have demonstrated differences in other (meristic, morphometric) characters which seem genetically determined and which change little from year to year. Certainly the possibility of such a genetic mechanism for the control of scale growth is a valid possibility.

Whatever the reason, we have found some degree of differentiation between our two stocks using this parameter. Separations using our preliminary training sets appears possible at the 65 % accuracy level. Larger sample sets, and the eventual discovery of additional scale parameters which do display regional variation should increase our levels of correct reclassification in this regard. Sophisticated biochemical and serological techniques for racial analysis have been applied to several fish, including the striped bass (Morgan, 1971). Use of scale analysis as an important part of an integrated effort involving biochemical, electrophoretic, and meristic techniques may provide a coordinated solution to the difficult problem of striped bass

source stock analysis.

RECOMMENDATIONS

1. Modal fork lengths in our sample tended to large (> 700 mm) for Hudson males and to shorter (300-400 mm) for Chesapeake specimens. Analysis of 300-500 mm males from both regions would provide a clearer indication as to those differences in scale structure which could be attributed directly to regional effects on scale growth. Texas Instruments personnel will be collecting additional striped bass this spring as part of their Phase II research effort. They will use these fish,collected from major spawning rivers to verify their present techniques of regional identification. A sample size of 200 fish (100 from each region) would permit better reclassification accuracies through a reduction of intra- population variability.

2. Combination of our results with those derived from Texas Instruments study of meristic and growth variation; URI study of fatty acid variation (a biochemical analysis program being pusued by W. Johnson, q.s., investigating fatty acid composition of striped bass muscle tissue, which has been shown to be useful in detecting genetic differences between spawning populations) (Gruger, 1964); may provide greater separation accuracies than those obtainable by any single method. The linear discriminant program permits combinations of up to 75 variables to be used simultaneously to permit separation between the two regions. It is recommended that

studies to evaluate separation accuracies obtainable through the use of such combined data sets be initiated.

3. Development of a method for automatic recognition of annular marks on scales appears possible on the basis of limited trials with present materials. Such a technique would permit study on an automatic basis of yearly growth increments and age structure for the different populations. This ability would be very useful in general fisheries research, as well as for the specific problem of striped bass source stock analysis.

Appendix 1. Error Analysis

Sources of Error

In assessing the value of any new and previously untried technique, it is necessary to determine the statistical reliability and repeatibility of values obtained. Several types of error may be introduced at various points in this new technique, and their separate magnitudes must be determined in order to measure the validity with which results must be accepted.

System Error

The first likely source of error which required investigation was the degree of noise or "jitter" arising from the equipment itself. This would represent an irreducible minimum noise value, to which other error sources would add. In order to ascertain the noise levels present within the undisturbed system, identical replicate runs were performed for several separate scales. Since the Dektak scale analyzer has automatic limit stops for both forward and reverse motion of the sample stage, in order to repeat a run over any given scale, it was merely necessary to raise the

sensor stylus, return the stage to zero (initial) position, and replace the surface sensor stylus. In this fashion, as many replicate runs as were desired could be performed. For the present investigation, duplicate runs were made for each test scale. When the two series of profiles were analyzed by the "WHGHT" technique, the values obtained for TBAR as well as HBAR were compared. Average differences in HBAR between the samples were extremely minute, averaging approximately 0.1 micron (less than the wavelength of visible light). In addition, visual inspection of primary and replicate profiles indicated successful detection of identical features, down to the smallest noticeable detail. This was encouraging evidence of the low noise inherent within the scale analyzer, as well as across the entire magnetic tape transcription system.

Resolution in the vertical direction is thus seen to be approximately 0.1 micron. Resolution in the horizontal direction has been calculated to be 1.7 microns, ascribable to the finite radius of curvature (r = 12 microns) of the sensor stylus tip. Features at this low level are beneath the limit of accuracy imposed by the 381.4 sample/mm sampling rate. This sampling threshold equals 1000 micron/mm divided by one-half the sampling rate, or some 5.2 microns. We are able to reduce this error by means of the linear interpolation capability of the WHGHT program.

To prevent errors from amplifier or oscillator drift, equipment was calibrated at the beginning and end of each analysis session. Gains were normalized to provide standard

digital height values for all profiles. Equipment was always given a one hour warm- up period, to assure complete system stabilization. In the later stages of analysis, fish scales from different regions were always run alternately, in order to compensate for any possible long- period gain changes. No major shifts in gain levels were ever observed.

