FINAL REPORT OF THE SYNOPTIC SUBPOPULATION ANALYSIS, PHASE I: REPORT ON THE FEASIBILITY OF USING INNATE TAGS TO IDENTIFY STRIPED BASS (*Morone saxatilis*) FROM VARIOUS SPAWNING RIVERS

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SUMMARY

During 1974, Texas Instruments Incorporated performed a study to assess the feasibility of using meristic, morphometric, and biochemical characters as innate tags to segregate striped bass from various spawning rivers. Representative samples of the spawning populations of four Chesapeake tributaries (Potomac, Rappahannock, Choptank, and Elk Rivers) and the Hudson River were collected. Despite extensive sampling efforts in the Delaware River, only three striped bass were collected. Laboratory analyses provided data on 41 meristic and morphometric characters, 45 protein characters, and 28 enzyme systems involving 52 genetic loci. Linear and quadratic discriminant function analyses were employed to evaluate the discriminatory power of the meristic and morphometric characters. The discriminatory power of the biochemical characters was evaluated with univariate techniques.

Twenty-one meristic and morphometric characters were highly correlated with length and were rejected from the character sets used in discriminant analyses. Two additional characters were highly cross correlated and similarly rejected from further analysis, as was the number of spines on the first dorsal fin (a constant in 849 of the 857 fish examined). The remaining 17 characters possessed discriminatory power, but the addition of more than 10 characters in a discriminant function failed to provide additional separation.

It was not possible to separate the spawning populations within Chesapeake Bay due to overlap of the character sets. The sample values from the Chesapeake tributaries were pooled and entered in a discriminant function with values from the Hudson River population. Four characters

(first annulus to second annulus distance/focus to first annulus distance ratio, snout length/head length ratio, internostril width/head length ratio, and number of scales along lateral line) provided maximum separation between Chesapeake Bay and Hudson River striped bass. Discriminant analysis with all fish collected in the study resulted in 80% correct classification of fish into the appropriate spawning populations.

Protein and isozyme analyses have shown striped bass to be one of the most genetically homogeneous species ever studied. Of the 52 loci examined, only two were variant. The variant gene frequencies were low in all populations but were clinal. Isocitrate dehydrogenase was fixed in the Hudson River population; consequently, all variant alleles uniquely classified fish of non-Hudson origin. All fish classified as Hudson in the discriminant analysis which possessed a variant allele were redefined as non-Hudson, and overall correct classification increased to 83%.

An experimental design has been developed to sample the Atlantic fishery from Cape Hatteras to Maine and to generate an estimate of the relative contribution of Hudson River striped bass to the Atlantic fishery. The spawning populations previously sampled will be sampled again to verify the discriminant functions. The Delaware and Roanoke River populations will be sampled to establish discriminant functions for their respective spawning populations.

A spatial and temporal stratified sampling design will be employed to collect a representative sample of the Atlantic fishery. Meristic, morphometric, and biochemical analyses will be performed on the collected fish. Discriminant function analyses will be employed to assign a spawning origin to each fish, and an estimate of the relative contribution of the Hudson River population will be made.

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INTRODUCTION

Most published work on striped bass has concluded that the Chesapeake Bay system is the major contributor to the mid-Atlantic fishery (Merriman, 1941; Vladykov and Wallace, 1952; Alperin, 1966; Schaefer, 1968; Porter and Saila, 1969; and Raney, 1972). Recent challenges to those works (Clark, 1972, and Goodyear, 1974) have suggested that the Hudson River may also contribute significantly.

Assessment of the relative contribution of Hudson River striped bass requires identification of individuals in the Atlantic fishery that originated in the Hudson River. Studies by Raney et al (1953, 1954) demonstrated that meristic characters allowed separation of a high percentage (70-80%) of striped bass originating in the Hudson from those originating in the tributaries of Chesapeake Bay. Furthermore, several studies (Moller, 1966; Drilhan et al, 1967; Jamieson, 1967; Fugino, 1969; Morgan et al, 1973) have shown that biochemical characters allow identification of fish from various sources of origin.

In February 1974, Texas Instruments began a study to determine the feasibility of using biochemical, meristic, and morphometric characters as innate tags to identify striped bass subpopulations among the major spawning areas of the Hudson River and tributaries of the Delaware and Chesapeake Bay systems.

The study objectives were to

- Identify a set of characters (innate tags) that would characterize a typical fish from each spawning area

- Formulate probability statements concerning the degree of certainty with which those character sets would identify the origin of a particular fish

- Design a sampling regime to collect a representative sample of the Atlantic striped bass fishery which will facilitate assessment of the Hudson River contribution

SECTION II

METHODS

A. FIELD COLLECTION

During the spring spawning season of 1974, 150 to 250 striped bass were collected from the spawning areas of each of the following: Rappahannock, Potomac, Elk, Choptank, and Hudson Rivers (Figure II-1). Sampling was restricted to the Delaware spawning grounds above the Chesapeake and Delaware Canal entrance to ensure that those striped bass collected were spawning in the Delaware River rather than migrating to the canal, which was sampled as part of the Elk River system, to spawn. Very little commercial fishing is directed toward striped bass in the Delaware River. Two commercial fishermen were employed to collect striped bass, but their efforts provided only three specimens (two specimens were immature). Based on the hypothesis that striped bass, like salmon, home to their natal stream to spawn, an assumption was made that a sexually ripe striped bass collected in the spawning area of a particular river during the spawning season originated in that river. Immature fish were occasionally collected but were not used in the study.

All fish from the Chesapeake region were purchased from commercial fishermen. Specimens from the Hudson River were obtained from commercial fishermen and sampling by Texas Instruments. Various types of fishing gear were used to collect the specimens: pound nets in the Rappahannock, stake gill nets in the Potomac, haul seines in the Choptank, drift gill nets in the Elk, and stake and anchor gill nets in the Hudson.

B. SPECIMEN PROCESSING IN FIELD

A numbered jaw tag was attached, and blood, liver, and muscle tissue samples were obtained from each fish in the field for isozyme analysis.

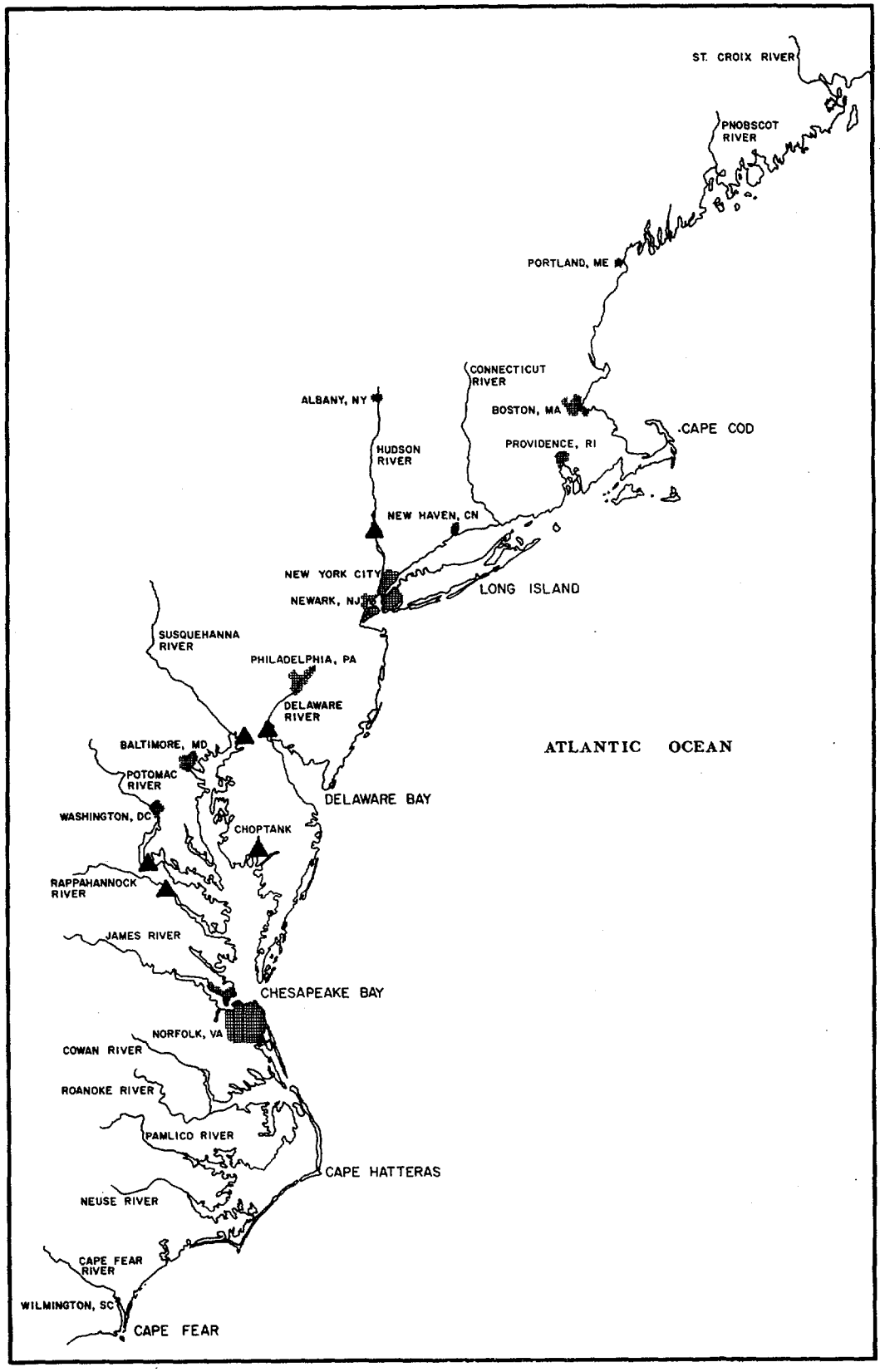

Figure II-1. Spawning River Collection Sites (indicated by ▲)

Using a syringe, 1 to 2 mℓ of blood was collected from the cardiac region, placed in a test tube, and centrifuged to separate the cellular and serum fractions. The serum was transferred to another test tube. Both fractions were stored on ice as long as 2 weeks while in the field and afterwards refrigerated in the laboratory at 40°F until processed. A strip of muscle tissue excised from the region between the first and second dorsal fins across the lateral line and a lobe of liver tissue were placed in separate whirl pack bags and stored in liquid nitrogen until processed. The samples from each fish were labeled with the number of the jaw tag. The biochemical samples were processed at Johns Hopkins University by Dr. Dennis A. Powers.

Scale samples for age and growth analysis were obtained from a key location above the lateral line between the first and second dorsal fins. The scale samples were stored in labeled envelopes. Jaw tag number, sex, and state of maturity were recorded for each fish. The fish were then preserved in 20% formalin and transferred to the Verplanck (New York) laboratory where meristic and morphometric analyses were performed.

C. SPECIMEN PROCESSING IN LABORATORY

After approximately 1 month's storage in formalin, the fish were soaked from 1 to 2 days in water to reduce the preservation fumes prior to processing. Thirty meristic counts and morphometric measurements were made on each fish. Scales along lateral line, scales above lateral line, scales below lateral line, scales around caudal peduncle, spines on first dorsal fin, and soft rays on the second dorsal and anal fins were enumerated in the manner prescribed by Hubbs and Lagler (1947). Formalin obscured the last two rays on the second dorsal and anal fins, causing the last three ray elements to appear as one; consequently, reported anal and second dorsal soft ray counts are one less than actual. For the left and right pectoral fins, all the rays including the rudiments were counted.

Gill rakers were enumerated on the first arch both excluding and including rudimentary rakers. A gill raker was considered complete if its height was greater than the diameter of its base; otherwise, it was considered rudimentary. A gill raker straddling the arch was counted with the lower arm.

Total length, standard length, snout length, length of upper jaw, head length, orbit to angle of preopercle length, length of orbital, interorbital width (least fleshy width), predorsal length, length of caudal peduncle, depth of caudal peduncle, length of base of second dorsal fin, length of first spine of second dorsal fin, length of base of anal fin, and length of first spine of anal fin were measured as prescribed by Hubbs and Lagler (1947). Fork length was measured from the most anteriorly projecting part of the head to the deepest fork of the caudal fin. The internostril width was the least fleshy distance between the excurrent nares.

Measurements of total, fork, and standard length were taken to the nearest millimeter on a fish-measuring board mounted with a metric rule. The remaining morphometric measurements were taken to the nearest millimeter with a pair of dividers and a metric rule.

Three scales (nonregenerated) were cleaned and mounted. Some scales were mounted wet between glass microscope slides. Most scales were mounted permanently on 6-in. x 3-in. x 0.02-in. acetate strips with a heat press. Mounting specifications were: temperature of upper heating plate, 220°F; temperature of lower heating plate, 180°F; pressure, 6000 psi; duration of pressing, 1 min. Differences in measurements from the focus to the first and second annuli between wet mounts and acetate mounts were found to be nonsignificant ($P = 0.95$) with a paired t-test.

The mounted scales were placed on a scale projector that magnified the scale image 47.5 times. Age determinations and measurements from the focus to the first and second annuli were made on the projected scale image.

D. ISOZYME ANALYSES

Starch gel electrophoresis was employed to identify all protein characters useful in discriminating among striped bass subpopulations. Forty-five protein systems, including 16 serum proteins and hemoglobins, were examined. In addition, 28 enzyme systems involving 52 loci were elucidated (Table II-1).

E. ANALYTICAL PROCESSING

The choice of characters to be used in segregating subpopulations of striped bass followed three stages of statistical analysis: analysis of correlation between each character and length, discriminant analyses, and analyses of the effects of sex and time of capture on each character. Only specimens with a complete set of measures for the characters of interest were used in the discriminant analyses; all specimens were used in the other analyses.

1. Correlation Analyses

Spawning populations contain fish of all mature age classes. Sampling gear differentially captures the various age classes. Year-class strength and gear selectivity would cause bias if age-specific meristic and morphometric characters were used to characterize the population. Length is a good indication of age (Mansueti, 1961); therefore, all characters were correlated with length. The difficulties presented by year-class strength and gear selectivity were avoided by eliminating from further analysis any character correlated with length above a minimal degree.

Table II-1

Enzyme Systems

Enzyme System	Abbreviation	No. of Loci
α-napthyl acetate esterase	α-nap·acetate-EST	1
α-napthyl butyrate esterase	α-nap·but-EST	4
Serum esterase	ser-EST	1
Phosphoglucomutase	PGM	1
Phosphohexoseisomerase	PHI	2
Isocitrate dehydrogenase	IDH	1
Alcohol dehydrogenase	ADH	2
Glucose 6-phosphate dehydrog.	G6PDH	1
Alkaline phosphatase	ALK PHOS.	1
Acid phosphatase	ACID PHOS.	1
Glucokinase	GK	1
Glutamate dehydrogenase	GDH	2
α-glycerophosphate dehydrog.	α-GPDH	2
6-phosphogluconate dehydrog.	6PGDH	2
Lactate dehydrogenase	LDH	2
Superoxide dismutase	SOD	2
Leucine aminopeptidase	LAP	1
Fructose 1,6 diphosphatase	F1,6DiPhos	2
Creatine kinase	CK	2
Adenylate kinase	AK	2
Aspartate aminotransferase	AAT	3
Xanthine dehydrogenase	XDH	1
Sorbitol dehydrogenase	SDH	2
Glyceraldehyde 3 phosphate dehydrogenase	G3-P DH	2
Monoamine oxidase	MO	2
Malate dehydrogenase	MDH	2
Peroxidase	Per	5
β-hydroxybutyrate dehydrogenase	βOHbutDH	2
Total systems - 28	Total loci	- 52

A computer program for linear correlation analysis (BMD03D, UCLA Biomedical Program) provided the correlation coefficients between each character and fork length and also between every pair of characters. Data from each river were analyzed separately. A pooled correlation coefficient was calculated by using the Fisher "z" transformation for each character. The test for homogeneity of correlation coefficients (Steel and Torrie, 1960) was used to determine if a pooled correlation coefficient was valid. A character was considered independent of length (i) if its pooled correlation coefficient indicated less than 5% variation attributable to length ($r = 0.224$) or (ii), in cases where the pooled correlation coefficient was invalid, if the variation attributable to length in any river was less than 10% ($r = 0.316$). If two characters were highly correlated with each other ($r = 0.70$), then only the character showing a lower correlation with length was retained.

2. Discriminant Analyses

Discriminant function analysis provides a method of classifying individuals from a mixed sample into their respective subpopulations. A set of meristic counts and morphometric measurements from individuals of a subpopulation are used to determine a discriminant function for that subpopulation. The number of discriminant functions determined equals the number of subpopulations.

In classifying an individual of unknown origin from a mixed population, the individual's values for these characters are applied to each function. A posterior probability is determined for each function which states the degree of certainty with which that function will identify the origin of the individual. The individual is classified as belonging to the subpopulation for which the posterior probability is greatest. The term posterior probability refers to that probability based on the data, rather than, for example, a probability obtained before the data are taken based on parameters of the underlying distribution.

Two types of discriminant functions are available: a linear discriminant function and a quadratic discriminant function. The linear discriminant analysis requires that the data come from multivariate normal distributions with common variance-covariance matrices within spawning populations to which individuals are classified and that each individual originate in one of the spawning populations to which individuals are assigned. The quadratic discriminant analysis is more general and does not require common variance-covariance matrices within spawning populations. Thorough treatments of linear and quadratic discriminant analyses are found in Anderson (1958) and Kendall and Stuart (1968) respectively.

Three stages were involved in the discriminant analyses: (i) discriminant functions were determined for a randomly selected subsample of specimens; (ii) the validity of these functions was tested with the remaining independent sample of specimens; (iii) discriminant functions were determined with the entire set of specimens.

In the first stage, 100 specimens from the Rappahannock, Potomac, Elk, and Hudson Rivers and 50 specimens from the Choptank were randomly subsampled. The assumption of common variance-covariance matrices within the five rivers and within the Chesapeake and Hudson regions was tested with a chi-square statistic for those characters that fulfilled the correlation criteria. When the assumption was not satisfied on a particular set of characters, a smaller set of characters as determined from the stepwise linear discriminant analysis program (BMD07M, UCLA Biomedical Program) was tested. The stepwise linear discriminant analysis program, run for the five and then for the two regions, entered characters in the order of their discriminating potential, thus providing information on the importance of each character. The quadratic discriminant program did not have this stepwise potential; therefore, the quadratic analysis was performed on various sets of characters in the order of importance in the stepwise linear discriminant program. The quadratic discriminant analysis program was also run for five rivers and then for the two regions.

After the discriminant functions were established, each program reanalyzed the specimens of known origin which had determined the functions and classified these specimens into various subpopulations. The percentage of specimens misclassified provided a measure of the classification error inherent in the functions. This procedure provided the percentage of correct classification shown in the results.

In the second stage, the remaining independent specimens were applied to the functions determined in the first stage to test their validity. These discriminant functions included only those characters that provided virtually all of the discriminating power. The final stage used the entire set of specimens to determine the coefficients of our final discriminant functions.

3. Sex and Time-of-Capture Analyses

Extraneous variations in the final set of characters used in the discriminant analyses due to sex and time of capture within each river were investigated using univariate techniques. Mean and variances for each capture period of a given sex and river were calculated for each discriminant character. Homogeneity of variance between capture periods within rivers and sexes was tested with Bartlett's test (Winer, 1971). When the assumption of common variance was satisfied, the effect of time of capture was tested with analysis of variance; but, when it was not satisfied, the Kruskal-Wallis nonparametric analysis (Winer, 1971) was used. The effect of sex on a character was tested with a paired t-test for each river and the Chesapeake and Hudson regions.

SECTION III

RESULTS

A. CORRELATION ANALYSES

A total of 41 potential meristic and morphometric discriminating characters was generated (Table III-1). The set of 41 characters was reduced by those 21 highly correlated with length; i.e., those characters highly correlated with length were not entered in a discriminant function analysis.

The scale ratio character (41) slightly exceeded the limits of correlation criteria for the Rappahannock and Potomac samples; however, it was retained because the Hudson River striped bass exhibited a compensatory growth strategy during their first 2 years and the Chesapeake striped bass did not, thus making it a potentially good discriminatory character.

High correlations occurred between the snout/fork length (12) and snout/head length (13) ratios and between the internostril width-fork length (23) and internostril width/head length (24) ratios, but characters 12 and 23 had a higher correlation with length and therefore were removed.

The number of spines on the first dorsal fin was nine in 849 of the 857 specimens (one had eight spines and seven had 10 spines); therefore, character 7 was removed.

B. DISCRIMINANT ANALYSES

1. Analyses for Five Subpopulations

A stepwise linear discriminant analysis for the five subpopulations was performed on the 450 randomly selected specimens for the remaining 17 characters (1, 2, 3, 4, 5, 6, 8, 9, 13, 16, 24, 32, 35, 36, 38, 39, and 41). Fifteen specimens with an incomplete measure for character 41 were

Table III-1

List of Characters Generated from Meristic Counts and Morphometric Measurements

Character Code	Character Description
* 1-LL	Scales along lateral line
* 2-AL	Scales above lateral line
* 3-BL	Scales below lateral line
* 4-ACP	Scales around caudal peduncle
* 5-LP	Rays on left pectoral fin
* 6-RP	Rays on right pectoral fin
7-FDOR	Spines on first dorsal fin
* 8-DOR	Soft rays on second dorsal fin
* 9-ANL	Soft rays on anal fin
10-TL/F	Total/fork length ratio
11-SL/F	Standard/fork length ratio
12-SNT/F	Snout/fork length ratio
*13-SNT/H	Snout/head length ratio
14-UJAW/F	Upper jaw/fork length ratio
15-UJAW/H	Upper jaw/head length ratio
*16-H/F	Head/fork length ratio
17-OP/F	Orbit-preopercle/fork length ratio
18-OP/H	Orbit-preopercle/head length ratio
19-ORB/F	Orbit/fork length ratio
20-ORB/H	Orbit/head length ratio
21-INO/F	Interorbital width/fork length ratio
22-INO/H	Interorbital width/head length ratio
23-NOS/F	Internostril width/fork length ratio
*24-NOS/H	Internostril width/head length ratio
25-PDOR/F	Predorsal/fork length ratio
26-CAUL/F	Caudal/fork length ratio
27-CAUW/CAUL	Caudal width/caudal length ratio
28-CAUL/F	Caudal width/fork length ratio
29-BDOR/F	Base second dorsal fin/fork length ratio
30-SDOR/F	First spine second dorsal/fork length ratio
31-SDOR/BDOR	First spine second dorsal/base second dorsal length ratio
*32-BANL/F	Base anal fin/fork length ratio
33-SANL/F	Second spine anal fin/fork length ratio
34-SANL/BANL	Second spine anal fin/base anal fin length ratio
*35-UGC	Upper arm gill rakers excluding rudimentary rakers
*36-UGR	Upper arm gill rakers including rudimentary rakers
37-LGC	Lower arm gill rakers excluding rudimentary rakers
*38-LGR	Lower arm gill rakers including rudimentary rakers
*39-FA	Focus to first annulus measure
40-SA	First annulus to second annulus measure
*41-SA/FA	First annulus to second annulus/focus for first annulus measure ratio

Note: Those characters fulfilling the criteria of the correlation analysis (Appendix A) are designated by an asterisk preceding the character code.

included in the analysis for 17 characters but, since these specimens account for only 3.3% of the sample size, their effect on the results was minimal. The variance-covariance matrices were significantly different at the 99% confidence level among the five subpopulations for four or more characters. A quadratic discriminant analysis was performed on the 4, 5 and 10 most important characters as determined by the stepwise linear discriminant analysis. The percentages of correct classification for the five subpopulations of the striped bass are shown in Table III-2.

Table III-2

Correct Classification of the Five Subpopulations
by Linear and Quadratic Discriminant Analyses

Characters Entered	Character Codes (in order of importance)	Type of Analysis	Percent Correct Classification						
			Hudson	Rappahannock	Potomac	Choptank	Elk	Overall Chesapeake	Overall Total
4	41, 36, 13, 24	Linear	70.0	7.0	21.0	48.0	43.0	27.1	36.7
		Quadratic	69.0	15.0	18.0	48.0	52.0	31.1	40.0
5	41, 36, 13, 24, 2	Linear	69.0	37.0	21.0	46.0	36.0	33.4	41.3
		Quadratic	68.0	28.0	21.0	52.0	54.0	36.9	43.8
10	41, 36, 13, 24, 2,	Linear	71.0	39.0	44.0	54.0	41.0	43.1	49.3
	3, 1, 16, 6, 39	Quadratic	71.0	57.0	33.0	74.0	39.0	47.4	52.7
16	41, 36, 13, 24, 2, 3, 1, 16, 6, 39, 8, 4, 38, 35, 9, 32	Linear	70.0	45.0	45.0	54.0	33.0	42.9	48.9

The linear discriminant analysis showed that addition of more than 10 characters did not improve the overall discrimination among the five subpopulations. The quadratic discriminant analysis improved the overall correct classification, but only a few percentage points. The low probability of correct classification within the Chesapeake region indicated that, based on the data available, discrimination between the four subpopulations was not possible for classification purposes.

2. Analysis for Two Regions

The specimens used in the previous analyses from the four Chesapeake rivers were combined to form a sample for the Chesapeake region. A stepwise linear discriminant analysis for the Hudson and Chesapeake regions was performed for the same 17 characters. The variance-covariance matrices were significantly different at the 95% confidence level between the two regions for six or more characters. A quadratic discriminant analysis was performed in a stepwise manner on the two through eight most important characters as determined by the stepwise linear discriminant analysis. The percentages of correct classification for the subpopulations of striped bass from the two regions are shown in Table III-3.

Table III-3

Correct Classification of Hudson and Chesapeake Subpopulations by Linear and Quadratic Discriminant Analyses

Characters Entered	Character Codes (in order of importance)	Type of Analysis	Percent Correct Classification		
			Hudson	Chesapeake	Overall
2	41, 13	Linear	71.0	72.9	72.4
		Quadratic	73.0	72.3	72.4
3	41, 13, 24	Linear	76.0	75.1	75.3
		Quadratic	77.0	74.9	75.3
4	41, 13, 24, 1	Linear	79.0	75.1	76.0
		Quadratic	70.0	77.1	77.6
5	41, 13, 24, 1, 36	Linear	76.0	74.6	74.9
		Quadratic	79.0	74.3	75.3
6	41, 13, 24, 1, 36, 39	Linear	77.0	75.4	75.8
		Quadratic	83.0	72.3	74.7
7	41, 13, 24, 1, 36, 39, 16	Linear	77.0	74.6	75.1
		Quadratic	86.0	74.9	77.3
8	41, 13, 24, 1, 36, 39, 16, 32	Linear	76.0	74.9	75.1
		Quadratic	85.0	76.0	78.0
13	41, 13, 24, 1, 36, 39, 16, 32, 8, 9, 35, 38, 5	Linear	79.0	77.4	77.8

When six or more characters were used, the assumptions of the linear discriminant analysis were not satisfied and the quadratic discriminant provided better classification within regions. Both discriminant techniques showed that four characters were the "best" discriminators between the two spawning populations and that additional characters did not improve the overall discrimination between the Hudson and Chesapeake spawning striped bass.

A new data file containing mature specimens with a complete set of measures for the four "best" discriminant characters was created. A new random sample of 450 specimens was processed in linear and quadratic discriminant analyses for these four characters. The percentages of correct classification for the Hudson and Chesapeake spawning populations were:

	Hudson	Chesapeake	Overall
Linear	80.0	80.0	80.0
Quadratic	83.0	79.4	80.2

The linear and quadratic discriminant functions were:

Linear for Hudson:

$$F_h = 12.70508W + 1359.47080X + 803.47649Y + 10.95542Z - 691.81492$$

Linear for Chesapeake:

$$F_c = 12.91537W + 1440.07970X + 737.84938Y + 8.69158Z - 715.01360$$

Quadratic for Hudson:

$$F_h = -765.792737 - (0.090488W^2 + 3181.385707X^2 + 4454.296082Y^2 + 2.385884Z^2 + 4.428150WX - 0.051704WY + 0.199158WZ - 2661.672704XY - 0.034823XZ + 12.836756YZ) + 12.796752W + 1731.546908X + 954.514043Y + 21.529989Z$$

Quadratic for Chesapeake:

$$F_c = -693.390444 - (0.107606W^2 + 2531.472727X^2 + 3719.231750Y^2 + 3.191504Z^2 + 0.313982WX - 2.259715WY + 0.028192WZ - 1704.280787XY + 2.516731XZ - 22.544127YZ) + 13.173173W + 1341.307945X + 716.629158Y + 4.562999Z$$

where

F = discriminant score

W = lateral line scale count

X = snout/head length ratio

Y = internostril/head length ratio

Z = first annulus to second annulus/focus to first annulus measure ratio

The validity of these functions was checked with an independent file of specimens which were not used in determining the function. The percentages of correct classification for the Hudson and Chesapeake spawning populations were:

	Hudson	Chesapeake	Overall
Linear	73.6	80.0	78.6
Quadratic	72.4	78.1	76.9

The overall correct classification was lower than that for the specimens used to determine the functions, but the agreement between the two sets of data has shown that the techniques used were valid.

The entire set of 857 specimens having a complete set of measurements for the four characters was processed in linear and quadratic discriminant analyses and the final discriminant functions obtained. The percentages of correct classification for the Hudson and Chesapeake spawning populations were:

	Hudson	Chesapeake	Overall
Linear	77.5	78.5	78.3
Quadratic	79.7	78.2	78.5

The final linear and quadratic discriminant functions were:

Linear for Hudson

$$F_h = 11.37071W + 1449.33620X + 970.99374Y + 4.96371Z - 676.70158$$

Linear for Chesapeake

$$F_c = 11.55886W + 1517.35630X + 919.73629Y + 2.80536Z - 698.73614$$

Quadratic for Hudson

$$F_h = -725.111712 - (0.084818W^2 + 2984.908970X^2 + 3972.379025Y^2 + 2.297438Z^2 + 4.281602WX - 0.475769WY + 0.121288WZ - 1912.330302XY - 22.722956XZ + 0.689404YZ) + 11.839444W + 1729.269530X + 943.759217Y + 6.963189Z$$

Quadratic for Chesapeake

$$F_c = -675.634936 - (0.094820W^2 + 2547.820634X^2 + 3634.695748Y^2 + 2.720402Z^2 - 0.053239WX - 1.786265WY + 0.014482WZ - 1071.465753XY - 4.935841XZ - 15.112246YZ) + 11.512824W + 1442.712280X + 923.493087Y + 1.838425Z$$

where

F = discriminant score

W = lateral line scale count

X = snout/head length ratio

Y = internostril/head length ratio

Z = first annulus to second annulus/focus to first annulus measure ratio

C. ISOZYME ANALYSES

Isozyme analyses were completed following the completion of the multivariate discriminant analyses and were analyzed with univariate techniques. Isozyme analyses have shown striped bass to be one of the most homogeneous species ever studied. Of the 52 isozyme loci examined, only isocitrate dehydrogenase (IDH) and α-glycerophosphate dehydrogenase (α-GPDH) were variant. Serum transferrin was also variant but was too labile to be used.

Both variant enzyme systems showed clinal changes in gene frequency with latitude (Table III-4). Although the gene frequencies were low, each population had a unique value. IDH appeared to be fixed at a frequency of 1.00 in the Hudson River population, while its degree of variation increased in southern populations. For α-GPDH, the degree of variation was greater in the Hudson River than in southern populations.

Table III-4

Gene Frequencies for Most Common Allele in Enzymes α-GPDH and IDH

River	Gene Frequency	
	α-GPDH	IDH
Hudson	0.883	1.000
Elk	0.910	0.945
Choptank	0.932	0.977
Potomac	0.976	0.966
Rappahannock	0.983	0.944

Fixation at the IDH locus in the Hudson River striped bass provided a mechanism to uniquely classify a fraction of fish as "non-Hudson" in origin. All specimens characterized with a variant IDH allele came from rivers other than the Hudson. Consequently, all fish classified as Hudson in the final discriminant analysis and possessing a variant IDH allele were re-defined as fish of Chesapeake, and overall correct classification increased 3%. Results from α-GPDH can be used in a similar manner but the analyses are statistical and have not been performed.

D. SEX AND TIME-OF-CAPTURE ANALYSES

The effects of time of capture and sex on the final four discriminant characters are given in Tables III-5 and III-6 respectively and are coded as follows:

		Variances	
		Equal	Unequal
Means	Equal	A	C
	Unequal	B	D

The letters A and C indicate that, at the 95% confidence level, no significant difference occurred in a character's values between different capture periods or sexes within spawning populations.

Table III-5

Effects of Time of Capture on Discriminant Characters within the Five Subpopulations

River	Sex	Capture Periods Analyzed	Characters (Coded) 1-LL	13-SNT/HL	24-NOS/HL	41-SA/FA
Rappahannock	Male	1, 2, 3	A	D	B	C
	Female	1, 2, 3	A	A	B	A
Potomac	Male	1, 2, 3, 4	A	C	A	A
	Female	2, 3, 4	A	A	A	A
Choptank	Male	1, 2, 3	A	A	A	A
	Female	2 3	?	A	A	A
Elk	Male	1, 2, 3, 4	A	B	B	A
	Female	3, 4	A	A	A	B
Hudson	Male	1, 2, 3	C	A	C	A
	Female	1, 2, 3	A	A	A	A

[1]A indicates equal variances and equal means
B indicates equal variances and unequal means
C indicates unequal variances and equal means
D indicates unequal variances and unequal means

Table III-6

Effects of Sex on Discriminant Characters within the Five Subpopulations and the Chesapeake Region

River	Characters (Coded) 1-LL	13-SNT/HL	24-NOS/HL	41-SA/FA
Rappahannock	A	*	*	B
Potomac	B	C	D	D
Choptank	*	A	A	A
Elk	A	*	*	*
Hudson	B	A	C	B
Chesapeake	A	C	D	B

[1]A indicates equal variances and equal means
B indicates equal variances and unequal means
C indicates unequal variances and equal means
D indicates unequal variances and unequal means
* indicates that test could not be made since time of capture was significant for either male or female for that river

The time of capture was significant for characters within the rivers of Chesapeake Bay. In the final discriminant analyses, these four rivers were pooled together and these differences were not critical.

Significant character value difference occurred between male and female striped bass in each river and region, but the results are inconclusive. Only the scale ratio character (41) had a significant difference within both the Hudson and Chesapeake regions. Since the discriminant functions deal with a mean vector of character values rather than individual character values, the use of univariate techniques may produce misleading results.

SECTION IV
DISCUSSION

Multivariate discriminant analyses were applied to meristic and morphometric characters, first and second year growth rates, and appropriate transformations of those characters to assess the feasibility of using such characters to identify subpopulations of striped bass. A "best" set of characters was identified to characterize a typical fish from each sampled spawning area. That character set was established independent of size, sex, and time of capture, producing the most generally applicable set of characters for fish in the Hudson and fish spawning in the Chesapeake area. It was not possible to segregate among striped bass sampled from the tributaries of Chesapeake Bay.

Comparisons of linear and quadratic discriminant functions produced similar results when six or fewer characters were used. For more than six characters, the quadratic function produced better results because the assumptions of the linear models were not met. Four characters provided virtually all the discriminatory power contained in the character sets for fish of Hudson or Chesapeake origin; these characters were first annulus to second annulus distance/focus to first annulus distance ratio; snout length/head length ratio; internostril width/head length ratio; and number of scales along lateral line. The probability of correctly classifying a fish with four characters from a mixed population from the Chesapeake and Hudson subpopulations was 80%.

The use of discriminant function analysis with meristic characters for separating populations of fish has been successful in numerous studies. Hill (1959) correctly classified 81% of shad into their respective Hudson and Connecticut River populations based on six meristic characters. Fukuhara et al (1962) correctly classified 77% of sockeye salmon into their respective Asian and North American populations based on seven meristic characters.

Amos et al (1963) correctly classified 72% of pink salmon into their respective Asian and North American populations based on only three meristic characters. Most recently, Parsons (1972) contrasted autumn and spring herring, correctly classifying from 80.6 to 86.2% for autumn herring spawners and from 79.4 to 90.7% for spring herring spawners based on three meristic characters.

The results for striped bass compare favorably with those of previous stock discrimination studies in which meristic and morphometric characters were employed. However, there are potential limitations in using the results to assess the relative contribution of the Hudson River to the mid-Atlantic fishery.

Fish at large in the fishery come from spawning areas other than those sampled in the current study. Indeed, striped bass spawn in most of the major rivers from Florida north to the Hudson (Raney, 1972). Results of tagging studies (Vladykov and Wallace, 1952; Alperin, 1966; Shaefer, 1968; Florence, 1974; Campbell et al, in preparation; and ongoing American Littoral Society programs) suggest that fish from all spawning sources north of Cape Hatteras utilize the entire coast north of their respective spawning areas to Maine. The relative contribution of all other spawning areas to the Atlantic fishery is unknown and undoubtedly will affect the probability of correctly identifying fish of Hudson River origin. Fish from any spawning population that has a significant overlap in the character sets with Hudson River fish will cause incorrect classification as a fish of Hudson origin, thereby inflating the estimate of Hudson contribution. Consequently, to minimize the potential problems, the following steps have been taken:

- The sampling regime for 1975 collections in the Atlantic fishery (see Section V for a full discussion) has been designed to maximize sampling efforts in areas where fish of Hudson River origin are expected to provide maximum contribution. In addition, sampling will occur over the entire fishery from Cape Hatteras to Maine.

- Samples of the spawning populations in the Roanoke and Delaware Rivers will be collected to provide specimens to characterize fish of these two other important spawning sources.

- Samples from the spawning areas previously sampled during 1974 will be collected to verify past results.

- Measurements of 10 useful meristic and morphometric characters will be made for all fish collected to allow identification of fish from sources other than those characterized in the current study.

- Isozyme analyses will be performed for IDH and α-GPDH in all fish collected.

The biochemical genetic structure of striped bass is one of the most homogeneous ever studied. However, the fixation of IDH and the clinal nature of both IDH and α-GPDH provide discriminating power beyond the meristic and morphometric characters. IDH fixation allows unique "non-Hudson" classification of specimens with variant alleles. The clinal nature of enzyme frequencies, when extrapolated to more southerly populations, allows correct identification of a greater number of "non-Hudson" fish originating in the more southerly spawning area.

In addition to morphometric, meristic, and biochemical analyses, a critical scale analysis has been performed at the University of Rhode Island (Taub, 1975). Scales from all striped bass collected during this study have been forwarded to the University of Rhode Island for analysis. Results from this study will be incorporated into the multivariate discriminant functions.

In summary, character sets have been established which characterize typical fish in the Hudson River and Chesapeake spawning populations. The probability of correctly identifying a fish from a mixed sample using

those characters was 80-83%. The experimental design for sampling the Atlantic fishery and performing analyses on specimens collected from the fishery has maximized the probability of correctly classifying fish of Hudson River origin.

SECTION V

1975 EXPERIMENTAL DESIGN

Assessment of the relative contribution of the Hudson River striped bass population to the Atlantic fishery requires collection of striped bass samples representative of the composition of the fishery. The sampling regime must provide samples from the entire fishery and must consider the migratory nature of the striped bass. To fulfill the above objectives, a spatially and temporally stratified sampling design will be employed. Geographic stratification reduces variance and thereby provides more precise estimates of the composition of the populations within the geographic strata of interest. Furthermore, if the results warrant such analysis, stratification provides a precise estimate across strata (i.e., the composition of the entire Atlantic fishery). Geographic strata are defined based on three criteria:

- Stratification is based on the availability of accessory data useful for the objectives. Commerical catch data, in particular, are reported by state, and strata generally are aligned along state boundries.

- Stratification is based on real habitat differences which may lead to differences in the composition of the populations occupying those habitats. The mouths of the Chesapeake and Delaware Bays, Cape Cod, and Long Island are geographic barriers which may separate populations. Consequently, some strata are defined along geographic barriers.

- Finally, stratification is based on area-specific gear use. In certain areas, due to regulation or habitat, the commercial fishery uses only one type of gear (e.g., the eastern Long Island Sound fishery has no pound nets, and the New England fishery uses only hook and line).

Figure V-1 illustrates the strata to be sampled during 1975. In general, state boundaries describe the strata. Since is is reasonable to assume that the majority of the Hudson River striped bass inhabit the areas adjacent to the mouth of the river, a greater number of finer strata are defined in the New York Bight and Long Island areas. Collections within strata will be taken from two to three substrata to assess the variation in the composition of each stratum.

Temporal stratification is necessary due to the migratory nature of the striped bass. The year is generally stratified into six 2-month periods to provide precise estimates of the composition of the populations of each stratum at various times of the year and to demonstrate potential changes in composition throughout the year. Due to the behavior of the striped bass, certain geographic-time strata will be unfilled (e.g., striped bass do not frequent New England waters during the winter months). The sampling schedule is shown in Table V-1.

Table V-1

Number of Fish To Be Collected Per Geographic Stratum and Per Time Stratum

STRATUM	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
I					100				100			
II					100				100			
III			100		100		100		100		100	
IV			100		100		100		100		100	
V			100		100		100		100		100	
VI			100		100		100		100		100	
VII	100		100		100		100		100		100	
VIII	100		100		100		100		100		100	
IX	100				100		100				100	

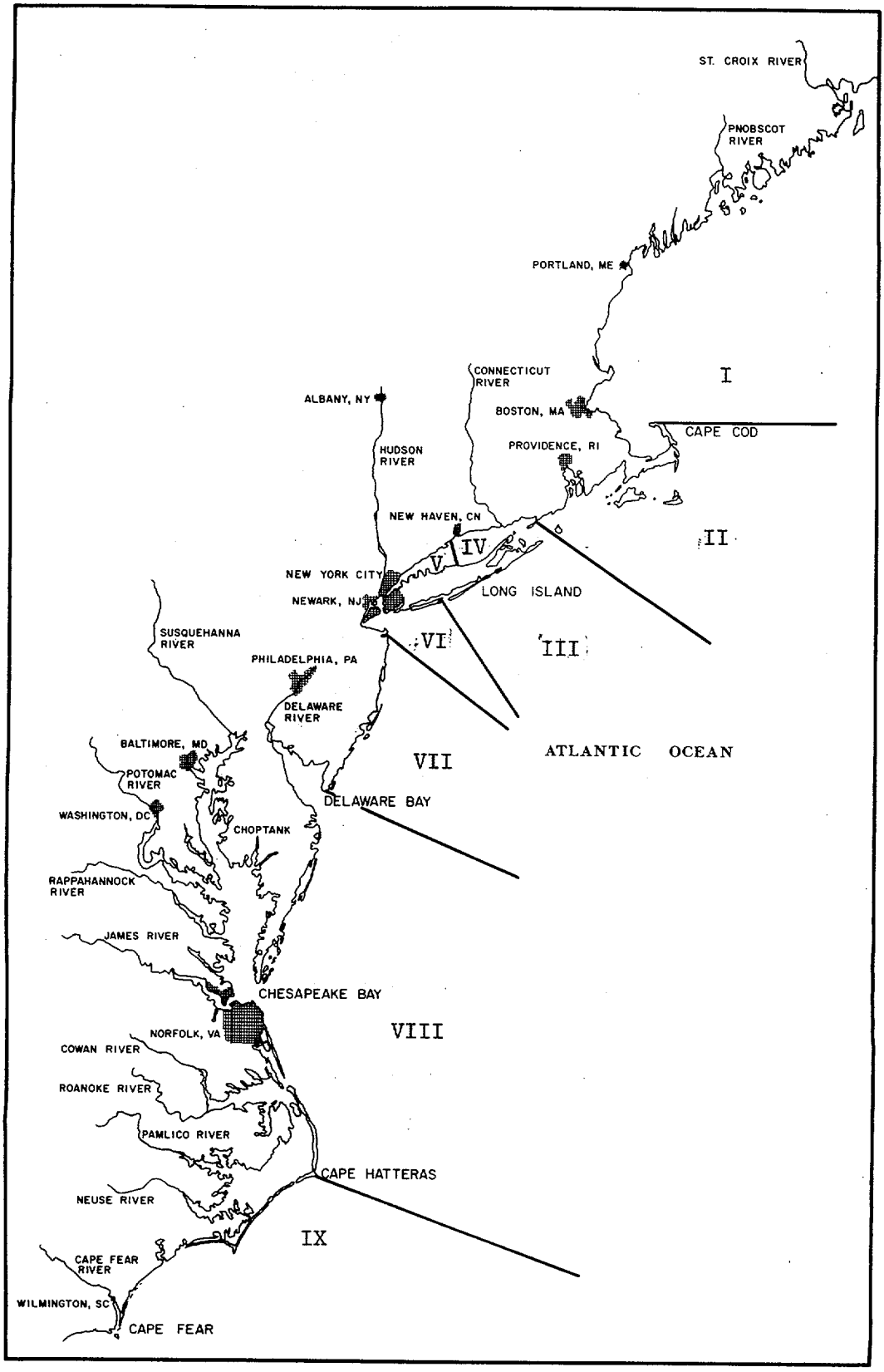

Figure V-1. Atlantic Sampling Strata

In addition to coastal sampling, some collections will be made from spawning areas. The populations of the Roanoke and Delaware Rivers represent potentially major contributors to the Atlantic fishery, and samples of the spawning populations will be collected to characterize those populations. Samples will be collected also from the spawning areas sampled in the current study to provide verification of the character sets.