Re- orientation Error

A further source of error which required investigation was the effect of lack of consistency in scale orientation by the operator on scale measurement accuracies. As mentioned earlier, a small fixed mark in the 40x Dektak analyzer eyepiece was used to orient the scale, by placing the left edge of the rostral field against this small mark. In this fashion, the track of the sensor was directed over the central portion of the lateral field. In order to assess the level of error arising from lack of consistency in scale orientation by the operator, a series of duplicate scale runs with scale re- positioning after the primary run,were performed. 8 scales were analyzed in this manner. T- test analyses of these interscale comparisons , using TBAR and HBAR as indices of scale measurement, demonstrated no significant differences between re- oriented scale pairs ($t = 0.75$, 7 d.f., N.S.). This was a strong indication that consistent operator error was not a major cause for concern,

and would not contribute significantly to inter- scale variation.

Between- Scale Error

All scales collected for use in this study were obtained from a single area on the body of the fish. Scales were taken from the scale rows immediately above the lateral line, along a perpendicular leading upward toward the posterior insertion of the first (spinous) dorsal fin (pers. comm., T. Berggren). This was undertaken in order to standardize our technique, since it is known (Clutter & Whitesel, 1956) that scales from widely differing regions on the body of the fish provide varying indications of fish growth. The aforementioned location represents a "preferred" region for striped bass scale collection, since the scales are generally symmetrical, and possess maximal growth information. Such standardization would certainly tend to reduce inter- scale differences. Further study was nonetheless required to assure that minimal variation actually existed between multiple scales from the same fish. For this reason, 4 pairs of scales, each pair from a single fish, were compared, in terms of HBAR, and TBAR. On the basis of 16 profiles (2 per scale, 8 scales) from 4 fish, no major differences were observed. Results once again tend to reassure us that intra- fish variation is not a significant contributor to

observed scale growth differences.

Independent Confirmation of System Accuracy Using Scanning Electron Microscopy

As mentioned above preliminary light microscopy measurement provided limited confirmation of system accuracy. Rough manual circulus frequency counts of 18 circ/mm are in good agreement with circulus frequency counts output by the Fourier power spectral analysis of recorded scale profiles. In order to more critically evaluate analyzer accuracy, Scanning Electron Microscopy was performed on a scale from a selected Rappahannock R. fish. After mounting the scale on an electron microscope specimen stub (using Eastman 910 adhesive) a Gold- Palladium coating was deposited on the scale to enhance its electron beam reflectivity.

Two photographs of this scale were produced. The first, at 20x magnification (fig. 1) displays the lateral field, which was the primary site of investigation for this entire study. A more detailed view, at 110x magnification (fig. 2) provided sufficient enlargement to permit accurate measurements of spacing between individual circuli (white ridges). Seventeen circuli were measured, along a path corresponding to the track of the surface profile analyzer stylus. These values are tabulated and a mean value for

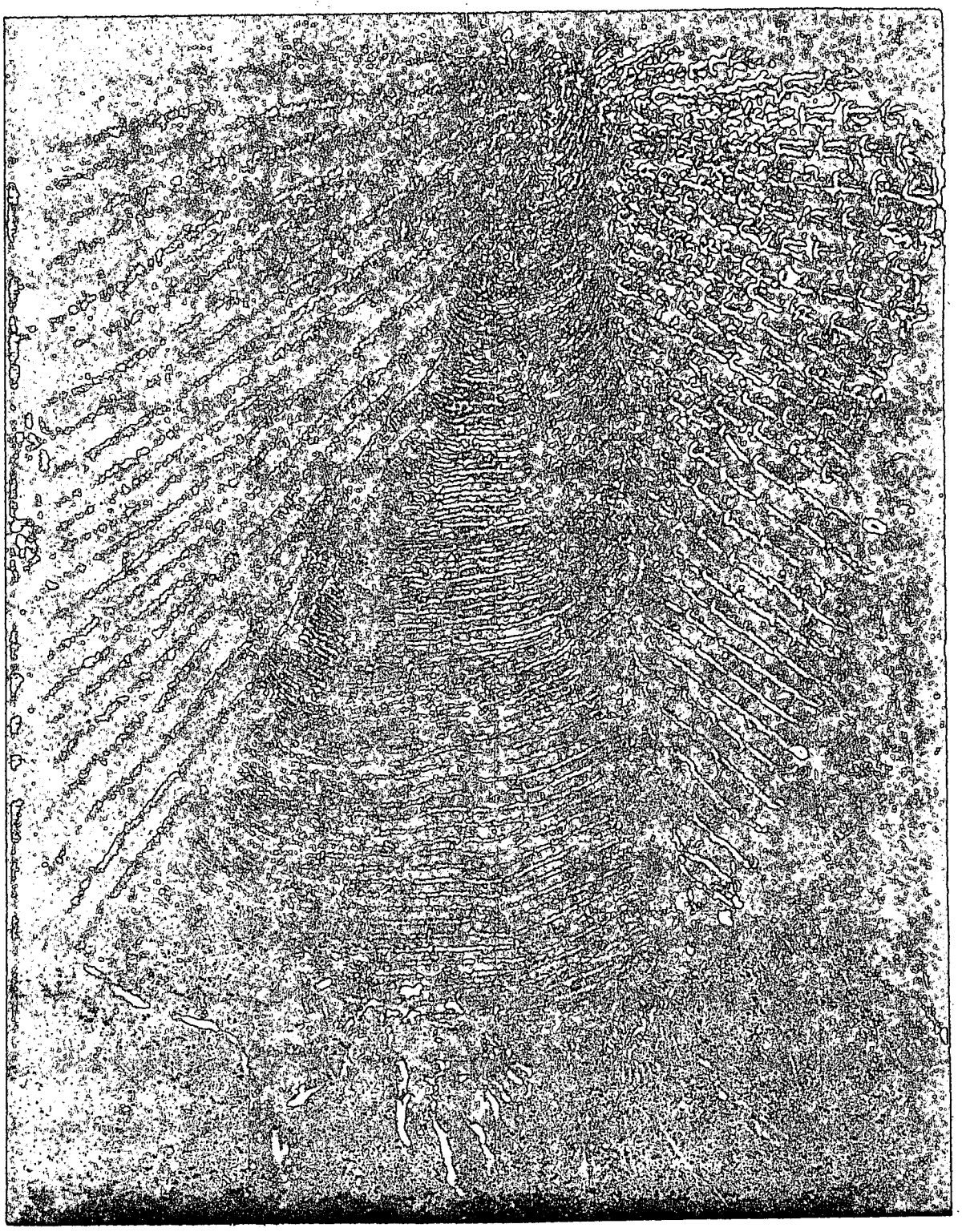

Figure 1. Scanning Electron Micrograph of striped bass scale (20x).