Collections in the field will be obtained primarily by purchase of fish from sport and commercial fishermen. Supplementary collections will be provided by TI fishing efforts. Haul seines and gill nets will be used in areas where commercial fisheries do not exist.

Upon collection, all meristic and morphometric characters identified as having discriminatory power will be quantified in the field. Subsequently, the same analyses will be performed on a subsample of preserved fish. Scale samples will be taken for subsequent age and growth rate determinations in the laboratory, and a duplicate set of scales will be forwarded to the University of Rhode Island. Tissues samples will be collected, frozen, and forwarded to Dr. Powers at Johns Hopkins University for isozyme analyses.

The character values obtained from the samples collected during 1975 will be entered in a multivariate discriminant function. The results of the function will identify the fish, within the associated probabilities, as typical of the Hudson River population or the Chesapeake Bay population or as atypical of either. All atypical fish will be classified as having an "other" source of origin.

SECTION VI

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APPENDIX A

CORRELATION ANALYSIS RESULTS

Table A-1 (Contd)

LOCALITY	13-SNT/H	14-UJAW/F	15-UJAW/H	16-H/F	17-OP/F	18-OP/H
Rappahannock	.01099 (156)	-.39791 (156)	-.30694 (156)	-.21426 (156)	.40102 (156)	.50033 (156)
Potomac	-.06693 (202)	-.35304 (202)	-.39139 (202)	.06104 (202)	.52723 (202)	.43343 (202)
Choptank	.01366 (92)	-.38126 (92)	-.31288 (92)	-.12649 (92)	.33657 (92)	.33700 (92)
Elk	.13079 (250)	-.22254 (250)	-.23518 (250)	-.02266 (250)	.32014 (250)	.31169 (250)
Hudson	.23349 (192)	.06623 (192)	.00250 (192)	.08296 (192)	.08387 (192)	.01447 (192)
x^2	10.778	28.384	18.565	44.305	25.401	29.836
x^2 .95 (4 d.f.)	9.49	9.49	9.49	9.49	9.49	9.49
Average r[a]	NV	NV	NV	NV	NV	NV
Average r^2						
Percent variation attributable to length[b]	m5.45	m15.83	m15.32	m4.59	m27.80	m25.03

LOCALITY	19-ORB/F	20-ORB/H	21-INO/F	22-INO/H	23-NOS/F	24-NOS/H
Rappahannock	-.84533 (156)	-.84551 (156)	.64971 (156)	.69914 (156)	.07158 (156)	.15368 (156)
Potomac	-.93763 (202)	-.93304 (202)	.80519 (202)	.76958 (202)	.34512 (202)	.29886 (202)
Choptank	-.86556 (92)	-.87108 (92)	.77051 (92)	.73732 (92)	.00816 (92)	.07237 (92)
Elk	-.83422 (250)	-.82746 (250)	.62664 (250)	.62864 (250)	.04339 (250)	.05802 (250)
Hudson	-.75906 (192)	-.77679 (192)	.46259 (192)	.37121 (192)	.04607 (192)	-.00862 (192)
x^2	55.385	46.126	41.907	44.030	15.396	11.513
x^2 .95 (4 d.f.)	9.49	9.49	9.49	9.49	9.49	9.49
Average r[a]	NV	NV	NV	NV	NV	NV
Average r^2						
Percent variation attributable to length[b]	m87.92	m87.06	m64.83	m59.23	m11.91	m8.93

a. The letters NV designate that an average correlation coefficient is not valid.

b. The letter m designates that the value shown is the maximum variation attributable to length observed in any of the localities.

Table A-1 (Contd)

LOCALITY	25-PDOR/F	26-CAUL/F	27-CAUW/CAUL	28-CAUW/F	29-BDOR/F	30-SDOR/F
Rappahannock	.40958 (156)	.22369 (156)	-.45006 (156)	-.47829 (156)	.27158 (156)	-.56349 (154)
Potomac	.50958 (202)	.31989 (202)	-.61825 (202)	-.66535 (202)	.28729 (202)	-.74492 (198)
Choptank	.16608 (92)	.09170 (92)	-.26278 (92)	-.27546 (92)	.40475 (92)	-.75998 (92)
Elk	-.0505 (250)	.12975 (250)	-.23839 (250)	-.22789 (250)	.27485 (250)	-.53784 (249)
Hudson	.17144 (192)	.02187 (192)	-.19009 (192)	-.23957 (192)	.15342 (192)	-.63207 (191)
x^2	48.628	10.757	37.326	46.889	4.948	21.380
x^2 .95 (4 d.f.)	9.49	9.49	9.49	9.49	9.49	9.49
Average r[a]	NV	NV	NV	NV	.26550	NV
Average r^2					.07049	
Percent variation attributable to to length[b]	m25.97	m10.23	m38.22	m44.27	7.05	m57.76

LOCALITY	31-SDOR/BDOR	32-BANL/F	33-SANL/F	34-SANL/BANL	35-UGC	36-UGR
Rappahannock	-.57988 (154)	.15851 (156)	-.70016 (154)	-.68851 (154)	.23190 (146)	.16226 (146)
Potomac	-.76464 (198)	.09015 (202)	-.85247 (201)	-.85303 (201)	.15609 (189)	.02401 (189)
Choptank	-.78935 (92)	.20023 (92)	-.85290 (89)	-.87172 (89)	.29059 (82)	.33484 (82)
Elk	-.56593 (249)	-.00320 (250)	-.66689 (250)	-.64805 (250)	.01381 (245)	-.07827 (245)
Hudson	-.68021 (191)	-.18187 (192)	-.61966 (188)	-.55697 (188)	.10624 (167)	-.11463 (167)
x^2	23.940	14.887	43.908	61.029	7.367	16.807
x^2 .95 (4 d.f.)	9.49	9.49	9.49	9.49	9.49	9.49
Average r[a]	NV	NV	NV	NV	.13132	NV
Average r^2					.01724	
Percent variation attributable to length[b]	m62.31	m4.01	m72.74	m75.99	1.72	m11.21

a. The letters NV designate that an average correlation coefficient is not valid.

b. The letter m designates that the value shown is the maximum variation attributable to length observed in any of the localities.

Table A-1 (Contd)

LOCALITY	37-LGC	38-LGR	39-FA	40-SA	41- $\frac{SA}{FA}$
Rappahannock	-.04930 (156)	.00613 (156)	.11862 (152)	.46084 (152)	.32790 (152)
Potomac	-.29223 (201)	-.21385 (201)	.16310 (185)	.50421 (185)	.34368 (185)
Choptank	-.43053 (92)	-.25052 (92)	-.12408 (88)	.19126 (88)	.20050 (88)
Elk	-.15544 (250)	-.17369 (250)	.10319 (245)	.23899 (245)	.12828 (245)
Hudson	.09411 (192)	.04909 (192)	.00054 (187)	-.01800 (187)	-.04992 (187)
x^2	25.941	12.072	6.358	37.995	20.010
x^2 .95 (4 d.f.)	9.49	9.49	9.49	9.49	9.49
Average r[a]	NV	NV	.07382	NV	NV
Average r^2			.00545		
Percent variation attributable to length[b]	m18.54	m6.28	0.54	m25.42	m11.81

a. The letters NV designate that an average correlation coefficient is not valid.

b. The letter m designates that the value shown is the maximum variation attributable to length observed in any of the localities.

USE OF ISOZYMES TO IDENTIFY STRIPED BASS STOCKS

Final Report Prepared for
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475

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(Final Report Prepared for Texas Instruments Incorporated)

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INTRODUCTION

In order to survive, a population must adapt to its environment. Most population biologists agree that individuals possess genotypes and phenotypes (see glossary of terms) which permit survival. A population must be able to adapt or alter its genetic composition, at the expense of individuals, so that the species can persist. As a consequence, a population must produce and reproduce new genotypes and phenotypes. The extent of this latter phenomenon is a measure of the genetic flexibility or plasticity of the population.

Genetic plasticity is maintained differently in different kinds of organisms. Microorganisms have enormous, rapidly reproducing populations, where there are enough new mutations at any time to "preadapt" the species to many unique situations. Sexually reproducing organisms, however, have smaller populations and must rely on some innate genetic variance within the populations.

It is of value here to summarize the Darwinian concept of how evolution occurs by natural selection:

1. Organisms produce far more offspring than can ever survive to become reproducing individuals.

2. Because the numbers of individuals in species are for the most part constant, there must be a high death rate.

3. Individuals are not identical, but vary in their characterizatics.

4. Those variants that naturally fit their environments will be favored over those less fit. By inheritance those individuals better fit will pass their characteristics on to the next and future generations in greater

numbers than those less fit.

5. The following generations of individuals will exhibit, and continue to improve, the adaptations realized in gradual changes by their ancestors.

The Nature of Variation:

In nature two kinds of differences can be designated: variations among populations, races, or species; and variations among individual members of a population.

All sexually reproducing organisms vary. No two such individuals are exactly alike. A variety of factors contribute to the particular identity or specific measurements of a given individual. Individual variations result from non-genetic as well as genetic influences.

Before going further, it is useful to distinguish between genotype and phenotype in denoting individual traits or characteristics. Genotype refers to the sum or totality of the genetic attributes of an individual. Phenotype refers to the totality of characteristics which comprise the appearance of an individual; it results from an interaction between an individual's genotype and its internal as well as external environment. The two definitions are not natural opposites. Both genotype and phenotype are concerned in adaptation by natural selection, but only differences in genotypes are sources of heritable variations.

Sources of New Variation:

We have seen that variations in genotype associated with variations in phenotype provide the materials upon which natural selection operates immediately for adaptation and ultimately for evolution. All sources of variation arise from:

1. Mutation:

a. of genes

b. of chromosomes

2. Recombination:

a. of genes by crossing-over and segregation in meiosis.

b. of chromosomes by conjugation of gametes with unlike chromosomes.

True genetic variations arise only by mutation. Mutation is a random process which provides de novo changes in genes and in chromosomes. Recombination provides new individual variations within populations to a much greater degree than does mutation. Indeed most of the individual genetic variations observed within populations are of a recombinational origin. But these are new variations limited to a range set by a pre-existing genetic theme. New genetic themes arise only by mutation. To generalize, recombination provides ad hoc genetic variations of great importance in immediate adaptation and short-term evolution, whereas mutations provide de novo genetic variations of great importance in long-range, as well as short-term adaptation and evolution of populations.

Hardy-Weinberg Law:

In the study of Mendelian consequences in continuous populations, certain principles or laws have emerged. Perhaps the most famous is the Hardy-Weinberg Law of population equilibrium (see glossary for any unfamiliar terms).

In what follows we shall assume that there are only two alleles (A,a) at a particular locus. We shall suppose that there are N diploid individuals, of which D are dominant (AA), H are heterozygous (Aa), and R are recessive (aa), where $D + H + R = N$. To designate such a group of individuals, we shall subsequently adopt, for the sake of brevity, the following symbol:

$$(D,H,R),$$

where the three numbers are always understood to be in the order of AA, Aa, aa. Although there are three types of individuals in this group, there are only two kinds of genes: A and a. These N individuals have 2N genes altogether. Since each AA individual has two A genes, and each Aa individual has one A gene, the total number of A genes in this group is 2D + H. Therefore, the proportion of A genes in this group is

$$P = \frac{2D + H}{2N} = \frac{D + 1/2\ H}{N}$$

This proportion is known as the gene frequency of A in this group. Similarly, the frequency of the gene a in this group is $q = (H + 2R)/2N = (1/2\ H + R)/N$, so that $p + q = 1$. For example, in a group of 40 individuals: (2, 12, 26), $p = (2 + 6)/40 = 0.20$ and $q = (26 + 6)/40 = 0.80$.

Frequently the three genotypes are given in percentages instead of in actual numbers, especially when the group is large. Then we may let $D + H + R = 1$, where D is now the proportion of AA individuals in this group, etc. Hence, $p = D + 1/2\ H$ and $q = 1/2\ H + R$. Adopting this notation, our previous population (2, 12, 26) takes the form (.05, .30, .65), in which $p = 0.15 + 0.65 = 0.80$, as before. In subsequent pages we shall deal with large populations and use D, H, R to denote the proportions of the three genotypes in the population.

Many organisms in nature, both animals and plants, seem to reproduce at random or nearly so. In the case of bisexual organisms, the formal definition of random mating is that any one individual of one sex is equally likely to mate with any individual of the opposite sex. In other words, the frequency of a particular mating is dictated by chance. It should be emphasized however, that the theoretical frequencies will be realized only in very large populations.

The term "panmixia" is often used as a synonym of random mating and the population is said to be panmictic.

Let us consider a large panmictic population with p of A and q of a, where $p + q = 1$. The proportions of the three genotypes with respect to this pair of genes in the population are

$$D = p^2, \quad H = 2pq, \quad R = q^2$$

this follows from the fact that the probability of bringing two A gametes together at fertilization (to make an AA individual) is simply $p \times p = p^2$. Similarly the probability of making an aa individual is q^2. Examination of the diagram below reveals that the probability of the heterozygote, Aa, is 2pq.

		EGGS	
		p (A)	q (a)
SPERM	p (A)	p^2 (AA)	pq (Aa)
	q (a)	pq (Aa)	q^2 (aa)

Summing up the entries in the matrix, and recalling that the total must equal unity, one obtains

$$p^2_{AA} + 2pq_{Aa} + q^2_{aa} = 1$$

or

$$(p_A + q_A)^2 = p^2_{AA} + 2pq_{Aa}\ 2\ q^2_{aa} = 1$$

since $p + q = 1$ and $1^2 = 1$.

If the genotype proportions in the next generation are the same as those of the preceding generation, then the population (p^2, $2pq$, q^2) is then said to be in equilibrium under the system of random mating. By "equilibrium" we mean that there is no change in genotypic proportions in a population from generation to generation. This implies no changes in gene frequencies either. There are many possible types of equilibrium conditions. The particular equilibrium condition under random mating is known as the Hardy-Weinberg law because it was discovered independently by Hardy and by Weinberg in the same year, 1908. It follows that in all subsequent generations the genetic composition of such a population will remain the same provided that there are no disturbing forces at work. This equilibrium is due to the symmetry of the Mendelian mechanism for bisexual reproduction.

As a numerical example, consider a large random-mating population (.04, .32, .64) in which $p = 0.2$ and $q = 0.8$. The following shows that the proportions of the three genotypes in the next generation do indeed remain the same as those of the parent-generation.

Matings and Offspring in an Equilibrium Random-Mating Population

Type of Mating	Frequency of Mating	Offspring AA	Offspring Aa	Offspring aa
AA x AA	p^4	p^4	---	---
AA x Aa	$4p^3q$	$2p^3q$	$2p^3q$	---
Aa x Aa	$4p^2q^2$	---	$2p^2q^2$	p^2q^2
AA x aa	$2p^2q^2$	---	$2p^2q^2$	---
Aa x aa	$4pq^3$	---	$2pq^3$	$2pq^3$
aa x aa	q^4	---		q^4
Total...	1.00	p^2	$2pq$	q^2

Note that $p^2 + 2pq + q^2 = (p + q)^2 = 1.00$.

The Assumptions of Hardy-Weinberg Equilibrium are:

1) Random mating (panmictic)
2) Genetically static (no mutations or selection)
3) Isolated (no migration)
4) All individuals reproduce at the same time
5) No repetitive matings
6) Infinitely large populations

Thus, a characteristic of ideal panmictic Mendelian systems is that no matter what the starting frequencies for the diploid genotypes, they will conform to the binomial distribution (p^2, $2pq$, q^2) in the next generation and in each generation thereafter.

Example: The organic compound phenylthiocarbamide (PTC) tastes very bitter to most people. The inability to tast PTC is controlled by a single recessive gene. In the American white population, about 70 percent can taste PTC, whereas 30 percent cannot. Estimate the frequencies of the taster (T) and nontaster (t) genes in this population as well as the frequencies of the diploid genotypes.

Answer: The necessary information which is given is that q^2, the frequency of tt persons, is 0.30. The square root of 0.30 is $q = 0.55$, which is the estimated frequency of the t allele. The frequency of the T allele can then be estimated as $p = 1 - q = 0.45$. The frequency of the homozygous tasters (TT) is $p^2 = 0.20$ and the frequency of the heterozygote tasters (Tt) is $2pq = 0.50$. Furthermore, the frequencies p and q will not change from generation to generation if the population is ideal.

Heterozygosity H, hP:

The concept of heterozygosity can be a difficult one to follow since

a variety of symbols are often used to define the same idea and it is not always clear whether an author speaks of heterozygosity for a species as a whole or for a single trait. "h", heterozygosity for a single locus is determined by measuring the gene frequencies for a locus in a population. For example, if a normal random mated diploid population were sampled and examined for a protein which possessed two alleles, then it might be the case that 400 are homozygous dominate (A,A), 900 homozygous recessive (a,a) and 1,200 heterozygous (A,a). "h" would be calculated as $\frac{\#\text{ of het.}}{\text{Total}} = \frac{1200}{1200 + 900 + 400} = .48$. This refers to a single trait only. If next one were to repeat this for many loci, different values for various traits would be obtained, including zero for some. The average of all these "h's" is called "H", the average heterozygosity/locus. If a value of H = .20 were obtained, which is moderately high, then this would be interpreted-- one out of every five loci looked at in a "perfectly random" individual of the population would be heterozygous. Two individuals probably would not have the same set of heterozygous loci; however, for each of them, 20% of his loci would be heterozygous even though 30% of his loci might potentially be polymorphic.* This 30% or .30 is called "P", the proportion of loci which are polymorphic in the population and is equal to $\frac{\#\text{ of polymorphic loci}}{\#\text{ of loci tested}}$.

P,H, and h are all measures of genetic variation in a population and are determined by sampling various loci (usually enzyme loci). An important assumption in dealing with these values is that the loci sampled are representative of the organism as a whole. There will always be many more loci not sampled than sampled, and the types of loci tested should be taken into account in reading the literature. The many esterases, for example,

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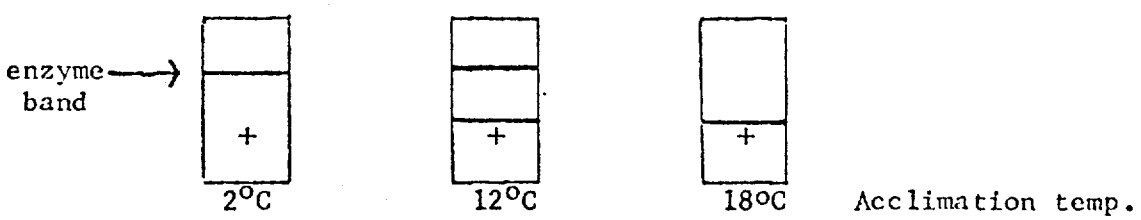
* see glossary for definition

are highly polymorphic. A study in which esterases constituted the bulk of the loci examined (they are easy to stain for) would yield biased estimates of P and H not accurate for the species as a whole.

Environmentally Induced Variation: Non-genetic Variation

Phenotypic change without genetic variation is a mechanism by which many organisms can compensate for environmental change. There are several means by which this can be accomplished and only a few of them will be discussed here. Three mechanisms under consideration to illustrate this non-genetic type variation are: 1) seasonal isozyme synthesis, 2) complex isozyme system, 3) variation of low molecular weight cofactors in response to an environmental change such as temperature.

Seasonal isozyme synthesis is known to occur in rainbow trout brain acetylcholinesterases (ACHE). If these fish are acclimated to 4° C, a "cold" variant is exclusively produced in the cells of these fish. At 18° C a "warm" variant is produced exclusively. These two isozymes appear to be distinct proteins produced in response to surrounding water temperature, each having their own temperature characteristics. At 12° C, both species are formed as evidenced by the electrophoretic data below.

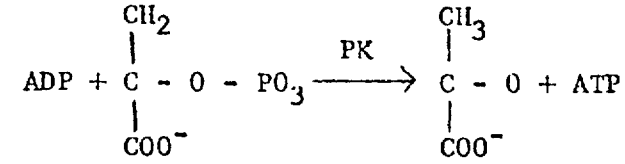


Data from Baldwin and Hochacka (1970), Biochemical Journal 116: 993-887.

Rather than turning the synthesis of an isozyme on or off, optimal isocitrate dehydrogenase (IDH) activity in some rainbow trout is maintained

by 3 simultaneously occurring isozymes. The precise characteristics of the reaction catalyzed by IDH depend on the ratio of the isozymes present. Individuals with these isozymes have a possible advantage over those with only a single IDH isozyme in that the former will maintain a more stable rate of IDH activity than those with a single isozyme. It is possible that single isozyme organisms have alternative regulatory mechanisms.

The process of genetic change occurs over generations. Even the induction of a genetically present but phenotypically absent variant is time consuming, occurring in weeks. In response to temperature change, rapid conformational change of an enzyme species to produce new phenotype is known to occur for certain enzymes. An example of this is pyruvate kinase (PK) in Alaskan King Crab leg muscle. In terms of function, this PK is similar to the "warm" and "cold" trout ACHE's mentioned previously, but in this case a single enzyme is involved. The three-dimensional conformation of the enzyme depends on the temperature, thus avoiding the processes of gene activation, transcription, translation, etc. One might ask whether this really represents a change in phenotype. PK catalyzes the reaction shown below



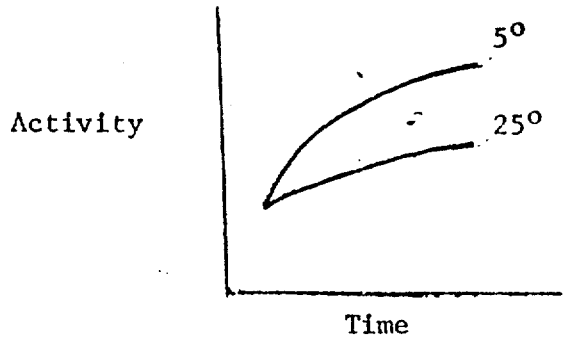
phosphoenolpyruvate (PEP)

pyruvate

The differences in phenotype are reflected by distinct differences in reaction velocity and enzyme-substrate affinity for the two conformers

of PK when measured at the same temperature.

The environment in which an enzyme resides can play a major role in modulating its activity. pH, ionic strength, and the presence or absence of various low molecular weight modifiers can all affect changes in activity. Many enzymes which are involved in oxidation reactions occurring in the mitochondria are membrane bound. One of these, succinic dehydrogenase, contains a lipid moiety necessary for enzymatic activity. In studies done by Hazel (1972), goldfish were acclimated to 5° and 25° and enzyme activity was measured. Figure (1) shows the results:



Significant differences in SDH activity were found.

The SDH's were then separated into their protein and lipid moieties and analyzed. For each enzyme, the protein components were "identical" as measured by electrophoresis. The enzyme was reconstituted by adding purified lipid fractions from the 5° and 25° acclimated fish.

Purified lipid fractions from the 5° fish caused greater activation than those from the 25° fish. The conclusion taken from these experiments was that SDH activity was dependent upon the specific lipid environment to which it found itself associated. Further studies indicated that this

activity was directly proportional to the degree of unsaturation of the lipid moiety, i.e., the more double bands found in the lipid component the greater the activity. Hazel, (1972); Comp. Biochem. and Physiol.; 43B: 837-861, 863-882.

Another example of environmental interaction with genotypes is the height differences among immigrants to North America. Hulse, (1968) investigated the difference between Italian-Swiss born in California and in Switzerland. He found almost a 4 cm difference between the two groups (California born were taller) with a standard error small enough to make this 4 cm difference significant. Oddly enough, no significant difference in height existed between immigrants and their brothers who stayed in Italy discounting a possible artifactual relation between height and migration tendency. The possible environmental factors involved include better food, decreased occurrence of disease, and decreased progeny size. Other factors currently not being considered might play a role in the observed difference (increase stature due to heterosis caused by migration).

These mechanisms illustrate how the environment, in these cases, temperature and nutrition, can affect the phenotypic, i.e., the physical expression of the genetic information. The next section will discuss a more central issue; the variation of genetic material.

Genotype vs. Phenotype:

Genotype, according to Mayr, is the totality of genetic factors that make up the genetic constitution of an individual. One can consider genotype to be the blueprint upon which the organism is built. It can also be used to refer to a single trait. The genotype refers to the precise

sequence of DNA bases which make up the heritable material of the organism, i.e., the gene. Any change in this sequence either by mutation or recombination changes the genotype, but does not necessarily change the physical manifestation of the information which is known as the phenotype.

As an aid to understanding the distinction between genotype and phenotype, note that the word phenotype is related to the Latin work "phaenomenon" which means appearance. But phenotype means more than just appearance such as blue eyes or brown hair. It refers to growth rates, enzyme velocities, fecundities, etc. It can also be thought of as the result of an interaction between genotype and the environment, the actualization of genetic potential.

Many people underestimate the importance of phenotypic considerations. Dr. Richard C. Lewontin illustrates this point by commenting that, "population geneticists, in their enthusiasm to deal with changes, have often forgotten that what are ultimately to be explained are the myriad and subtle changes in size, shape, behavior, and interaction with other species that constitute the real stuff of evolution."

Parameters Affecting Genetic Variation:

Since not all mutations that occur are acquired by a given species, there must be some sort of guide lines that influence the assimilation of a new mutation into the population. J. Valentine (in Ayala, 1976) has suggested such parameters to include: age of environment, physical environmental stability, spatial heterogeneity of the environment, trophic resource levels and trophic stability.

The age of the environment is an obvious consideration when examining the diversity of life within a region. New environmental regimes caused by say, natural catastrophe would naturally be expected to support less

diverse forms. It is impossible to specify the exact period of time necessary to produce genetic diversity, but the time scale is on the order of hundreds of millions of years between species to hundred of thousands of years between enzyme variants. Time is not the sole factor operating--for example, the deep sea environment has been compared with that of the high latitudes in cooling characteristics and age. In the high latitudes there is very little diversity yet, in the deep sea communities are extremely diverse.

A common sense approach would demand that physical environmental stability would play a role in determining the diversity of species. The theory is that the more the variability of the environment in terms of temperature, frost, salinity, etc., the greater the stress that is placed on an organism. Consequently fewer species should be able to adapt to demands of the environment. Unfortunately, observations do not fit the theoretical prediction. Comparison of the very stable marine environments of both high and tropic latitudes with variable climates of Temperate Zone waters show that the amount of diversity does not correlate well with the physical stability of environment.

Food supply or trophic resource level is often implicated in the maintainance of population diversity. It is easy to see the difference between a tropical forest as opposed to the barren Mojave desert. However, in the oceans the correlation breaks down completely. Again deep sea communities do not lack diversity despite a low supply of trophic (nutritional) resources. A more favorable correlation exists between the stability of the resources and the diversity present. Stability of the food supply rather than the absolute level would permit the continued existence of species

regardless of diet and habitat restrictions. Small population size might also result. The best adaptive strategy would be for an organism to eat a large variety of food types or be able to exist in a large variety of habitats in the event of low trophic resource region.

The conclusion from these parameters is that genetic diversity should be the greatest in a region of high spatial heterogeneity and greatest trophic stability. Such conditions are found, for example, in coral reefs, and indeed the diversity of life found there is great.

Sources of New Genetic Variation:

Ultimately all new genetic variation arises from mutation i.e., changes in the base sequence of the DNA. Estimates of mutation rates vary. King and Jukes (1969) in the journal Science (164: 788-798), estimate 1.6×10^{-9} mutation per codon per year. Assuming 3 generations per year and a length of 130 codons for a protypical polypeptide, Lewontin (1974); page 222, calculates a substitution rate per locus of 6.4×10^{-7} per year. Studies by Mukai; (1970) and Tobani and Kojama, (1972) seem to substantiate this as at least a reasonable estimate for mutation rate. Essential questions of population genetics that must be answered are:

1. Which mutations are fixed into a population and why?
2. How many mutations and of what types must be accumulated for speciation to occur?

Recombination, chromosomal inversion, and translocation may be thought of as pseudo-genetic variation. New combinations of genetic information via recombination might produce new phenotypic expressions but do so only within a previously existing framework. Most genomic variation occurs by this route.

Translocation, inversion and even polyploidy can produce at least phenotypic change. The most striking example of this is Down's Syndrome or trisome 21 (referring to existence of 3 #21 chromosomes). The genetic information is identical to that of a normal person with the exception that the afflicted individual has some extra repetitive information.

Natural Selection:

Darwin thought that one of the driving forces behind natural selection was the tendency for living organisms to multiply if left unrestrained. For an example of this idea, it has been shown that a pair of house flies would leave behind 191,000,000,000,000,000,000 descendants in 5 months if all lived to breeding age. On the other end of the spectrum one finds the elephant. Assuming that it begins breeding when thirty years old, and goes on breeding until ninety years old, bringing forth 6 young in the interval, and surviving until one hundred years old; if this be so, after a period of 740-750 years there would be nearly nineteen million elephants alive descended from the first pair.

But it is not the case the case that we find houses and buildings overridden with flies or forests overflowing with animal life. This is because for each species of organisms, constraints exist which prevent unchecked growth. The most important of these constraints is a limited food supply. From this factor alone, one can easily see the rationale for Darwin's "struggle for existence". Another limiting factor is the existence of predatory animals. Disease is yet another reason why unrestricted growth does not occur. Epidemics can occur among animals. A less obvious check on unlimited growth is space restrictions. Over-crowding promotes disease and starvation. Furthermore, it is known that animals need room

to roam so that their reproductive habits may be satisfied thus allowing them to breed normally. This is why many animals in captivity do not produce young.

Finally, the environment itself plays a major role in limiting the size of the population. Severe heat, cold, flooding, etc., all take their toll on living organisms. Even man with all of his technology cannot always protect himself from the forces of nature.

Selection as Darwin originally envisioned it, is the struggle by organisms to overcome these forces and to reproduce.

a. Stabilizing Selection

The term natural selection can be used to encompass a variety of selective forces. With reference to a given trait, there are 3 ways in which selection acts: stabilizing, directional, and disruptive.

Stabilizing selection serves to cut down variation by removing from the population those phenotypes, and thus those genes, which stray too far from the population norm. Its effects can be seen in the evolution of hemoglobin (Ayala, 1976). Phylogenetic trees have been constructed describing the evolution of globin amino acid sequences. Evolution, as measured by nucleotide replacements, proceeded initially at a rapid rate (46 nucleotide replacement per 100 codons per 10^8 years = 46NR%). However, for the next 300 million years the rate decreased to about 15NR%. The early evolution of vertebrate hemoglobins included separation by gene duplication from myoglobin followed by the H and B hemoglobin lines which also arose by gene duplication. Evolution of hemoglobin has slowed considerably in late mammalian development. Selectionists argue that the slow-down of hemoglobin evolution is due to positive natural (directional)

selection . Is there any logical basis for these statements? In terms of environmental demand, the answer is yes. The organisms of say, the pre-Cambrian era possessing monomeric, non-Bohr effect hemoglobin (opposed to our heterotetrameric pH-sensitive form) exhibited hyperbolic kinetics with a strong oxygen affinity which probably satisfied their O_2 needs. The demands of these small, creeping animals were less than those of the larger and much more mobile organisms. Positive natural selection would then favor new forms of hemoglobin that would better meet the needs of the new animals. This would account for the rapid development of the Hb molecule. The ensuing slow down in evolutionary rate caused by stabilizing selection is the result of successful development of the molecule. Fewer and fewer subsequent mutation in the Hb molecules could improve upon the existing structure. Many mutations along the line were obviously deleterious and were eliminated continuously throughout the process.

Another, (Mayr, 175) perhaps more straight-forward, example of stabilizing selection, is found among snake and lizard populations. Samples taken from adult and juvenile populations show that in many cases the variability in the number of scales is far lower in adult than in the juvenile. Since these creatures do not possess the ability to change the number of scales on their body, one must presume that it is some selective force (stabilizing) affecting the number of their scales which acts against younger members of the population. This results in a higher mortality rate in the younger individuals with unfavorable scale numbers.

b. Directional Selection

In contrast to stabilizing selection, directional selection does not change the variance of the gene frequencies in a population. It is the averages of the

phenotypic distribution which are affected. Directional selection has been utilized for centuries by breeders for a variety of purposes: faster horses, fatter swine, sweeter roses, etc. Often this was accomplished by purposely mating the male and female of the species best displaying the desired trait. Extensive artificial selection has been demonstrated in Drosophila (Lewinton 1974; pg. 91) for the following traits: size, bristle number, developmental rate, fecundity, egg size, behavior, bristle pattern, resistance to DDT, and ability to reproduce parthenogenetically as well as several others. However, natural stabilizing selection often results in poor viability of these animals by selecting against extreme phenotypes. The idea of artificial directional selection (it wasn't called that) was known to Darwin and in his concern for natural populations, he queried. "How was selection carried on in nature with no breeder to pick and chose and no record book?"

One of the best known cases of directional selection occurring in a natural population is the fate of the peppered moth, Biston betularia, and its adaptation to the "catastrophic" effect of industrialization of Manchester, England around the mid 1800's . About this time a second form of the moth was discovered. Instead of being greyish with irregular dark markings, it was all black and is known as the melanic form. This moth was named B. betularia carbonaria and was estimated to compose less than 1% of the B. betularia population. By 1900 carbonaria population was estimated at 90%. In short, within 50 or so years, severe directional selection had taken place. It is now known that the cause of this phenomenon was the fact that the hiding place of the grey peppered moth, greyish green lichen,was polluted by soot, a by product of industrialization. The light grey moth was an easy target for birds against the now darkened lichen which before provided camouflage for the light colored moth. It was then the black moth that profited from lichen camouflage.

Directional selection served to reduce selective forces against one form and increase them against the other. These facts were substantiated by researchers in the 1950's who brought black and grey moths to polluted and unpolluted environments and recorded the results on film in an attempt to recreate the selective processes.

c. Disruptive Selection

Disruptive or diversifying selection is a process by which two or more phenotypes are selected for simultaneously. This form of selection would occur most often in an environment which possesses multiple habitats. This form of selection might also appear as a possible mechanism for sympatic (see glossary) speciation or subspeciation. Clearly if niches were to become isolated this would be plausible.

Disruptive selection can take place artificially. Both high and low bristle number in Drosophila have been selected for concurrently, producing a biomodal distribution in a short period of time. However, the price of this selective event was reduced viability, presumably through stabilizing selection. Here again we have no one selective force acting without consequences to the others.

On diversity selection Theodosius Dobzhansky (1961), a famous population geneticist, once wrote "Instead of one perfect genotype, diversifying selection favors many genotypes; it favors genetic polymorphism. A population which abounds in genetic variety has a better grip on a complex environment than a genetically uniform population; polymorphism is one way of exploiting the environment more fully."

Genetic Drift:

Genetic drift is the alteration of gene frequencies through sampling

error. It operates to some degree in all finite populations, but can probably be significant as an evolutionary force only in populations that are relatively very small. To gain an immediate intuitive understanding of what sampling error means, consider the following simple experiment in probability theory. Suppose that we were asked to take a random sample of 10 marbles from a very large bag containing exactly half black and half white marbles. Despite the 1:1 ratio in the bag, we would not expect to draw exactly five white and five black marbles each time. In fact, we know from the binomial probability distribution that the probability of obtaining a perfect ratio is

$$\frac{10!}{5!5!}\ \frac{1}{2}\ \ 10 = 0.\dot{2}46$$

On the other hand, there is a small probability $[2(1/2)^{10} = 0.002]$ of drawing a sample of either all white or all black. This situation is analogous to sampling in a small population.

In theory, three situations have been envisaged in which genetic drift might play an effective role in the evolution of small natural populations.

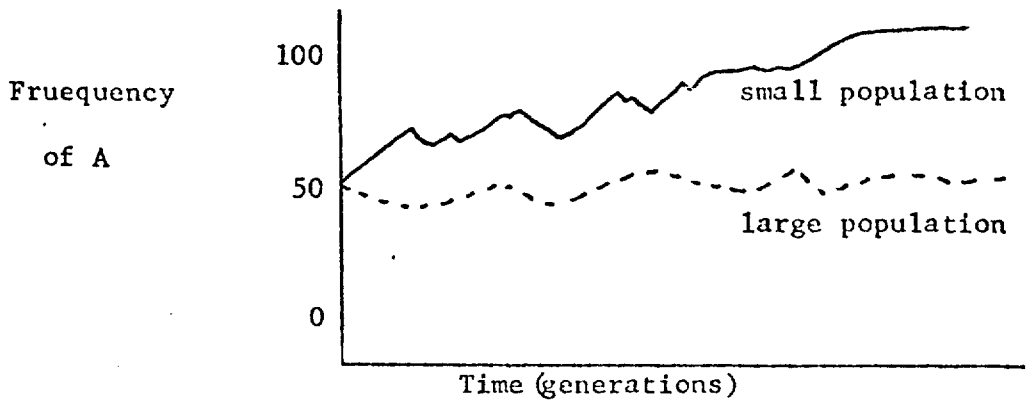
1. Continuous drift. The population remains small in size, and sampling error is effective each generation.

2. Intermittent drift. The population is occasionally reduced to a size small enough to allow drift to operate. Reduction can be effective in one or the other of two ways: (a) if mortality is random at the time of reduction, the sample of survivors can have a different genetic composition due to chance alone (the "bottleneck effect"); (b) if the populations remain small over at least two generations, the process of continuous

drift is then initiated.

3. The founder principle. New populations are often started by small numbers of the individuals, which carry only a fraction of the genetic variability of the parental population and hence differe from it. If chance operates in the selection of the founder individuals (and it almost certainly does to some extent), new populations will tend to differ from the parent population and from each other. The founder principle (or founder effect, as it has also been called) is of potential importance in the origin of species.

Computer simulation has been used to simulate hypothetical populations. Fig. (2) shows the result of two mythical populations and the effects of sampling on the frequency of a theoretical gene, A.



Taken from : Human Populations Genetic Variation and Evolution; L. N. Morris, p. 5443.

This is of course assuming that no selection is taking place on the small population, a factor which can be calculated for even in computer simulation. As shown in the figure, gene A is fixed (i.e., 100%) in the small population by the random processes of genetic drift.

Drift and Selection

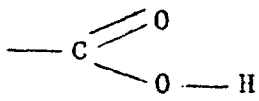
Evidence from natural selection tells us that deleterious phenotypes should be removed from the population. However, evidence from drift studies shows us that these can be maintained in the population. How can this dilemna be reconciled? In absolute terms it can't be, not yet anyway. This is still a growing science. Intricate relationships between animals, plants, and their environment are not well understood. The genetics of natural wild populations are not well characterized. Natural selection and genetic drift might be thought of as partners. Whether or not chance may be operative in determining gene frequencies in the early stages of evolution depends on the final fate of the gene as a contributor to evolution.

ELECTROPHORETIC TECHNIQUES

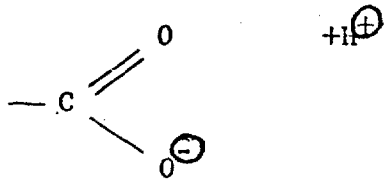
Principles of Starch Gel Electrophoresis:

Population biologists are often interested in separating and identifying allozymes of a particular locus since this allows a polymorphism present in a natural population to be studied in a quantitative manner. Starch gel electrophoresis is a powerful technique for the analytical separation of proteins and is widely used in biochemical population genetics.

The two basic physical properties of proteins which allows them to be separated by starch gel electrophoresis are their size (or shape) and their net electrical charge. The protein molecules are separated according to their size and shape as they pass in a 'sieving' process through the holes in the starch gel matrix. These holes, which are of many sizes, originate from the branching and intertwining of the carbohydrate chain of the gel. It is the net charges of the proteins, however, which are primarily responsible for their ability to separate from one another during electrophoresis. The net charge of a protein is determined by the various pK's of the dissociable groups on the side chains of the amino acids, which comprise the macromolecule. In a population of one type of protein molecule, the pK of a dissociable group on a certain amino acid residue is the pH at which 50% of those particular groups have a proton (H^+ ion) on them. Thus, if the group in question is acidic, such as a carboxyl group:



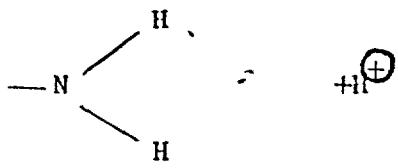
at pH = $pK_{carboxyl}$ the other 50% of these groups in the population have lost (dissociated) their proton. They will possess a net negative charge since the group becomes a carboxylate ion:



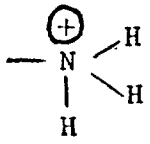
carboxylate ion
charge = - 1

proton (dissociated)
charge = ⊕ 1

If the side chain contains a basic group such as an amino group:



At pH = pK_{Amino} 50% of the groups will have gained a proton,



and thus increased their net charge by +1. Therefore, at pH = pK one-half of the particular groups are charged and the remaining half are electrically neutral. Whether the net charge is positive or negative depends upon whether the group in question is basic or acidic. Since there are many different amino acid side chains on the surface of a protein there will be a large number of such dissociations, involving many different pK's, taking place at a certain pH value. The algebraic sum of

negative plus positive charges on the protein determines the net charge at that pH. If these protein molecules are now placed within a starch gel and set in an electric field, they will move through the gel matrix, by virtue of their net charges, thus contributing to an electric current in this field.

The basic equipment for starch gel electrophoresis is illustrated in Figure 1. A starch gel is placed between two reservoirs containing buffer of desired pH and ions to carry the current. An electrode (one positive and one negative) is placed into each buffer reservoir and connected to a suitable power supply.[1] Current (ions) will flow from the "negative" reservoir through the gel to the "positive" reservoir. Proteins, carrying their share of the current, will move through the gel according to their size, shape and net charge. The result is that a mixture of proteins originally applied to the gel have been separated from one another due primarily to differences in net charge.

In choosing the electrolyte buffers, the best results are obtained at a pH on the alkaline side of the isoelectric point[2] of the proteins in the mixture. This will diminish adsorption of proteins to the negatively charged groups present in the starch gel matrix. The pH in the region of the gel in which separation occurs should also be such that the maximum difference in mobility (therefore net charge) exists between the different molecular species undergoing separation. The ionic strength and thus conductivity of the buffer must be as low as possible because

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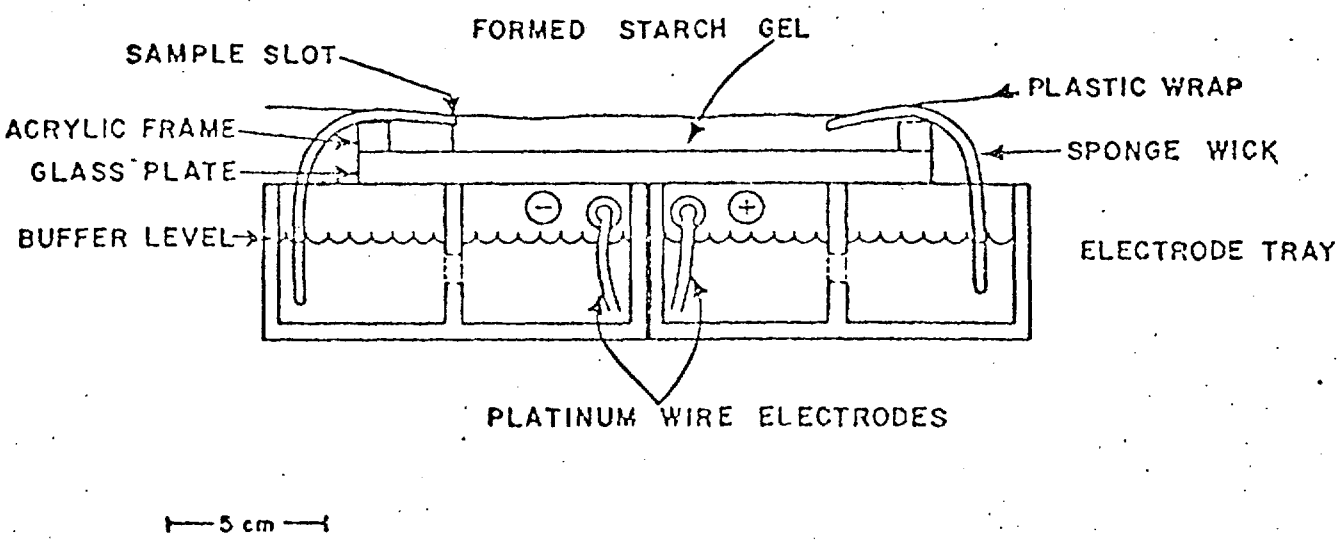
[1] Constant current power supplies are preferred as this avoids heating of the gel during electrophoresis.

[2] The pH at which the net charge on the protein is zero.

SIDE VIEW



TOP VIEW

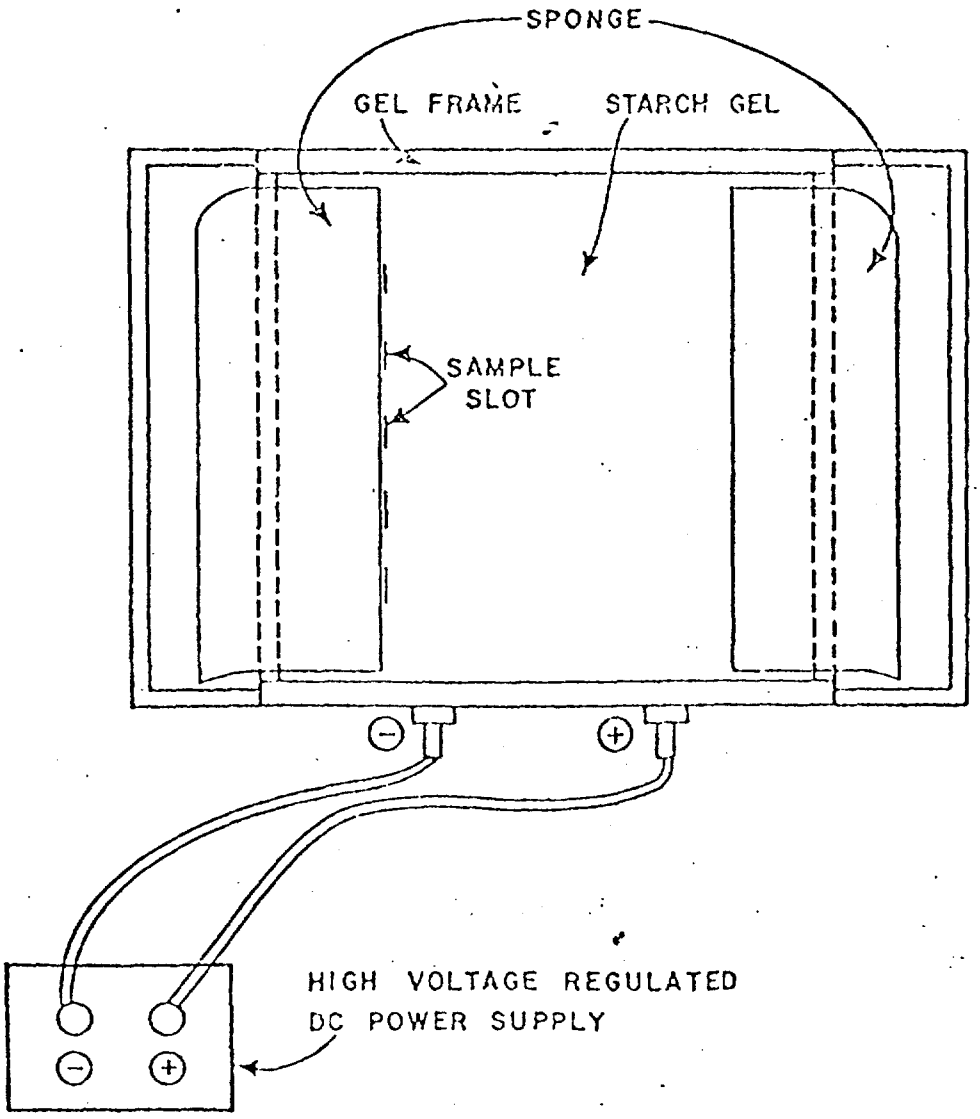


Figure 3 Apparatus for horizontal starch gel electrophoresis

at low conductivity, the protein carries a greater share of the current. Therefore, higher mobilities can be achieved with minimum production of heat. Although buffers of low conductivity have advantages due to the high potential gradients which can be employed and thus the rapidity of the separations which can be achieved, the degree to which the buffers can be diluted is strictly limited. This is because on dilution of the buffer in which the proteins are dissolved, the probability of molecular association increases. Also at dilute concentrations adsorption effects are a possibility. Therefore, the buffer should be chosen which minimizes the possibility of interactions between proteins (as already mentioned, this is less likely in stronger buffers). The chelating agent, ethylene diamine tetracetic acid (EDTA) is an example of a specific reagent used to minimize such problems. Finally, the buffer should be such that interaction with the proteins undergoing separation does not lead to formation of more than a single band after the gel is stained (see Methods). The addition of reducing agents (such as β-mercaptoethanol) to the gel often help alleviate this difficulty. These agents prevent the appearance of oxidation products which will cause a single protein to migrate through the gel as more than one oxidation state of the same molecule. The addition of enzyme cofactors such as TPN or DPN will often prevent multiple band formation from a single protein species. The reason for this will be explained later.

Most starch gel electrophoresis methods employ a discontinuous buffer system whereby the buffer present in the gel differs from that in the electrode reservoirs. The effect of the discontinuous buffer is that the proteins commence migration in the gel buffer, but complete

migration in the electrode buffer due to the fact that the electrode buffer ions have a higher migration than proteins in the gel and so overrun and pass them. The value of this procedure is that there is a voltage discontinuity at the buffer interface which results in a sharpening of the rear side of each protein band (i.e. increased resolution of the proteins).

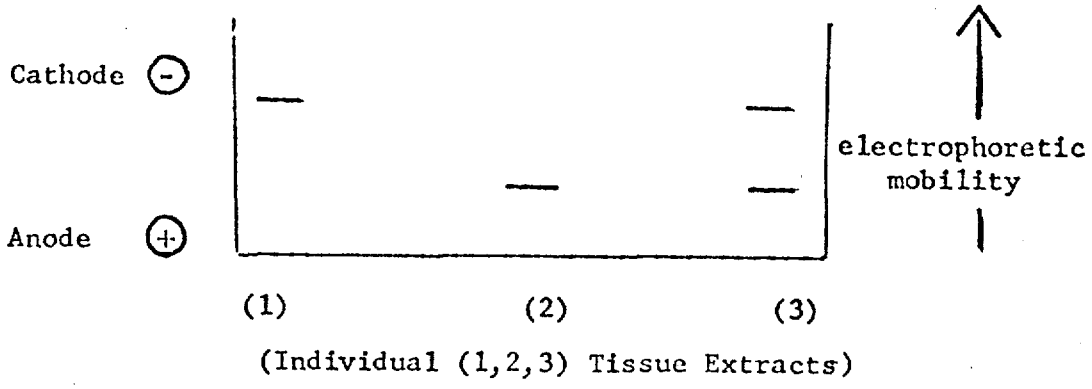
Applications of Starch Gel Electrophoresis

Starch gel electrophoresis has wide applications in the biochemical analysis of genetic variation in fish populations. The tremendous advantage of the technique is that one can use small amounts of a crude extract from any tissue of the animal and stain specifically for certain proteins following electrophoresis (as explained in Methods). This removes the costly and time-consuming burdens of enzyme purification.

Variants of a given enzyme[3] fall into two broad classes; (1) isozymes, or variants of the same enzyme which are genetically coded for by different genes or loci; (2) allozymes, or variations of the same enzyme arising through genetic alteration (mutation) at the same gene. Since different isozymes are often expressed in different tissues, starch gel electrophoresis affords a convenient method of identifying such variants. Several different tissues (eye, liver, heart, muscle, etc.) are excised from the animal and, after extraction of the enzymes, the tissue samples are electrophoresed. If the isozymes differ from each other in net charge, they will be easily identified after histochemical staining and categorized as to their expression, or appearance, in a particular tissue. The most common application of starch

[3] An enzyme is a protein which acts as a specific catalyst for the many chemical reactions of metabolism which are necessary for the life of the cell.

gel electrophoresis to the study of fish populations is, however, the separation and identification of allozymes. If the allozymes differ from each other in net charge they will be easily distinguishable on starch gel electropherograms. One can quickly determine if a given enzyme system is polymorphic, i.e. possesses genetic variants. This variation can then be quanitifed since one can readily determine the percentage of homozygotes for each allele and the percentage of heterozygotes in a sampled population of fish. The gene frequency for each allele is then easily calculated. Perhaps this can be best explained with the following example: Suppose we sample 100 individuals of a certain fish species. Electrophoresis is performed on the liver extracts from each fish. After specific histochemical staining of the electrophoresed gel, we discover that a certain enzyme present in the liver of these fish is polymorphic. Any particular fish will have a staining pattern on a starch gel which could be one of the following three possibilities (i.e., for a monomeric enzyme (see glossary):



(Individual (1,2,3) Tissue Extracts)

Individuals who stain as (1) are arbitrarily called homozygous AA (phenotype AA); those who stain as in (2) are homozygous aa (phenotype aa).

Individuals who stain as in (3) are heterozygotes (phenotype Aa). Note that the heterozygote Aa, which has a copy of both genes A and a, possess each enzyme since the enzymes are direct products of the genes.

In the theoretical 100 individuals examined, 30 are AA, 50 are Aa, and 20 are aa. The gene frequency of A is then:

$$P(A) = \frac{30 + 25}{100} = .55$$

and that of a is:

$$P(a) = \frac{20 + 25}{100} = .45$$

Note that $P(A) + P(a) = 1$ as it must.

As soon as genetic variation is quantified, as in the above example, one can investigate the behavior of the genes involved in fish populations from different environments. For example, are fish from warmer waters enriched for phenotype aa? These sorts of studies provide anwers to the question of whether some fish populations are better adapted or 'genetically armed' for one environment vs. another. For example, fish with the aa phenotype might be better able to survive in warmer water than fish from a cooler environment possessing the AA phenotype. Heterozygotes may do well in warm and cold water since they have both enzymes present in their liver cells (e.g., see Powers and Powers, 1975).

Electrophoresis is believed useful in identifying innate biochemical tags in fish. Suppose, for example, that fish from one spawning area are 'fixed' for gene A in the preceding example (i.e. 100% of all fish examined

are phenotype AA). Fish from other spawning areas, on the other hand, are fixed for other allozymes (a, B, b, etc.). Fish could then be identified as to their home source simply by examining starch gel electropherograms of liver extracts from these animals. This is of great potential use in fisheries managment since migrating fish could be easily identified as to home spawning ground on the basis of their innate genetic differences. This latter approach is particularly useful in differentiating between fish of closely related species like striped bass and white perch (Sidell, Otto and Powers, 1977).

Working Assumptions for the Interpretation of electrophoretic data.

There are a number of inherent assumptions one must be aware of in interpreting data obtained from starch gel electrophoresis. Population geneticists are often interested in knowing the amount of genetic variation, or percent heterozygosity, present in natural populations. If a number of enzyme systems are examined and a certain percentage of those systems are found to be polymorphic (i.e. possessing genetic variants), this degree of polymorphism can be quantitatively expressed as percent of loci examined which were polymorphic. These estimates are usually based on no greater than twenty enzyme systems. A basic assumption in using this calculation is that the loci which were investigated are a fair representation of the entire genome, or collection of genes, of the individual. There are many other proteins besides these 20-odd enzymes present in the cell. The loci which code for these proteins may not be genetically variable or invariable as those enzyme loci which were examined. The estimate of percent polymorphism, or genetic variability, of the entire genome of the organism may thus be incorrect. Another source of error in this regard is the assumption that a single band on a gel corresponds to a single enzyme. A genetic variant of a given enzyme (allozyme)

results from a(n) amino acid substitution(s). If this change in amino acid composition is electrically neutral, i.e. does not result in a net charge which differs from the original protein, it will not be distinguishable by electrophoresis. In this case, a single band would be misinterpreted to indicate monomorphism, or lack of variation. Such a error would also effect the estimate of percent polymorphism.

As was alluded to previously, a protein may generate multiple bands on starch gels which originate from a variety of sources (see below also). Thus, the appearance of more than one band does not necessarily imply the existence of a true genetic variant. This problem can be overcome by performing genetic crosses, or mating experiments to determine if the alleles in question are inherited by the offspring in a manner which is consistent with Mendel's classical genetic laws of independent assortment. If so, the presence of a true polymorphism is indicated. These genetic crosses also allow one to distinguish allozymes from isozymes.

Limitations of Starch Gel Electrophoresis

The primary limitation of starch gel electrophoresis, as with most biochemical methods which study enzymes, is instability of the enzyme molecules. The protein may be damaged or modified in a variety of ways which may occur either during sample storage or preparation.

Storing the sample may result in the appearance of artifact bands on the gel. These bands may be caused by a number of processes such as aggregation of the protein molecules, etc. This problem can usually be avoided by storing tissue samples under liquid nitrogen (-180° C). In our experience this storage method has maintained enzymes stable for periods exceeding two years.

The amount of time between removal of the tissue from the live fish and storage is also critical. Proteases[4] may begin to act quickly on the enzymes soon after tissue excision. This leads to a loss of activity of the enzyme thus making it impossible to visualize the enzyme on the gel with the histochemical staining procedure.

Proteases are also a problem during preparation of the sample prior to electrophoresis since they are extracted during this procedure along with the enzymes of interest. Oxidation products of an enzyme may also be generated at this step. As was mentioned before, addition of reducing agents to the extraction buffer help alleviate this problem, but does not always eliminate it.

The presence of low molecular weight cofactors on the enzyme may change the net charge on the protein. During sample preparation some enzyme molecules may have a cofactor molecule attached to them since these cofactors are also present in the cell. This will change the net charge on the protein thus conferring a different mobility to it than other identical protein molecules with no cofactor bound. Therefore, more than one band will appear for the enzyme when the gel is stained. Such cofactor modification can often be 'swamped out' by adding an excess of cofactor to the extraction buffer or to the gel.

The process of sonication (see METHODS) during sample preparation may also damage enzymes. It may disrupt them physically, cause them to aggregate, or remove lipids which are necessary for enzyme activity. If this occurs, homogenization of the tissue in buffer may be the preferred extraction procedure.

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[4] Proteases are enzymes which 'chew up' or digest other protein molecules.

MATERIALS AND METHODS FOR SAMPLE PREPARATION

Liver and white muscle tissues in liquid nitrogen were supplied by Texas Instruments. Individual livers and muscle samples were suspended in equal volumes of distilled water while kept on ice. Samples were subjected to ultrasonic disruption for 10 seconds using a Bronwill Biosonik III fitted with a microprobe and at an intensity setting of 30. The reducing agent β-mercaptoethanol was added to give a final concentration of one millimolar. These homogenates were then centrifuged at 10,000 xg for 15 minutes in a Sorvall RC2-B refrigerated centrifuge. The liver supernatant was then removed to separate it from the fat layer.

A. Preparation of Constituents

The total amount of constituents employed in making a starch gel varies from one apparatus to another. The plexiglas frame (15 x 15 cm) and glass trays employed each hold a total volume of approximately 200 ml. For most electrophoresis, gels of 11% starch concentration were used.

The instructions given below are for making two gels and will be stated in the present tense.

B. Preparation of Gel

After selecting the desired buffer system[5], prepare a total of 500 ml. Weigh out 56 gms of hydrolyzed starch[6] and place in a 500 ml Erlenmeyer flask. From the total 500 ml solution of buffer, add 250 ml to the hydrolyzed starch and thoroughly disperse the starch. Place the remaining buffer into a 2000 ml

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5. A list of the most commonly used buffer systems and their formulae can be found in the Appendix.

6. We used hydrolyzed potato starch purchased from Electrostarch Co., Madison, Wisc.

Erlenmeyer flask and heat.

Once the buffer is boiling, the starch suspension is added directly into the boiling buffer, the mixture is shaken for 15 or 20 seconds so that even mixing of the starch suspension is facilitated. (Note: In some enzyme studies additional constituents are added to the gel, triphosphopyridine nucleotide, β-mercaptoethanol, etc. It is at this point in gel preparation that these constituents should be added.) Degas the gel solution.

Once the gel is totally degassed it should be poured slowly into the gel mold. The mold should be filled only until there is a small meniscus on top of the form. If more than a single gel is being prepared, care should be taken so that all gels are the same thickness, or differential rates of migration will result during electrophoresis. Once the gel has semi-solidified, it may be placed under refrigeration for the duration of the cooling time, a period of about 30 minutes.

C. Introduction of Samples into the Gel

Once the gel has completely cooled, cut the frame loose from the gel by sliding a scalpel around the edge and lift the frame off the glass plate. A slice is made in the gel about one and 1/4 inches from one edge and protein samples are introduced into the gel by small paper wicks. When all samples have been introduced into the gel, carefully push the narrower starch strip tightly against the paper wicks.

D. Electrophoresis

The glass plate carrying the gel with samples is placed between two plexiglas electrophoresis trays, each containing the appropriate buffer. The electrical contacts with the gels are made with sponge strips. These should be placed on both edges of the gel from the two trays so that they overlap the

gel about 1/2 inch on each end. It is important that the edges of the sponges be absolutely parallel with the sample slit or differential migration rates will occur across the gel. Once the electrode sponges have been placed on the gel, carefully cover the gel and sponge strips with a sheet of Saran Wrap.

The connected power supply is turned on and the voltage adjusted until the current is about 30-50 milliamperes. If the buffer system being used is a discontinuous one (like that used for IDH and αGPDH in this study), let the buffer front migrate almost completely across the gel, a period usually about two to three hours. If a continuous buffer system is being used, electrophoresis time may vary anywhere from 2-16 hours (see Appendices B and C).

E. Cutting the Gel for Staining

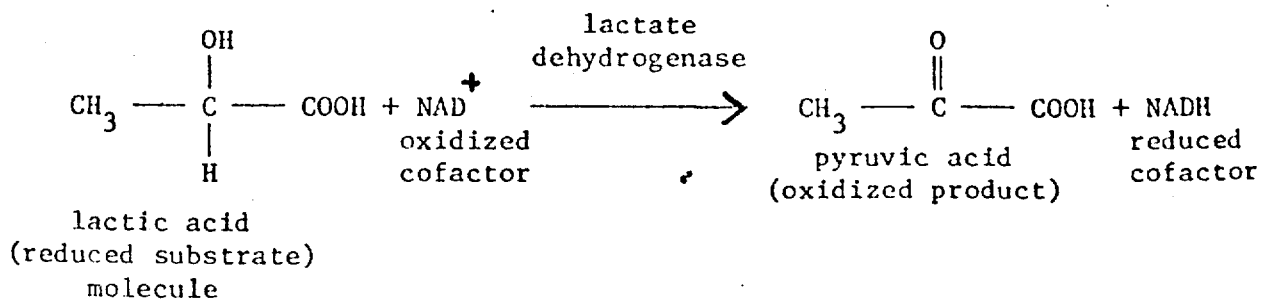
When the desired electrophoresis time has expired, shut off the power supply and remove the Saran Wrap and the electrode sponges and place the glass plate and gel on the table. Remove the cathodal portion of the gel, unless a low pH gel buffer was employed. Trim away about an inch of the anodal gel edge, place the two plexiglas cutting strips on either side of the gel to be cut.

Cut a piece of nylon line approximately 15 inches in length. Gripping both ends tightly, one in each hand, slowly pull the nylon line horizontally through the gel maintaining tension in the line and forcing it down against the plexiglas cutting strips. Remove the plexiglas cutting strips and again pull the line underneath the gel to free it from the glass plate.

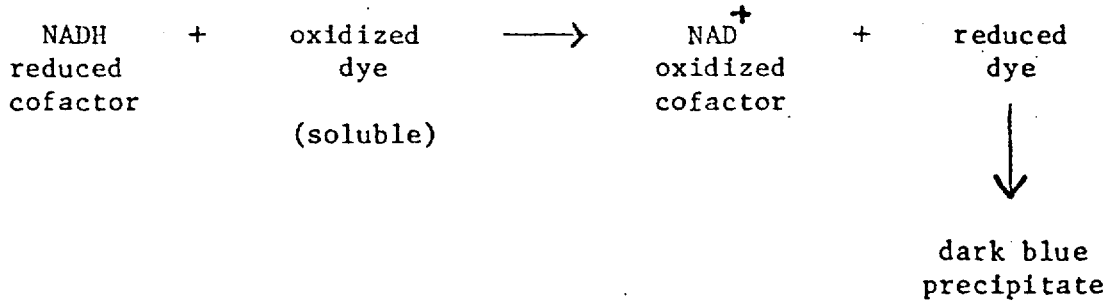
Carefully lift the top half of the gel off and place into a staining tray. Similarly place the bottom half in a staining tray.

The histochemical stains which are employed to locate an enzyme in the gel operate on the basic principle of the enzyme's specificity for its substrate. An enzyme is a specific catalyst which will recognize only the one chemical compound(s) (substrate) which it converts to the product compound(s) during the enzymatic reaction.

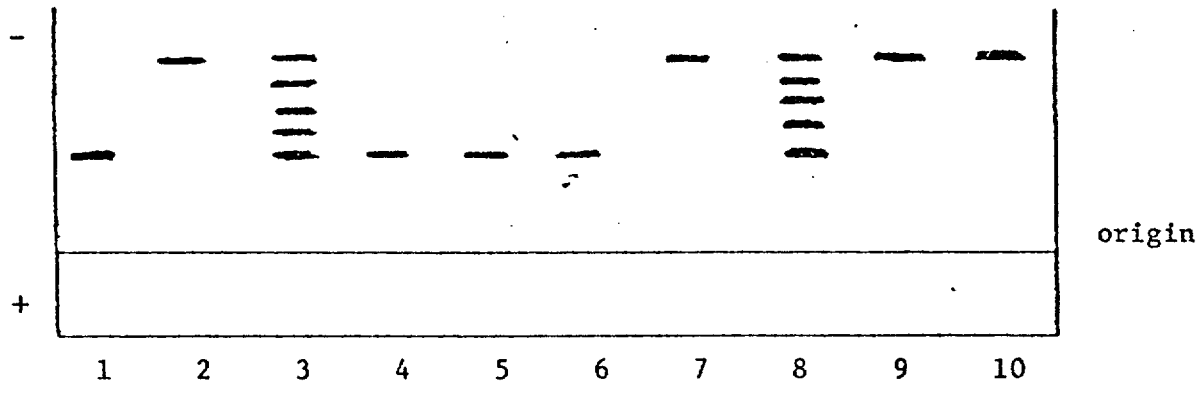
A large number of the enzymatic reactions catalyzed by enzymes involve the transfer of electrons to cofactor molecules such as NAD (DPN) or NADP (TPN). Normally, the cofactor is reduced (gains an electron) or oxidized (loses an electron) during the reaction. Certain compounds exist which are soluble when they are oxidized but when reduced, precipitate as a dark blue dye. Some of these dyes, such as phenazine methosulfate (PMS) or nitro blue tetrazolium (NBT) are able to accept the electrons from the reduced cofactor molecules (NADH (DPNH) or NADPH (TPNH)). These dyes are dissolved in a solution with all the other special compounds required for the specific enzymatic reaction. When an electrophoresed starch gel is placed in this staining solution the presence of a specific enzyme can then be easily detected as a blue band resulting from the precipitated dye. Despite the fact that there are hundreds of different enzymes in the gel, only the specific enzyme of interest appears, since the staining solution contains the special substrate compound that is specific for the enzyme of interest. The enzyme lactate dehydrogenase (LDH) is given below as an example.



then:



Thus, the location of LDH is evidenced by a blue band. If we load the liver extracts from 10 fish onto a starch gel and stain for LDH we may see:



Individuals 1, 4, 5 and 6 are homozygous AA. Individuals 3 and 8 are heterozygotes. Numbers 2, 7, 9 and 10 are homozygous aa. Thus gene frequencies for LDH can be calculated as explained previously.

A series of enzyme stains were used in the original screening of the stripped bass populations and these specific stains are detailed in appendix C. The two genetically variant systems were IDH and αGPDH and these stains (see appendix C) were both resolved on the LiOH buffer system found in appendix B and the Results and Discussion section.

RESULTS AND DISCUSSION

The raw results of our study have been supplied directly to the Texas Instruments Corporation. The following is a summary and overview of the results previously supplied.

Our analysis shows Morone saxatilis to be one of the most genetically homogeneous species ever studied in a natural population. We have successfully resolved two serum proteins, several isohemoglobins accounting for 4 loci, and 28 enzyme systems that account for at least 52 loci (see Table 1). As has been previously reported (Morgan, 1971; Morgan et al., 1973), we found serum transferrin to be polymorphic, but technical difficulties kept us from using transferrin for the geographical distribution studies. Among the enzyme loci studied, only two were polymorphic α-glycerophosphate dehydrogenase, (α-GPDH) and isocitrate dehydrogenase (IDH) and both were in liver tissue.

A conservative estimate of protein polymorphism in striped bass is about 9%. Morgan (1971) reported that out of a total of 31 serum proteins, only one (transferrin) showed genetic variation. This lowers the estimate of protein polymorphism to 4.5% - one of the lowest recorded for a natural population. Johnson et al. (1973) found low levels of polymorphism in the genus Sabastes (rockfishes).

Spawning Populations: Allelic frequencies of the two variable enzyme systems are presented in Table 2 for each of the spawning rivers. The phenotypic frequencies of the polymorphic loci agree with the expectations derived from a multinomial distribution for a randomly mating population. Therefore, we have assumed that each of the 2 variable enzyme loci is controlled by a polymorphic gene locus.

Both α-glycerophosphate dehydrogenase (αGPDH) and isocitrate dehydrogenase (IDH) show subtle changes in allelic frequency with latitude (see Table 2). The IDH-B allele appears to be fixed in the Hudson River, while its frequency is less than unity in southern populations. For αGPDH-B the opposite is true: its frequency is greater in the Hudson River than in the southern populations.

Two findings are particularly noteworthy. A third allele (IDH-A) occurred in the Roanoke River population (Table 2). In the late spring of 1975, the two rare IDH alleles (A and C) suddenly appeared in Hudson River specimens. Striped bass collected in the Hudson River through 23 May had an allelic frequency for IDH and α-GPDH similar to that of the 1974 collections from the Hudson spawning stock and collections of striped bass overwintering in the Hudson River (Table 2). However, after 23 May, allelic frequencies for both IDH and α-GPDH within the Hudson spawning stock were similar to the southern spawning stocks.

Oceanic Migrating Populations: Isozyme analyses of oceanic populations indicated that three IDH alleles (A,B, and C) were present in most samples while the third α-GPDH allele (α-GPDH-C) was only present in three samples (see Table 3). From these data alone, it is impossible to detect unique Hudson populations of striped bass. However, if the IDH-A allele is really absent in the majority of Hudson populations, then the data in Table 3 suggest a large portion of the oceanic populations are from southern rivers.

Striped bass spawn in most of the major rivers from Florida north to the Hudson (Porter et al., 1969). Tagging studies (Alperin, 1966; Florence, 1974; Schaefer, 1968; Vladykov et al., 1952) suggest that fish from all spawning sources north of Cape Hatteras migrate along the entire U.S. coast but always north of their respective spawning areas.

Despite the low variability of striped bass isozymes, electrophoretic variation may be useful in determining the composition of spawning populations. The IDH-A-allele found in the Roanoke River population could be useful as a genetic marker in determing the origin or lack thereof for oceanic migrating populations. The sudden appearance of the IDH-A allele in Hudson River populations, where it did not exist days before, could be evidence for the appearance of migratory "Roanoke like" striped bass. The IDH-A and -C alleles were not present in 1974 Hudson spawning populations (Table 2). Furthermore, overwintering striped bass did not have the A and C alleles. Similar results were obtained in 1975 through May 23 (Table 2). The α-GPDH-B allele was present in the 1975 stock with a frequency similar to 1974 values (0.894 and 0.883) through May 23. The overwintering striped bass had a similar α-GPDH-B (i.e., 0.880). However, after May 23, the α-GPDH-B frequency increased to 0.969, a value similar to that in more southerly stocks, and there was a simultaneous appearance of the A and C IDH alleles.

If the frequency of the IDH-A-allele is relatively high south of the Roanoke River, but is not present in northern populations, then the fish appearing in the Hudson during late spring could be from a southern population. It is notable that the other IDH-C appears

simultaneously with the A-allele. The presence of both rare IDH alleles (A and C) in migratory oceanic populations strengthens this possibility.

Since we do not know the gene frequencies for spawning populations south of Virginia, we cannot be sure of the relative contribution of the Hudson populations to the oceanic fishery. If the gene frequencies are similar to those of oceanic sites 7,8,9 and 10 (see Table 3), then we would expect the contribution by Hudson to be small, but if the gene frequencies of IDH-A and IDH-C are high for southern populations, then the Hudson's contribution would be substantial.

Table 1. Enzyme systems investigated.

Enzyme System	Abbreviation	No. of Loci
α-napthyl acetate esterase	α-nap. acetate-EST	1
α-napthyl butyrate esterase	α-nap. but-EST	4
Serum esterase	ser-EST	1
Phosphoglucomutase	PGM	1
Phosphohexoseisomerase	PHI	2
Isocitrate dehydrogenase	IDH	1
Alcohol dehydrogenase	ADH	2
Glucose 6-phosphate dehydrogenase	G6PDH	1
Alkaline phosphatase	ALK PHOS.	1
Acid phosphatase	ACID PHOS.	1
Glucokinase	GK	1
Glutamate dehydrogenase	GDH	2
α-glycerophosphate dehydrogenase	α-GPDH	2
6-phosphogluconate dehydrogenase	6PGDH	2
Lactate dehydrogenase	LDH	2
Superoxide dismutase	SOD	2
Leucine aminopeptidase	LAP	1
Fructose 1,6 diphosphatase	F1,6DiPhos	2
Creatine kinase	CK	2
Adenylate kinase	AK	2
Aspartate aminotransferase	AAT	3
Xanthine dehydrogenase	XDH	1
Sorbitol dehydrogenase	SDH	2
Glyceraldehyde 3 phosphate dehydrogenase	G3-P DH	2
Monoamine oxidase	MO	2
Malate dehydrogenase	MDH	2
Peroxidase	Per.	5
β-hydroxybutyrate dehydrogenase	βOHbutDH	2
Total systems-28	Total loci – 52	

Table 2. Allelic frequencies at two polymorphic loci in spawning populations of Morone saxatilis from six different river systems.

Site	Year	Season	Location	Sample Size (n)	Gene Frequencies IDH P(A)	IDH P(B)	IDH P(C)	αGPDH P(A)	αGPDH P(B)
1	1974	Spring	Hudson River	200	0	1.000	0	.117	.883
		Winter+	"	62	0	1.000	0	.120	.880
	1975	Spring, Early*	"	79	0	1.000	0	.106	.894
		Late#	"	46	.065	.924	.011	.031	.969
2	1974	Spring	Elk River	253	0	.945	.055	.046	.954
	1975	"	"	57	0	.991	.009	.018	.982
3	1974	"	Choptank River	101	0	.970	.030	.057	.943
	1975	"	"	52	0	.990	.010	.038	.962
4	1974	"	Potomac River	206	0	.964	.036	.027	.973
	1975	"	"	46	0	.978	.022	.011	.989
5	1974	"	Rappahannock River	156	0	.944	.056	.022	.978
	1975	"	"	77	0	.948	.052	.019	.981
6	1975	"	Roanoke River	99	.025	.960	.015	.010	.990

* Early -- Early spring sampling (5/14/75 - 5/23/75).
# Late -- Late spring sampling (5/24/75 - 6/5/75).
+ Winter -- (12/6/74 - 1/10/75).

Table 3. Allelic frequencies at two polymorphic loci in Oceanic populations of Morone saxatalis from ten different localities in 1975

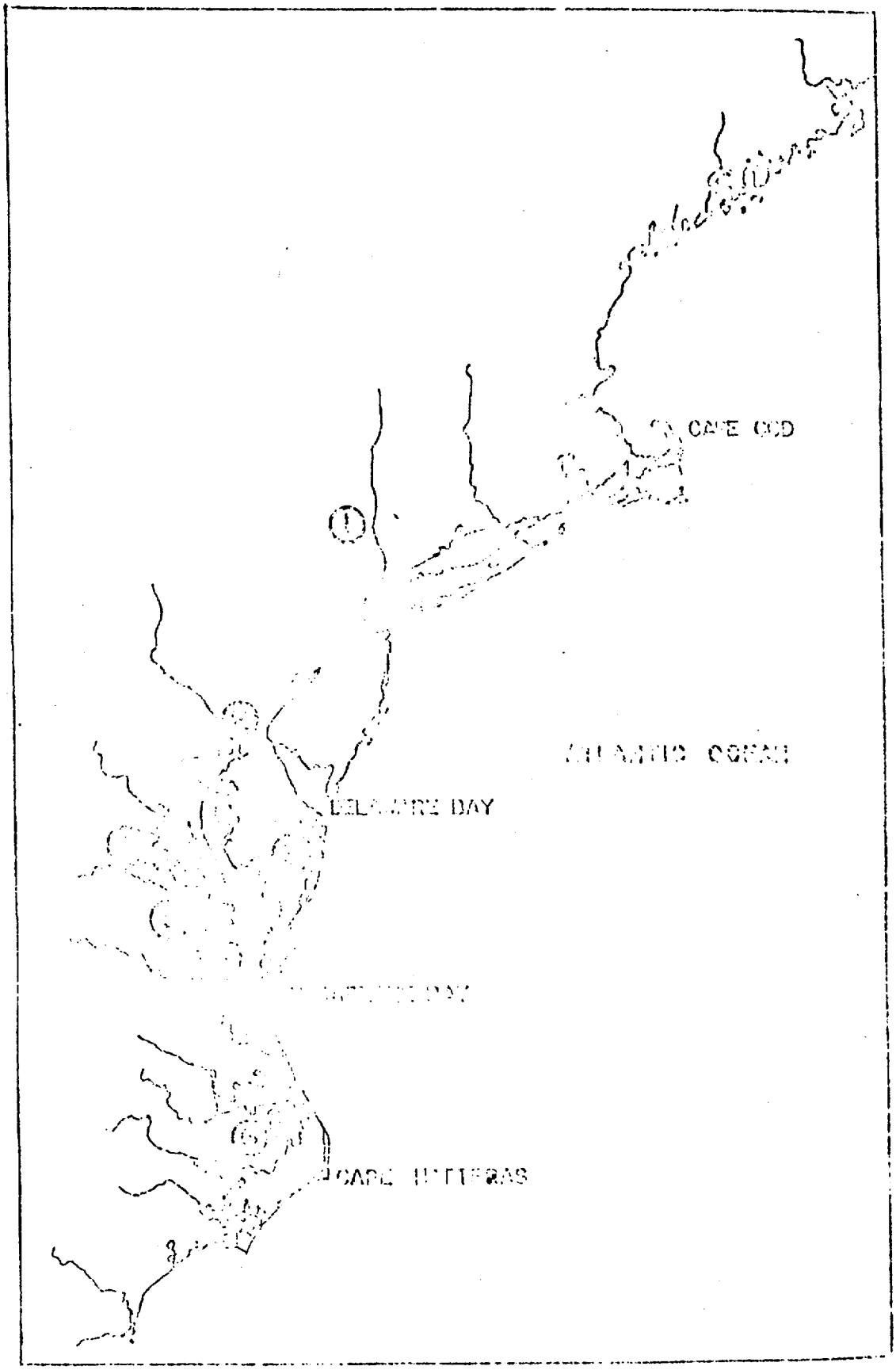
Site[+]	Period[#]	Sample size (n)	Gene Frequencies IDH P(A)	IDH P(B)	IDH P(C)	Sample size (n)	OGPDH P(A)	OGPDH P(B)	OGPDH P(C)
	3	78	.071	.897	.032	78	.051	.949	0
1	4	56	.009	.964	.027	58	.026	.974	0
	5	71	.021	.972	.007	71	.007	.993	0
	3	93	.011	.968	.021	93	.016	.984	0
2	4	81	.068	.926	.006	81	.019	.981	0
	5	76	.007	.960	.033	75	.027	.973	0
	3	52	.027	.973	0	55	.009	.991	0
3	4	41*	.110	.866	.024	40	.038	.962	0
	5	53	0	.962	.038	53	.057	.943	0
	3	77	.045	.948	.007	74	.027	.973	0
4	5	97	.026	.948	.026	107	.028	.971	0
	6	92*	.011	.984	.005	92	.016	.984	0
	2	34	0	.971	.029	32	.047	.953	0
5	3	45	.011	.989	0	45	.067	.933	0
	4	80	.006	.981	.013	80	.063	.937	0
	5	77	.013	.981	.006	77*	.026	.961	.013
	3	62	.040	.927	.032	62	.040	.952	.008
6	4	106*	.094	.887	.019	10[illegible]	.028	.972	0

	5	97	0	.995	.0005	97	.031	.969	0
	6	100	0	.970	.030	100	.015	.985	0
7	2	29	0	1.000	0	29	0	1.000	0
	3	60	.033	.950	.017	59	.042	.958	0
	4	76	.026	.967	.007	76	.039	.961	0
	5	89*	.022	.961	.017	89	.034	.966	0
	6	120	.008	.983	.008	120	.029	.971	0
8	2	32	.016	.984	0	32	.016	.984	0
	3	97	.005	.990	.005	97	.026	.974	0
	4	24	.062	.917	.021	24	0	1.000	0
	5	57	.009	.965	.026	57	.018	.982	0
	6	104	.005	.976	.019	104	.038	.962	0
9	2	62	.016	.976	.008	63	.032	.960	.008
	4	40*	.025	.950	.025	40	.050	.950	0
	6	89	0	.966	.034	89	.034	.966	0
10	1	26	.019	.981	0	26	0	1.000	0
	6	23	0	.978	.022	23	0	1.000	0

+ Sites are according to T.I. Geographical Stratification.

Periods are according to T.I. Collecting Schedule.

* Chi square value greater than 30.


CAPE COD
ATLANTIC OCEAN
DELAWARE BAY
CAPE HATTERAS

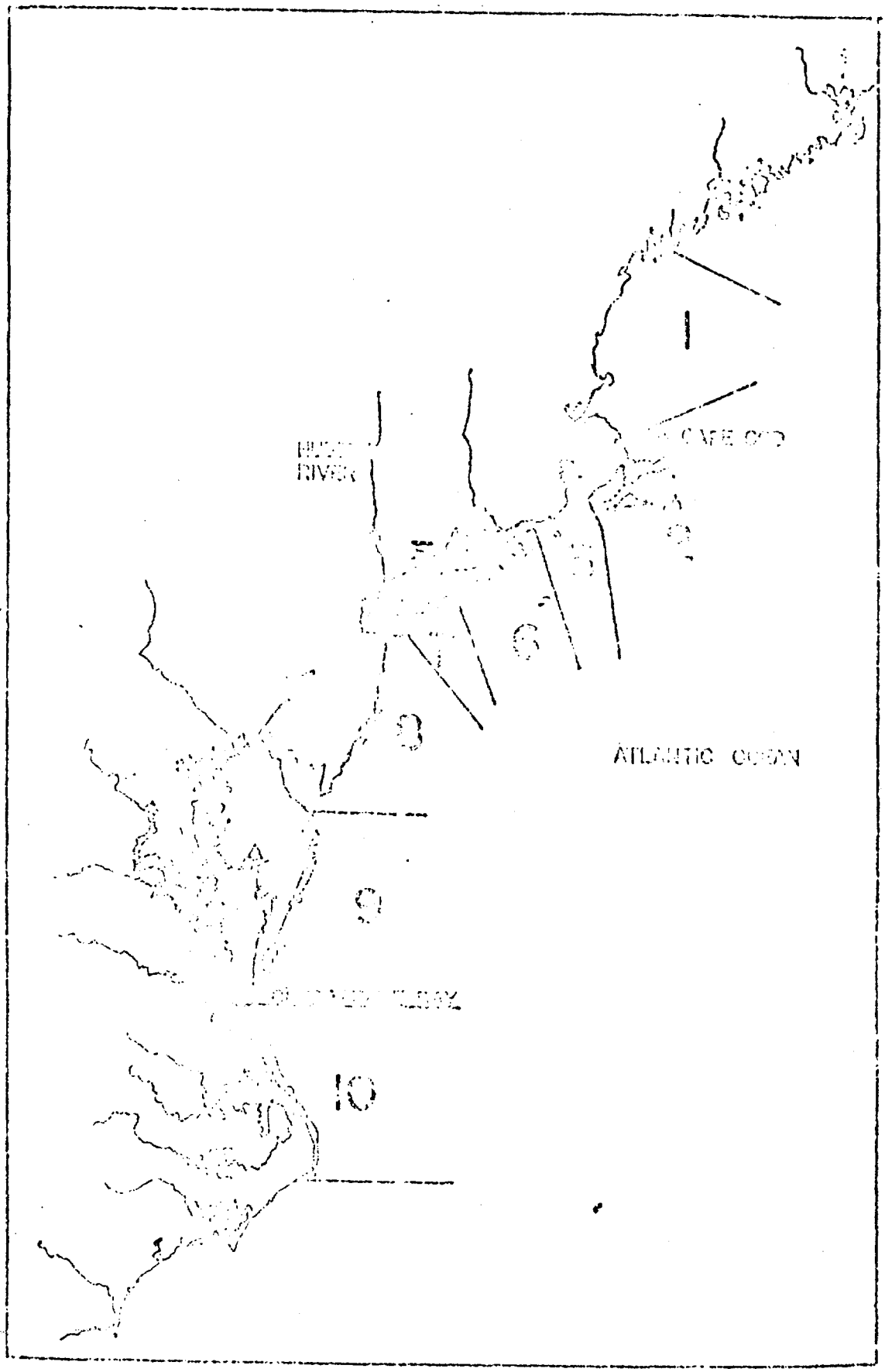


FIGURE 5 OCEANIC COLLECTION AREAS

APPENDIX A: Important Terms in Population Genetics from Mayr's Populations, Species and Evolution (1974).