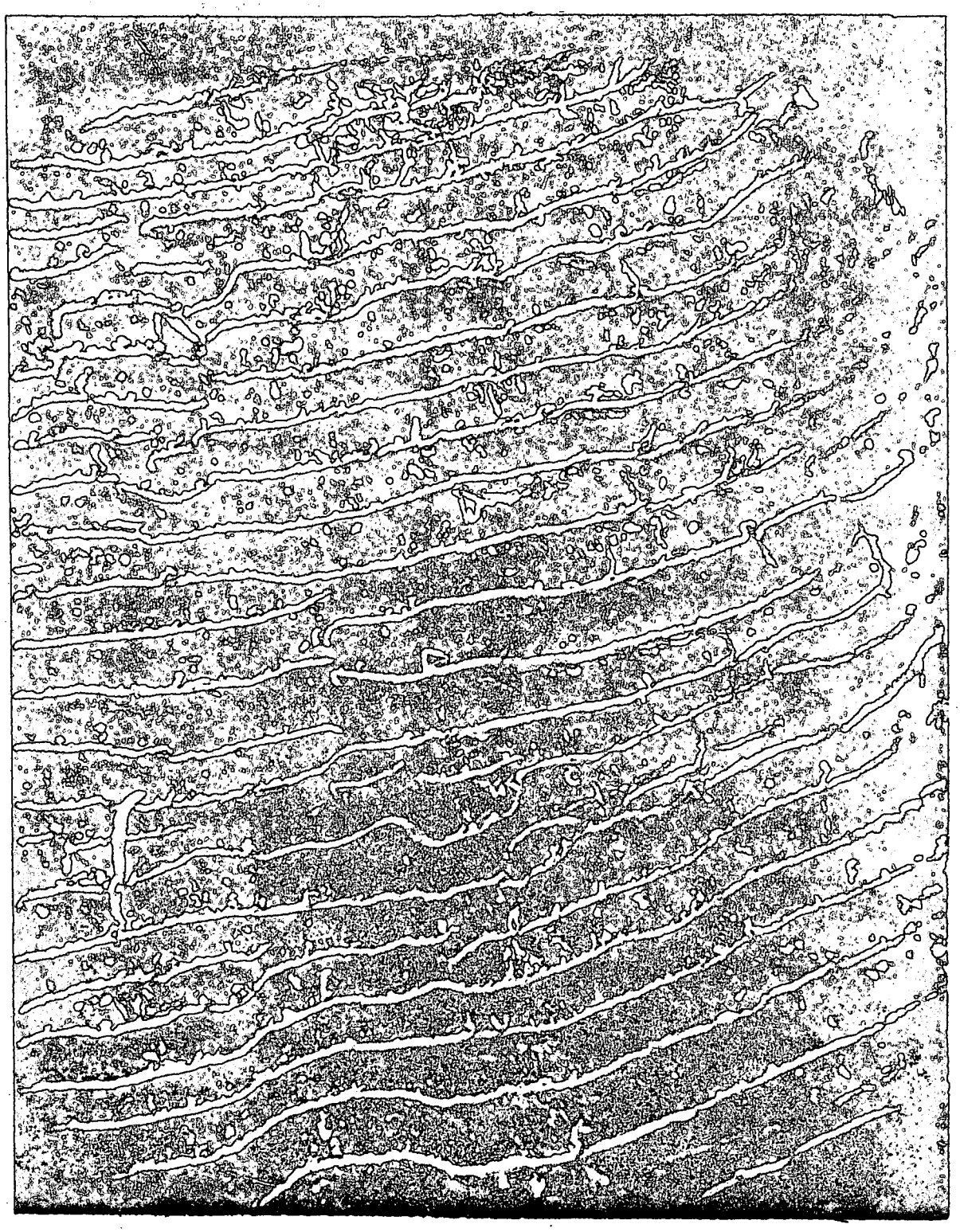

Figure 2. Scanning Electron Micrograph of lateral field of striped bass scale (110x).

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intercirculus spacing of 54.12 microns was derived (Table 1).

Selected comparable circulus periods from a special WHGHT run were next analyzed, and yielded a mean intercirculus distance of 57.167 microns. It is of interest to note that not only were the mean values comparable (t-value of 0.82104,d.f.=32, N.S.) but the variation of the individual circuli about the mean were quite similar in both cases, as measured by the standard deviations in each case (S.D.SEM = 12.72, S.D.ANALYZER = 12.63). This provides us with further reassurances as to the fundamental accuracy of measurement obtainable by use of our scale surface profile analyzer system.

Independent investigations have probed the question of scale circulus height values. Koo and Finn (1963) embedded scales of the sockeye salmon (O. nerka) in a plastic matrix and used light microscopy to examine the subsequent scale cross- sections. Circulus height values of between 9 and 18 microns were obtained. This is in excellent agreement with results of our investigation, as well as results described by Wallin (1957) who reported values of circulus height between 12 and 15 microns for the roach (Rutilus rutilus). It is taken as dual confirmation of our technique that our results parallel so closely the results of previous investigations into the morphology and development of scale surface microstructure.

Table 1. Comparison of Intercirculus Spacing: Electron Microscopy vs. Profile Analysis

SEM Spacing (μ)		Profile Analyzer Spacing (μ)	
45 μ	68 μ	62 μ	57 μ
55	73	87	56
50	23	42	56
64	62	31	52
45	41	73	53
68	45	47	74
53	55	53	62
64	64	57	59
45		62	
TBAR = 54.1 μ		TBAR = 57.7 μ	
S.D. = 12.7 μ		S.D. = 12.6 μ	

REFERENCES CITED

Anas, R.E. and S. Murai. 1968. Use of scale characters and a discriminant function for classifying sockeye salmon (O. nerka) by continent of origin. Int. North Pac. Fish. Comm. Bull. 26: 157-179.

Bendat, J. G. and A. G. Piersol. 1971. Random Data: Analysis and Measurement Procedures. Wiley-Interscience, New York. 407 p.

Bergland, G. D. 1969. A guided tour of the fast Fourier transform. I.E.E.E. Spectrum 6 (7): 41-52.

Bilton, H. T. 1974. Effects of starvation and feeding on circulus formation on scales of young sockeye salmon. In T. P. Bagenal, ed. Ageing of Fish, Unwin Bros. Ltd., Surrey. 234 p.

Blackman,R. B. and J. W. Tukey. 1958. The Measurement of Power Spectra. Dover, New York.

Bogoiavlenskaia, M. P. 1960. A study of calcium metabolism with a view to utilizing Ca^{45} as a mark for fish. Fish. Res. Bd. Can. Transl. Ser., No. 276.

Clutter, R. I. and L. E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Int. Pac. Salmon Fish. Comm. Bull. IX: 159 p.

Colbert, T. 1974. Acquisition and analysis of ocean wave data recorded on a spar buoy mounted digital cassette recorder. M.S. Thesis Univ. of Rhode Island.

Dixon, W. 1973. B.M.D. Biomedical Computer Programs. Univ. California Press, Berkeley.

Fisher, R.A. 1936. The use of multiple measurements for taxonomic problems. Ann. Eugen. 7(2): 179-188.

Kan, E. P. F. 1972. The latest version of ISODATA(A)/ISOCLS. Lockheed Electronics Co. Inc. , HASD, Houston. Tech. Memo. T642570.

Koo, T. S. Y. and E. Finn. 1963. Circulus height and related morphology of sockeye salmon scales as determined from sections. U. Wash. Coll. Fish. Contrib. 166: 15-18.

Lanzing, W. J. and D. R. Higginbotham. 1974. Scanning microscopy of surface structures of Tilapia mossambica (Peters) scales. J. Fish. Biol. 6: 307-310.

Lee, P. J. 1971. Multivariate Statistics and the fisheries biology. Fish. Res. Bd. Can. Tech Rept. No. 244.

Lewis, R. M. 1957. Comparative studies of populations of the striped bass. U.S. Fish & Wildlife Serv. Spec. Sci. Rept.- Fisheries, No. 204.

Lund, W. A. 1957. Morphometric study of the striped bass (Roccus saxatilis). U.S. Fish & Wildlife Serv.- Spec. Sci. Rept.- Fisheries, No. 216.

Marr, J. C. 1952. Contributions to the study of subpopulations of fishes. U.S. Fish & Wildlife Serv. Spec. Sci. Rept.- Fisheries, No. 208.

Mason, J. E. 1974. A semi-automatic machine for counting and measuring circuli on fish scales. In T. P. Bagenal, ed., op. cit.

Merriman, D. 1941. Studies on the striped bass (Roccus saxatilis) of the Atlantic coast. U.S. Fish & Wildlife Serv. Fish. Bull. 50(35): 77p.

Messinger, H. B. and H. T. Bilton. 1974. Factor analysis in discriminating the racial origin of sockeye salmon (O. nerka) J. Fish. Res. Bd. Can. 31(1): 1-10.

Minter, R. 1972. Computer program documentation ISOCLS iterative self-organizing clustering program. Lockheed Elect. Co. Inc., HASD Houston. Program C094.

Morgan, R. 1971. Comparative electrophoretic studies on the striped bass, Morone saxatilis. Ph. D. thesis, Univ. of Maryland. 92p.

Mosher, K. H. 1963. Racial analysis of red salmon by means of scales. Int. North Pac. Fish. Comm. Bull. 11: 31-56.

Murawski, W.S. 1958. Comparative study of populations of striped bass Roccus saxatilis, based on lateral line scale counts. M.S. thesis Cornell, 80 p.

Nichols, P. R. and R. V. Miller. 1967. Seasonal movements of striped bass, Roccus saxatilis (Walbaum), tagged and released in the Potomac River, Maryland, 1959-61. Ches. Sci., 8(2): 102-124.

Raney, E. C. 1952. The life history of the striped bass, Roccus saxatilis (Walbaum). Bull. Bingham Ocean. Coll., 14(1): 5-97.

_____, 1957. Subpopulations of the striped bass Roccus saxatilis (Walbaum) in tributaries of Chesapeake Bay. In Marr, J.C., ed. op. cit.

Raney, E. C. and D. P. DeSylva. 1953. Racial investigation of the striped bass, Roccus saxatilis (Walbaum). J. Wildl. Mgt., 17(4):495-509.

Rao, C.R. 1952. Advanced Statistical Methods in Biometric Research. Wiley, New York. 390 p.

Scofield, E. C. 1931. The striped bass of California (Roccus lineatus). Cal. Fish & Game Fish Bull. No. 29: 1-84.

Van Utrecht, W. L. and E. J. Schenkkan. 1972. On the analysis of the periodicity in the growth of scales, vertebrae and other varied structures in a teleost. Aquaculture, 1: 293-316.

Vladykov, V. D. and D. H. Wallace. 1952. Studies of the striped bass Roccus saxatilis (Walbaum) with special reference to the Chesapeake Bay region, during 1936-1938. Bull. Bingham Ocean. Coll. 14(1): 132-177.

Wallin, O. 1957. On the growth structure and developmental physiology of the scales of fishes. Rep. Inst. Freshwater Res., Drottningholm, No. 38: 385-445.