Important Terms in Population Genetics

Adaptation. The condition of showing fitness for a particular environment, as applied to characteristics of a structure, function, or entire organism; also the process by which such fitness is acquired.

Adaptive radiation. Evolutionary divergence of members of a single phyletic line into a series of different niches or adaptive zones.

Allele. Any of the alternative expressions (states) of a gene (locus).

Allopatric. Of populations or species occupying mutually exclusive (but usually adjacent) geographical areas (i.e., not overlapping territories).

Autopolyploid. A polyploid originating by the doubling of a diploid chromosome set.

Autosome. A chromosome other than a sex chromosome.

Balanced load. The amount by which the overall fitness of a population is depressed owing to the segregation of inferior genotypes the component genes of which are maintained in the population because they add to fitness in different combinations (for instance, as heterozygotes).

Balanced polymorphism. A polymorphism maintained by a selective superiority of the heterozygotes over either type of homozygotes.

Cline. A gradual and essentially continuous change of a character in a series of contiguous populations; a character gradient.

Clone. All the individuals derived by asexual reproduction from a single sexually produced individual.

Closed population. A population with no genetic input other than that by mutation.

Coadaption. The harmonious epistatic interactions of genes brought together by natural selection.

Competition. The simultaneous seeking by two or more individuals of an essential resource of the environment that is in limited supply.

Competitive exclusion. The principle that no two species can coexist at the same locality if their ecological requirements are identical.

Deme. A local population.

Density-dependent factors. Causes of mortality and fecundity that become more effective as the density of a population increases.

Diploid. Having a double set (2n) of chromosomes; the normal chromosome number of the cells (except of mature germ cells) in any individual derived from a fertilized egg.

Disruptive (diversifying) selection. Selection for phenotypic extremes in a population (until a discontinuity is achieved).

Electrophoresis. A technique that separates mixtures of molecules (particularly proteins and polypeptides) by their different rates of travel in an electric field.

Epistatic interaction. An interaction of genes at different loci.

Ethological. Behavioral, particularly with reference to species-specific components of behavior the phenotypic expression of which is largely determined genetically.

Ethological barriers. Isolating mechanisms caused by behavioral incompatibilities of potential mates.

Fecundity. Reproductive potential as measured by the quantity of gametes, particularly eggs, produced.

Fitness. The relative appropriateness or suitability of genes, individuals, populations or species.

Founder principle. The principle that the founders of a new colony (or population) contain only a small fraction of the total genetic variation of the parental population (or species).

Gene flow. The exchange of genetic factors between populations.

Gene pool. The totality of the genes of a given population existing at a given time.

Genetic drift. Genetic changes in populations caused by random phenomena rather than by selection.

Genetic homeostasis. The property of the population of equilibrating its genetic composition and of resisting sudden changes.

Genetic load. The depression of fitness (from a theoretical optimum) caused by deleterious genes (for example, not yet eliminated recessives).

Geographic barrier. Any terrain that prevents gene flow between populations.

Geographic isolate. A population or group of populations prevented by an extrinsic barrier from free gene exchange with other populations of the species.

Geographic race. A geographically delimited race, usually a subspecies.

Geographic speciation. The acquisition in a population -- while it is geographically isolated from other populations of its parental species -- of characters that promote or guarantee reproductive isolation after the external barriers break down.

Geographic variation. The differences between spatially segregated populations of a species; population differences in the space dimension.

Geographic vicariance. Geographic replacement of populations or species by each other (i.e., the moving out of one species followed by the intrusion of a new species).

Hardy-Weinberg law. The fact that, owing to particulate inheritance, the frequency of genes in a population remains constant in the absence of selection, or nonrandom mating, and of accidents of sampling.

Heterosis. Selective superiority of heterozygotes (see: overdominance).

Heterozygote. An individual with different genetic factors (alleles) at the homologous (corresponding) loci of the two parental chromosomes.

Homozygous. Having identical alleles at the two homologous loci of a diploid chromosome set.

Hybridization. The crossing of individuals belonging to two unlike natural populations that have secondarily come into contact.

Inbreeding. Crossing with genetically similar individuals, particularly with close relatives.

Inbreeding depression. A reduction of fitness owing to severe inbreeding; often manifested by loss of fertility, growth anomalies, and metabolic disturbances.

Industrial melanism. The evolution of a darkened population owing to selection of melanistic individuals that better blend with their substrate in the sooty surroundings of an industrial area.

Linkage. The occurrence of genes on the same chromosome. The closer they are on the chromosome, the more tightly they are linked, that is, the less likely they will be separated by crossing over.

Locus. The location of a given gene on a chromosome.

Meiotic drive. A force able to alter the mechanics of meiotic cell division in such a manner that the two kinds of gametes produced by a heterozygote do not occur with equal frequency.

Melanism. An unusual darkening of color owing to increased amounts of black pigment - sometimes a racial character, sometimes restricted to a certain percentage of individuals in a population, giving rise to polymorphism.

Morph. Any of the genetic forms (individual variants) that account for polymorphism.

Mutation. A change in the genetic material; most often a change in a single gene (gene mutation), consisting of a replacement, duplication, or deletion of one or several base pairs in the DNA.

Neo-Darwinism. Weismann's theory of evolution; sometimes, any modern evolutionary theory featuring natural selection.

Niche (ecological). The constellation of environmental factors into which a species fits; the outward projection of the needs of an organism, its specific way of utilizing its environment.

Normalizing selection. The removal by selection of all genes that produce deviations from the normal (= average) phenotype of a population.

Ontogeny. The development of the individual, particularly the embryogenesis.

Open population. A population freely exposed to gene flow and subject to much input of alien genes owing to immigration.

Outbreeding. Crossing with genetically different, not closely related individuals, particularly with members of different populations.

Overdominance. Superiority of the heterozygote over both kinds of homozygotes.

Panmictic. Of populations, randomly interbreeding, the whole population or species forming a single deme.

Phenotype. The totality of characteristics of an individual (its appearance) as a result of the interaction between genotype and environment.

Ploidy. A term referring to the number of chromosome sets.

Poikilothermal. Ectothermal.

Polygenes. Genes that jointly with several or many other genes control a character.

Polymorphism. The simultaneous occurrence of several discontinuous phenotypes or genes in a population, with the frequency even of the rarest type higher than can be maintained by recurrent mutation.

Population, local. A group of potentially interbreeding individuals at a given locality.

Selfing. Self-fertilizing in hermaphrodites or monoecious plants.

Sibling species. Morphologically similar or identical populations that are reproductively isolated.

Sicke-cell anemia. An anemia due to a hemoglobin mutation found mostly in tropical areas and lethal to homozygotes.

Speciation. The splitting of a phyletic line; the process of the multiplication of species; the origin of discontinuities between populations caused by the development of reproductive isolating mechanisms.

Species. A reproductively isolated aggregate of interbreeding populations.

Species group. A group of closely related species, usually with partially overlapping ranges.

Stabilizing selection. The elimination by selection of all phenotypes deviating too far from the population mean, and hence also of genes producing such deviating phenotypes.

Stochastic process. A process the various outcomes of which can be predicted with a specified probability (versus deterministic process).

Subspecies. An aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species and differing taxonomically from other populations of the species.

Sympatric speciation. Speciation without geographic isolation; the acquisition of isolating mechanisms within a deme.

Synthetic theory. The current evolutionary theory, which is a synthesis of the best components of many previously proposed theories, with mutation and selection as the basic elements.

Tetraploid. A polyploid with four haploid chromosome sets, normally the result of the doubling of the diploid chromosome number.

Variance. A sample statistic relating to deviations from the mean. The variance on the mean square is: Variance = $\varepsilon y^2/n$.

Variety. An ambiguous term of classical (Linnaean) taxonomy for a heterogeneous group of phenomena including nongenetic variations of the phenotype, morphs, domestic breeds, and geographic races.

Vector. Carrier, particularly an animal that transmits a pathogen (protozoan, bacterium, virus) from one host to another.

Zygote. A fertilized egg; the cell (individual) that results from the fertilization of an egg cell.

APPENDIX B: Buffers used in Gel Electrophoresis Screening for Electrophoretic Variants in Striped Bass Populations

Buffers for Starch Gel Electrophoresis

1. Lithium Hydroxide (pH 8.0): This was the system used for the two electrophoretic variant enzymes IDH and αGDPH (see Results).

Solution A	18 gm. LIOH (hydrated)	
	177 gm. Boric Acid	
	Both in 15 liters distilled water	
Solution B	6.4 gm. Citric Acid	
	24.2 gm. Tris	
	Both in 4 liters distilled water	

9 parts B and 1 part A used for gel buffer; A is used for the electrolyte bath.

2. Tris-Borate EDTA (disodium salt : pH 8.6)

.436 gm. Tris
123 gm. Boric Acid in 4 liters distilled water
29.8 gm. EDTA

Buffer is diluted 1:20 for gel buffer, 1:5 for anode compartment and 1:7 for cathode compartment.

3. Tris-citrate (pH 6.9)

Tris	0.75 molar
Citric Acid	0.25 molar

Buffer is diluted 1:60 for gel and 1:20 for electrolyte bath.

APPENDIX C: List of Recipes for Staining Solutions of Enzymes used in the Original Screening of Stripped Bass Populations.‡

‡Since variants were only found for two enzyme systems (αGDPH and IDH), the readers' attention should be directed particularly to those stains.

ALCOHOL DEHYDROGENASE (1.1.1.2)

Add 12 mg DPN to starch before degassing

Stain Buffer: 0.5 M Phosphate, pH 7.0

Constituents	Agar	50 ml Liquid
DPN	25 mg	25 mg
NBT	15 mg	15 mg
PMS	1 mg	1 mg
Ethanol (95%)	2.5 ml	2.5 ml
Buffer	5 ml	5 ml
Water	20 ml	45 ml

Incubate at 37°C until bands appear. Our experience has indicated very low activity of this enzyme and therefore longer incubation times are necessary.

AMINOPEPTIDASE (3.4.1.2)

Stain Buffer: 022 M Tris-HCl, pH 8.0

Constituents	Agar	50 ml Liquid
L-Leucyl-L-Alanine	40 mg	40 mg
Snake Venom (Bothrops or Crotalus)	10 mg	10 mg
Peroxidase	20 mg	20 mg
O-Dianisidine, 2HCl	10 mg	10 mg
0.25 M $MnCl_2$	0.5 ml	0.5 ml
Buffer	25 ml	50 ml

Add Tris-HCl and $MnCl_2$ just before staining. Incubate at 37°C. until yellow-brown bands appear, usually about 1 hour. Fix for only 2 hours, since bands will disappear upon continued soaking in fixative.

ESTERASE (3.1.1.1)

Stain Buffer: Any pH 7.0 buffer

Substrate solution:

alpha-naphthyl acetate*	1 g
Acetone	50 ml
Water	50 ml

Stain:

Substrate solution	1.0 ml
Fast Blue RR	50 mg
Buffer	50 ml

Incubate at room temperature or 37°C if necessary.

* Other esters may be substituted.

CREATINE KINASE (2.7.3.2)

Stain Buffer: 0.5 M Tris-HCl, pH 7.0

Constituents	Agar	50 ml Liquid
Creatine phosphate	365 mg	365 mg
ADP	35 mg	35 mg
Glucose (dextrose)	45 mg	45 mg
$MgCl_2$, $6H_2O$	10 mg	10 mg
TPN	12 mg	12 mg
PMS	1 mg	1 mg
NBT	10 mg	10 mg
Buffer	5 ml	5 ml
Water	20 ml	45 ml
Hexokinase	80 units	80 units
Glucose-6-Phosphate Dehydrogenase	40 units	40 units

Incubate at 37°C until bands appear.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (1.1.149)

Add 12 mg TPN/600 ml gel before degassing.

Stain Buffer: 0.5 M Tris-HCl, pH 7.0

Constituents	Agar	50 ml Liquid
TPN	15 mg	15 mg
NBT	10 mg	10 mg
PMS	1 mg	1 mg
Disodium glucose-6-phosphate, hydrate	100 mg	100 mg
Buffer	10 ml	10 ml
Water	15 ml	40 ml

Incubate at 37°C until bands appear

GLUTAMATE OXALOACETATE TRANSAMINASE (2.6.1.1)

(Aspartate Aminotransferase)

Stain Buffer: 0.1 M Phosphate, pH 7.0

Constituents	Agar	50 ml Liquid
L-aspartic acid	230 mg	230 mg
Ketoglutaric acid	130 mg	130 mg
Fast Blue B (or Fast Violet B)	200 mg	200 mg
Pyrodoxal phosphate	1 mg	1 mg
Buffer	25 ml	50 ml

Adjust to pH 7.4 with 5 N NaOH.

Incubate gels at 37°C until bands appear. Bands begin to fade after about 3 hours.

GLYCEROL-3-PHOSPHATE DEHYDROGENASE (1.1.1.8)

Stain Buffer: 0.1 M Phosphate, pH 7.0

Substrate solution:

Sodium glycerolphosphate 21.6 gm

Dissolve in 50 ml water, adjust to pH 7.0 with 1 N HCl and dilute to 100 ml

Constituents	Agar	50 ml Liquid
DPN	25 mg	25 mg
NBT	15 mg	15 mg
PMS	1 mg	1 mg
Substrate solution	5 ml	5 ml
Buffer	5 ml	5 ml
Water	15 ml	40 ml

Incubate at 37°C until bands appear

INDOPHENOL OXIDASE

Electrophoretic Buffer: As required

Stain:

Tris-HCl, pH 8.0 - 100 ml

$MgCl_2$ - .2 g

PMS - 10 mg

MTT - 10 mg

Light bands appear on blue background.

ISOCITRATE DEHYDROGENASE (1.1.1.41-42)

Stain Buffer: 0.2 M Tris-HCl, pH 8.0

Constituents	Agar	50 ml Liquid
TPN (1.1.1.42) or DPN (1.1.1.41)	10 mg	10 mg
NBT	10 mg	10 mg
PMS	2 mg	2 mg
0.1 M Sodium isocitrate, hydrate	2.5 ml	2.5 ml
0.25 M Manganese Chloride, hydrate	0.2 ml	0.2 ml
Buffer	23 ml	50 ml

Incubate at 37°C until bands appear.

LACTATE DEHYDROGENASE (1.1.1.27)

Stain Buffer: 0.5 M Tris-HCl, pH 7.0

Substrate solution:

85 percent DL-lactic acid	10.6 ml
1 M Sodium carbonate	49 ml

Carefully add sodium carbonate to lactic acid in 50 ml of water. Adjust to pH 7.0 with .5 M sodium carbonate. Dilute to 100 ml.

Constituents	Agar	50 ml Liquid
DPN	25 mg	25 mg
NBT	15 mg	15 mg
PMS	1 mg	1 mg
Substrate solution	5 ml	5 ml
Buffer	5 ml	5 ml
Water	15 ml	40 ml

Incubate at 37°C until bands appear.

LEUCINE AMINOPEPTIDASE (3.4.1.1)

Stain Buffer: 0.5 M Phosphate, pH 7.0

Constituents	Agar	50 ml Liquid
L-leucyl-B-naphthylamide	5 mg	5 mg
Fast Garnet GBC	10 mg	10 mg
Buffer	25 ml	50 ml

Incubate at 37°C until bands appear.

MALATE DEHYDROGENASE (1.1.1.37)

Stain Buffer: 0.5 M Tris-HCl, pH 7.0

Substrate solution:

L-malic acid	13.4 g
2 M Sodium carbonate	49 ml
water to make up as for LDH	1000 ml

Constituents	Agar	50 ml Liquid
DPN	25 mg	25 mg
NBT	15 mg	15 mg
PMS	1 mg	1 mg
Substrate Solution	5 ml	5 ml
Buffer	5 ml	5 ml
Water	10 ml	40 ml

Incubate at 37°C until bands appear.

PEROXIDASE (1.11.1.7)

Stain Buffer: .5% Glacial Acetic Acid

Constituents	50 ml Liquid
Benzidine dihydrochloride	100 mg
30% hydrogen peroxide	0.1 ml
Buffer	50 ml

Incubate at room temperature. Bands appear within a few seconds and continue to become darker. When desired intensity is reached, fix gels. Bands will fade completely within 2 - 3 hours after fixing.

PHOSPHOGLUCOMUTASE (2.7.5.1)

Stain Buffer: 0.2 M Tris-HCl, pH 8.0

Constituents	Agar	50 ml Liquid
Glucose-1-phosphate, hydrate	70 mg	70 mg
TPN	5 mg	5 mg
PMS	1 mg	1 mg
MTT	5 mg	5 mg
0.1 M Magnesium Chloride	5 ml	5 ml
Buffer	.5 ml	5 ml
Water	15 ml	40 ml
Glucose-6-phosphate Dehydrogenase	40 units	40 units

Incubate at 37°C until bands appear.

PHOSPHOHEXOSE ISOMERASE (5.3.1.9)

Stain Buffer: 0.05 M Tris-HCl, pH 8.0

Constituents	Agar	50 ml Liquids
Sodium fructose-6-phosphate	5 mg	5 mg
Magnesium chloride, hydrate	100 mg	100 mg
DPN	5 mg	5 mg
MTT	5 mg	5 mg
PMS	2 mg	2 mg
Buffer	25 ml	50 ml
Glucose-6-phosphate Dehydrogenase	40 units	40 units

SORBITOL DEHYDROGENASE (1.1.1.14)

Stain Buffer: 0.05 M Tris-HCl, pH 8.0

Constituents	Agar	50 ml Liquid
Sorbitol	250 mg	250 mg
DPN	5 mg	5 mg
MTT	8 mg	8 mg
PMS	1 mg	1 mg
Buffer	25 ml	50 ml

Incubate at 37°C until bands appear

XANTHINE DEHYDROGENASE (1.2.3.2)

Stain Buffer: 0.5 M Tris-HCl, pH 7.0

Constituents	Agar	50 ml Liquid
0.5 M Hypoxanthine	5 ml	5 ml
DPN	15 mg	15 mg
NBT	10 mg	10 mg
PMS	1 mg	1 mg
Buffer	5 ml	5 ml
Water	20 ml	45 ml

Incubate at 37°C until bands appear

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TI 89

Use of Isozymes to Identify Striped Bass Stocks

Final Report Prepared for
Texas Instruments
Incorporated

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December 2, 1976

Mr. Lorenzo Rodriguez
Consolidated Edison Company of New York, Inc.
P.O. Box 237
Buchanan, New York 10511

Dear Mr. Rodriguez:

Texas Instruments Incorporated is pleased to submit to Consolidated Edison Company of New York, Inc. 200 finalized copies of "Report on Relative Contribution of Hudson River Striped Bass to the Atlantic Coastal Fishery."

Your attention is invited to the fact that additional statistical techniques have been utilized since the drafting of the enclosed report. These techniques were instituted to reduce the bias in the estimates of stock contribution. As a result of applying these techniques, the relative contribution estimates of the Hudson River are now concluded to be approximately 15% to the NRC-defined Inner Zone; 0% to the NRC-defined Outer Zone; and 7% to the Atlantic Fishery. These techniques will be described in detail in the TI portion of the report which is concurrently in preparation for submission early in 1977.

Very truly yours,

TEXAS INSTRUMENTS INCORPORATED

John T. Major
Program Manager

JTM:job
cc; Dr. Kenneth Marcellus

REPORT ON RELATIVE CONTRIBUTION OF HUDSON RIVER STRIPED BASS TO THE ATLANTIC COASTAL FISHERY

DECEMBER 1976

Prepared for

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4 Irving Place
New York, NY 10003

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INTRODUCTION

Striped bass (*Morone saxatilis*) is an important sport and commercial fish in the estuaries and coastal waters of the Atlantic seaboard from Maine to North Carolina (Koo, 1970). Recruitment to the striped bass fishery is from various stocks (i.e., genetically isolated populations) of striped bass whose individuals are spawned and developed in the rivers and estuaries along the Atlantic coast. Recapture locations of tagged striped bass suggest that individuals from all spawning areas north of Cape Hatteras, North Carolina, utilize much of the Atlantic coast north of their respective spawning areas during a northward migration in the spring and a southern migration in the fall (Merriman, 1941:36-42; Raney et al., 1954:385-395; Alperin, 1966:105-106; Schaefer, 1968a:13-37; Florence, 1974; Appendix A). The tagging results appear to indicate that the area south of the Chesapeake Bay region contributes little to the migratory Atlantic coastal population (Raney, 1972:12) and that most striped bass stocks found southward from southern North Carolina probably are more or less indigenous to that area (Raney and Weller, 1972:29). The major spawning areas which potentially contribute individuals to the fisheries operating during the northward and southward migrations are the tributaries of the Chesapeake Bay system, and the Roanoke, Delaware, and Hudson Rivers.

Although tagging data have not led to precise quantative estimates of relative contribution of the major stocks to the coastal striped bass fishery, they have led to conflicting ideas as to which

stock predominates in the fishery: the Hudson stock or the Chesapeake stock. Studies on striped bass migratory behavior have generally concluded that the striped bass stock of the Chesapeake Bay system is the major contributor to the fisheries north of Chesapeake Bay (Merriman, 1941:47-52; Vladykov and Wallace, 1952:172-177; Alperin, 1966:96-105, 110; Schaefer, 1968a:38-43; Porter and Saila, 1969:4-5; Raney, 1972:10-13). These studies have indicated movements of striped bass between Chesapeake Bay and New England and relationships between exceptionally good production of young-of-the-year fish in Chesapeake Bay in 1934, 1958, and 1961 and the occurrence of dominant year classes of striped bass in northern waters two and more years later.

Clark (1972:4) and Goodyear (1974) have taken exception to the conclusion that the Chesapeake stock is the major contributor in the middle Atlantic striped bass fishery (primarily New York and New Jersey). Although tagging studies showed that few 2-year-olds left the Bay, studies by Merriman (1941:26) and Schaefer (1968a:38) showed that 2-year-olds were predominant in collections from New York and Connecticut waters in 1936 and 1963. Clark and Goodyear decided that the number of striped bass, especially 2-year-olds, tagged in Chesapeake Bay and recaptured outside the Bay was too low to indicate a large contribution of Chesapeake stock to that fishery. Therefore, because of apparent inconsistencies in the tagging studies and the closer proximity of the Hudson River to middle Atlantic and New England fisheries, they concluded that the striped bass stock of the Hudson River is the major contributor to the coastal fishery from New Jersey to Massachusetts.

Estimates of impact on this fishery of electric-generating plants on the Hudson River require precise, quantitative estimates of relative contribution of the major stocks. Due to the lack of such estimates, rough estimates of Hudson contribution (i.e., relative percentage) were assumed by the U.S. Nuclear Regulatory Commission (NRC 1975:V-166 to V-178) in their impact statement. They assumed a value of 90 percent Hudson contribution to the waters of the Hudson River, the western half of Long Island Sound, and the New York Bight ("inner" zone) and values of 10 and 50 percent to the remaining waters extending from Maine to Cape May, New Jersey ("outer" zone). However, the controversy still existed as to which stock predominated.

Reliable estimates of the relative proportion of the major stocks in the fishery were therefore needed to settle the controversy of which stock predominates and provide a basis for making meaningful estimates of plant impact on the Atlantic fishery of striped bass. Texas Instruments conducted a study to obtain such estimates using innate tags, i.e., inherited traits. A previous study (TI 1975:Appendix B) demonstrated the feasibility of using innate tags to idstinguish between Hudson and Chesapeake spawning stocks with approximately 80 percent accuracy. In the present study, collections were made during the 1975 spawning season from the Hudson River, major tributaries of Chesapeake Bay and from the Roanoke River and discriminant functions based on similar innate tags were established to segregate the stocks. Using the discriminant functions obtained with the 1975 spawning stock specimens, TI classified, by area of origin, striped bass obtained during 1975 from the Atlantic coastal (oceanic) fishery extending from southern Maine to Cape Hatteras, North Carolina. The proportion of the

oceanic catch allocated to the Hudson River spawning stock was used to estimate the percentage contribution of that stock to the Atlantic coastal fishery.

SECTION II

SUMMARY AND CONCLUSIONS

Texas Instruments Incorporated (TI) conducted a study to identify the origin of striped bass collected in the Atlantic coastal fishery, thus providing a basis for estimating the relative contribution of the Hudson River stock to that fishery. TI applied a quadratic discriminant routine on values of five meristic and morphometric characters obtained from Hudson, Chesapeake and Roanoke spawning stock specimens to determine functions which best separated the stocks. Correct classification of 76.8, 67.7, and 85.9% for the Hudson, Chesapeake, and Roanoke spawning stocks, respectively, were obtained, resulting in an overall correct classification of 74.4%. Most of the misclassifications occurred between the Hudson and Chesapeake stocks, as indicated by overlaps of 24.06, 4.44, and 10.94% obtained for the Hudson-Chesapeake, Hudson-Roanoke, and Chesapeake-Roanoke spawning-stock pairs respectively. The character set was the most generally applicable set of characters that distinguished among fish spawning in the Hudson, Chesapeake, and Roanoke estuaries as indicated by a cross-validation procedure.

Assessment of the relative contribution of various stocks of striped bass to the Atlantic coastal fishery was made on collections of striped bass from geographical and temporal strata. The geographical stratification consisted of ten strata from southern Maine to Cape Hatteras, North Carolina. Temporally, the year was divided into six two-month periods. Mean estimates of contribution were obtained by averaging the estimates within the strata. Since the assessment of contribution to the coastal

fishery required that the individuals were actually part of the fishery, collections of striped bass of sublegal size and those overwintering in the Hudson River were analyzed separately.

Oceanic samples were classified by the discriminant functions, and mean estimates of 23.0% Hudson, 66.0% Chesapeake, and 11.0% Roanoke stocks were obtained within the Atlantic coastal fishery over the entire year. The relative contribution to the fishery of the Hudson stock exceeded 30% only in Rhode Island, western Long Island Sound and the New York Bight during certain periods. Approximately 52% of the specimens collected in the 1975 oceanic sampling program were from the 1970 year class and 77% of these were classified as Chesapeake fish, overshadowing the Hudson and Roanoke classification percentages.

Biochemical data provided evidence that either (1) an influx of southern migrants or (2) a unique late-breeding segment of Hudson stock occurred in the Hudson River after 23 May 1975. Estimates of the relative contribution of Hudson River stock to the 1975 oceanic collections increased or decreased depending on which hypothesis was accepted. Differences up to 16 percentage points between the estimates obtained from the two hypotheses occurred during the summer months; however, the yearly estimates varied < 5 percentage points.

The relative contribution of the Hudson stock exceeded that of the Chesapeake stock for sublegal-sized striped bass (fork length < 406.5 mm) captured in western Long Island Sound and the New York Bight and all striped bass captured overwintering in the Hudson River. In collections from western Long Island Sound and the New York Bight, the Hudson's contribution of sublegal fish was $\geq 80\%$; for Hudson River

collections of overwintering fish a contribution of 76.3% Hudson stock was estimated.

Estimates of relative contribution of Hudson stock to commercial landings during the 2-month periods in 1975 ranged from 10.4 to 28.3%, with a 20.4% weighted average contribution for the entire year. The year exhibited 68.1% Chesapeake and 11.4% Roanoke stock contributions; these were similar to the yearly estimates of unweighted mean relative contributions of 23.0% Hudson, 66.0% Chesapeake, and 11.0% Roanoke stocks. Based on the average of 1973 and 1974 weighting factors for commercial landings, the mid-Atlantic region yielded approximately three times more poundage than did either the New England region, coastal Chesapeake region or coastal South Atlantic region north of Cape Hatteras, North Carolina.

Estimates of the relative contribution of the Hudson River stock to the "inner" and "outer" zones defined by the United States Nuclear Regulatory Commission (USNRC) in its environmental statement were 31.7 and 19.2% respectively. This was substantially lower for the "inner" zone than the estimated 90% contribution currently used by USNRC. For the "outer" zone, however, the estimate is intermediate between the USNRC values of 10 and 50% contribution.

To date, this study concludes that

- The Chesapeake stock is the major contributor to the Atlantic coastal striped bass fishery from southern Maine to Cape Hatteras, North Carolina.
- The Chesapeake stock is also the major contributor of legal-sized fish in the near vicinity of the Hudson River-western Long Island Sound and the New York Bight.

- Sublegal striped bass collected in the vicinity of western Long Island Sound and the New York Bight are predominately of Hudson origin.

- Striped bass overwintering in the Hudson River are predominately of Hudson origin.

- Based on expected correct classification of 76.8, 67.7, and 85.9% of the Hudson, Chesapeake, and Roanoke specimens in the oceanic collections, the relative contribution of Hudson River striped bass to the Atlantic coastal fishery is approximately 20%.

- Since this estimate is within the range where bias becomes important, techniques to reduce misclassification and/or bias in the estimates of relative contribution need to be investigated.

SECTION III

METHODS AND MATERIALS

A. COLLECTING SPECIMENS FROM SOURCE RIVERS

A prerequisite for adequate discrimination of different stocks is the collection of representative specimens that are actually members of the particular stock in question. Although young-of-the-year and yearling fish are considered to have originated from the rivers from which they are collected (Raney and deSylva, 1953:496), spawning stock collections from many year classes were used in this study since the estimation of relative contribution necessitated sampling from the ocean which is composed primarily of two-year-old and older striped bass. Collections from the source rivers were based on the spawning segment of each stock since it was assumed that mature striped bass collected on the spawning grounds of a river during the spawning season originated from that river, i.e., that striped bass like salmon and other anadromous fishes, home to their natal stream to This assumption was supported by tagging studies in which striped bass tagged on spawning grounds were recaptured on the same spawning grounds in successive years (Nichols and Miller, 1967; Mansueti, 1961).

During the spring spawning season of 1975, mature striped bass were collected from the source rivers of stocks potentially comprising the largest proportions of the Atlantic coastal fishery. The selection of source rivers within the Chesapeake Bay system and North Carolina was based on recommendations by local state agencies and documented landings in commercial catch statistics*.

* Courtesy of National Marine Fisheries Service (NMFS), Statistics and Market News Division, Washington, D.C. and Easton, Maryland

The collections were composed of 70 mature striped bass from the spawning area of the Rappahannock River, 53 from the Potomac River, 52 from the Choptank River, 57 from the Elk River and Chesapeake and Delaware Canal, 168 from the Hudson River, and 99 from the Roanoke River (Figure III-1). Despite an extensive sampling effort in the Delaware River above the entrance to the Chesapeake and Delaware Canal, only 19 sexually ripe striped bass were captured, which appears to confirm findings by Chittenden (1971) that spawning in the Delaware River is insignificant.

To assure an adequate representation of the sexes and multiple year classes in the spawning-stock collections sampling was designed to obtain nearly equal numbers of male and female striped bass and a minimum of 10 individuals in each of the following length categories: $\leq$ 399 mm, 400-549 mm, 550-699 mm, 700-849 mm, and $\geq$ 850 mm.

Collections were made primarily during April in the Chesapeake Bay tributaries and during May in the Hudson River, which are periods of peak spawning when both sexes are adequately represented. Collections were made in the Roanoke River primarily in late April when there was adequate representation of both sexes migrating toward the spawning grounds. All specimens were either purchased from commercial fishermen or netted by Texas Instruments personnel. Various types of fishing gear were used: pound nets, haul seines, and drift, stake, and anchor gill nets.

B. FIELD AND LABORATORY PROCESSING OF SOURCE-RIVER SPECIMENS

In the field, an identification tag was attached to each specimen, and liver tissue, scale samples, meristic counts, morphometric

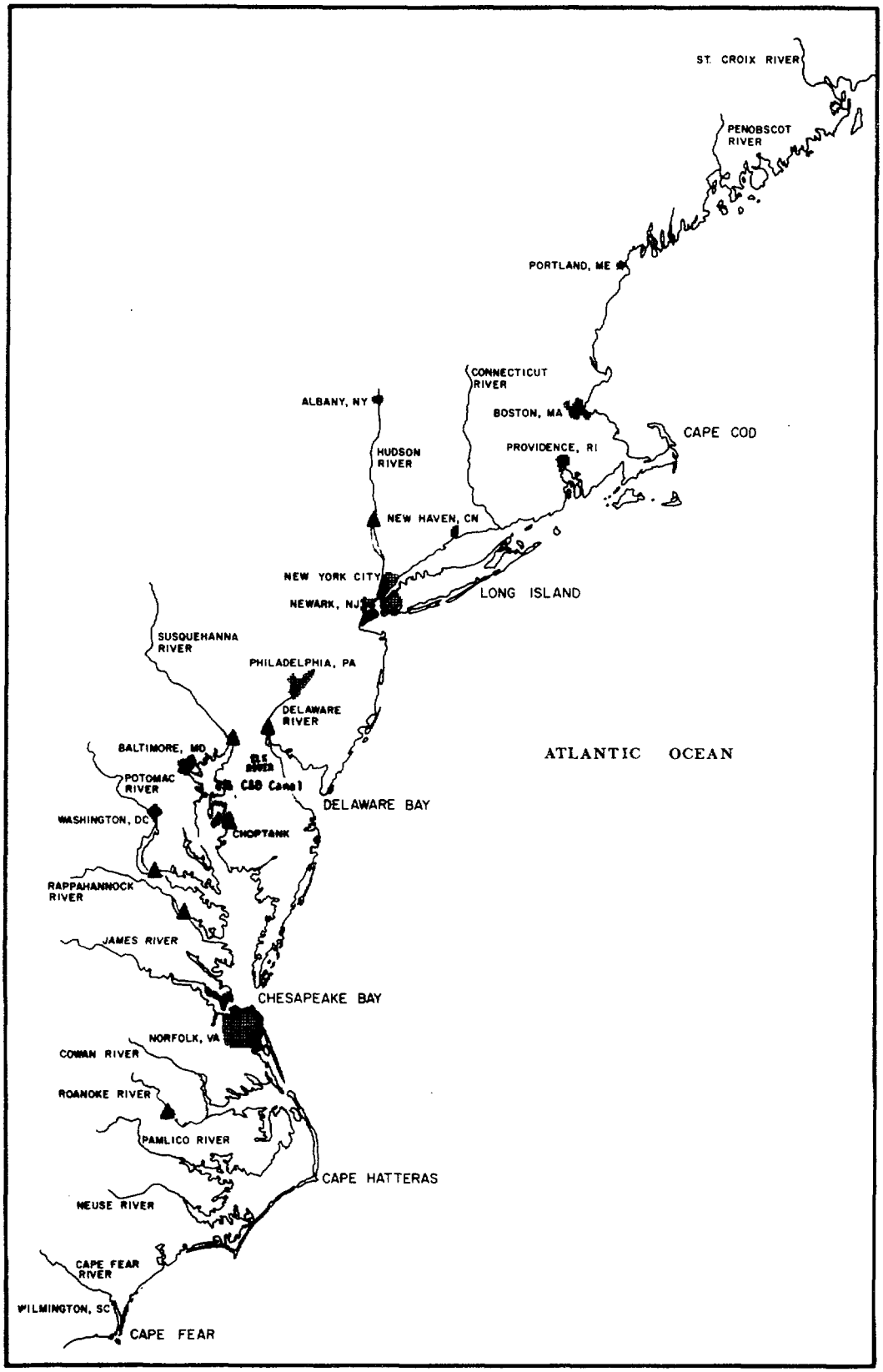

Figure III-1. Source Rivers Selected for Study. (Collection sites indicated by triangles)

measurements, sex and state of maturity (gonad development) were obtained from each fresh specimen for subsequent analysis. The liver tissues were stored in liquid nitrogen and delivered to Johns Hopkins University for biochemical analysis of two enzymes, α-glycerophosphate dehydrogenase (α-GPDH) and isocitrate dehydrogenase (IDH), using starch gel electrophoresis. The scale samples were obtained from above the lateral line between the first and second dorsal fins and were pressed on acetate cards. Ages were determined by the scale annulus method (Mansueti, 1961). Measurements from the focus to the first and second annuli were made on magnified scale images projected by a calibrated microprojector. The ten meristic and morphometric determinations were: number of lateral line scales; left pectoral ray count; right pectoral ray count; second dorsal ray count; anal ray count; upper-arm gill-raker count; fork length; snout length; head length; and internostril width. The methods used were those discussed by Hubbs and Lagler (1946) and Texas Instruments (1975). The measurements were made with dividers and a metric rule mounted on a fish measuring board.

The precision of the data collected in 1975 (Appendix C) was increased by having a second observer replicate counts, measurements, and age determinations. A set of tolerances on counts and measurements (Table III-1) was established to reduce human observation errors; when the difference between replicated observations exceeded the tolerances, the observations were rechecked by both observers and the rechecked values were added to the data set of values which were within the tolerances. The means of the replicated counts and the means of the ratios of the measurements from this "resolved" data set were used in the statistical analyses for segregation of spawning stocks, and will hereafter be referred to as the values for the character sets.

Table III-1

Tolerance Limits for Meristic, Morphometric and Scale Observations

Observation	Range (mm)	Acceptable† Variation (mm)
Fork length	≤499	± 2
	500-699	± 3
	700-899	± 4
	≥900	± 5
Snout length and internostril width	≤74	± 1
	≥75	± 2
Head length	≤149	± 1
	150-249	± 2
	≥250	± 3
All meristic counts		0
Age determinations		0
Scale ratio*		± 0.15

†Between replicate observations

* Scale ratio difference = $\frac{SA_2 - FA_2}{FA_2} - \frac{SA_1 - FA_1}{FA_1}$

where

SA_n = focus to second annulus measurement for reader n

FA_n = focus to first annulus measurement for reader n

C. ANALYTICAL PROCESSING OF SOURCE-RIVER SPECIMENS

The analytical procedures that led to the final set of characters used to discriminate the major spawning stocks in the Atlantic coastal fishery emanated from 2 years of investigation of source-river data. In a previous study; Texas Instruments (1975) demonstrated the feasibility of using biochemical, meristic, and morphometric characters as innate tags to distinguish between striped bass spawning stocks of the Hudson River and Chesapeake Bay system. In the present study, additional sampling was conducted to verify the 1974 results, to increase the number of rivers investigated to include the Roanoke, and to determine discriminant functions based on fresh specimens rather than on preserved specimens as were used in 1974. A comparison between the source-river data from the two studies is presented in Appendix B.

The choice of meristic and morphometric characters for segregation of spawning stocks of striped bass followed three stages of statistical analysis: correlation analysis between each character and fork length; analysis of the effects of sex and age on each character; and discriminant analysis. Analysis involved only those specimens with observations for all meristic counts and morphometric and scale-annulus measurements.

Following analyses on meristic and morphometric characters, enzyme characters were investigated for their discriminative potential. The gene frequencies for IDH and α-GPDH were calculated to determine if unique genotypes exist for the Hudson stock. An approach for using unique genotypes, discussed in a previous study by Texas Instruments Incorporated (1975), was used to assign striped bass, misclassified by

the discriminant functions, to their proper spawning stock.

1. Correlation Analysis

To verify that character differences among stocks were caused by racial differences and not by differences in length distributions among stocks which can result from variations in year-class strength and gear selectivity, each character was correlated with length. Characters were considered to be independent of length when variations attributable to length, i.e., coefficient of determination (r^2), in any stock were less than or equal to 0.10 ($r^2 \leq 0.10$, $r \leq 0.316$). Along with those characters that satisfied this criterion, characters that were not independent of length were used in further analyses when the distribution of the character values had small overlap among the spawning stocks. Such characters could still provide good potential for proper identification of the natal stock of the fish.

2. Sex and Age Analyses

Sex and age structure within each spawning stock were investigated with multivariate statistical analyses since discriminant procedures assumed that each stock was homogeneous in the character set. Differences in the character set among ages for males or females and between sexes within each region were tested with a procedure that combined tests of equality of means and equality of covariance matrices (Anderson, 1958). Assuming equal covariance matrices, rejection of the null hypothesis of equal distributions indicated that one or more of the character means differed among ages or between sexes. In order to find which characters differed among ages, statistical methodology called

"Simultaneous confidence intervals for double linear combinations" (Miller, 1966; Morrison, 1967) was used.

3. Discriminant Analyses

Since individual meristic and morphometric characters exhibited only small differences between striped bass stocks, simultaneous analysis of many characters with a statistical technique known as discriminant analysis was essential in order to gain adequate separation among stocks. The set of meristic and morphometric characters from individuals of each spawning stock was used to determine a discriminant function for that stock. The values of the character set for an individual were applied to each function to allocate the individual to one of the stocks. For each function, a posterior probability (Ferguson, 1967) stating the degree of certainty that the individual came from the spawning stock was determined. The individual was allocated to the spawning stock with the largest posterior probability.

Two types of discriminant functions were used: a linear discriminant function and a quadratic discriminant function (Anderson, 1958; Kendall and Stuart, 1967). The linear discriminant analysis required that the character set of spawning stocks has a multivariate normal distribution with a common covariance matrix among spawning stocks. The quadratic discriminant analysis was more general in that it did not require a common covariance matrix among spawning stocks.

Discriminant functions were determined for the Hudson, Chesapeake, and Roanoke stocks. It was not possible to segregate spawning stocks within the Chesapeake system because of the extent of overlap among the discriminant functions of its tributaries (Appendix B); therefore, the Chesapeake tributaries were analyzed as a unit. The extent of overlap among the discriminant functions of the spawning stocks provided a measure of the misclassification probabilities inherent in the functions. A measure of the effectiveness of the discriminant functions to separate stocks of striped bass was provided by the probabilities of correct classification of the spawning-stock specimens that were used to determine the discriminan functions.

Cross-validation procedures were used to independently assess the effectiveness of the discriminant functions. The spawning-stock collections were randomly divided in half and specimens from one of the halves used to obtain quadratic discriminant functions. These functions were applied to the specimens from the other half of the collection to estimate correct-classification percentages and the relative proportions of spawning stocks within the collections. These estimates of relative contribution were compared with the known proportions of spawning stocks within the collection to evaluate the ability of discriminant analysis to estimate precisely the relative contribution of spawning stocks for collections of striped bass from the fishery.

D. COLLECTION, PROCESSING, AND ANALYSIS OF ATLANTIC COASTAL SPECIMENS

Assessment of the relative contribution of various stocks of striped bass to the Atlantic coastal fishery requires a sampling scheme that

provides samples from the entire coastal fishery and considers the migratory nature of striped bass. To fulfill the above objectives, a geographically and temporally stratified sampling design was used. The geographical stratification consisted of ten strata from southern Maine to Cape Hatteras, North Carolina, with two to four substrata within each stratum to compensate for variations in the fishery composition within the stratum (Figure III-2). The Rhode Island stratum was not subdivided because of its small size. Temporally, the year was divided into six two-month periods to obtain more precise estimates of the composition of each stratum at various times of the year and to demonstrate potential changes in composition throughout the year.

Collections of striped bass from the coastal fishery were accomplished primarily by purchasing fresh fish from sport and commercial fishermen; in areas in which adequate sport and commercial fisheries did not exist, TI personnel used haul seines and gill nets to collect specimens. Collections were limited to striped bass caught during the same day (i.e., within 24 hours) to assure freshness. In many instances, the entire catch was collected from the sport and commerical fishermen; however, when sub-sampling was required due to the size of the catch, a stratified random sample proportional to the number of small (< 550 mm), medium (550-850 mm), and large (>850 mm) striped bass caught was obtained.

Oceanic specimens were processed in the same manner as the spawning stock specimens. Two replicates of 10 meristic counts and morphometric measurements were taken from each specimen, and scale samples were obtained for subsequent age and growth-rate determinations in the laboratory. A total of 2737 oceanic specimens with a complete set

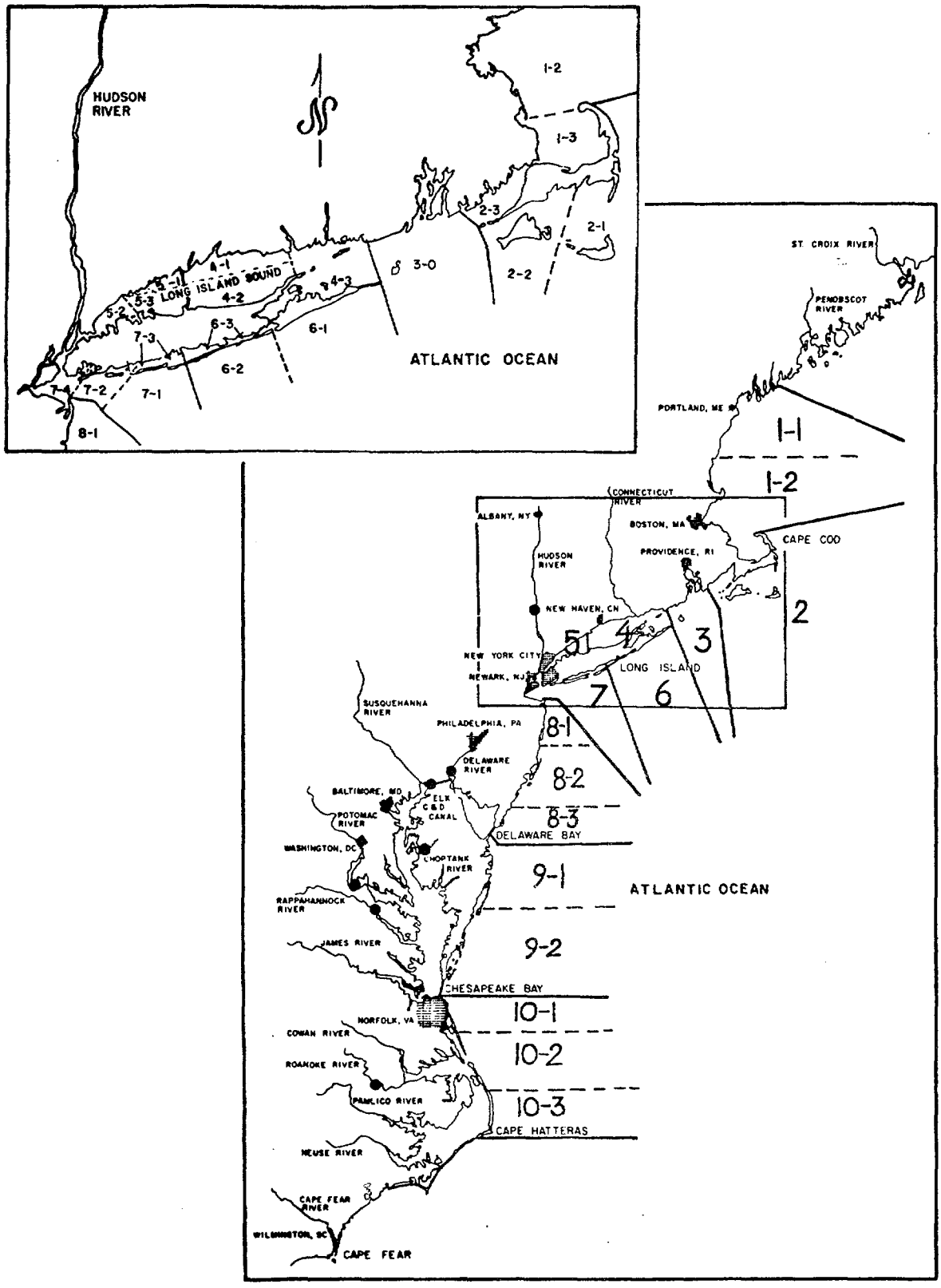

Figure III-2. Collection Regions for the Atlantic Coastal Fishery Showing Geographical Stratification and Substratification; Collection Sites for Spawning-Stock Specimens Indicated by Dots on Source Rivers.

of meristic, morphometric and scale characters was analyzed (Table III-2). Additionally, 43 specimens obtained from Chesapeake Bay during July were analyzed. Samples of liver tissue were collected, frozen in liquid nitrogen, and forwarded to Johns Hopkins University for isozyme analysis.

The relative contribution of each stock to the Atlantic coastal striped bass fishery was calculated by geographical and temporal stratum. Each specimen's character-set values were entered into the spawning-stock discriminant functions and probability statements were formulated assessing the degree of certainty with which each function would identify the origin of the specimen. Each fish was allocated to the spawning stock with the largest posterior probability. The proportion of striped bass allocated to a spawning stock from the oceanic collections in a geographical and temporal region provided an estimate of that stock's relative contribution to the striped bass fishery there. Mean estimates of relative contribution were calculated for the year by averaging the estimates over geographical strata within periods followed by averaging the mean estimates over the six periods. Mean estimates were not used for the analysis of selected age groups; instead, the number of Hudson-, Chesapeake-, and Roanoke-classified fish within three age categories (i.e., 2-4, 5, and 6+) were summed across all geographical and temporal strata before the determination of the relative contribution estimates for the selected age groups and the aggregate collection was made. Sub-legal-size (fork length < 406.5 mm) striped bass collected in New York waters by study personnel were analyzed separately from the oceanic specimens of legal size; sublegal-sized fish cannot be considered to be a part of the coastal fishery prior to their recruitment to the fishery.

Table III-2

Number of Striped Bass with Complete Character Sets Collected by Spatial Stratum and Period from Atlantic Coastal Fishery in 1975

Spatial Stratum	Locality	Legal/ Sublegal*	Period 1 Jan-Feb	2 Mar-Apr	3 May-Jun	4 Jul-Aug	5 Sep-Oct	6 Nov-Dec	Total
1	S. Maine N. Massachusetts	Legal			82	58	74		214
2	S. Massachusetts	Legal			91	90	82		263
3	Rhode Island	Legal			60	43	56		159
4	E. Long Island Sound	Legal			96		140	99	335
		Sublegal			5			1	6
5	W. Long Island Sound	Legal	1	38	14	15	89		157
		Sublegal		2	42	85	10		139
6	E. Long Island S. Shore	Legal		1	89	102	86	106	384
		Sublegal			8	17	19		44
7	W. Long Island S. Shore	Legal		30	58	93	120	124	425
		Sublegal		4	11				15
8	New Jersey	Legal		34	113	28	73	117	365
9	Delaware-Maryland-N. Virginia	Legal		71	3		6	100	180
10	S. Virginia-N. Carolina	Legal	27					24	51
Total			28	180	672	531	755	571	2737

* Sublegal (fork length < 406.5mm) striped bass from New York waters (strata 4, 5, 6, and 7) are analyzed separately.

Table III-3

Estimates of Averaged 1973 and 1974 Proportions of Periodic Commercial Poundage Landed Within Each Stratum and of Yearly Poundage Landed Within Each Period*

NMFS Region	Stratum	Period Jan-Feb (1)	Mar-Apr (2)	May-Jun (3)	Jul-Aug (4)	Sep-Oct (5)	Nov-Dec (6)	Regional Proportion of Yearly Poundage
New England	1	0	0	0.061	0.119	0.053	0	
	2	0	0	0.023	0.001	0.004	0	
	3	0	0.040	0.240	0.194	0.242	0.049	0.144
	4	0	0.027	0.297	0.144	0.305	0.112	
	5†	---	---	---	---	---	---	
Mid-Atlantic	6	0.003	0.043	0.218	0.145	0.249	0.124	
	7	0.004	0.033	0.105	0.350	0.097	0.038	
	8	0.005	0.035	0.055	0.045	0.045	0.373	0.529
Chesapeake (coastal)	9	0.229	0.461	0.001	0.001	0.002	0.180	0.135
South Atlantic ‡	10	0.759	0.361	0	0	0.002	0.124	0.191
Periodic proportion of yearly poundage		0.159	0.064	0.107	0.096	0.194	0.379	0.999

* Mean weighing factors derived in Appendix G.

† No commercial fishery exists in stratum 5.

‡ Region north of Cape Hatteras, North Carolina, to Chesapeake Bay Inlet in Virginia

To provide an estimate of the stock composition for the 1975 commercial landings, estimated stock proportions from each geographical and temporal stratum were weighed by the corresponding averaged 1973 and 1974 proportion of the periodic commercial poundage landed within the stratum and by the averaged 1973 and 1974 proportion of the yearly poundage landed within the period (Table III-3). The weighting factors were calculated (Appendix G) using 1973 and 1974 striped bass commercial-catch statistics by state, county, and body of water.* Only poundage from coastal waters was used to insure proper weighting of contribution estimates of coastal strata. New York and New Jersey landings comprised over 50 percent of the annual commercial poundage landed in coastal waters from southern Maine to Cape Hatteras, North Carolina. However, the coastal landings of 4,645,060 lbs (1973) and 3,702,670 lbs (1974) were 37.1% (1973) and 38.1% (1974) of total landings from Massachusetts to North Carolina including Delaware Bay, Chesapeake Bay, Albemarle Sound, Pamlico Sound and coastal waters of North Carolina souh of Cape Hatteras. No weighting factors were available for the western half of Long Island Sound (stratum 5) since in that area no commercial fishery existed, therefore, that area's stock composition was not used. Elsewhere, the stock proportions were based on the striped bass collected from both the sport and commercial fisheries.

An estimate of the proportion of Hudson, Chesapeake, and Roanoke stocks in the NMFS regional commercial landings during each two-month interval was calculated by multiplying the commercial weighting factor and stock proportions within each stratum and summing the results within each NMFS region for each period. An estimate of relative contribution

*Courtesy NMFS, Statistics and Market News Division, Washington, D.C. and Easton, Maryland.

Table III-3

Estimates of Averaged 1973 and 1974 Proportions of Periodic Commercial Poundage Landed Within Each Stratum and of Yearly Poundage Landed Within Each Period*

NMFS Region	Stratum	Period Jan-Feb (1)	Mar-Apr (2)	May-Jun (3)	Jul-Aug (4)	Sep-Oct (5)	Nov-Dec (6)	Regional Proportion of Yearly Poundage
New England	1	0	0	0.061	0.119	0.053	0	
	2	0	0	0.023	0.001	0.004	0	
	3	0	0.040	0.240	0.194	0.242	0.049	0.144
Mid - Atlantic	4	0	0.027	0.297	0.144	0.305	0.112	
	5†	---	---	---	---	---	---	
	6	0.003	0.043	0.218	0.145	0.249	0.124	
	7	0.004	0.033	0.105	0.350	0.097	0.038	
	8	0.005	0.035	0.055	0.045	0.045	0.373	0.529
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Periodic proportion of yearly poundage		0.159	0.064	0.107	0.096	0.194	0.379	0.999

* Mean weighing factors derived in Appendix G.

† No commercial fishery exists in stratum 5.

‡ Region north of Cape Hatteras, North Carolina, to Chesapeake Bay Inlet in Virginia.

for the period was calculated by summing the weighted proportions of each stock for the four NMFS regions, and dividing by the sum of weighting factors with corresponding stock proportions (i.e., proportion of landings investigated, which standardized the relative percentages in terms of 100%). This standardization was required because the sum of weighting factors with corresponding classification percentages in various time periods was less than unity due to lack of collections in every stratum with an associated weighting factor. The stock composition of the commercial landings for the year was estimated by multiplying the periodic weighting factors with the associated weighted stock proportions, then summing the results across periods.

The relative contribution of Hudson, Chesapeake, and Roanoke stocks was assessed for the "inner" and "outer" zones designated by the U.S. Nuclear Regulatory Commission (1975). The inner zone encompassed western Long Island Sound (TI stratum 5), the New York Bight (TI stratum 7), and northern New Jersey (TI stratum 8, substratum 1); whereas the outer zone encompassed the remaining waters from Cape May, New Jersey to Maine (TI strata 1, 2, 3, 4, 6, and 8, substrata 2 and 3). There was a minor difference between the eastern boundary of the inner zone at Moriches Inlet and the boundary of stratum 7 at Davis Park, Fire Island; however, stratum 6 west of Moriches Inlet yielded few fish. Estimates of relative contribution for the inner and outer zones were calculated for each period by summing the number of Hudson-, Chesapeake-, and Roanoke- classified fish within the appropriate TI strata. Estimates were also obtained for three age groups (i.e., 2-4, 5, and 6+) of striped bass. Mean estimates of contribution within each zone were calculated for the year by averaging the estimates of the periodic contribution.

E. COLLECTION, PROCESSING, AND ANALYSIS OF OVERWINTERING HUDSON RIVER STRIPED BASS

Striped bass overwintering in the Hudson River were collected and the percentage contribution of the major coastal stocks to this population was estimated. Nylon multifilament gill nets with stretch meshes of 5 and 6 in. were used to collect 79 striped bass from Croton Bay on the Hudson River from 6 December 1974 through 20 March 1975. The fish collected were processed and the data analyzed in the same manner as for specimens from the oceanic collections.

SECTION IV

RESULTS AND DISCUSSION

A. SOURCE-RIVER SPECIMENS

1. Correlation Analysis

The five characters (Table IV-1) established as the best potential character set for discriminating among the Hudson, Chesapeake, and Roanoke spawning stocks (Appendix B) satisfied the criterion for lack of correlation with fish length ($r \leq 0.316$ and $r^2 \leq 0.100$), in each region, with one exception (Table IV-2). The snout length/internostril width ratio for the Roanoke River stock had a coefficient of determination of nearly 0.20 but was nevertheless retained because it had the least population overlap of all characters, thus making it a potentially good discriminator (Table IV-3).

2. Age and Sex Analysis

The spawning stock from the Hudson River was found to be homogeneous in the discriminative characters among ages and between males and females, but significant differences were found in the characters among ages and between sexes in the Chesapeake stock and among ages in the Roanoke stock. The combined test of equality of means and covariance matrices supported the assumption of a homogeneous Hudson River population but revealed differences among ages for Chesapeake and Roanoke males and females (Table IV-4). Tests for equal covariance matrices among ages for Chesapeake and Roanoke males and females detected only one significant difference (Table IV-5). Ninety-five percent simultaneous confidence

Table IV-1

Variables Used in Discriminant Function Analysis
to Classify Striped Bass from Atlantic Fishery

No.	Code	Character Description
1	LL	Later line scale count
2	CI	Character index* = sum of rays on left pectoral, right pectoral, second dorsal, and anal fins
3	UGR	Upper arm gill raker count including redimentary rakers
4	SA/FA	First to second annulus/focus to first annulus measure ratio
5	SNT/NOS	Snout length/internostril width ratio

* Raney and deSylva (1953)

Table IV-2

Correlation of Final Characters with Fish Length

Locality (region)	Sample Size	Character 1 (LL)	2 (CI)	3 (UGR)	4 (SA/FA)	5 (SNT/NOS)
Roanoke	99	0.03563	0.01862	0.13959	0.15514	− 0.43805*
Chesapeake	232	0.06578	− 0.09853	0.03421	0.10341	− 0.16912
Hudson	168	− 0.28979	0.08543	0.15854	0.00955	0.20474

* Exceeds the criterion: $r^2 \leq 0.10$, $r \leq 0.316$

Table IV-3

Mean and Standard Deviation of Snout Length/Internostril Width Ratio for Hudson, Chesapeake, and Roanoke Stocks

Stock	Sample Size	Mean	Standard Deviation
Hudson	168	1.616	0.064
Chesapeake	232	1.701	0.068
Roanoke	99	1.825	0.062

Table IV-4

Test for Equality of Means and Covariance Matrices Among Ages for Source-River Specimens

Stock	Sex	Ages†	Degrees of Freedom	χ^2- Value
Hudson	Males	4, 5, 6, 10	60	62.82
Hudson	Females	9, 10, 11, 12	60	54.71
Chesapeake	Males	3, 4, 5	40	72.11*
Chesapeake	Females	5, 6, 8, 9	60	90.36*
Roanoke	Males	3, 4	14‡	33.82*
Roanoke	Females	4, 5	20	37.10*

†Sample size >10.

‡Upper gill raker excluded because its constant value in 4-year-old Roanoke males prevented the test from being used.

*Significant at $\alpha = 0.05$.

Table IV-5

Test for Equality of Covariance Matrices Among Ages for Source-River Specimens

Stock	Sex	Ages†	Degrees of Freedom	χ^2- Value
Chesapeake	Males	3, 4, 5	30	33.02
Chesapeake	Females	5, 6, 8, 9	45	59.69
Roanoke	Males	3, 4	10†	17.88
Roanoke	Females	4, 5	15	25.40*

†Upper gill raker excluded because its constant value in 4-year-old Roanoke males prevented the test from being used.

*Significant at $\alpha = 0.05$.

intervals for differences in means were formed to find which characters differed among ages for Chesapeake males and females and Roanoke males. Out of 54 intervals, only the scale-ratio comparison between 3- and 4-year-old Roanoke males was found to be significantly different from zero. However, additional significant differences may have existed since the confidence intervals for double linear combinations are somewhat longer than simultaneous confidence intervals on pairwise contrasts (Miller, 1966). Differences in the character set between males and females were found only in the Chesapeake system (Table IV-6). The differences found in the Chesapeake spawning stock may have resulted from the pooling of collections from its four major tributaries. However, Texas Instruments Incorporated (1975) found that this pooling was necessary for adequate discrimination between Hudson and Chesapeake stocks. Although differences were found and can be partially explained, their effect on the ability to distinguish among stocks needed assessment (see results of cross-validation study in Appendix A).

Table IV-6

Test for Equality of Means and Covariance Matrices between Males and Females for Source-River Specimens

Stock	Degrees of Freedom	X^2-Value
Hudson	20	21.76
Chesapeake	20	33.99*
Roanoke	20	27.40

* Significant at $\alpha = 0.05$.

3. Discriminant Analyses

The correct classification percentages of spawning stocks using quadratic discriminant functions closely agreed with results obtained by Raney and deSylva (1953) and Raney et al. (1954). The quadratic approach was used since the assumption of equal covariance matrices among stocks needed for linear discriminant analysis was not met ($\alpha = 0.05$). Correct classification of 76.8, 67.7, and 85.9% for the Hudson, Chesapeake, and Roanoke spawning stocks respectively were obtained, resulting in an overall correct classification of 74.4%. Most of the misclassifications occurred between the Hudson and Chesapeake stocks, as indicated by overlaps of 24.06, 4.44, and 10.94% obtained for the Hudson-Chesapeake, Hudson-Roanoke, and Chesapeake-Roanoke spawning-stock pairs respectively.

Cross-validation procedures, used to independently measure the effectiveness of the discriminant functions, indicated close agreement with classification results obtained from total collections (Table IV-7).

Table IV-7

Correct Classification Percentages of Collection Sets of Spawning Stocks Based on Quadratic Discriminant Functions

	Spawning Stock			
Collection Set	Hudson	Chesapeake	Roanoke	Overall
Function set†	81.0	69.8	87.8	75.9
Independent set ‡	72.6	68.1	86.0	73.2
Total set ǂ	76.8	67.7	85.9	74.4

† Randomly sampled half of total spawning-stock collections used to determine quadratic functions for cross-validation.

‡ Remaining half of spawning-stock collections classified by quadratic functions obtained from function set.

ǂ All specimens from spawning-stock collections; percentages obtained from quadratic functions based on total set.

Quadratic discriminant functions determined from the randomly sampled specimens (Appendix D, Quadratic Equations 7-9), correctly classified 73.2% of the remaining specimens in the collections, which was near the 75.9% correct classification of the specimens used to determine these functions and the 74.4% correct classification of the total collections.

Comparisons between estimates of spawning-stock proportions and the known proportions in the collection sets indicated that misclassification percentages tended to cancel, thus providing accurate estimates of spawning-stock proportions (Table IV-8). Fukuhara et al. (1962) discussed bias in relative-contribution estimates and suggested methodology for correction. These investigators stated that misclassification did, in fact, tend to cancel when the mixture of stocks in the coastal fishery contained equal proportions of specimens from the stocks but that bias increased substantially as spawning-stock contributions became more disproportionate. Results of the comparisons between estimated and actual relative proportions from spawning-stock collections were based on proportions of 0.34 (Hudson), 0.46 (Chesapeake), and 0.20 (Roanoke) in the total spawning-stock sample. In collections from the coastal fishery should any stock contribute a large majority (e.g., 80%) in a stratum, the bias in relative contribution estimates might be larger than the bias indicated in Table IV-8.

Table IV-8

Comparison of Estimated Relative Proportions with Known Relative Proportions of Spawning Stocks within Collection Sets Based on Quadratic Discriminant Functions

Collection Set	Type of Proportion	Spawning Stock		
		Hudson	Chesapeake	Roanoke
Function set †	Known	33.7	46.6	19.7
	Estimated	36.6	40.2	23.3
Independent set ‡	Known	33.6	46.4	20.0
	Estimated	35.2	42.4	22.4
Total set ǂ	Known	33.7	46.5	19.8
	Estimated	36.9	40.3	22.9

† Randomly sampled half of total spawning-stock collections used to determine quadratic functions for cross-validation.

‡ Remaining half of spawning-stock collections classified by quadratic functions obtained from function set.

ǂ All specimens from spawning-stock collections; percentages obtained from quadratic functions based on total set.

4. Enzyme Analysis

The gene frequencies of isocitrate dehydrogenase (IDH) and α-glycerophosphate dehydrogenase (α-GPDH) indicated a low level of variation occurring among Hudson, Chesapeake, and Roanoke stocks on a north to south cline with the exception of collections from the Hudson River after May 23, 1975 (Table IV-9). Striped bass collected in the Hudson River through 23 May had an allelic frequency for IDH and α-GPDH similar to that of the 1974 collections from the Hudson spawning stock and collections of striped bass overwintering in the Hudson River. However, after 23 May, allelic frequencies for both IDH and α-GPDH within the Hudson spawning stock were similar to the southern spawning stocks, especially that of the Roanoke. Specimens collected from the Hudson River after 23 May and from the Roanoke River possessed the A allele of IDH.

Table IV-9

Gene Frequencies for Each Allele of α-GPDH and IDH within Hudson, Chesapeake, and Roanoke Spawning Stocks

Region/Date	Sample Size	Gene Frequency α-GPDH A	α-GPDH B	IDH A	IDH B	IDH C
Hudson						
1974	192	0.12	0.88		1.00	
Dec 1974-Mar 1975	71	0.12	0.88		1.00	
May 14-23/75	73	0.10	0.90		1.00	
May 24-Jun 5/75	44	0.03	0.97	0.08	0.91	0.01
Chesapeake						
1974	688	0.04	0.96		0.96	0.04
1975	189	0.03	0.97		0.97	0.03
Roanoke						
1975	92	0.01	0.99	0.03	0.95	0.02

The changes in allelic frequencies of IDH and α-GPDH in the spawning-stock collections from the Hudson River after 23 May indicated that these collections included individuals from either (a) a southern spawning stock or (b) a unique late-breeding segment of the Hudson stock. The hypothesis that southern migrants entered the Hudson River during the latter portion of the spawning run was plausible for two reasons. First, the 44 striped bass which were analyzed for IDH and α-GPDH from the collections made in the Hudson River following 23 May represented less than 12 percent of all specimens that were collected from the Hudson River analyzed for IDH and α-GPDH. This small sample could easily be influenced by an influx of southern migrants. The remaining 88 percent, which included striped bass from the 1974 and early 1975 spawning runs and from the overwintering population (demonstrated in Appendix C to be primarily of Hudson River origin using meristic and morphometric characters), were genetically representative of a single stock. Second, multivariate analysis (Anderson 1958:101-125) indicated significant differences ($\alpha = 0.05$) in the mean values of the meristic and morphometric characters between the early (through 23 May) and late (after 23 May) segments of the spawning run in the Hudson River, thus supporting the hypothesis for southern migrants. However, when discriminant functions were determined for specimens collected before 23 May and applied to specimens collected after 23 May, all fish except two were classified as they had been by the discriminant functions based on all Hudson collections. Therefore, the early-and late-spawning striped bass collected from the Hudson River were similar enough in their values of the meristic and morphometric characters to be classified as a single stock. An alternative hypothesis of a unique "late-breeding" segment of the Hudson stock which appears similar to the major segment in its mean values for the

meristic and morphometric characters but different in its allelic frequencies for the two enzymes was equally plausible.

Based on meristic and morphometric characters, the difference between early- and late-spawning fish in the Hudson River was not pronounced. The correct classification percentages of the discriminant functions based on early Hudson specimens were similar to those based on all Hudson specimens (Table IV-10). Likewise, both sets of functions provided similar classification results for oceanic collections (Appendix Table E-1).

Table IV-10

Correct Classification Percentages of Hudson, Chesapeake, and Roanoke Spawning-Stock Specimens with 1975 Character Set

Type of Analysis*	Correct Classification Percentage		
	Hudson	Chesapeake	Roanoke
QBH	75.9	63.4	85.9
QTH	76.8	67.7	85.9

* QBH designates quadratic discriminant analysis with early (through 23 May 1975) Hudson, Chesapeake, and Roanoke spawning-stock specimens. QTH designates quadratic discriminant analysis with total Hudson, Chesapeake, and Roanoke spawning-stock specimens.

An increase of approximately 2% in the overall correct classification percentages was possible with the assumption that IDH was fixed at the B allele in the Hudson River (hypothesis A). Cheaspeake and Roanoke spawning-stock specimens with the C allele of IDH that were misclassified as Hudson in origin could be reclassified as "non-Hudson"; likewise, Roanoke spawning-stock specimens with the A allele of IDH that were misclassified as Chesapeake or Hudson in origin could be reclassified as "non-Hudson-non-Chesapeake." Assigning these misclassified specimens to the spawning stock from which they were collected resulted in 2% increase in correct classification for 1974 and 1975 collections. If IDH were not fixed at the B allele in the Hudson River (hypothesis B), then there would be no change in the correct classification percentages for the spawning-stock collections.

B. ATLANTIC COASTAL SPECIMENS

1. Contribution Estimates for TI Strata

a. Classification of Legal Fish

Relative-contribution estimates indicated that Chesapeake stock predominated in 34 of the 35 spatial and temporal strata shown in Table IV-11. The Hudson River stock contribution to western Long Island Sound and the New York Bight ranged from 21.0 to 52.6% during the year but elsewhere from 7.8 to 30.2%. When the geographical strata percentages were averaged, only minor changes in the contribution of each stock occurred throughout the year (Table IV-12). The Hudson stock ranged from 16.7 to 27.0%, Chesapeake from 63.0 to 71.4%, and Roanoke from 7.9 to 15.0%. The estimated yearly average for 1975 within the

Table IV-11

Estimates of Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to 1975 Oceanic Collections by Period and Spatial Stratum*

Period	Stratum	Sample Size †	Classification Percentage ‡ Hudson	Chesapeake	Roanoke
Jan-Feb (1)	10	27	25.9	63.0	11.1
Mar-Apr (2)	5	38	52.6	42.1	5.3
	7	30	23.3	73.3	3.3
	8	34	23.5	67.6	8.8
	9	71	8.5	77.5	14.1
May-Jun (3)	1	82	11.0	68.3	20.7
	2	91	14.3	71.4	14.3
	3	60	30.0	60.0	10.0
	4	96	21.9	69.8	8.3
	5	14	35.7	57.1	7.1
	6	89	25.8	65.2	9.0
	7	58	41.4	51.7	6.9
	8	113	23.9	67.3	8.8
Jul-Aug (4)	1	58	19.0	67.2	13.8
	2	90	7.8	72.2	20.0
	3	43	30.2	65.1	4.7
	5	15	26.7	66.7	6.7
	6	102	22.5	63.7	13.7
	7	93	33.3	65.6	1.1
	8	28	21.4	71.4	7.1
Sep-Oct (5)	1	74	13.5	77.0	9.5
	2	82	12.2	58.5	29.3
	3	56	25.0	58.9	16.1
	4	140	16.4	64.3	19.3
	5	89	41.6	46.1	12.4
	6	86	15.1	73.3	11.6
	7	120	23.3	63.3	13.3
	8	73	16.4	76.7	6.8
	9	6	16.7	66.7	16.7
Nov-Dec (6)	4	99	21.2	66.7	12.1
	6	106	16.0	69.8	14.2
	7	124	21.0	76.6	2.4
	8	117	21.4	72.6	6.0
	9	100	8.0	80.0	12.0
	10	24	12.5	62.5	25.0

* Not included are striped bass < 407-mm fork length from New York waters.

† Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum 5 (1 Chesapeake); period 2, stratum 6 (1 Chesapeake); period 3, stratum 9 (2 Chesapeake and 1 Roanoke).

‡ Classified with quadratic (QTH) functions.

Table IV-12

Estimate of Mean Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to 1975 Oceanic Collections by Period and Overall Year*

Period	Sample Size†	No. of Stratum Averaged	Mean Classification Percentage‡ Hudson	Chesapeake	Roanoke
1	27	1	25.9	63.0	11.1
2	173	4	27.0	65.1	7.9
3	603	8	25.5	63.8	10.6
4	429	7	23.0	67.4	9.6
5	726	9	20.0	65.0	15.0
6	570	6	16.7	71.4	12.0
Overall average‡‡			23.0	66.0	11.0

* Mean is average of spatial strata classification percentages within periods. Not considered are striped bass <406.5-mm fork length from New York waters.

† Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum (1 Chesapeake); period 2, stratum 6 (1 Chesapeake); period 3, stratum 9 (2 Chesapeake and 1 Roanoke).

‡ Classified with quadratic (QTH) functions.

‡‡ Average of the six periodic mean classification percentages.

Atlantic coastal fishery was Hudson, 23.0%; Chesapeake, 66.0%; and Roanoke, 11.0%.

Observations of the migratory nature and seasonal distribution of striped bass in this study were similar to those by Schaefer (1968a), Koo (1970), and other investigators: a southern range during winter and early spring (periods 1, 2, and 6) and a northern range during late spring, summer, and fall (periods 3, 4, and 5). Hudson River tag recapture data (Appendix A) showed a northern migration and no recaptures south of northern New Jersey. However, classification results indicated that fractions of the Hudson River stock may have migrated south during late fall and winter. Clark (1968) discussed the possibility of the existence of a Hudson contingent that spends the winter and summer with the large migrating Atlantic population but moves into the river in spring for spawning.

One must be cautious in considering the significance of the Hudson-classified fish collected off Cape Hatteras, North Carolina in February and December 1975 because of the small sample size involved and the age structure (age 8+) of the sample. The discriminant function for the Roanoke spawning stock was derived from fish (all but two) having a fork length of <800 mm; however, all but two specimens collected from Cape Hatteras were >800 mm. Since snout length/internostril width (the best discriminatory character) for the Roanoke stock had a negative correlation with length, there was a potential for misclassification of large Roanoke fish as Hudson fish (Table IV-2). Results from other collections in southern waters have indicated a much lower Hudson contribution. In collections from Maryland's and Virginia's coastal

waters, only 8.0-8.5% of the March and December specimens were classified as Hudson in origin. However, these specimens were younger than the Cape Hatteras specimens. A more diverse age structure (3 to 11 years old, with 37.9% of the fish age 6+) appeared in a collection from upper Chesapeake Bay during July 1975; of 29 specimens, the percentage contribution was Hudson, 3.4%; Chesapeake, 75.9%, and Roanoke, 20.7%. Additionally, adult striped bass tagged in the Hudson River during the spawning season have never been recaptured south of northern New Jersey Appendix A). Therefore, until tagged striped bass from the Hudson River are recaptured in southern waters, caution must be taken in interpreting these low Hudson contributions. A logical explanation is that these specimens were part of the misclassification errors inherent in the discriminant functions (Table IV-10).

Aside from demonstrating that the Chesapeake stock was the major contributor to the Atlantic coastal fishery in 1975, this study has provided additional information, upon pooling the specimens from all periods and strata and analyzing selected age groups, on the importance of dominant year classes of striped bass. Part of the rationale used by investigators of striped bass migratory behavior in concluding that the Chesapeake Bay system was the major contributor to the Atlantic coastal fishery was the relationship between good production of young-of-the-year fish in Chesapeake Bay and the occurrence of dominant year classes of striped bass in the northern waters two and more years later (see Section I). Schaefer (1972) stated that the production of young-of-the-year striped bass in Chesapeake Bay during 1970 was the largest ever recorded and that if survival remains high and migration follows normal patterns, then this year class should provide excellent fishing in New York waters for 6 to 8 years after

recruitment. This appears to have occurred since approximately 52% of the specimens collected from the coastal fishery in 1975 were from the 1970 year class, and 77% of this year class were classified with the discriminant functions as Chesapeake fish (Table IV-13). The presence of this dominant year class of Chesapeake fish confirms the rationale of a predominance of the Chesapeake stock in the coastal fishery. A summary of the occurrence of dominant year classes in the Atlantic coastal fishery has been given by Schaefer (1968a:38-40).

The proportion of males in the 1975 collections was only 14.4%; the male proportion of the Hudson stock was nearly double that of the Chesapeake and Roanoke stocks in all age groups analyzed (Table IV-13). Studies by Merriman (1941) and Schaefer (1968b) indicated that the proportion of male striped bass among the fish that migrate northward is low. TI's study confirmed these findings and additionally indicated that relatively fewer males migrated from Chesapeake Bay and Albemarle Sound than from the Hudson River.

The estimated proportion of males decreased with age in the combined 1975 oceanic collections as well as within each spawning stock (Table IV-13). A chi-square test (Maxwell, 1961) found trends in the overall male proportions with age to be significant ($\alpha = 0.05$). Similar results occurred when this technique was applied to the data in Schaefer (1968b). Also, Merriman (1941) demonstrated a decrease in male abundance from age 2 to age 3 and older. In summary, there are fewer males than females among the older age groups in the Atlantic coastal fishery. However, more of the older males are classified into the Hudson River spawning stock than into the spawning stocks of the Chesapeake

Table IV-13

Estimates by Selected Age Groups of Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to 1975 Oceanic Collections and Proportion of Male Fish in Overall Collections and in Each Stock*

Age Group	Sample Size	Overall Male Proportion	Hudson		Chesapeake		Roanoke	
			Classification Percentage†	Proportion of Males	Classification Percentage†	Proportion of Males	Classification Percentage†	Proportion of Males
All	2533	0.144	21.0	0.231	67.2	0.124	11.8	0.101
2-4	449	0.238	20.0	0.522	60.6	0.176	19.4	0.138
5	1328	0.146	14.7	0.224	76.6	0.135	8.7	0.112
6+	756	0.083	32.7	0.130	54.8	0.063	12.6	0.053

*Not included are striped bass 406.5-mm fork length from New York waters.

†Classified with quadratic (QTH) functions.

or Roanoke systems.

b. Enzyme Influence of Contribution Estimates for Hudson River Stock

Because of the two hypothesis discussed previously (p. IV-8), there are two approaches to using the enzyme data to reassign striped bass misclassified by the discriminant functions to their proper stock. The reclassification of striped bass specimens from oceanic collections using hypothesis A resulted in a decrease in the relative proportion of specimens classified as Hudson stock; the use of hypothesis B resulted in an increase in the estimated relative proportion of Hudson stock specimens (Table IV-14). Under hypothesis A, specimens from the coastal waters with an A or C allele were "non-Hudson"; also those specimens with an A allele were "non-Chesapeake" since the A allele did not occur in spawning-stock specimens from the Chesapeake Bay system. Striped bass classified as Hudson or Chesapeake fishes which violated these assumptions were reclassified. As a result, the use of hypothesis A decreased the relative percentages of specimens classified into the Hudson and Chesapeake stocks and increased that of the Roanoke stock (or that of another southern stock not investigated in this study). Under hypothesis B, specimens from the coastal waters with an A or C allele were potentially Hudson; however, those specimens with an A allele were still "non-Chesapeake". The majority of those specimens with an A allele classified into the Chesapeake stock may be of Hudson origin for two reasons. First, the A allele occurred in 16 percent of the Hudson spawning-stock specimens in the 1975 spawning runs. Second, the overlap between the Hudson and Chesapeake spawning stocks was double that between the Roanoke and Chesapeake spawning stocks. Therefore, all specimens from the coastal waters with an A allele classified as Chesapeake fishes were reclassified

Table IV-14

Changes by Period and Spatial Stratum in Relative Contribution of Legal Hudson River Stock to 1975 Oceanic Collections Using IDH Assumptions A and B†

Period	Stratum	Sample Size‡	Classification Percentages‡ Case A	QTH#	Case B
1	10	27	25.9	25.9	29.6
2	5	38	50.0	52.6	52.6
	7	30	23.3	23.3	23.3
	8	34	23.5	23.5	26.5
	9	71	8.5	8.5	8.5
3	1	82	6.1	11.0	14.6*
	2	91	12.1	14.3	14.3
	3	60	28.3	30.0	33.3*
	4	96	19.8	21.9	27.1*
	5	14	35.7	35.7	42.9*
	6	89	23.6	25.8	27.0
	7	58	37.9	41.4	43.1*
	8	113	23.0	23.9	24.8
4	1	58	17.2	19.0	20.7
	2	90	5.6	7.8	15.6**
	3	43	23.3	30.2	39.5**
	5	15	26.7	26.7	26.7
	6	102	18.6	22.5	32.4**
	7	93	33.3	33.3	37.6
	8	28	21.4	21.4	32.1**
5	1	74	12.2	13.5	16.2
	2	82	12.2	12.2	12.2
	3	56	25.0	25.0	25.0
	4	140	15.7	16.4	18.6
	5	89	38.2	41.6	41.6
	6	86	15.1	15.1	15.1
	7	120	23.3	23.3	25.0
	8	73	15.1	16.4	16.4
	9	6	16.7	16.7	16.7
6	4	99	21.2	21.2	21.2
	6	106	15.1	16.0	16.0
	7	124	20.2	21.0	21.8
	8	117	20.5	21.4	23.1
	9	100	8.0	8.0	8.0
	10	24	12.5	12.5	12.5

†Not included are striped bass <406.5-mm fork length from New York waters.

‡Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum 5 (1 Chesapeake); period 2, stratum 2, stratum 6 (1 Chesapeake); period 3, stratum 9 (2 Chesapeak and 1 Roanoke).

‡Case A assumes IDH classification of AA, AB, BC, or CC to be non-Hudson; case B assumes IDH classification of AA, BB, BC, or CC to be Hudson and AA or AB misclassified as Chesapeake to be Hudson.

#Classified with quadratic (QTH) functions.

*Indicates variation between cases A and B of >5%.

**Indicates variation between cases A and B of >10%.

to be of Hudson origin. As a result, the use of hypothesis B increased the relative percentage of specimens classified into the Hudson stock without influencing that of the Roanoke stock.

Although the relative proportion of specimens classified into the Hudson stock using hypothesis A or B differed from the original as-classified estimate, the difference on the average was slight. The yearly estimates of Hudson contribution, obtained by averaging strata within periods then averaging the periods, were 22.0 percent for hypothesis A, 23.0 percent as-classified, and 25.6 percent for hypothesis B (Table IV-15). Although differences between 10 and 16 percentage points (Table IV-14) among the as-classified estimates using hypotheses A and B occurred during the summer for Massachusetts (stratum 2), Rhode Island (stratum 3), eastern Long Island (stratum 6), and New Jersey (stratum 8), differences of this magnitude occurred in less than 12 percent of temporal and geographical strata investigated.

c. Classification of Sublegal Fish

Relative-contribution estimates of sublegal striped bass (fork length <406.5 mm) captured in New York waters indicated that Hudson contribution was greatest in strata 5 and 7 and that Chesapeake and Roanoke contributions equalled or dominated the Hudson in strata 4 and 6 in all time periods (Table IV-16). However, the effort expended in capturing sublegal specimens in strata 4, 6, and 7 was minimal. The large May-October contribution to strata 4 and 6 of age 2 Roanoke fish (85.7% of all sublegal specimens classified as Roanoke stock) must be viewed cautiously. A collection of 13 striped bass from the Rappahannock River during July 1975 contained one age 5 Chesapeake fish and 12 age 2

Table IV-15

Estimate of Changes by Period and Overall Year in Mean Relative Contribution of Legal Hudson River Stock to 1975 Oceanic Collections Using IDH Assumptions A and B†

Period	Sample Size‡	No. of Strata Averaged	Mean Classification Percentage‡‡ Case A	QTH	Case B
1	27	1	25.9	25.9	29.6
2	173	4	26.3	27.0	28.4
3	603	8	23.3	25.5	28.4*
4	429	7	20.9	23.0	29.2*
5	726	9	19.3	20.0	20.8
6	570	6	16.2	16.7	17.1
Overall Average#	2528		22.0	23.0	25.6

†Mean is average of spatial strata classification percentages within periods. Not considered are striped bass <406.5-mm fork length from New York waters.

‡Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum 5 (1 Chesapeake); period 2, stratum 6 (1 Chesapeake); period 3, stratum 9 (2 Chesapeake and 1 Roanoke).

‡‡Case A assumes IDH classification of AA, BB, BC, or CC to be non-Hudson; case B assumes IDH classification of AA, AB, BC, or CC to be Hudson and AA or AB misclassified as Chesapeake to be Hudson. QTH indicates classification with quadratic functions.

#Average of the six periodic mean classification percentages.

*Indicates variation between cases A and B of 5%.

Table IV-16

Estimate of Relative Contribution of Sublegal Hudson, Chesapeake and Roanoke Stocks to New York Waters in 1975 by Period and Spatial Stratum*

Period	Stratum	Sample Size†	Classification Percentage‡ Hudson	Chesapeake	Roanoke
3	4	5	---	80.0	20.0
	5	42	92.9	7.1	---
	6	8	12.5	50.0	37.5
	7	11	81.8	18.2	---
4	5	85	88.2	11.8	---
	6	17	41.2	41.2	17.6
5	5	10	80.0	20.0	---
	6	19	26.3	36.8	36.8

*Sublegal refers to striped bass 406.5-mm fork length from New York waters.

†Sample sizes of 4 specimens or less in any stratum not included: period 2, stratum 5 (2 Hudson) and stratum 7 (1 Hudson and 3 Chesapeake); period 6, stratum 4 (1 Hudson).

‡Classified with quadratic (QTH) functions.

Roanoke fish (all either truly Roanoke or members of a year class of Rappahannock striped bass similar to Roanoke stock). The potential error in the classification of legal-sized Roanoke striped bass would be minimal since <0.5% of legal Roanoke specimens were age 2.

A large percentage of sublegal Hudson River striped bass was indicated in strata 5 and 7 (Table IV-16). In the May-October collections from stratum 5 (primarily Little Neck Bay), the Hudson's contribution of sublegal fish ranged from 80 to 93%. Hudson stock also contributed 81.8% to sublegal collections from stratum 7 during May-June. To what extent and in what locations will these sublegal fish contribute to the coastal Atlantic fishery in future years when they become recruited to the fishery is an important but still unanswered question.

2. Contribution Estimates for Commercial Fishery

Results of the application of weighting factors to obtain estimates of the stock composition of the 1975 commercial fishery indicate little difference from the unweighted estimates. Estimates of relative contribution of Hudson stock to commercial landings during the two-month periods ranged from 10.4 to 28.3%, with a 20.4% contribution for the entire year (Tables IV-17 and IV-18). The year exhibited 68.1% Chesapeake and 11.4% Roanoke stock contributions; these were similar to the yearly estimates of unweighted mean relative contributions of 23.0% Hudson, 66.0% Chesapeake, and 11.0% Roanoke stocks, which included classification percentages from stratum 5 (Table IV-12).

Table IV-17

Estimates of Relative Contribution of Hudson, Chesapeake, and Roanoke Stocks to Atlantic Coastal Commercial Landings by NMFS Regions and Periods

Period	NMFS Region*	TI Stratum**	Sample Size***	Mean Weight	Classification Proportions‡			Weighted Proportion in NMFS Region‡			Proportion of Landings Investigated#
					Hudson	Chesapeake	Roanoke	Hudson	Chesapeake	Roanoke	
1	New England	1,2&3	0	0	--	--	--	--	--	--	--
	Mid-Atlantic	4	0	0	--	--	--	--	--	--	--
		6	0	.003	--	--	--	--	--	--	--
		7	0	.004	--	--	--	--	--	--	--
		8	0	.005	--	--	--	--	--	--	--
	Chesapeake	9	0	.229	--	--	--	--	--	--	--
	South Atlantic	10	27	.759	.259	.630	.111	.197	.478	.084	.759
Total weighted contribution (%)								26.0	63.0	11.1	
2	New England	1&2	0	0	--	--	--	--	--	--	--
		3	0	.040	--	--	--	--	--	--	--
	Mid-Atlantic	4	0	.027	--	--	--	--	--	--	--
		6	1	.043	--	--	--	--	--	--	--
		7	30	.033	.233	.733	.033	--	--	--	--
		8	34	.035	.235	.676	.088	.016	.048	.004	--
	Chesapeake	9-1	22	.118	.091	.909	0	--	--	--	--
		9-2	49	.342	.082	.714	.204	.039	.351	.070	--
	South Atlantic	10	0	.361	--	--	--	--	--	--	.528
Total weighted contribution (%)								10.4	75.6	14.0	
3	New England	1	82	.061	.110	.683	.207	--	--	--	--
		2	91	.023	.143	.714	.143	--	--	--	--
		3	60	.240	.300	.600	.100	.082	.202	.040	--
	Mid-Atlantic	4	96	.297	.219	.698	.083	--	--	--	--
		6	89	.218	.258	.652	.090	--	--	--	--
		7	58	.105	.414	.517	.069	--	--	--	--
		8	113	.055	.239	.673	.088	.178	.441	.056	--
	Chesapeake	9	3	.001	--	--	--	--	--	--	--
	South Atlantic	10	0	0	--	--	--	--	--	--	.999
Total weighted contribution (%)								26.0	64.4	9.6	
4	New England	1	58	.119	.190	.672	.138	--	--	--	--
		2	90	.001	.078	.722	.200	--	--	--	--
		3	43	.194	.302	.651	.047	.082	.207	.025	--
	Mid-Atlantic	4	0	.144	--	--	--	--	--	--	--
		6	102	.145	.225	.637	.137	--	--	--	--
		7	93	.350	.333	.656	.011	--	--	--	--
		8	28	.045	.214	.714	.071	.160	.354	.027	--
	Chesapeake	9	0	.001	--	--	--	--	--	--	--
	South Atlantic	10	0	0	--	--	--	--	--	--	.855
Total weighted contribution (%)								28.3	65.6	6.1	

Period	NMFS Region*	TI Stratum**	Sample Size***	Mean Weight	Classification Proportions‡ Hudson	Chesapeake	Roanoke	Weighted Proportion in NMFS Region‡ Hudson	Chesapeake	Roanoke	'Proportion of Landings Investigated#
5	New England	1	74	.053	.135	.770	.095	--	--	--	--
		2	82	.004	.122	.585	.293	--	--	--	--
		3	56	.242	.250	.589	.161	.069	.185	.045	--
	Mid-Atlantic	4	140	.305	.164	.643	.193	--	--	--	--
		6	86	.249	.151	.733	.116	--	--	--	--
		7	120	.097	.233	.633	.133	--	--	--	--
		8	73	.045	.164	.767	.068	.118	.475	.104	--
	Chesapeake	9	6	.002	.167	.667	.167	<.0005	.001	<.0005	--
	South Atlantic	10	0	.002	--	--	--	--	--	--	.997
Total weighted contribution (%)								18.8	66.3	14.9	
6	New England	1	0	0	--	--	--	--	--	--	--
		2	0	0	--	--	--	--	--	--	--
		3	0	.049	--	--	--	--	--	--	--
	Mid-Atlantic	4	99	.112	.212	.667	.121	--	--	--	--
		6	106	.124	.160	.698	.142	--	--	--	--
		7	124	.038	.210	.766	.024	--	--	--	--
		8	117	.373	.214	.726	.060	.131	.461	.054	--
	Chesapeake	9-1	50	.122	.100	.800	.100	--	--	--	--
		9-2	50	.058	.060	.800	.140	.016	.144	.020	--
	South Atlantic	10	24	.124	.125	.625	.250	.016	.078	.031	.951
Total weighted contribution (%)								17.1	71.8	11.0	

*The NMFS considers Delaware in the mid-Atlantic region, but TI's study included Delaware collections in strata 9. Only oceanic waters were considered for Chesapeake collections. The south Atlantic region included only the area north of Cape Hatteras (North Carolina) to Chesapeake Bay inlet.

**Stratum 5 is omitted because it has no commercial fishery. Stratum 9 was analyzed at substratum level in period 2 and 6 because of large differences in substratum weights.

***Not considered were sample sizes of 5 specimens or less in any stratum.

†Mean weighting factors are derived in Appendix G. New England weights are based on poundage from Rhode Island and Massachusetts, middle Atlantic weights on poundage from New York and New Jersey, Chesapeake weights on poundage from Maryland and Virginia north of Chesapeake Bay inlet, and South Atlantic weights on poundage from Virginia south of Chesapeake Bay inlet and North Carolina north of Cape Hatteras.

Classification resulted from application of quadratic (QTH) functions.

The proportion of the periodic poundage landed within each NMFS region, allocated each stock, eg., the weighted proportion of the Hudson stock in the mid-Atlantic landings is

$$W_4P_{H4} + W_6P_{H6} + W_7P_{H7} + W_8P_{H8}, \text{ where } W_i = \text{mean weight in } i^{th}$$

stratum, and P_{Hi} = Hudson proportion in i^{th} stratum.

The sum of the weights in every period equals 1.00; however, due to

Table IV-18

Estimates of Relative Contribution of Hudson, Chesapeake, and Roanoke Stocks to Commercial Landings (Poundage) by Period and Overall Year

Period (ℓ)	Sample Size	Percent of Landings Investigated	Weight † (W_ℓ)	Classification Weighted Proportion					
				Hudson		Chesapeake		Roanoke	
				(P_ℓ)‡	($P_\ell \bar{W}_\ell$)	(P_ℓ)	($P_\ell \bar{W}_\ell$)	(P_ℓ)	($P_\ell \bar{W}_\ell$)
1	27	75.9	.159	.260	.041	.630	.100	.111	.018
2	135	52.8	.064	.104	.007	.756	.048	.140	.009
3	589	99.9	.107	.260	.028	.644	.069	.096	.010
4	414	85.5	.096	.283	.027	.656	.063	.061	.006
5	637	99.7	.194	.188	.036	.663	.129	.149	.029
6	570	95.1	.379	.171	.065	.718	.272	.110	.042
Cumulative weighted proportion#					.204		.681		.114

* Stratum 5 has no commercial fishery, so its sample size is not included. Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum 5 (1 Chesapeake); period 2, stratum 6 (1 Chesapeake); and period 3, stratum 9 (2 Chesapeake and 1 Roanoke).

† Sum of weights in every period equals 1.00; however the sum of weights with corresponding collections is 1.00. The percent of landings investigated equals this sum which is 1.00.

‡ Equals periodic proportion of yearly total poundage (Table IV-17).

‡ Equals total weighted contribution by period in (Table III-3).

\# Equals $\sum_{\ell}^{6} P_\ell \bar{W}_\ell$

3. Contribution Estimates for USNRC Zones

Although the mean estimate of the Hudson stock contribution in 1975 was low, i.e., 23.0%, its contribution (Table IV-19) to western Long Island Sound and the New York Bight was higher than 23.0% during most periods, thus indicating a higher yearly Hudson contribution within the strata adjacent to the Hudson River (USNRCinner zone) and a lower contribution elsewhere (USNRC outer zone). The opposite was true during most time periods for the contributions of Chesapeake and Roanoke stocks; they were greater in the outer zone than in the inner zone. Although the Chesapeake stock was the predominant contributor to both the inner and outer zones, the Hudson stock's contribution exceeded that of the Roanoke stock in the inner zone, but was less than that of the Roanoke stock in the outer zone during most periods. Analysis of selected age groups (2-4, 5, 6+) indicated that within the inner zone the percentage of six-year-old and older striped bass classified into the Hudson stock was larger than that classified into Chesapeake stock from March through October. This demonstrated that a dominant Chesapeake year class (see Section IV) can overshadow the contribution of six-year-old and older fish classified into the Hudson stock in the strata adjacent to the Hudson River.

The mean estimates of relative contribution of the Hudson River stock to the inner and outer zones were 31.7% and 19.2%, respectively, for the year (Table IV-20). The mean estimate of 31.7% Hudson contribution for the inner zone was higher than its mean contribution to the entire Atlantic coastal fishery; however, it was substantially lower than the 90% contribution used by the USNRC. The mean estimate of 19.2% Hudson contri-

Table IV-19

Estimates of Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to 1975 Oceanic Collections within NRC Zones by Period and Select Age Groups*

		Inner-Zone Classification Percentages†				Outer-Zone Classification Percentages			
Period	‡ Age Group	Sample Size	Hudson	Chesapeake	Roanoke	Sample Size	Hudson	Chesapeake	Roanoke
2	All	69	35.3	60.3	4.4	34	22.9	68.6	8.6
	2-4	37	35.1	56.8	8.1	6	33.3	66.7	0
	5	28	39.3	60.7	0	25	16.0	76.0	8.0
	6+	4	75.0	25.0	0	3	66.7	0	33.3
3	All	112	36.9	56.8	6.3	491	20.0	67.8	12.2
	2-4	17	23.5	64.7	11.8	58	6.9	72.4	20.7
	5	59	38.1	60.3	1.6	193	12.4	78.2	9.3
	6+	36	50.0	38.9	11.1	240	29.2	58.3	12.5
4	All	136	30.2	66.9	3.0	293	18.4	67.2	14.3
	2-4	17	23.5	76.5	0	74	14.9	60.8	24.3
	5	101	27.7	68.3	4.0	133	13.5	76.7	9.8
	6+	18	50.0	50.0	0	86	29.1	58.1	12.8
5	All	252	28.6	59.5	11.9	468	16.0	67.1	16.9
	2-4	63	39.7	46.0	14.3	91	13.2	54.9	31.9
	5	137	15.3	73.7	11.0	256	8.2	78.9	12.9
	6+	52	50.0	38.5	11.5	121	35.5	50.4	14.0
6	All	161	22.4	75.8	1.8	285	18.6	69.5	11.9
	2-4	32	25.0	71.9	3.1	30	26.7	50.0	23.3
	5	109	21.1	77.1	1.8	150	8.7	81.3	10.0
	6+	20	25.0	75.0	0	105	30.5	58.1	11.4

* Not considered were striped bass <406.5-mm fork length from New York waters. Nuclear Regulatory Commission inner zone corresponds to TI strata 5, 7, and 8-1 and outer zone to TI strata 1, 2, 3, 4, 6, 8-2, and 8-3 (substrata designated in stratum 8).

† Classified with quadratic (QTH) functions.

‡ Only one striped bass (classified Chesapeake) collected in inner zone (stratum 5) during period 1.

bution for the outer zone was intermediate between the 10 and 50% contribution used by the USNRC.

Throughout the year, the percentage of males in inner-zone collections was greater than in outer-zone collections (Table IV-21) presumably because of the greater percentage of Hudson fish in the inner zone than in the outer zone; earlier analyses (Table IV-13) indicated that the proportion of Hudson males was nearly double that of Chesapeake or Roanoke males in oceanic collections. The large percentage of males collected in the inner zone during March-April may have been the result of male migrations to the vicinity of the Hudson River for spawning during May.

Table IV-20

Estimates of Mean Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to 1975 Oceanic Collections within USNRC Zones*

Zone	Sample Size	Classification Percentages†		
		Hudson	Chesapeake	Roanoke
Inner	729	31.7	62.9	5.5
Outer	1572	19.2	68.0	12.8

*Mean refers to average of (5) periodic classification percentages. Not considered were striped bass <406.5-mm fork length from New York waters. U.S. Nuclear Regulatory Commission inner zone corresponds to TI strata 5, 7, 8-1 and outer zone to TI strata 1, 2, 3, 4, 6, 8-2, and 8-3 (substrata designated in stratum 8).

†Classified with quadratic (QTH) functions.

Table IV-21

Percentage of Legal Male Striped Bass by Period within 1975 Oceanic Collections from USNRC Zones*

Period†	Percentage Males Inner Zone	Outer Zone
2	51.5	2.9
3	16.1	9.6
4	17.6	10.3
5	24.6	13.8
6	16.9	11.9

* Mean refers to average of (5) periodic classification percentages. Not considered were striped bass <406.5-mm fork length from New York waters. U.S. Nuclear Regulatory Commission (USNRC) inner zone corresponds to TI strata 5, 7, 8-1 and outer zone to TI strata 1, 2, 3, 4, 6, 8-2, and 8-3 (substrata designated in stratum 8).

† Only one striped bass (classified Chesapeake) collected in inner zone (stratum 5) during period 1.

The predominance of the Hudson stock in the overwintering collections was further substantiated by the results of the enzyme analysis, since the gene frequencies obtained were almost identical to those of the 1974 and early 1975 Hudson spawning stock (see Table IV-9).

The proportion of males for the Hudson and Chesapeake stocks in the overwintering collections (Table IV-22) was double that for each stock in the oceanic collections (Table IV-14); as in the oceanic collections, the proportion of Hudson males (0.543) was double that of Chesapeake males (0.278) in the overwintering collections. The higher male proportion (0.48) in the winter collections from the Hudson River is in agreement with studies by Vladykov and Wallace (1952) and Trent and Hassler (1968) which indicated that proportions of male striped bass in the Chesapeake and Roanoke systems were greater than 50%.

Table IV-22

Estimates of Relative Contribution of Legal Hudson, Chesapeake, and Roanoke Stocks to Hudson River Collections of Overwintering (6 December 1974-20 March 1975) Striped Bass and Proportion of Males in Each Stock*

	Classification		
Stock	Number	Percentage †	Proportion of Males †
Hudson	58	76.3	0.543
Chesapeake	18	23.7	0.278
Roanoke	0	----	----

* Not considered were 3 sublegal fish <406.5-mm fork length classified as Hudson.

† Classified with quadratic (QTH) functions.

† One hermaphroditic striped bass (age 6, 497-mm fork length) which was classified as a Hudson River fish was counted as 1/2 male.

SECTION V

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APPENDIX A

MOVEMENTS OF STRIPED BASS (*Morone saxatilis*) TAGGED IN HUDSON RIVER, 1972-75

APPENDIX A

MOVEMENTS OF STRIPED BASS (*Morone saxatilis*) TAGGED IN HUDSON RIVER, 1972-75

A. INTRODUCTION

Determination of the area of the Atlantic in which Hudson River striped bass may be found is a subset of a major study problem, that of determining the proportional contribution of Hudson River striped bass to various strata within the Atlantic fishery. Texas Instruments Incorporated (TI) conducted tagging studies between 1972 and 1975 to obtain data that could be used for this determination.

Early studies suggested that Hudson River striped bass are relatively nonmigratory, being restricted largely to the western end of Long Island Sound and the extreme southwestern end of Long Island (Neville, 1940; Raney and deSylva, 1953; Raney et al, 1954; Alperin, 1966). Subsequent studies (Clark, 1968; Clark and Smith, 1969) indicated more extensive migrations by Hudson River striped bass: two fish tagged in the Hudson River during the first week of March had moved as far as Newburyport, Massachusetts. However, the origin of these overwintering fish may be in some doubt since Raney et al (1954) suggested that some striped bass from Chesapeake Bay may overwinter in the Hudson. Clark (1968) also hypothesized the existence of three "contingents" or "groups of fish that engage in a common pattern of seasonal migration between feeding areas, wintering areas, and spawning areas" that can be identified as (1) the Hudson Estuary Contingent, (2) the Hudson-West Sound Contingent, and (3) the Hudson-Atlantic Contingent (summers in the New York Bight area and Southern New England).

TI personnel were able to tag and release large striped bass not only during the overwintering period but also during the spring spawning season. The recovery of fish tagged during the spawning season (April-June) has provided conclusive data on the Atlantic areas in which Hudson River striped bass may be found.

B. MATERIALS AND METHODS

From April 1972 through June 1975, TI tagged and released 1820 striped bass in the Hudson River between the George Washington Bridge (river KM 19 [measured from the southernmost tip to Manhattan]) and Troy Dam (river KM 245). Many large fish were tagged during the spawning season (April-June) in areas of the river in which striped bass eggs are routinely collected (KM 48-192) (TI, 1975a); several were tagged in the major spawning area between river KM 74 and 96 (Rathjen and Miller, 1957). Most of the large fish ($\geq$ 200 mm) were captured in gill nets; smaller fish (<200 m) were generally taken in beach seines and box traps.

Tag number, date, time, location, and gear were recorded for each tagged fish. Total length was recorded for most tagged fish. From many of the large fish, a scale sample was taken for age determination by the annulus method (Mansueti, 1961).

The study utilized four types of tags: the vast majority of all fish (98.8%) were tagged with either fingerling tags (Floy No. FTF-69) or nylon internal anchor tags (Floy No. FD-67C), depending on fish length; spaghetti (Floy No. FT-4) or Carlin tags were attached to 21 individuals in 1972 and 1973 (1.2% of the total number tagged).

Fingerling or Carlin tags were applied primarily to fish ≤15 cm in 1972 and 1973 and to fish ≤20 cm in 1974 and 1975, while larger fish received internal anchor or spaghetti tags. All tags were applied through the back of the fish between the first and second dorsal fins. Return address, serial number, and indication of reward ($1 for each tag returned) were printed on each tag.

The most frequently tagged total-length category was 10-20 cm (Table A-1); however, 20% of all measured tagged fish were > 50 cm. There were approximately seven times as many releases in spring and fall as in winter and summer.

Table A-1

Length Distribution of Striped Bass Tagged and Released in Hudson River (1972-75) between River Kilometers 19 and 245

Total Length (mm)	Number of Fish Tagged				
	Winter (Jan-Feb-Mar)	Spring (Apr-May-Jun)	Summer (Jul-Aug-Sep)	Fall (Oct-Nov-Dec)	Totals
≤ 100	0	95	1	28	124
101-200	0	352	162	443	957
201-300	0	103	12	101	216
301-400	0	19	6	18	43
401-500	18	21	0	30	69
501-600	34	52	1	35	122
601-700	34	43	0	8	85
701-800	6	27	0	4	37
801-900	2	29	0	0	31
901-1000	0	62	0	0	62
>1000	1	25	0	0	26
Not measured	0	0	2	46	48
Totals	95	828	184	713	1820

C. RESULTS

From 1 April 1972 through 31 December 1975, there were 48 tag returns (2.6% return rate) (Table A-2). Return rates were higher for the larger classes, with > 40-cm fish yielding a 9.3% return rate:

Length Group (cm)	Percent Return (%)
≤ 20.0	0.6
20.1-40.0	0.4
40.1-60.0	8.9
60.1-80.0	10.7
80.1-100.0	8.6
>100.0	7.7

Of the returns, 22 (46%) were from outside the Hudson River and its tributaries (Figure A-1): nine from Long Island Sound, five from the south side of Long Island, and eight from points east (Montauk, New York, to Boston, Massachusetts). The most distant and also the largest recapture occurred in Boston Harbor, Massachusetts. Larger fish recaptured outside the Hudson River had a tendency to move the greatest distances. The eight fish > 80 cm in total length recovered outside the Hudson averaged 354 km in distance traveled; the 15 fish < 80 cm in total length averaged 119 km. The mean distance traveled was significantly different for these two size groups ($t = 6.82$, $P < 0.001$). Fish recovered outside the Hudson that had moved < 200 km averaged 63 cm in total length, but those traveling > 200 km averaged 89 cm. The mean sizes of these two groups were significantly different ($t = 4.12$, $P < 0.001$).

D. DISCUSSION

TI's data on striped bass migration from wintering-spawning areas in the Hudson River to areas outside the river indicate

Table A-2

Release and Recovery Data for Recaptured Striped Bass
Tagged in the Hudson River, 1972-74

Release Data				Recovery Data				
Date	Location (River KM)	Total Length (mm)	Age	Date	Location	Days at Large	Approximate Distance (km) from Tagging Site	Recapture Gear
1972								
Dec 28	54	555	---	5/1/73	Long Island Sound, Rye, NY	123	104	SF
	54	605	---	5/25/73	Long Island Sound, Greenwich, CT	147	112	SF
1973								
Jan 3	54	567	---	4/23/73	Hudson River Kilometer 54	110	<1	CF
	54	588	---	3/30/73	Long Island Sound, Little Neck Bay, NY	86	80	SF
Mar 9	53	645	VI	4/23/73	Hudson River Kilometer 54	45	1	CF
	53	570	V	4/27/73	Hudson River Kilometer 62	49	9	PS
	53	670	V	7/21/73	Long Island Sound, Mamaroneck, NY	34	95	SF
13	51	610	V	5/1/73	Hudson River Kilometer 62	49	11	PS
	51	650	V	6/20/73	Long Island Sound, Glen Cove, NY	99	104	SF
	51	650	V	7/27/73	Long Island Sound, Stamford, CT	36	112	SF
14	54	552	---	3/14/73	Hudson River Kilometer 54	1	<1	SF
15	53	870	VIII	7/5/73	Nantucket Sound, Nantucket MA	112	400	SF
26	54	575	---	6/7/73	Great South Bay, R. Moses Bridge, NY	73	136	SF
	54	660	---	9/5/74	Long Island Sound, Stamford, CT	528	112	SF

Table A-2 (Contd)

Date	Location (River KM)	Total Length (mm)	Age	Date	Location	Days at Large	Approximate Distance (KM) from Tagging Site	Recapture Gear
Apr 3	53	492	IV	4/13/73	Hudson River Kilometer 50	10	3	CF
19	96	903	VII	10/12/73	Block Island Sound, Montauk Pt., NY	174	296	SF
20	94	975	IX	6/19/73	Buzzards Bay, New Bedford, MA	60	389	SF
	91	1040	VIII	7/15/74	Boston Harbor Boston, MA	451	512	SF
26	94	745	VII	9/17/73	Lower NY Bay Rockaway Pt., NY	145	128	SF
Nov 12	62	645	VI	5/10/74	Stockport Creek, Tributary to Hudson River at Kilometer 192	179	144	SF
1974								
Apr 23	67	908	IX	5/2/74	Hudson River Kilometer 53	9	14	SF
24	22	120	I	8/1/74	Hudson River Kilometer 46	99	24	SF
30	88	808	---	6/15/75	Gravesend Bay Coney Island, NY	411	105	SF
May 16	67	1035	X	6/27/74	Cape Cod Bay Orleans, MA	42	416	SF
Sep 18	46	145	---	10/29/74	Hudson River Kilometer 46	41	<1	PS
20	86	144	---	5/29/75	Hudson River Kilometer 86	251	<1	PS
Oct 9	56	238	---	6/23/75†	Hudson River Kilometer 19	258	37	SF
25	67	165	---	11/13/74	Hudson River Kilometer 67	19	<1	PS
Nov 4	64	174	---	11/15/74	Hudson River Kilometer 64	11	<1	PS

Table A-2 (Contd)

Date	Location (River KM)	Total Length (mm)	Age	Date	Location	Days at Large	Approximate Distance (KM) from Tagging Site	Recapture Gear
1975								
Apr 8	54	572	---	8/1/75	Housatonic River, Stratford, CT	115	156	SF
15	56	578	---	4/29/75	Hudson River Kilometer 43	14	13	CF
16	56	607	VI	4/17/75	Hudson River Kilometer 56	1	<1	PS
16	56	591	VI	8/21/75	Atlantic Beach Long Island, NY	127	98	SF
17	56	567	VII	4/18/75	Hudson River Kilometer 56	1	<1	PS
17	61	586	V	4/18/75	Hudson River Kilometer 61	1	<1	PS
17	61	661	VI	4/18/75	Hudson River Kilometer 61	1	<1	PS
17	56	590	VI	4/29/75+	Hudson River Kilometer 46	12	10	SF
23	56	592	---	4/24/75	Hudson River Kilometer 56	1	<1	PS
29	56	624	VII	4/30/75	Hudson River Kilometer 43	1	13	CF
29	61	568	---	4/30/75	Hudson River Kilometer 43	1	18	CF

Table A-2 (Contd)

Date	Location (River KM)	Total Length (mm)	Age	Date	Location	Days at Large	Approximate Distance (KM) from Tagging Site	Recapture Gear
30	56	660	---	6/2/75	Long Island Sound, Mamaroneck, NY	33	99	SF
30	56	847	IX	6/22/75	Fallmouth Heights, MA	53	367	SF
May 1	61	846	X	5/2/75	Hudson River Kilometer 61	1	< 1	PS
7	53	473	---	6/2/75	Block Island Sound, Shagwong Point, NY	26	248	CF
14	67	535	---	6/10/75	Verrazano Bridge Lower Bay, NY	27	78	SF
23	66	944	---	6/4/75	Cuttyhunk, MA	12	349	SF
27	64	174	---	6/10/75	Hudson River Kilometer 64	14	< 1	PS
Jun 10	64	192	---	6/23/75	Hudson River Kilometer 64	13	< 1	PS

* SF Sport fishing
CF Commercial fishing
PS Project sampling

† Date represents postmark date; no more accurate recapture data are available.

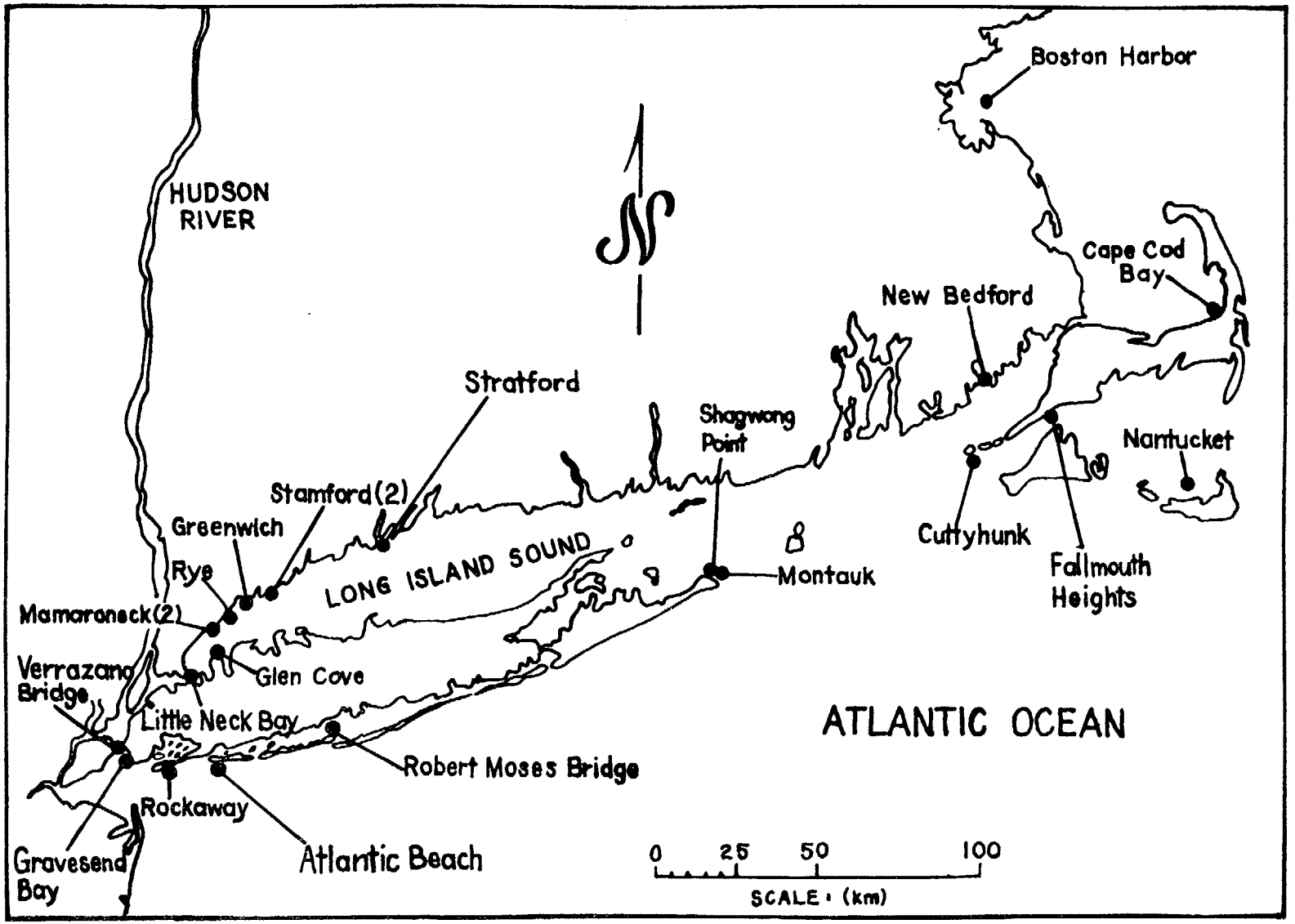

Figure A-1. Returns of Striped Bass to the Area Outside the Hudson River and its tributaries.

that some large individuals make extensive northeast movements into New England waters and that larger fish migrate farther. Clark's (1968) Hudson-Atlantic contingent is confirmed by fish tagged in the spring on known spawning grounds which were recaptured as far away as Boston Harbor, and seems to be composed primarily of very large striped bass (> 80 cm). Large striped bass reportedly make extensive oceanic migrations (Chapoton and Sykes, 1961; Schaefer, 1968) as did those tagged in the Hudson River. Fish moving < 200 km (e.g., those taken in western Long Island Sound [Clark's Hudson-West Sound contingent]) tend to be smaller (50-80 cm). Individuals < 40 cm were not recovered outside the Hudson estuary, indicating the relatively nonmigratory status of smaller striped bass as reported for other estuarine systems (Vladykov and Wallace, 1938, 1952; Merriman, 1941; Raney, 1952, 1957; Mansueti, 1961; Massman and Pacheco, 1961; Nichols and Miller, 1967).

A recurring question of fundamental importance in a study such as this is the origin of the tagged fish: are they of the Hudson River stock? There is evidence that Hudson River fish are genetically distinct from those found in more southerly waters (Raney and deSylva, 1953; TI, 1975b). It has also been suggested (Raney and deSylva, 1953; Raney et al, 1954) that striped bass of other stocks may overwinter in the Hudson River. Hence, fish tagged during the winter (January-March) are of questionable origin. TI assumes that those fish tagged in the spring (April-June) on known spawning grounds (Rathjen and Miller, 1957; TI, 1975a) most likely are of Hudson River stock. In April and May, 13 such fish tagged at river KM 53-96 were recovered outside the Hudson; seven of these were recovered > 200 km from the point of release and as far

away as Boston Harbor. The proportion of the available population making large-scale movements cannot be determined based solely on tagging data; however, the larger, older individuals that engage in extensive coastal migrations are not likely to represent a very large portion of the Hudson River striped bass population. Smaller individuals (40-80 cm) appear to restrict their movements outside the Hudson primarily to western Long Island Sound and the southwestern shore of Long Island. Fish < 40 cm in total length were not recaptured outside the Hudson River.

A number of rivers along the Atlantic Coast produce striped bass, and most tagging studies have indicated the same general migratory patterns for all stocks that are well studied, i.e., a northward migration of older individuals along the coast in late spring and southward return migration in late fall (Merriman, 1941; Vladykov and Wallace, 1952; Raney et al, 1954; Massman and Pacheco, 1961; Chapoton and Sykes, 1961; Nichols and Miller, 1967). TI's study would seem to indicate a similar pattern of migration by Hudson River stock. Thus, the Hudson River may be expected to contribute some fish to fisheries as far away as Massachusetts.

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APPENDIX B

COMPARISON OF 1974 AND 1975 SOURCE-RIVER DATA

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COMPARISON OF 1974 AND 1975 SOURCE-RIVER DATA

In the comparison of the 1974 and 1975 data from the spawning-stock collections, the three best discriminant characters common to both years were used. These characters were obtained through the elimination of characters from the initial set of characters with the use of correlation and discriminant analyses..

A. CORRELATION ANALYSIS

Using adult striped bass collected in 1974 from the Hudson River and the Chesapeake Bay system, Texas Instruments established a set of 17 meristic and morphometric characters that, out of 41 characters initially generated, satisfied the correlation criterion (TI, 1975). In subsequent analyses, however, the number of characters in this set was reduced: three characters were removed from the set of 17 because scale-to-scale variation was observed when scale measures were not used as ratios and because bias was observed on the upper-arm gill-raker counts due to improper removal of the gill arch from preserved fish. Pairwise comparisons between observers indicated a correctable bias of 2 mm more in all internostril width measurements taken by one observer (person code 3). The affected internostril width measurements were corrected, as well as the second dorsal and anal fin ray counts that were documented as one ray less than actual (TI, 1975). An additional character, a ratio of snout length/internostril width, was generated. Consequently, 15 characters (Table B-1) were used in the subsequent discriminant analysis of 1974 data.

Table B-1

Initial 15 Characters Used in Discriminant Analysis of 1974 Data

Character Code		Description
1	LL	Scales along lateral line
2	AL	Scales above lateral line
3	BL	Scales below lateral line
4	ACP	Scales around caudal peduncle
5	LP	Rays on left pectoral fin
6	RP	Soft rays on second dorsal fin
7	DOR	Soft rays on second dorsal fin
8	ANL	Soft rays on anal fin
9	SNT/H	Snout/head length ratio
10	NOS/H	Internostril width/head length ratio
11	SNT/NOS	Snout length/internostril width ratio
12	H/F	Head/fork length ratio
13	BANL/F	Base anal fin/fork length ratio
14	LGR	Lower arm gill rakers including rudimentary rakers
15	SA/FA	First annulus to second annulus/focus to first annulus measure

Table B-2

List of Characters Generated from 1975 Meristic Counts and Morphometric Measurements

Character Code		Description
1	LL	Scale along lateral line
2	LP	Rays on left pectoral fin
3	RP	Rays on right pectoral fin
4	DOR	Soft rays on second dorsal fin
5	ANL	Soft rays on anal fin
6	UGR	Upper arm gill rakers including rudimentary rakers
7	SA/FA	First annulus to second annulus/focus to first annulus measure ratio
8	SNT/NOS	Snout length/internostril width ratio
9	SNT/L	Snout/fork length ratio
10	SNT/HL	Snout/head length ratio
11	NOS/FL	Internostril/fork length ratio
12	NOS/HL	Internostril/head length ratio
13	HL/FL	Head/fork length ratio

Using adult striped bass collected in 1975, TI generated 13 meristic and morphometric characters (Table B-2) and then removed those five characters highly correlated with fish length in more than two rivers (Table B-3). The snout length/internostril width ratio exceeded the correlation criterion in the Rappahannock and Roanoke stocks, but was retained because, of all the characters, its distribution within the Roanoke stock had the least overlap with that of Hudson and Chesapeake stocks, thus making it a potentially good discriminating character. The significance of the correlation within the Rappahannock was lost when the Chesapeake tributary data were pooled.

With the exception of ratios involving the internostril width within the Potomac stock, the five characters that had exceeded the correlation criterion with 1975 data had not done so with the 1974 data. Since fresh fish were measured in 1975 and preserved fish in 1974, the observed correlation differences apparently were caused by the differential shrinkage between the body and head sections. Fairly constant shrinkage between two head sections such as snout length and internostril width from population to population was expected, so the discriminatory potential of the character was expected to remain fairly constant between the 2 years even though each stock's snout length/internostril width ratio changed between 1974 and 1975 collections.

Of the remaining seven 1975 characters, only the right pectoral fin ray count for the Potomac and Rappahannock stocks slightly exceeded the correlation criterion (Table B-4). Because of the sample size and discrete nature of the fin ray distributions, this correlation with fish length was not considered significant. These seven characters,

Table B-3

Correlation of Six Morphometric Characters with Length of Fish (1974 and 1975 Data)

LOCALITY RIVER	YEAR	SAMPLE SIZE	Characters 1 (SNT/FL)	2 (SNT/HL)	3 (HL/FL)	4 (NOS/FL)	5 (NOS/HL)	6 (SNT/NOS)
Hudson	1974	192	.26452	.23349	.08296	.24701	.15477	.02874
	1975	168	.52866*	.67156*	-.34671*	.32168*	.53176*	.20420
Elk	1974	250	.09549	.13079	-.02266	.28157	.27083	-.12727
	1975	57	.48292*	.71462*	-.55966*	.38592*	.64136*	.10199
Choptank	1974	91	-.01890	.04100	-.12649	.30063	.32747*	-.26049
	1975	52	.21553	.54250*	-.53384*	.50029*	.66180*	-.23975
Potomac	1974	202	-.01862	-.06693	.06104	.59147*	.51492*	-.47506*
		53	.32786	.66106*	-.55761*	.42727*	.71766*	-.06155
Rappahannock	1974	156	-.10800	.01099	-.21426	.15556	.23027	-.20306
	1975	70	-.32645*	.17713	-.57900*	.29537	.64009*	-.49239*
Roanoke	1975	99	.25927	.13265	.22519	.60953*	.56942*	-.44120*

* Denotes values exceeding lack of correlation with fork length criterion i.e., $r \leq 0.316$, $r^2 \leq 0.100$

Table B-4

Correlation of Meristic and Scale Ratio Characters with Length of Fish (1975 Data)

LOCALITY RIVER	SAMPLE SIZE	Characters 1 (LL)	2 (LP)	3 (RP)	4 (DOR)	5 (ANL)	6 (UGR)	7 (SA/FA)
Hudson	168	-.28979	.14851	-.03015	.04451	.04815	.15854	.00955
Elk	57	.21008	.27188	.10127	-.09987	-.22581	.01847	.22439
Choptank	52	.07448	.04985	-.09077	-.15141	-.00240	-.00089	.17525
Potomac	53	.06575	-.31076	-.35624*	.05113	-.04977	-.09145	.07707
Rappahannock	70	-.13385	-.20330	-.31881*	.12123	.21480	.22818	-.04995
Roanoke	99	.03563	-.11103	.11037	-.03397	.01009	.13959	.15514

* Denotes values exceeding lack of correlation with fork length criterion, i.e., $r < 0.316$, $r^2 \leq 0.100$.

along with the one morphometric character, were used in the discriminant analyses of 1975 data.

B. DISCRIMINANT ANALYSIS

A low overall probability of correct classification occurred when segregation of five (1974) or six (1975) spawning stocks was attempted (Table B-5). Since adequate segregation of spawning stocks within the Chesapeake region was not possible, data from the Chesapeake rivers were pooled and new discriminant functions determined for two regions (Hudson and Chesapeake) using 1974 data and for three regions (Hudson, Chesapeake, and Roanoke) using 1975 data. When either two or three regions were analyzed, a stepwise linear discriminant analysis (Dixon, 1970) showed that three characters--snout length/internostril width ratio, first annulus to second annulus measure/focus to first annulus measure ratio, and lateral line scale count (in order of decreasing importance)--"best" discriminated spawning stocks and that additional characters did not significantly improve overall discrimination. Therefore, subsequent classification matrices refer only to discriminant analysis involving this set of three characters.

Table B-5

Correct Classification Percentages of Various Spawning Stocks by Quadratic Discriminant Analysis

Year	Characters Entered	Hudson	Chesapeake				Roanoke	Overall
			Rapp.	Pot.	Chop.	Elk		
1974	15	78.7	39.3	38.5	52.3	57.9		54.3
1975	8	50.6	37.1	26.4	73.1	21.1	68.7	48.7

Table B-6 indicates an overall correct classification of Hudson and Chesapeake spawning stocks of approximately 76%. Based on tests for common covariance matrices, the quadratic function was considered more valid for the 1974 data and the linear function more valid for the 1975 data. The close agreement between the 1974 and 1975 data indicated small year-to-year variation in the character set's discriminative potential.

Table B-6

Correct Classification Percentages of Hudson and Chesapeake Spawning Stocks for 2 Years of Data

Year	Type of Analysis	Hudson	Chesapeake	Overall
1974	Linear	78.1	77.3	77.5
	Quadratic	83.1	74.6	76.4
1975	Linear	78.6	74.6	76.2
	Quadratic	76.8	73.7	75.0

Table B-7 indicates an overall correct classification among Hudson, Chesapeake, and Roanoke spawning stocks of approximately 73% based on meristic and morphometric characters. Tests for common covariance matrices indicated the quadratic function to be more valid. When three regions were analyzed, the overall percentages of correct classification was reduced 4% primarily because Chesapeake fish were misclassified as Roanoke; the Chesapeake correct classification percentage dropped from approximately 74% to 63% with the addition of the Roanoke River spawning stock, whereas the Hudson correct classification percentage remained stable.

Table B-7

Correct Classification Percentages of Hudson, Chesapeake, and Roanoke Spawning Stocks

Type of Analysis	Hudson	Chesapeake	Roanoke	Overall
Linear	78.0	65.5	84.8	73.5
Quadratic	76.2	62.9	87.9	72.3

The individual fin ray characters did not add significant discriminatory ability to the foregoing analyses; therefore, the left and right pectoral, second dorsal, and anal fin rays were summed to form a character index (Raney and deSylva, 1953). Raney and de Sylva (1953) and Raney et al. (1954) had demonstrated that character indices and lateral-line scale counts allowed 70-80% separation between particular age-specific year classes of striped bass originating in the Hudson River and those originating in the tributaries of Chesapeake Bay. Separation among multiple year classes of striped bass originating in the Hudson, Chesapeake, and Roanoke was not nearly as pronounced; however, the character index was the third most important character in the stepwise linear discriminant routine, followed by the upper-arm gill-raker count and finally the lateral-line scale count. These characters, along with the two best discriminators--the snout length/internostril width ratio and scale ratio--formed the final set of discriminatory characters used to classify oceanic collections.

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APPENDIX C

RELIABILITY ANALYSIS

APPENDIX C

RELIABILITY ANALYSIS

Highly reliable data (values near 1.0) are important for adequate discrimination of overlapped populations. Extensive observer variation may cause large classification errors and lead to poor estimates of relative contribution: the larger the overlap, the greater is the probability of misclassification and the importance attached to resolution of the data.

The reliability of characters used in the discriminant analysis was estimated from replicated meristic counts and ratios of replicated morphometric measurements. The reliability index (Winer, 1971) measured the observers' variation for each character relative to that among fish: the smaller the variation between observers, the more reliable was the mean of the observations (i.e., the final character values). For a given variation between observers, the reliability of a character increased with an increase in the variation among fish in that character.

Results indicated close and consistent agreement between observers through time (Table C-1). The reliability of resolved data was estimated to be at least 0.990 for meristic and growth characters and 0.818 for the morphometric ratio. Reliability values were consistently high for the spawning river collections, the overwintering Hudson River collections, and the periodic oceanic collections; the minimum difference was 0.008 (upper gill raker) and the maximum difference was 0.113 (snout length/internostril width ratio). The tolerances that were set on the

Table C-1

Estimates of Reliability of Characters
Used in Discriminant Analysis

Collection Period	Month	Data Set	Character* SNT/NOS	SA/FA	LL	CI	UGR
Spawning	Apr-Jun	Resolved	0.9027	0.9973	0.9970	0.9984	0.9975
Overwintering	Dec-Mar	Resolved	0.8182	0.9955	0.9965	0.9969	1.0000
1, 2	Jan-Apr	Resolved	0.8853	0.9969	0.9901	0.9952	0.9925
3	May-Jun	Resolved	0.9311	0.9973	0.9942	0.9992	0.9959
4	Jul-Aug	Original	0.7635	0.9435	0.9837	0.9857	0.9256
		Resolved	0.8404	0.9974	0.9941	0.9996	0.9944
5	Sep-Oct	Original	0.7637	0.9116	0.9873	0.9889	0.9584
		Resolved	0.8669	0.9968	0.9953	0.9992	0.9967
6	Nov-Dec	Original	0.7975	0.9140	0.9693	0.9787	0.9417
		Resolved	0.8848	0.9971	0.9905	0.9989	0.9964

* SNT/NOS snout length/internostril width ratio
SA/FA first to second annulus/focus to first annulus measure ratio
LL lateral line scale count
CI character index
UGR upper arm gill raker count

characters increased the quality of the data--in many cases substantially--as additional readings corrected outlier observations (i.e., large discrepancies). The increase in reliability values from original to resolved data was as large as 13.54% and provided evidence that the use of tolerances improved the quality of the data. Differences in the reliability values for resolved data among the five characters in the character set were due, at least in part, to each character's variation among the fish collected. Reliability values of >0.99 for the lateral-line scale count, upper-arm gill-raker count, and scale ratio character were due to these characters' large variation among fish. On the other hand, reliability values for the snout length/internostril width ratio ranged from 0.81 to 0.90 because a relatively small variation in this character among fish.

Large reliability values were important for meristic counts, since the overlap among spawning stocks was large for these characters. Observers agreed less on the average on the true value of the snout length/internostril width ratio; nevertheless, it was sufficient for discrimination, since overlap among stocks in this character was the smallest of all characters.

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APPENDIX D

QUADRATIC DISCRIMINANT FUNCTIONS

APPENDIX D

QUADRATIC DISCRIMINANT FUNCTIONS

The quadratic discriminant functions* (QTH) determined with total Hudson, Chesapeake, and Roanoke spawning-stock specimens were as follows, where F = discriminant score, U = lateral line scale count, W = character index, X = upper-arm gill-raker count, Y = first to second annulus/focus to first annulus measurement ratio, and Z = snout length/internostril width ratio.

(1) • Hudson

$$F_{HUD} = -1489.070559 - (0.077516\ U^2 + 0.256954\ W^2 + 1.171065\ X^2 + 2.536320\ Y^2 + 123.907000\ Z^2 - 0.019058\ UW + 0.015160\ UX - 0.007057\ UY + 0.090968\ UZ + 0.047441\ WX + 0.023246\ WY + 0.164200\ WZ + 0.457365\ XY - 2.799760\ XZ - 2.861250\ YZ) + 8.776221\ U + 28.127772\ W + 24.321052\ X + 7.985031\ Y + 381.695141\ Z$$

(2) • Chesapeake

$$F_{CHES} = -1368.946420 - (0.089560\ U^2 + 0.242459\ W^2 + 1.122690\ X^2 + 2.155850\ Y^2 + 117.554000\ Z^2 - 0.007099\ UW + 0.005302\ UX + 0.015500\ UY + 0.321075\ UZ - 0.092151\ WX - 0.000861\ WY - 2.363980\ WZ + 0.381082\ XY + 3.623860\ XZ - 1.590090\ YZ) + 11.316822\ U + 21.749040\ W + 25.294896\ X + 7.014936\ Y + 323.469441\ Z$$

(3) • Roanoke

$$F_{ROAN} = -1650.902863 - (0.107062\ U^2 + 0.316254\ W^2 + 2.063540\ X^2 + 0.842590\ Y^2 + 139.577500\ Z^2 - 0.062826\ UW + 0.015703\ UX + 0.043640\ UY + 0.228873\ UZ - 0.293615\ WX$$

+ 0.129292 WY - 1.009790 WZ + 0.106776 XY - 0.606466 XZ

4.416000 YZ) + 10.320202 U + 27.000888 W + 25.512087 X

+ 22.351388 Y + 469.422957 Z

The quadratic discriminant functions* (QBH) determined with early Hudson (collected through 23 May 1975), Chesapeake, and Roanoke spawning-stock specimens were as follows, where F = discriminant score, U = lateral line scale count, W = character index, X = upper-arm gill-raker count, Y = first to second annulus/focus to first annulus measurement ratio, and Z = snout length/internostril width ratio.

(4) • Hudson

$$F_{BHUD} = - 1389.968674 - (0.073546\ U^2 + 0.236110\ W^2 + 1.278640\ X^2 + 2.997845\ Y^2 + 127.528000\ Z^2 - 0.005298\ UW - 0.015588\ UX - 0.026702\ UY + 0.207226\ UZ + 0.002679\ WX + 0.022327\ WY - 0.185918\ WZ + 0.395962\ XY - 3.942820\ XZ - 4.641430\ YZ) + 8.860940\ U + 25.617779\ W + 20.502111\ X + 4.254934\ Y + 368.349108\ Z$$

(5) • Chesapeake (same as Equation 2)

(6) • Roanoke (same as Equation 3)

Quadratic discriminant functions* determined with a random subset of half the Hudson, Chesapeake, and Roanoke spawning-stock specimens for cross-validation analysis were as follows, where F = discriminant score, U = lateral line scale count, W = character index, X = upper-arm gill-raker count, Y = first to second annulus/focus to first annulus measurement ratio, and Z = snout length/internostril width ratio.

(7) o Hudson

$$F_{HUD} = - 1320.886939 - (0.084786\ U^2 + 0.228129\ W^2 + 1.663295\ X^2 + 3.137505\ Y^2 + 149.395000\ Z^2 - 0.052682\ UW + 0.023791\ UX + 0.0164041\ UY - 0.274435\ UZ - 0.111029\ WX - 0.068202\ WY + 1.714310\ WZ + 1.307790\ XY - 9.375630\ XZ - 14.249100\ YZ) + 7.371288\ U + 23.519487\ W + 17.055562\ X - 3.557743\ Y + 442.685604\ Z$$

(8) o Chesapeake

$$F_{CHES} = - 1242.530599 - (0.095322\ U^2 + 0.207200\ W^2 + 0.921915\ X^2 + 1.982665\ Y^2 + 133.754000\ Z^2 - 0.021247\ UW - 0.014457\ UX + 0.091562\ UY - 0.232904\ UZ - 0.044647\ WX - 0.097993\ WY - 2.022570\ WZ + 0.395453\ XY + 4.208560\ XZ - 4.708530\ YZ) + 10.127539\ U + 17.864371\ W + 23.498883\ X + 0.726204\ Y + 364.976314\ Z$$

(9) o Roanoke

$$F_{ROAN} = - 1685.385783 - (0.108869\ U^2 + 0.616505\ W^2 + 2.577535\ X^2 + 1.090300\ Y^2 + 143.895000\ Z^2 - 0.164081\ UW + 0.157549\ UX + 0.029382\ UY - 0.539137\ UZ - 0.962390\ WX + 0.059099\ WY - 2.944660\ WZ + 0.057419\ XY - 3.972200\ XZ + 6.847480\ YZ) + 4.852745\ U + 44.237576\ W + 0.941652\ X + 22.254568\ Y + 296.185506\ Z$$

* except for a constant ($-2.5\ \ell n\ 2\pi$) common to each function

APPENDIX E

COMPARISON OF QUADRATIC QBH AND QTH FUNCTIONS IN DETERMINING RELATIVE CONTRIBUTION OF LEGAL HUDSON, CHESAPEAKE, AND ROANOKE STOCKS TO 1975 OCEANIC COLLECTIONS BY PERIOD AND STRATUM

APPENDIX E

COMPARISON OF QUADRATIC QBH AND QTH FUCTIONS IN DETERMING RELATIVE CONTRIBUTION OF LEGAL HUDSON, CHESAPEAKE, AND ROANOKE STOCKS TO 1975 OCEANIC COLLECTIONS BY PERIOD AND STRATUM*

				Classification Percentage		
Period	Stratum	Sample Size†	Function‡	Hudson	Chesapeake	Roanoke
1	10	27	QBH	29.6	59.3	11.1
			QTH	25.9	63.0	11.1
2	5	38	QBH	55.3	39.5	5.3
			QTH	52.6	42.1	5.3
	7	30	QBH	13.3	83.3	3.3
			QTH	23.3	73.3	3.3
	8	34	QBH,QTH	23.5	67.6	8.8
	9	71	QBH,QTH	8.5	77.5	14.1
3	1	82	QBH	9.8	69.5	20.7
			QTH	11.0	68.3	20.7
	2	91	QBH	14.3	70.3	15.4
			QTH	14.3	71.4	14.3
	3	60	QBH	35.0	55.0	10.0
			QTH	30.0	60.0	10.0
	4	96	QBH	22.9	68.8	8.3
			QTH	21.9	69.8	8.3
	5	14	QBH,QTH	35.7	57.1	7.1
	6	89	QBH	28.1	62.9	9.0
			QTH	25.8	65.2	9.0
	7	58	QBH,QTH	41.4	51.7	6.9
	8	113	QBH	24.8	66.4	8.8
			QTH	23.9	67.3	8.8
4	1	58	QBH	24.1	62.1	13.8
			QTH	19.0	67.2	13.8
	2	90	QBH	10.0	70.0	20.0
			QTH	7.8	72.2	20.0
	3	43	QBH,QTH	30.2	65.1	4.7
	5	15	QBH,QTH	26.7	66.7	6.7
	6	102	QBH	26.5	59.8	13.7
			QTH	22.5	63.7	13.7
	7	93	QBH	35.5	62.4	2.1
			QTH	33.3	65.6	1.1
	8	28	QBH,QTH	21.4	71.4	7.1

Period	Stratum	Sample Size†	Function‡	Classification Percentage		
				Hudson	Chesapeake	Roanoke
5	1	74	QBH	20.3	70.3	9.4
			QTH	13.5	77.0	9.5
	2	82	QBH,QTH	12.2	58.5	29.3
	3	56	QBH	35.7	48.2	16.1
			QTH	25.0	58.9	16.1
	4	140	QBH	17.9	62.8	19.3
			QTH	16.4	64.3	19.3
	5	89	QBH	44.9	42.7	12.4
			QTH	41.6	46.1	12.4
	6	86	QBH,QTH	15.1	73.3	11.6
	7	120	QBH	24.2	62.5	13.3
			QTH	23.3	63.3	13.3
	8	73	QBH	20.6	72.6	6.8
			QTH	16.4	76.7	6.8
	9	6	QBH,QTH	16.7	66.7	16.7
6	4	99	QBH	23.2	64.6	12.1
			QTH	21.2	66.7	12.1
	6	106	QBH	18.8	67.0	14.2
			QTH	16.0	69.8	14.2
	7	124	QBH	23.4	74.2	2.4
			QTH	21.0	76.6	2.4
	8	117	QBH	20.5	73.5	6.0
			QTH	21.4	72.6	6.0
	9	100	QBH	9.0	79.0	12.0
			QTH	8.0	80.0	12.0
	10	24	QBH,QTH	12.5	62.5	25.0

* Not considered were striped bass <406.5-mm fork length from New York waters.

† Sample sizes of 5 specimens or less in any stratum not included: period 1, stratum 5 (1 Chesapeake); period 2, stratum 6 (1 Chesapeake); period 3, stratum 9 (2 Chesapeake and 1 Roanoke).

‡ QBH designates quadratic discriminant functions determined with early Hudson (collected through 23 May 1975), Chesapeake, and Roanoke spawning-stock specimens. QTH designates quadratic discriminant functions determined with total Hudson, Chesapeake, and Roanoke spawning-stock specimens.

APPENDIX F

RELATIVE CONTRIBUTION OF LEGAL HUDSON, CHESAPEAKE, AND ROANOKE STOCKS TO 1975 OCEANIC COLLECTIONS BY PERIOD, STRATUM AND SUBSTRATUM

APPENDIX F

RELATIVE CONTRIBUTION OF LEGAL HUDSON, CHESAPEAKE, AND ROANOKE STOCKS TO 1975 OCEANIC COLLECTIONS BY PERIOD, STRATUM, AND SUBSTRATUM*

				Classification Percentage ***		
Period	Stratum	Substratum **	Sample Size	Hudson	Chesapeake	Roanoke
1	5		1	0	100	0
		5-2	1	0	100	0
	10		27	25.9	63.0	11.1
		10-3	27	25.9	63.0	11.1
2	5		38	52.6	42.1	5.3
		5-3	38	52.6	42.1	5.3
	6		1	0	100	0
		6-3	1	0	100	0
	7		30	23.3	73.3	3.3
		7-2	30	23.3	73.3	3.3
	8		34	23.5	67.6	8.8
		8-2	34	23.5	67.6	8.8
	9		71	8.5	77.5	14.1
		9-1	22	9.1	90.9	0
		9-2	49	8.2	71.4	20.4
3	1		82	11.0	68.3	20.7
		1-1	22	0	81.8	18.2
		1-2	30	10.0	70.0	20.0
		1-3	30	20.0	56.7	23.0
	2		91	14.3	71.4	14.3
		2-1	30	13.3	60.0	26.7
		2-2	30	3.3	86.7	10.0
		2-3	31	25.8	67.7	6.4
	3		60	30.0	60.0	10.0
	4		96	21.9	69.8	8.3
		4-2	20	15.0	80.0	5.0
		4-3	76	23.7	87.1	9.2
	5		14	35.7	57.1	7.1
		5-2	7	42.9	57.1	0
		5-3	7	28.6	57.1	14.3
	6		89	25.8	65.2	9.0
		6-1	35	43.0	57.0	0
		6-2	37	11.0	70.0	19.0
		6-3	17	23.5	70.5	6.0
	7		58	41.4	51.7	6.9
		7-2	5	60.0	40.0	0
		7-4	53	39.6	52.8	7.6
	8		113	23.9	67.3	8.8
		8-1	40	32.5	62.5	5.0
		8-2	38	10.5	73.7	15.8
		8-3	35	28.6	65.7	5.7
	9		3	0	66.7	33.3
		9-2	3	0	66.7	33.3

APPENDIX F (CONTD)

Period	Stratum	Substratum **	Sample Size	Classification Percentage*** Hudson	Chesapeake	Roanoke
4	1		58	19.0	67.2	13.8
		1-1	28	17.8	67.9	14.3
		1-2	30	20.0	66.7	13.3
	2		90	7.8	72.2	20.0
		2-1	30	13.3	56.7	30.0
		2-2	30	6.7	76.7	16.6
		2-3	30	3.3	83.3	13.3
	3		43	30.2	65.1	4.7
	5		15	26.7	66.7	6.7
		5-2	1	0	100	0
		5-3	14	28.6	64.3	7.1
	6		102	22.5	63.7	13.7
		6-1	15	6.7	66.7	26.6
		6-2	72	25.0	66.7	8.3
		6-3	15	26.7	46.7	26.6
	7		93	33.3	65.6	1.1
		7-1	29	27.6	72.4	0
		7-2	24	33.3	66.7	0
		7-4	40	37.5	60.0	2.5
	8		28	21.4	71.4	7.1
		8-1	28	21.4	71.4	7.1
5	1		74	13.5	77.0	9.5
		1-1	20	10.0	80.0	10.0
		1-2	29	20.7	72.4	6.9
		1-3	25	8.0	80.0	12.0
	2		82	12.2	58.5	29.3
		2-1	28	3.6	53.6	42.8
		2-2	24	20.8	62.5	16.7
		2-3	30	13.3	60.0	26.7
	3		56	25.0	58.9	16.1
	4		140	16.4	64.3	19.3
		4-2	55	16.4	61.8	21.8
		4-3	85	16.5	65.9	17.6
	5		89	41.6	46.1	12.4
		5-2	40	72.5	17.5	10.0
		5-3	49	16.3	69.4	14.3
	6		86	15.1	73.3	11.6
		6-1	20	5.0	85.0	10.0
		6-2	60	18.3	71.7	10.0
		6-3	6	16.7	50.0	33.3
	7		120	23.3	63.3	13.3
		7-1	30	13.3	66.7	20.0
		7-2	30	36.1	58.3	5.6
		7-3	30	16.7	66.7	16.6
		7-4	24	25.0	62.5	12.5
	8		73	16.4	76.7	6.8
		8-1	43	16.3	76.7	7.0
		8-2	30	16.7	76.7	6.6
	9		6	16.7	66.7	16.7
		9-1	6	16.7	66.7	16.7

Period	Stratum	Substratum **	Sample Size	Classification Percentage*** Hudson	Chesapeake	Roanoke
6	4		99	21.2	66.7	12.1
		4-2	51	29.4	54.9	15.7
		4-3	48	12.5	79.2	8.3
	6		106	16.0	69.8	14.2
		6-1	54	16.7	74.1	9.2
		6-2	52	15.4	65.4	19.2
	7		124	21.0	76.6	2.4
		7-1	44	11.4	84.1	4.5
		7-2	34	38.2	58.8	3.0
		7-5†	21	28.6	71.4	0
		7-6‡	25	8.0	92.0	0
	8		117	21.4	72.6	6.0
		8-1	37	27.0	73.0	0
		8-2	40	12.5	82.5	5.0
		8-3	40	25.0	62.5	12.5
	9		100	8.0	80.0	12.0
		9-1	50	10.0	80.0	10.0
		9-2	50	6.0	80.0	14.0
	10		24	12.5	62.5	25.0
		10-2	24	12.5	62.5	25.0

*Not considered were striped bass <405.6-mm fork length from New York waters.

**Substrata designations appear in Figures III-2 of main text. Substrata with no samples are not listed.

***Classified with quadratic (QTH) functions.

†Substratum 7-5 designates combined area of substrata 7-2 and 7-4.

‡Substratum 7-6 designates combined area of substrata 7-1 and 6-2.

APPENDIX G

PROCEDURES FOR INCORPORATING NATIONAL MARINE FISHERIES STATISTICS WITH RELATIVE CONTRIBUTION CALCULATIONS OF COMMERCIAL CATCH WEIGHTS

APPENDIX G

PROCEDURES FOR INCORPORATING NATIONAL MARINE FISHERIES STATISTICS WITH RELATIVE CONTRIBUTION CALCULATIONS OF COMMERCIAL CATCH WEIGHTS

The fishery was broken down by state for the applicable NMFS county code and water code representing the specific strata/substrata as presented in Table G-1. The analysis of statistics was conducted as follows:

Letting X = poundage

i = TI spatial strata (i = 1, 2, ...10)

j = spatial confines of commercial catch data that conform to specific combinations of TI spatial strata and substrata (j = 1, 2, ...16)

k = month (k = 1, 2, ...12)

ℓ = period (ℓ = 1[Jan-Feb], 2[Mar-Apr], ... 6[Nov-Dec])

and using dot notation to indicate summations, poundage was summarized by month and spatial confine (1) and totals calculated for months (2), periods (3), and year (4) as follows (Table G-2 and G-3):

(1) X_{jk} = poundage in j^{th} confine and k^{th} month

(2) $\sum_{j}^{16} X_{jk} = X._{k}$ = monthly poundage in all confines

(3) $X._{\ell}$ = sum of appropriate $X._{k}$'s

(4) $\sum_{\ell}^{6} X._{\ell} = X..$ = total poundage in all confines for year

The relative proportion of poundage was calculated for each spatial confine in relation to its appropriate periodic poundage (5) and for each period in relation to total yearly poundage (6) as follows (Table G-4):

(5) $\frac{X_{j\ell}}{X._{\ell}} = W_{j\ell}$, where $X_{j\ell}$ = sum of appropriate X_{jk}'s

(6) $\frac{X._{\ell}}{X..} = W_{\ell}$

Using 1973 and 1974 weighting proportions, the mean weighting proportion for each spatial confine within period (7) and for each period within year (8) was calculated as follows (Table G-5):

(7) $\frac{W_{j\ell\ (1973)} + W_{j\ell\ (1974)}}{2} = \overline{W}_{j\ell}$

(8) $\frac{W_{\ell(1973)} + W_{\ell\ (1974)}}{2} = \overline{W}_{\ell}$

Mean weighting proportion for each TI stratum within period (9) was calculated as follows (Table III-18):

(9) $\overline{W}_{i\ell}$ = sum of appropriate $\overline{W}_{j\ell}$'s.

Table G-1

Listing of NMFS State, County, and Water Codes as Utilized in Calculation of Commercial Landing Weights (Based on Poundages from 1973 and 1974 Catch Statistics) for Spatial Confines Conforming to TI Strata and Substrata

Stratum-Substratum	State	State Code	Water Code	County Code
1-2	Massachusetts	24	513,514	07
1-3	Massachusetts	24	514	01,13
2	Massachusetts	24	521,538	01,05,03
3†	Rhode Island	42	009,027, 526,537,538, 539,611,613	05,09
4	New York	35	036,611	15,35
6	New York	35	034,613	35
7	New York	35	612	11,15,35
7-4	New Jersey	33	027	25
8-1	New Jersey	33	612,613, 614,615	25,27
8-2	New Jersey	33	614,615, 616	01
8-3	New Jersey	33	614,631	09
8-3	New Jersey	33	621	01,09
9-1	Maryland‡			
9-2	Virginia	49	624,625, 626	01,18,45
10-1	Virginia	49	631,632, 635,636	13,18,51
10-2+3	North Carolina	36	024	17,19

†Considerable capture in other strata; monthly totals for all Rhode Island landings utilized.

‡Maryland landing obtained in 1973 from Commercial Catch Statistics (CFS) Nos. 6152, 6174, 6212, 6238, 6259, 6282, 6302, 6323, 6344, 6365 and 6385, and in 1974 from CFS Nos. 6431, 6451, 6472, 6492, 6512, 6532, 6553, 6582, 6602, 6622, 6641, and 6660.

Table G-2

Summary of 1973 NMFS Commercial Landings (Poundage) by Month and Spatial Confines Conforming to TI Strata and Substrata

State	Stratum-Substratum	Total Reported Landings (Thousand Pounds) Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Massachusetts	1-2	--	--	--	--	0.55	0.04	0.47	0.21	2.99	1.44	--	--
	1-3	--	--	--	--	2.88	13.96	30.51	22.54	23.08	13.88	0.14	0.02
	2	--	--	--	--	6.24	6.71	0.44	0.06	0.27	1.05	1.68	--
Rhode Island	3	0.62	--	--	3.56	99.53	52.77	52.07	40.04	47.25	217.54	109.99	0.05
New York	4	--	--	--	1.00	29.25	81.27	46.82	7.99	20.02	335.28	225.62	--
	5	--	--	--	--	--	--	--	--	--	--	--	--
	6	--	--	--	7.12	42.23	65.03	47.11	7.26	26.94	172.74	273.56	7.32
	7	--	--	--	--	--	15.10	51.37	73.35	41.38	9.85	86.38	--
New Jersey	7-4	--	--	--	1.00	5.23	4.68	3.28	--	0.25	0.36	--	--
	8-1	--	--	--	0.48	6.51	7.60	6.14	10.79	5.54	12.73	262.94	13.64
	8-2	--	--	0.30	1.10	5.22	--	--	3.52	--	4.74	125.60	19.30
	8-3	0.06	--	0.02	2.28	5.33	1.48	0.04	--	--	5.44	131.87	76.03
Maryland	9-1	11.70	9.76	12.19	4.88	0.16	0.06	0.01	0.61	0.04	0.26	31.33	161.23
Virginia	9-2	13.89	40.74	35.16	0.42	0.23	--	--	--	--	--	6.29	79.10
	10-1	7.74	--	--	1.36	0.03	--	--	--	--	0.05	10.52	10.75
North Carolina	10-2+3	500.50	182.28	103.53	30.44	0.16	--	--	--	--	--	35.38	204.19
Total by month	$X._k$	534.51	232.78	151.20	53.64	203.55	248.70	238.26	166.37	167.76	775.36	1301.30	571.63
Total by period	$X._\ell$	767.29		204.84		452.25		404.63		943.12		1872.93	
Total 1973	$X..$						4645.06						

--denotes no commercial landings

Table G-3

Summary of 1974 NMFS Commercial Landings (Poundage) by Month and Spatial Confines Conforming to TI Strata and Substrata

State	Stratum-Substratum	Total Report Landings (Thousand Pounds) Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Massachusetts	1-2	--	--	--	--	2.25	7.19	16.92	9.63	16.99	7.15	--	--
	1-3	--	--	--	0.22	4.34	22.75	10.09	4.12	8.27	10.22	0.12	--
	2	--	--	--	--	6.05	1.92	0.14	0.18	1.18	2.82	--	--
Rhode Island	3	--	--	--	19.49	18.11	44.06	48.93	12.89	7.97	132.13	51.98	0.87
New York	4	--	--	--	15.00	20.51	131.47	47.02	11.99	39.14	121.18	133.40	2.35
	5	--	--	--	--	--	--	--	--	--	--	--	--
	6	3.00	--	0.90	14.88	32.64	53.45	47.38	12.42	21.98	175.20	125.02	3.08
	7	4.85	--	0.90	17.81	20.38	44.25	74.80	72.09	31.73	60.92	32.17	6.58
New Jersey	7-4	--	--	--	0.15	2.02	0.24	1.00	--	1.44	2.00	--	--
	8-1	--	--	--	.60	3.43	4.28	8.13	6.88	3.99	6.85	201.42	0.39
	8-2	1.74	3.95	1.17	6.97	5.02	0.04	--	--	0.06	19.01	142.72	1.47
	8-3	0.14	--	--	6.84	9.21	0.40	--	--	--	11.82	155.48	37.86
Maryland	9-1	43.02	23.03	46.06	1.32	0.38	--	0.05	0.09	0.08	2.15	92.86	93.95
Virginia	9-2	107.95	29.84	152.13	6.18	0.06	0.02	--	--	--	0.04	19.73	71.69
	10-1	10.25	3.19	5.90	--	--	--	--	--	--	--	23.06	1.40
North Carolina	10-2+3	144.59	193.70	11.28	1.78	--	--	--	--	--	3.15	45.26	74.23
Total by month	$X._k$	315.54	253.71	218.34	91.30	124.40	310.07	254.46	130.29	132.83	554.64	1023.22	293.87
Total by period	$X._\ell$	569.25		309.64		434.47		384.75		687.47		1317.09	
Total 1974	$X..$						3702.67						

--denotes no commercial landings

Table G-4

Estimate of 1973 and 1974 Proportions of Periodic Commercial Poundage Landed within Each Spatial Confine and Yearly Poundage within Each 2-Month Period

Stratum-Substrata	1973 Weighting Factors						1974 Weighting Factors					
	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
1-2	--	--	.00130	.00168	.00470	--	--	--	.02173	.06901	.03511	--
1-3	--	--	.03724	.13111	.03919	.00009	--	.00071	.06235	.03693	.02690	.00009
2	--	--	.02863	.00124	.00140	.00090	--	--	.01834	.00083	.00582	--
3	.00081	.01738	.33676	.22764	.28076	.05875	--	.06294	.14309	.16068	.20379	.04013
4	--	.00488	.24438	.13546	.37673	.12046	--	.04844	.34981	.15337	.23320	.10307
5	--	--	--	--	--	--	--	--	--	--	--	--
6	--	.03476	.23717	.13437	.21172	.14997	.00527	.05096	.19815	.15554	.28682	.09726
7	--	--	.03339	.30823	.05432	.04612	.00852	.06062	.14876	.38178	.13477	.02942
7-4(NJ)†	--	.00488	.02191	.00811	.00065	--	--	.00048	.00520	.00260	.00500	--
8-1	--	.00234	.03120	.04184	.01937	.14767	--	.00194	.01775	.03901	.01577	.15322
8-2	--	.00683	.01154	.00870	.00503	.07737	.00999	.02629	.01165	--	.02774	.10948
8-3	.00008	.01123	.01506	.00010	.00577	.11100	.00025	.02209	.02212	--	.01719	.14679
9-1	.02797	.08333	.00049	.00151	.00032	.10281	.11603	.15302	.00087	.00036	.00324	.14184
9-2	.07120	.17370	.00051	--	--	.04559	.24205	.51127	.00018	--	.00006	.06941
10-1	.01009	.00664	.00007	--	.00005	.01136	.02361	.01905	--	--	--	.01857
10-2+3	.88986	.65402	.00035	--	--	.12791	.59476	.04218	--	--	.00458	.09072
W_{ℓ} ‡	.16518	.04410	.09736	.08711	.20304	.40321	.15374	.08363	.11734	.10391	.18567	.35571

† denotes New Jersey landing in stratum 7 substratum 4.

‡ denotes proportion of yearly poundage landed within each period.

--denotes no commercial landings.

Table G-5

Estimate of Averaged 1973 and 1974 Proportions of Periodic Commercial Poundage Landed within Each Spatial Confine and Yearly Poundage within Each 2-Month Period

Stratum-Substratum	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
1-2	--	--	.01152	.03534	.01990	--
1-3	--	.00036	.04980	.08402	.03304	.00009
2	--	--	.02348	.00104	.00361	.00045
3	.00040	.04016	.23992	.19416	.24228	.04944
4	--	.02666	.29710	.14442	.30496	.11176
5	--	--	--	--	--	--
6	.00264	.04286	.21766	.14500	.24927	.12362
7	.00426	.03031	.09108	.34500	.09454	.03777
7-4(NJ)†	--	.00268	.01356	.00536	.00282	--
8-1	--	.00214	.02448	.04042	.01757	.15044
8-2	.00500	.01656	.01160	.00435	.01638	.09342
8-3	.00016	.01666	.01859	.00005	.01148	.12890
9-1	.07200	.11818	.00068	.00094	.00178	.12232
9-2	.15662	.34248	.00034	--	.00003	.05750
10-1	.01685	.01284	.00004	--	.00002	.01496
10-2&3	.74231	.34810	.00018	--	.00229	.10932
W_ℓ‡	.15946	.06386	.10735	.09551	.19436	.37946

--denotes no commercial lands; no commercial fishery exists in stratum 5.

† denotes New Jersey landings in stratum 7 substratum 4.

‡ W_ℓ denotes averaged 1973 and 1974 proportions of yearly poundage landed within each period.

Institute of Environmental Medicine

CYTOGENETIC STUDY OF THE STRIPED BASS *Morone saxatilis* FROM THE HUDSON RIVER

REPORT
To

CONSOLIDATED EDISON COMPANY OF NEW YORK, INC.
4 Irving Place
New York, New York 10003

From

NEW YORK UNIVERSITY MEDICAL CENTER
A.J. Lanza Research Laboratories
Department of Environmental Medicine
Long Meadow Road
Sterling Forest, Tuxedo, New York 10987

September, 1976

New York University Medical Center
New York, N.Y. 10016

CYTOGENETIC STUDY OF THE STRIPED BASS, *Morone saxatilis* (WALBAUM) FROM THE HUDSON RIVER ESTUARY, NEW YORK.

ABSTRACT

Striped bass larvae of 10 mm total length were utilized in obtaining chromosome spreads from epithelial tissue. Karyotypic analysis revealed a diploid chromosome number of 48, consisting of all acrocentric chromosomes with a size range of 1.25 µm-2.75 µm. Pairing of homologous chromosomes was not accomplished due to the small size of the chromosomes.

Introduction

Fishes are the most varied of the vertebrates, comprising more than 20,000 species, or about one third of all living vertebrate species. Despite the variety, abundance and importance of fishes, chromosomes have been reported for less than 600 fish species and of this total, karyotypes have been reported for fewer than 300 species (Denton, 1973). Included among the species studied for chromosomes and karyotypes are several forms common to the Hudson River Estuary (Table 1) including the two Ichthyopercids, Morone saxatilis and M. americana (Kerby, 1972).

The majority of fishes which have been studied have 48 chromosomes (Roberts, 1967; Denton, 1973). Fifty percent of the species likely to be found in the lower Hudson Estuary have been identified as having 48 chromosomes. Ohno, et al. (1969) suggested that ancestral fishes had 48 chromosomes and that many forms have retained the ancestral, diploid complement throughout more than 400 million years of evolution.

Chromosome number, morphology and complete karyotypic analysis have been used extensively in population studies of fishes (Denton, 1973; Fisher and Rachlin, 1972; Arcement and Rachlin, 1976; Kerby, 1972; Chen and Ruddle, 1969). Evidence exists that karyotypic analysis may be used to discern genetic divergence of isolated populations (Arcement and Rachlin, 1976) and perhaps to identify different genetic stocks in mixed populations.

Table 1. Chromosome numbers for estuarine and euryhaline fish species common in the lower Hudson River.

Species	Chromosome Number 2N	N	Reference
Sea lamprey	168		Potter & Rothwell, 1970
American eel	38		Sick, 1962
Alewife	48	24	Mayers & Roberts, 1969
Rainbow trout	60		Simon & Dollar, 1963
Brown trout	80		Svardson, 1945
Goldfish	94 - 104	(47)	Post, 1965 Ohno & Atkin, 1966 Chiarelli, et al., 1969
Carp	104	52	Makino, 1939
Golden shiner	50		Leippman & Hubbs, 1969
Banded killifish	48	24	Chen & Ruddle, 1970 Arcement & Rachlin, 1976
Mummichog	48	24	Chen & Ruddle, 1970 Fisher & Rachlin, 1972
Striped killifish	48	24	Chen & Ruddle, 1970 Fisher & Rachlin, 1972
Fourspine stickleback	46	23	Chen & Ebeling, 1970
Redbreasted sunfish	48	24	Roberts, 1964
Pumkinseed	48	24	Roberts, 1964
Largemouth bass	46	23	Roberts, 1964
White crappie	48	24	Roberts, 1964
White perch	48		Kerby, 1972
Striped bass	48		Kerby, 1972

The present study was undertaken to establish a karyotype for striped bass of Hudson River origin, and to compare that karyotype with that of a population of striped bass studied by Kerby (1972) captured from the James River, Virginia.

The assistance of Dr. J.W. Rachlin, Dept. of Biological Sciences, Herbert Lehman College, C.U.N.Y. in carrying out these studies is greatly appreciated.

Materials and Methods

Karyotypic analysis was performed on striped bass larvae of average total length of 10 mm. Larvae were obtained from the Hudson River Striped Bass Hatchery operated by Texas Instruments, Inc. Verplanck, New York.

Entire larvae were used in the preparation of chromosome spreads for analysis, and the method of Arcement and Rachlin (1976) was followed throughout. Groups of larvae were pre-treated in an hypotonic solution for 1.0 to 1.5 hours. Following pre-treatment the larvae were fixed in a methanol/acetic acid solution for 1/2 hour, divided into three 10 minute intervals, using fresh cold fixative for each 10 minute interval.

Larvae were removed from the fixative with forceps and "flocked" onto xylene-cleaned glass slides. The epithelial cells which adhered to the slides, after removal of scales and other gross debris, were allowed to air dry prior to staining. The slides were stained with buffered (pH 9.0)

Giemsa stain (Patil, et al., 1971), cleared in xylol and mounted for examination. After initial screening, chromosomes were examined for number and types, and the best preparations were used both for camera lucida drawings and photography for more careful study.

Results

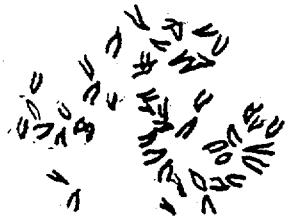
An examination of Figures 1, 2 and 3 show that the chromosome complement of these striped bass larvae consists of a total of 48 acrocentric chromosomes. This is in contrast with the preliminary work of Kerby (1972) who reported that the striped bass from the James River, Virginia, had a chromosome complement of 48 chromosomes of which 46 were acrocentric and two were metacentric.

An examination of the idiogram in Figure 1, prepared from the camera lucida drawing in Figure 1 also indicates that there are only acrocentric and no metacentric chromosomes associated with the chromosome complement prepared from the larvae obtained from the Hudson River Striped Bass Hatchery. Further, Table 2 demonstrates that the size range of these chromosomes is from 1.25 to 2.75 µm. A further examination of Table 2 demonstrates that one chromosome is 2.75 µm, four are 2.5 µm, 10 are 2.25 µm, nine are 2.2 µm, 12 are 2.0 µm, four are 1.75 µm, one is 1.7 µm, five are 1.5 µm and two are 1.25 µm long.

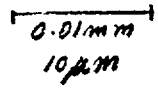
Figure 1

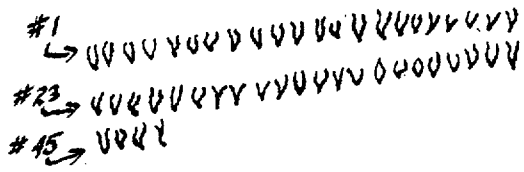
Camera Lucida drawing of Chromosome spread of Roccus saxatilis = Morone saxatilis (Walbaum)

Striped Bass
Roccus saxatilis = Morone saxatilis
Fish #14C
Larvae 10mm Total length
Epithelial spread



Chromsome Count
48 acrocentics





Idiogram of Above Chromosome Spread

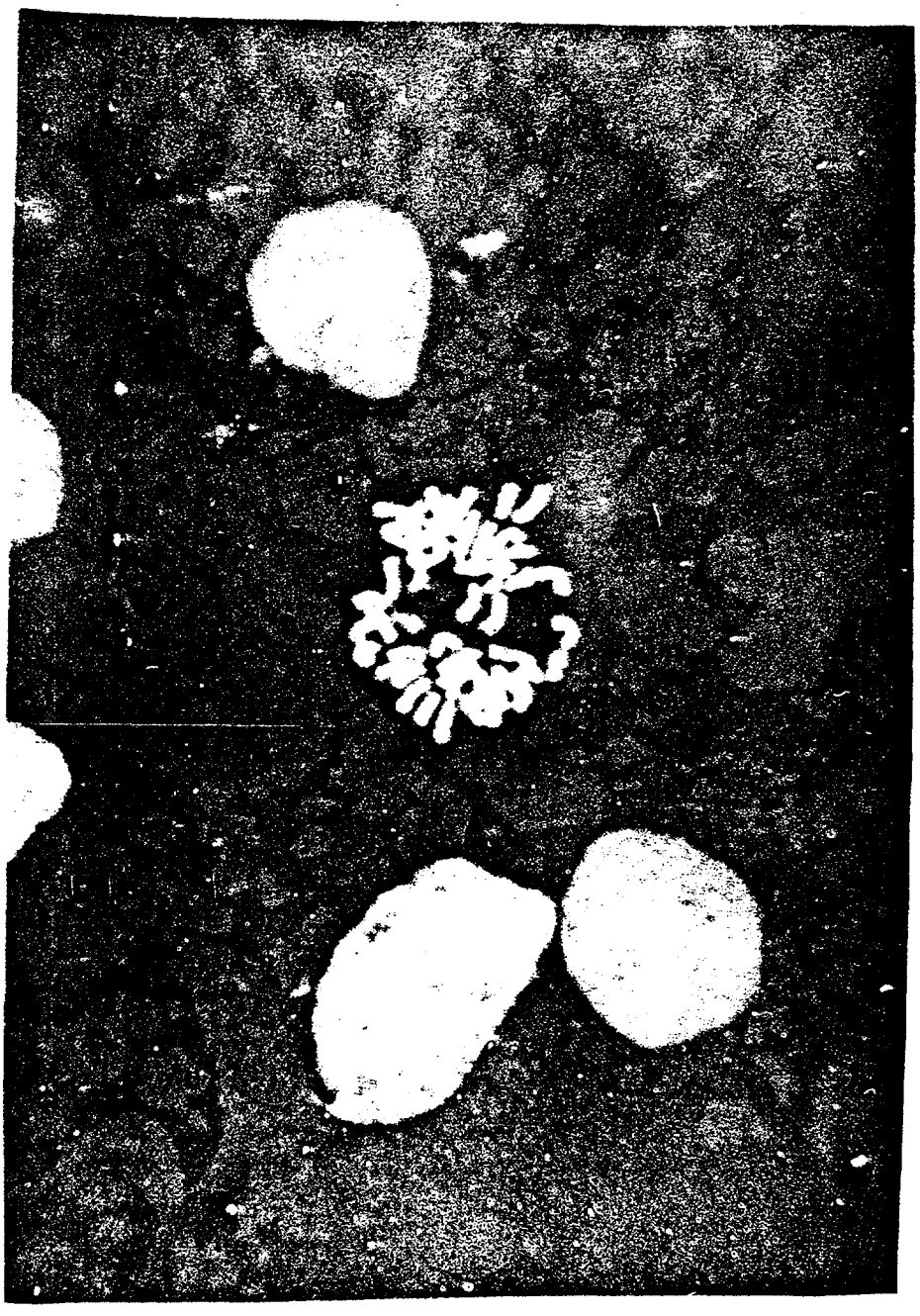
Figure 2

Metaphase chromosome spread from *Morone saxatilis*

10 μm

Figure 3

Metaphase chromosome spread from Morone saxatilis



10 μm

Table 2. Size of chromosomes of striped bass as measured from the idiogram in Figure 1.

Chromosome Number	Chromosome Size (microns)
15	2.75
14, 44, 47, 48	2.5
2, 4, 8, 9, 11, 27, 34, 37, 38, 40	2.25
1, 10, 12, 16, 22, 25, 26, 32, 33	2.2
3, 5, 7, 18, 28 29, 36, 39, 42, 43 45, 46	2.0
23, 24, 30, 35	1.75
31	1.7
6, 17, 20, 21, 41	1.25
13, 19	1.25

Examination of the slides indicated presumptive banding, however, due to the tightness of coiling of the chromosomes and their small size, one could not resolve the banding with enough precision to use the technique as a means of pairing the homologous chromosomes.

Discussion

A karyotype of striped bass larvae, derived by artificial spawning of adult striped bass captured in the Hudson River Estuary, has been prepared. This karyotype consists of 48 acrocentric chromosomes and resembles closely the published karyotype for the white perch, Morone americana (Kerby, 1972). It differs from the published karyotpye of striped bass, Morone saxatilis, from the James River, Virginia (Kerby, 1972), in that the James River striped bass were reported to have a karyotype consisting of 48 chromosomes of which 46 are acrocentric and two are metacentric. However, Kerby (pers. comm., June, 1976) has indicated that the initial classification of James Rivers striped bass chromosomes as consisting of 46 acrocentrics and 2 metacentrics may have been premature, and that further study of this karyotype is anticipated during the 1977 spawning season.

A difference of this magnitude between the chromosome complements of these two striped bass strains (Hudson River vs. James River) is possible, but unlikely between populations only partially isolated geographically for a relatively

brief period of time. It could be explained on the basis of Robertsonian inheritance, (Mayr, 1963) involving first a centromere division of the two metacentric chromosomes creating a karyotype consisting of 50 acrocentric chromosomes followed by a second event involving chromosome loss; perhaps by a non-disjunction, establishing, once again, a chromosome complement of 48 chromosomes, but now with only acrocentric chromosomes in the complement. Further discussion of this question depends upon additional information regarding karyotypes of striped bass from other populations.

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1496

RACIAL INVESTIGATION OF THE STRIPED BASS USING CRITICAL SCALE ANALYSIS

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Final Report Submitted to Consolidated Edison Co., May 1, 1977

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SUMMARY

The University of Rhode Island has conducted a two year study designed to determine if scale surface microstructure is a useful parameter for stock analysis of the striped bass (Morone saxatilis). A Dektak^{R} surface profile analyzer capable of detecting surface features as small as 1 micron (.001 mm) has been used to detect circulus (ridge) structure on the surface of scales. A Nova 1200 mini-computer has been programmed to quantify the analog signal derived from the profile analyzer. Digital values for height and width of each ridge on individual scales have been computed and placed in permanent magnetic disk storage.

Texas Instruments Ecological Services have provided all scales used in this study. Fish collected from five major spawning rivers (Rappahannock, Potomac, Choptank, Elk, and Hudson) in 1974 were used in URI Phase I digital profile analyses. Scale training libraries consisting of a set of profiles from each spawning region were submitted to analyses designed to detect inter-regional differences in scale surface structure, if present. Fourier (harmonic frequency) analyses detected no significant difference in modal circulus width for scales from the five rivers. Polymodal circulus width spectra were often found in male fish from the Potomac River.

Significant differences in mean circulus height (11 vs. 13 μ) were found between scales derived from Chesapeake Bay and Hudson regions. Using regression analysis, fork length and average circulus height were demonstrated to be significantly correlated. Results of discriminant analysis on 1974 profiles yielded 75% non-jackknifed separation between spawning groups, and 66% accuracy when mixed groups of scales derived from the two regions were classified using discriminant functions calculated from spawning river training sets.

In 1975 Texas Instruments provided 500 scale samples derived from six spawning rivers (Rappahannock, Potomac, Choptank, Elk, Hudson and Roanoke). In addition, over 3000 scales collected along the Atlantic Coast from Maine to North Carolina were provided.

Phase II of the URI Critical Scale Analysis Study has attempted to maximize utilization of recorded scale surface growth information. Normalized probability density functions (histograms) which describe the actual distribution of circulus heights and widths within the scale were calculated. The histogram approach resulted in improvements in stock separation levels when applied in a discriminant analysis to previously recorded Phase I scale data.

In Phase II, several new analytical techniques were also introduced. A moving baseline technique was incorporated into the circulus measurement program. This permitted, for the first time, the detection of circuli which deviated from the (fixed) scale baseline. Average circulus width was reduced from 80 to 55 μ. This was in better agreement with results derived from other measurement techniques.

A new program PROD/COMP was developed. This method generates a single base profile derived from all scales within a distinct river training group. Oceanic scale profiles are successively compared with each of the base river profiles. On the basis of maximum congruency measurements, oceanic scales are classified as being derived from the river whose base profile they most nearly resemble.

AVPLT is a program which produces an average profile for all scales within a given river. This program provides a measure of mean and dispersion values for circuli 1-60, i.e. those for which maximum inter-regional variability would be expected. Pairwise inter-region comparisons using AVPLT were performed to detect those specific segments of scale surface which differed maximally between regions.

CONCLUSIONS

1. The URI scale analysis method has proven accurate in the detection of scale surface structure. Accuracy to within 0.5% has been demonstrated using a calibration blank prepared by the National Bureau of Standards. Replicate runs over the same scale demonstrate consistent values to within an accuracy of 0.1%.

2. Size homogeneous samples derived from fish collected from the three natal regions (Chesapeake Bay, Roanoke River, Hudson River) in 1975 were analyzed. An overall separation level of 67% was realized for fish within the size range 400-600 mm. This suggests the existence of racial differences when size bias effects on scale growth are removed.

3. Extended size range samples (400-1000 mm) when analyzed using probability density function-discriminant analysis techniques provided 45-55% separation levels. Inter-region variability in scale growth appears too slight to permit totally reliable racial classifications to be based solely on best available scale growth analytic techniques. This lack of scale growth variability relative to environmental variability appears to confirm trends noted in other investigations (Hallin, 1957; Van Utrecht and Schenkkan, 1972; Messinger and Bilton, 1974).

4. Inter-validation studies between URI classification results and Texas Instruments classifications demonstrate significant lack of agreement in classification of individual fish. URI discriminant analysis offers best agreement of all techniques (44%) for 197 fish used in training sets. Both PROD/COMP height and width techniques failed to adequately separate training sets. PROD/COMP height functioned best (39% agreement) in URI-Texas Instruments inter-validation studies of oceanic fish.

5. The results of this study do not indicate that scale surface microstructure may be used to confidently assign striped bass to natal spawning regions. Two key assumptions underlie the URI scale analysis investigation. First, that fish collected while spawning in a particular spawning river were themselves native to that river during their juvenile stages of development (i.e. validity of homing hypothesis). Based on results of the Texas Instrument meristic study, this hypothesis appears valid for striped bass. The second necessary condition is that there be differences in early scale growth surface structures sufficiently marked to permit reliable reclassification based solely on these structures. At the present time, such marked differences do not appear to occur on scales of the striped bass.

INTRODUCTION

The problem of source stock analysis in the striped bass (Morone saxatilis) has been approached via meristic, morphometric and biochemical techniques (Raney and DeSylva, 1953; Morgan, Koo et al., 1973; Merriman, 1941; Lewis, 1957). More recently, a combined approach utilizing a quadratic discriminant function on five significant meristic and morphometric characters yielded 70-80 percent reclassification accuracies for striped bass collected from major spawning rivers along the Atlantic Coast (Texas Instruments, 1976). Such methods generally require retention of the entire fish, trained personnel, and laboratory facilities. In this study a sensitive surface profile analyzer (Sloan Instrument Dektak^{R}) in conjunction with a Data General Nova mini-computer have been utilized for the detection, recording, and analysis of scale surface microstructure. Previous studies have shown that variations in scale growth may be used as indicators of source region for migratory, mixed stock fish populations, such as the sockeye salmon (Onchorhynchus nerka) (Anas and Murai, 1968; Messinger and Bilton, 1974). The objective of this investigation is to assess the potential for differentiation between racial stocks of the striped bass on the basis of regional variation in scale growth structure.

Texas Instruments, as part of its synoptic subpopulation survey, collected fish in 1974 from major striped bass spawning rivers along the Atlantic Coast (Hudson River, Elk, Rappahannock, Choptank, and Potomac Rivers). Scales taken from these fish in 1974 were submitted to surface profile analysis. Analytic techniques applied to the digitally recorded surface profiles included Fourier transform analysis, circulus height and width determination, and linear discriminant analyses applied to summary statistics describing scale structure. Non-jacknifed reclassification accuracies (see p. 24) were approximately 80 percent for Hudson and Chesapeake training

sets and somewhat lower (66 percent) for Hudson and Chesapeake test sets. (Training set refers to those scales actually used to compute the discriminant functions. Test sets were not used in the computations, but were subjected to discriminant analysis using previously derived discriminant functions.)

The following statements represent the results of Phase I (1974-1975) of the Critical Scale Analysis Investigation. The analytical format used in Phase I is illustrated in Figure 1. Fourier (harmonic frequency) analysis indicated general similarity in scale circulus widths over the five rivers investigated. Polymodal width structures were detected for male fish from the Potomac River.

A program (WHGHT) originally developed to characterize and quantify sea state (Colbert, 1974) was used to derive the following five parameters for each scale:

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

These parameters were submitted to linear discriminant analysis using BMD07M (Dixon, 1973). Composition of training sets used in this analysis is illustrated in Table 1. Mean values for these parameters are displayed in Table 2. Population classification accuracies are summarized in Table 3.

Figure 2 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

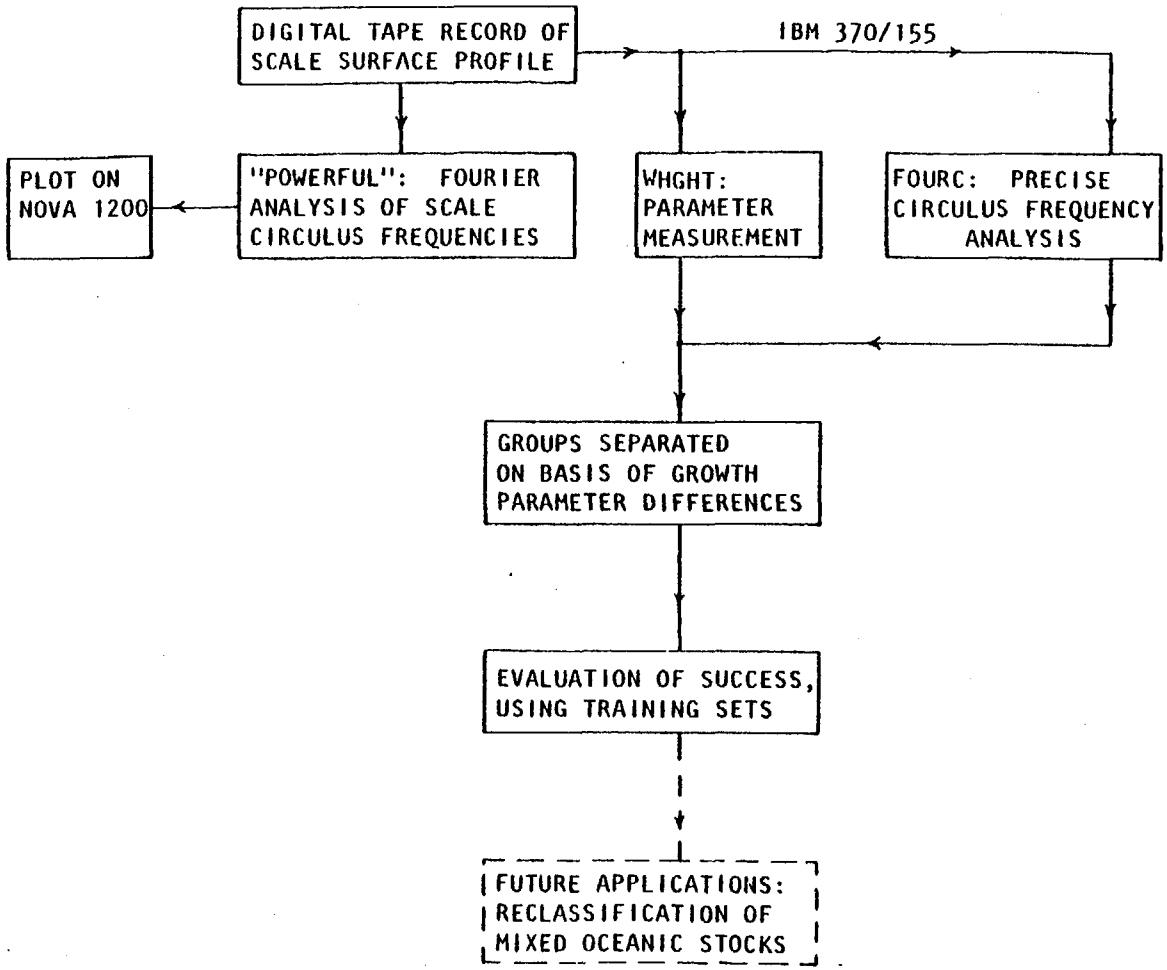


Figure 1. Flow chart of computer analyses conducted during Phase I of Critical scale analysis investigation, 1974-1975.

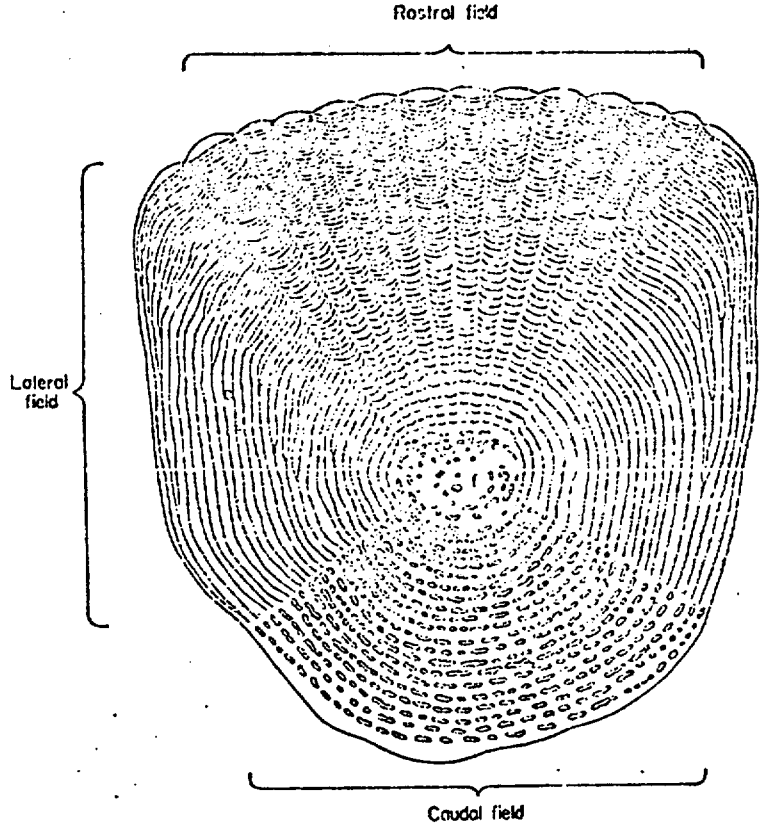


Figure 2. Structural surface morphology of a typical ctenoid scale. f represents the focus. (Lanzing and Higginbotham, 1974).

Table 2. WHGHT parameter summary: circulus height and spacing values.

River	$\overline{H}(\mu)$	$\overline{T}(\mu)$	H1/3(μ)	T1/3(μ)	TN	Sample Size
Hudson	13.66	76.98	19.67	93.10	31.9	N = 53
Rappahannock, Elk, Choptank, Potomac	11.08	77.98	16.87	99.22	32.1	N = 112

Table 3. Reclassification accuracies by region.

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

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.

At the close of Phase I of the Critical Scale Analysis Study the following results were noted. Striped bass fork length was significantly correlated with HBAR, the average circulus height. The different spawning grounds in 1974 possessed significantly different length frequency distributions among spawning fish. Chesapeake males collected were shorter in fork length (LBAR = 424 mm). Hudson males were larger in fork length (LBAR = 723 mm). Racial differences might have been due to scale differences based on size class variation between the regions. This could reduce the utility of circulus height as an indicator of racial origin. An analysis of covariance corrected for length effects. No significant differences in circulus height remained following such corrections. Further studies, using a carefully selected range of sizes of fish from both regions are described herein. These physically remove severe size bias as a racial parameter. This modification has been performed for the second part of the study, and results will be described below.

A second improvement to earlier analytic techniques was developed in this study to correct the fact that curved regions of scales which deviated from the (fixed) baseline were not adequately detected by our 1974 circulus measurement program. A moving baseline technique was incorporated into the 1975 circulus measurement program. Accuracy of circulus detection improved and results were in much better agreement with other independent measures of circulus width (e.g., Fourier Transform Analysis, Scanning Electron Microscopy). TBAR (i.e. average circulus width) was reduced from 80μ to 55μ for a test sample

of eight Chesapeake scales. This agrees with the 55-58μ values indicates from Fourier analysis. A final improvement was made in the area of data analysis. The 1974 surface profiles were collapsed into two numbers; HBAR, average circulus height, and TBAR, average circulus spacing. Two new techniques were developed to increase data analysis resolution capacity. Normalized probability density functions provide 44 information bearing variables instead of the previous two (see. p. 26). Different distributions of model heights and widths permit a much finer discrimination than do the cruder averages. Further, graphical techniques have been developed. These permit the display of group averaged profiles. Sequential circulus ordering is preserved. Marker regions along the scale profile may be detected on the basis of intergroup differences, if present. A new method (PROD/COMP) which utilizes this sequential ordering to categorize profiles by region represents an adaptation of manual growth curve analysis as originally applied by Koo (1963) to salmon smolts (see p. 30). Phase II analyses present results of more sophisticated analytical methods applied to more representative samples collected in 1975 by Texas Instruments from six spawning rivers (Rappahannock, Potomac, Choptank, Elk, Hudson, and Roanoke Rivers).

Fish collected from five oceanic strata have been classified into one of three natal regions (Chesapeake Bay, Roanoke River, Hudson River). Results for individual oceanic fish are compared with results of the Texas Instruments, meristic and morphometric study to assess validity of scale based classifications. An advantage of scale analysis is the potential for reduced handling of the individual fish. Scales may be collected without the necessity for sacrificing animals. Thus, lower accuracy than that obtained from meristic and morphometric analysis may be compensated for by a reduced time and effort over the course of a large sampling program.

METHODS AND MATERIALS

Scales used in Phase II of the Critical Scale Analysis Study have been provided by the Texas Instruments Ecological Services Group, Buchanan, NY. All scales were taken from a key location defined as follows: "...starting at the base of last spine on first dorsal, move down the diagonal posteriorly to row four (black) and five (white) located above the lateral line, and select four scales from each row, moving posteriorly." (personal communication, T. Berggren). Care in selection of such a consistent location is desirable, since previous studies have demonstrated a reduction in inter-scale variability arising from use of such a standardized location on the body of the fish (Clutter and Whitesel, 1956).

Scales provided for use in this study consisted of two distinct segments. The first group of scales was derived from fish collected during the 1975 spawning season at known spawning rivers along the Atlantic Coast. These included the Hudson River, the Elk, Choptank, Rappahanock, Potomac and Roanoke Rivers (Fig. 3). These fish composed the training set, and were used in derivation of discriminant classification functions. The second group of fish represents a sample of oceanic fish, taken from ten geographic strata (Fig. 5) over the course of an entire year, the year being divided into six sequential two-month periods. Effort was exerted to assure adequate samples of fish, with respect to such variables as size, sex and regional distribution.

Data was provided by Texas Instruments Ecological Services, concerning fork length, sex, and age associated with each of the scales in the data base. This data was necessary in order to insure that the scale training samples possessed an adequate, unbiased sex and fork length distribution.

The basic component of the critical scale analysis system (outlined schematically in Fig. 4) is the Sloan Instruments Dektak^{R} surface profile

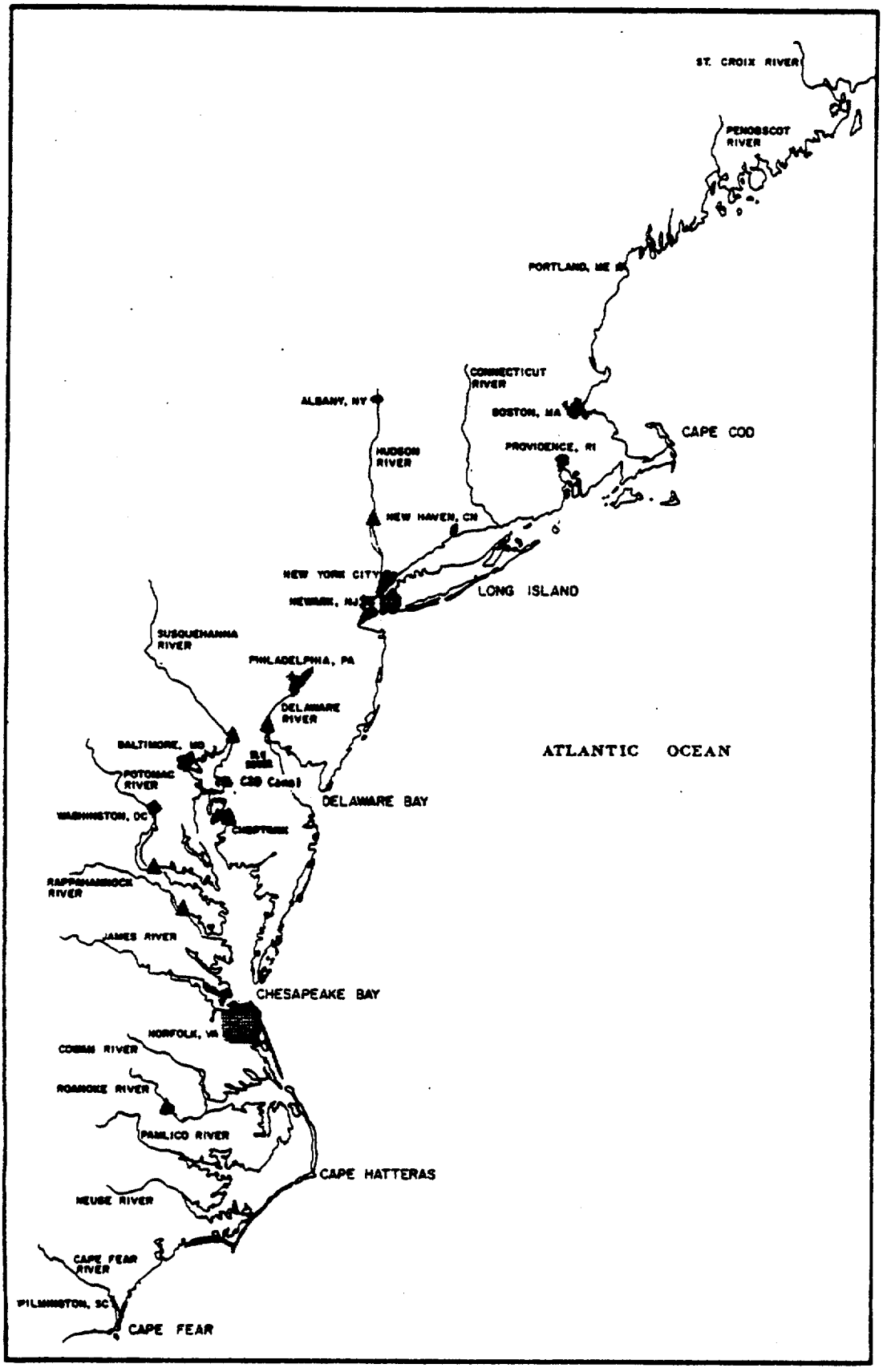


Figure 3. Source Rivers Selected for Study. (Collection sites indicated by triangles) (Texas Instruments, 1976).

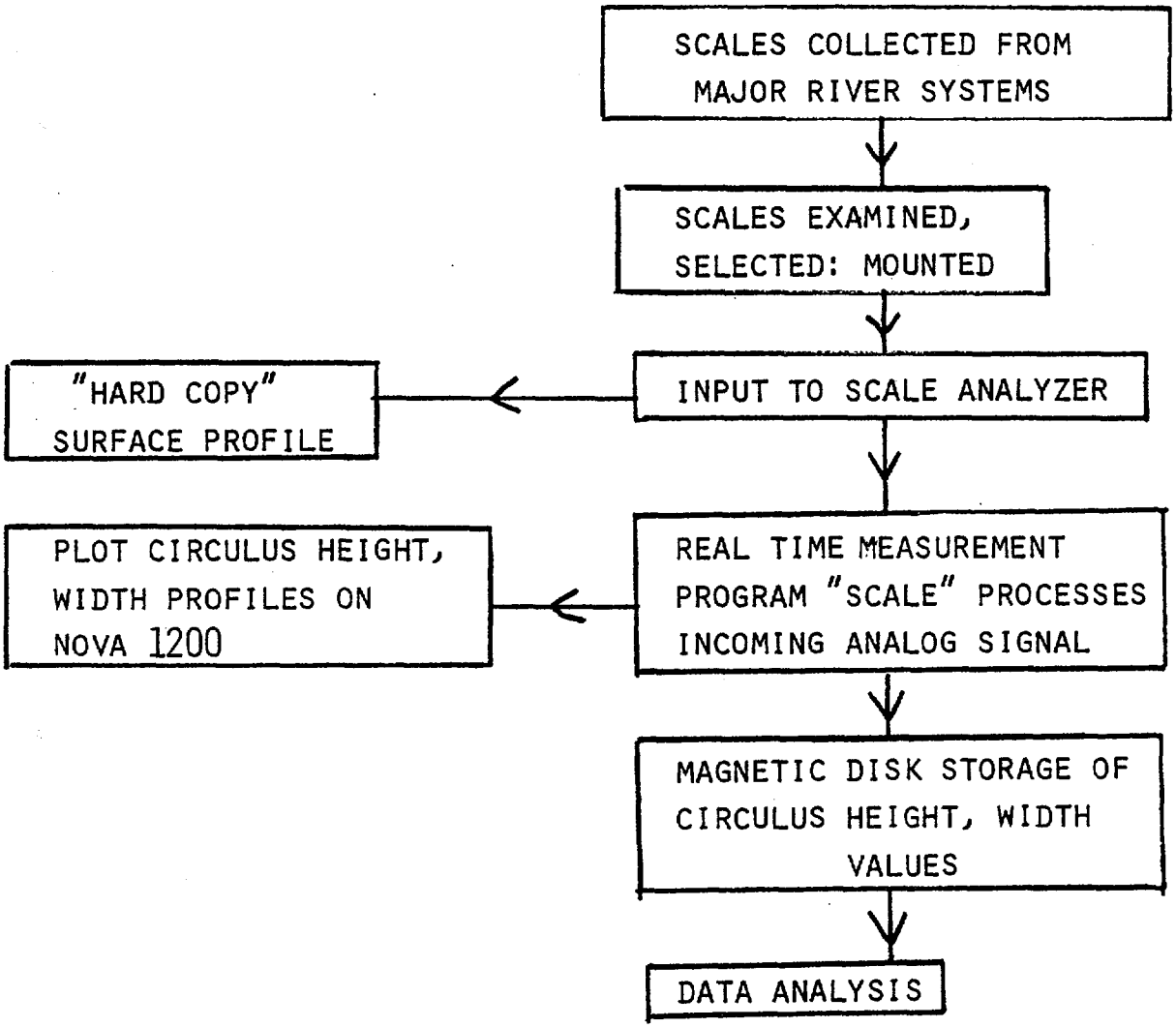


FIGURE 4. SCALE ANALYSIS SYSTEM

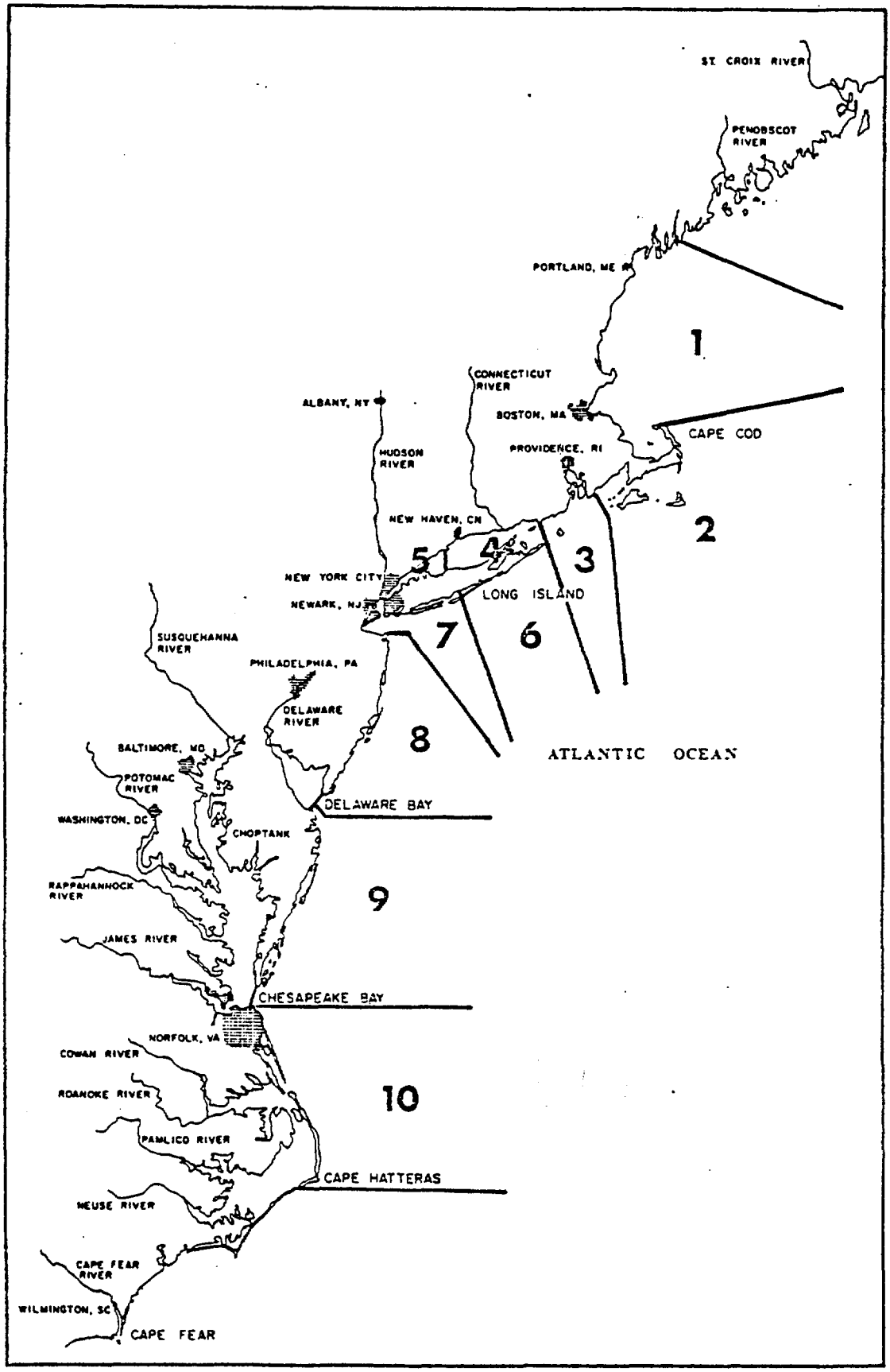


Figure 5. Atlantic Sampling Strata

analyser. 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.

A typical surface profile is illustrated in Figure 6. Beginning at the left, the area near the focus (f) displays several irregular low ridges. Within one mm of the origin, however, the regular pattern of circuli ridges characteristic of the lateral field becomes apparent. This record represents surface structure taken along a 4 mm track outward from the focus.

Thermoplastic impressions of all scales collected were received from Texas Instruments, Inc. Separate validation studies were performed to assess levels of fidelity of accurate reproduction of original scale growth. Reproduction to within 10% (i.e., 10% difference between actual scale and impression) was encountered for all extra-focal circuli. A four to five fold increase in overall processing speed arose from the reduction in scale preparation time. Larger sample size would compensate for reduced accuracy per scale. Mounting of each scale via the old method (Eastman 910 adhesive plus pressure) required an average of 20 minutes per scale for selection, examination, cleaning and mounting. This was in addition to the 10 to 15 minutes required to position and analyze each scale using the Dektak-Nova system. Use of plastic impressions permitted scales to be run simultaneously with the mounting of the next scale impression. Impressions were mounted by stapling the desired impression (inspected for damage previously under a 40x binocular microscope)

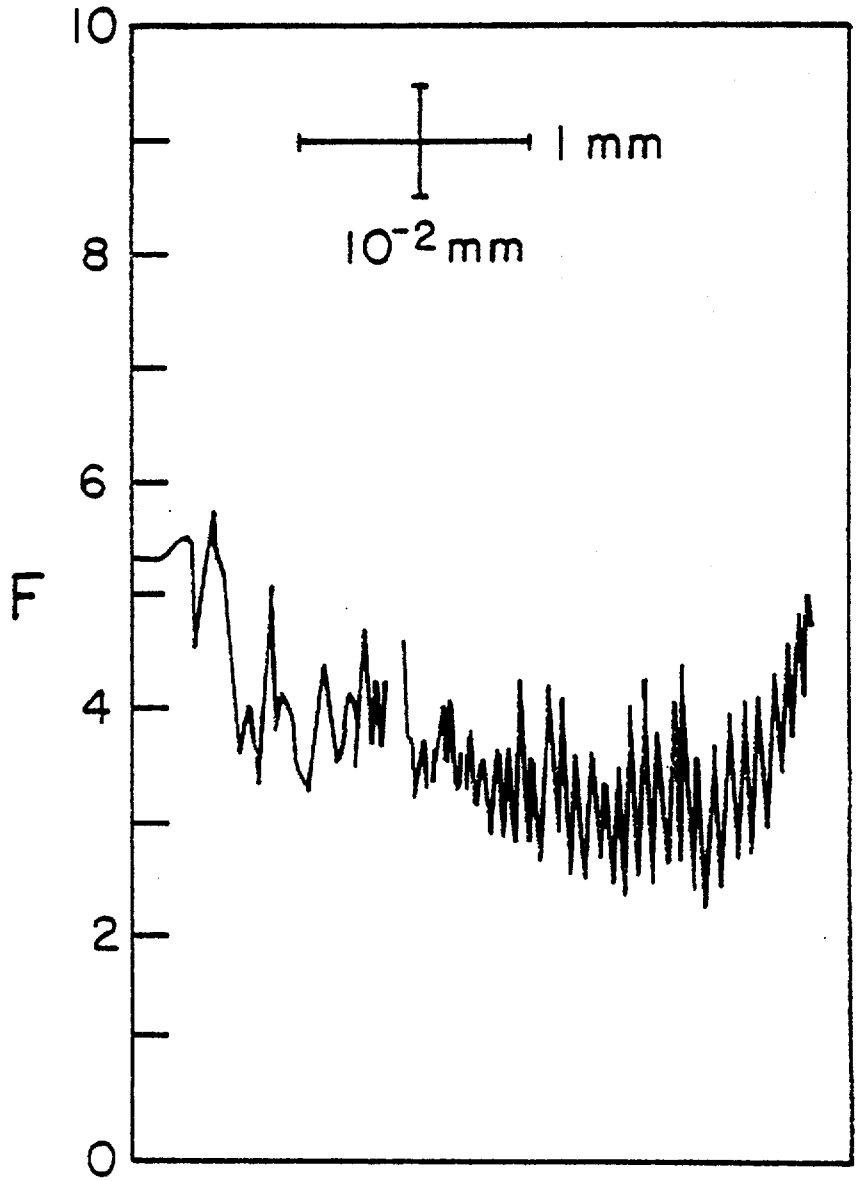


Figure 6. Surface profile of lateral field of scale of striped bass (Morone saxatilis). F represents scale focus. Scale used in this analysis; Hudson R. 1407 (female).

to a flat sheet of 1/4 inch plywood. This step was required to reduce residual curl in the plastic scale card as well as to maximally expose the lateral field circulus impressions, which had a tendency to close up if not stapled flat. Using two pieces of plywood, one scale could easily be examined and mounted while the other was undergoing surface profile analysis. This increased the rate of throughput of scale, to the maximum rate of processing. This was determined by the amount of time required to produce the desired scale profile.

The major innovation in the analytic system relative to the Phase I system has been the incorporation of a real time measurement program "SCALE" (S. Milligan, prog.) into the analytical loop. Acquisition of a DR-1200 32K Memory Board under Phase II extension has permitted application of a new, powerful extended FORTRAN capability.

Scale: Circulus Measurement Program

The real time circulus measurement program "SCALE" incorporates the moving baseline technique. This feature enhances the ability to detect all circuli, even when such circuli are on curved portions of scale which deviate sharply from the general slope of scale surface. A listing of this program appears in Appendix 1.

The scale is oriented so the sensor stylus will move along a track over the lateral field. This field possesses less extraneous surface noise and should permit maximum detection of regional growth variation. Calibration range signals are next requested by the computer for delineation of extreme signal limits to be accepted by the computer. The upper and lower values are used to recalibrate the system before each run. This eliminates any possible effects of systematic drift, either in the amplifier system, or in the analyzer itself. Drift would reduce comparability of scales recorded at different times. The computer is programmed to automatically terminate data collection if the sensed signal moves outside the linear region defined by

the upper and lower calibration signals. Signals exceeding the specified limits are invalid, and not recorded, otherwise they would destroy the value of the entire profile for use in subsequent analysis.

The basic format for processing of scales is as follows: scale samples are oriented by aligning the left edge of the radial field with a small fixed mark in the Dektak microscope eyepiece. Surface profile data has been collected along a straight line track 4 mm out into the lateral field. This would be expected to be the region of maximum regional growth variation, since it represents growth occurring during the first two years of life (Merriman, 1941), a period during which the fish is normally resident within its native river.

Tracking of the sample is begun using the stage motion switch on the analyzer. The sensor stylus is positioned at the scale focus, and stage motion is begun. An operator-actuated 6 VDC trigger signal is transmitted to the computer. The computer now begins to sample the analog scale signal at 10 Hz, as determined by the computer's internal crystal clock. This sampling rate is well above the 1.67 Hz, Nyquist criterion for avoidance of errors due to aliasing (i.e. sufficient samples are taken to detect scale structure, without missing significant high frequency components [Bergland, 1969]). As the periodic signal rises and falls above the locally averaged baseline, height and width are automatically calculated for each circulus. To terminate the run, a second 6 VDC trigger signal is transmitted to the computer. The computer responds by plotting a circulus by circulus depiction of the data as it has been calculated for that profile. A typical scale surface profile is illustrated in Figure 7. A typical circulus height-width display is illustrated in Figure 8. In addition to the display feature, circulus heights and widths for the just-computed profile are placed onto magnetic disk storage.

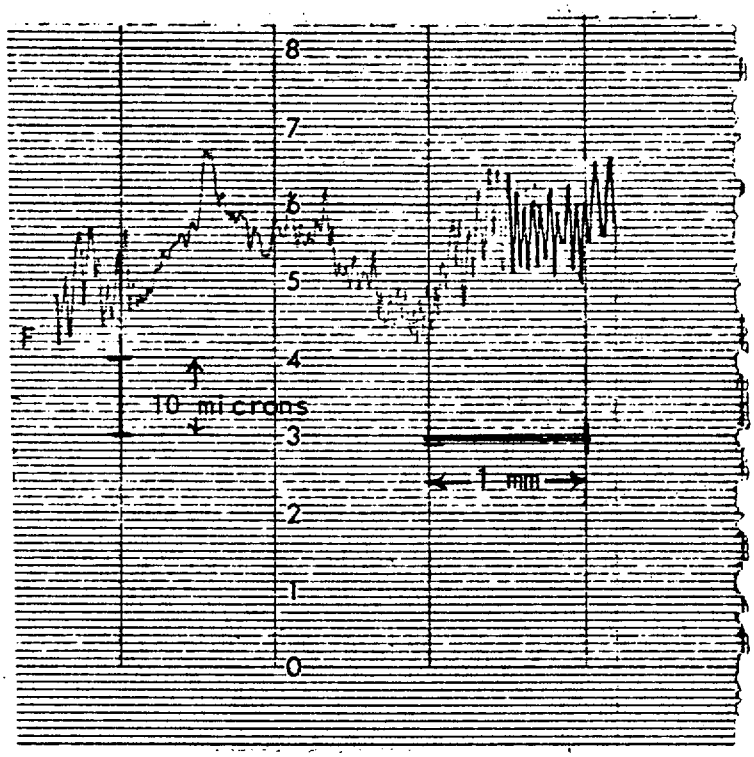


Figure 7. Scale surface profile, Potomac 733 female. (F:focus). Tracked over 4 mm into lateral field. Note irregularity of peri-focal circulus structure, relative to dominant regularity of circuli greater than 1-2 mm from focus.

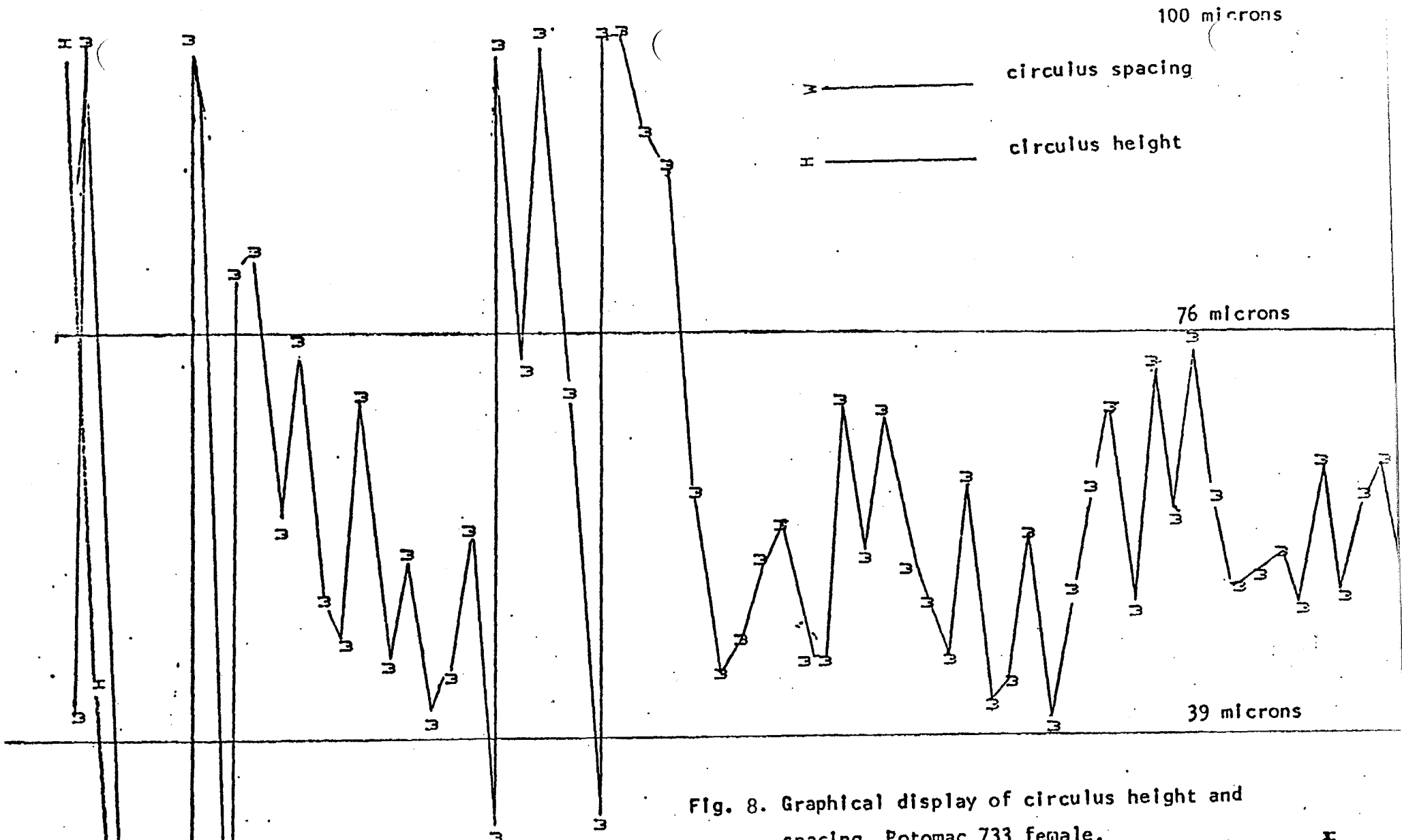
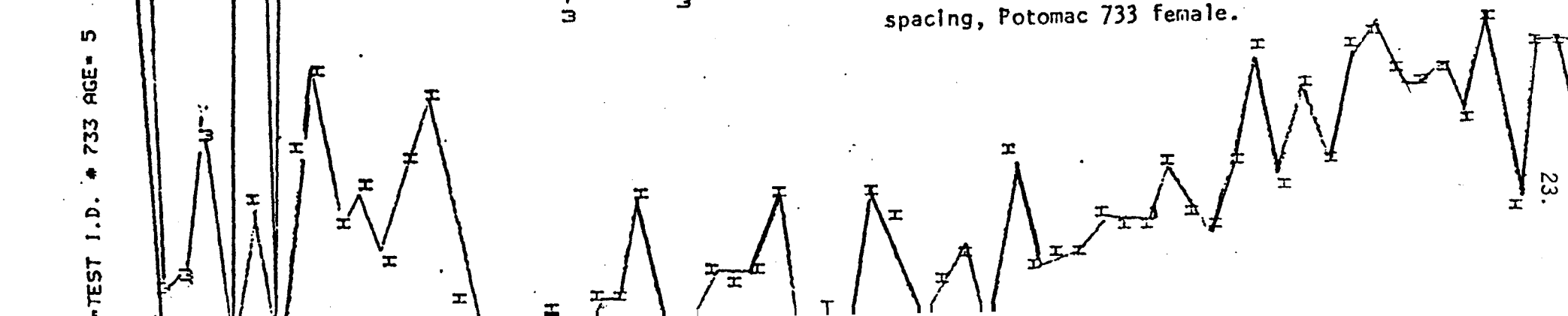


Fig. 8. Graphical display of circulus height and spacing, Potomac 733 female.



Associated ID numbers, age, sex, fork length, and regional data, entered at the beginning of the profile run are stored along with height and width data for use in the data analysis.

Circulus Height-Width Profile Data Analysis

The primary goal of this study has been to assess potential for classifying striped bass into their respective source stocks on the basis of regional variation in scale growth structure. The previous section has given a description of the preliminary processing to which each scale is subjected, in order to produce detailed measurements of growth structures present on the scale. Figure 9 illustrates the analytical scheme adopted for use in extraction of the maximum information from the recorded profiles. Three inter-related branches form the data analysis flow diagram. The principal branch is represented by the "PDF"-linear discriminant analysis pathway. This technique uses the growth information calculated for each scale in a multivariate fashion in order to produce optimal separation between the known spawning regions. The program selected (BMDP7M), stepwise linear discriminant analysis (Dixon, 1973), produces a jackknifed reclassification table for each group used in the analysis. "Jackknife" refers to the successive removal of individual profiles from the given group, the subsequent re-evaluation of discriminant coefficients and classification of the given (excluded) case. This permits a more realistic evaluation of the separabilities of the training sets from known spawning regions. Further advantages to BMDP7M, not available in BMD07M, are the relative ease with which groups may be selected on the basis of similar age, length, sex, or regional values associated with the profile data values (Dixon, 1973).

To extract information present in the profile, instead of using summary statistics (e.g. HBAR, average circulus height; TBAR, average circulus width)

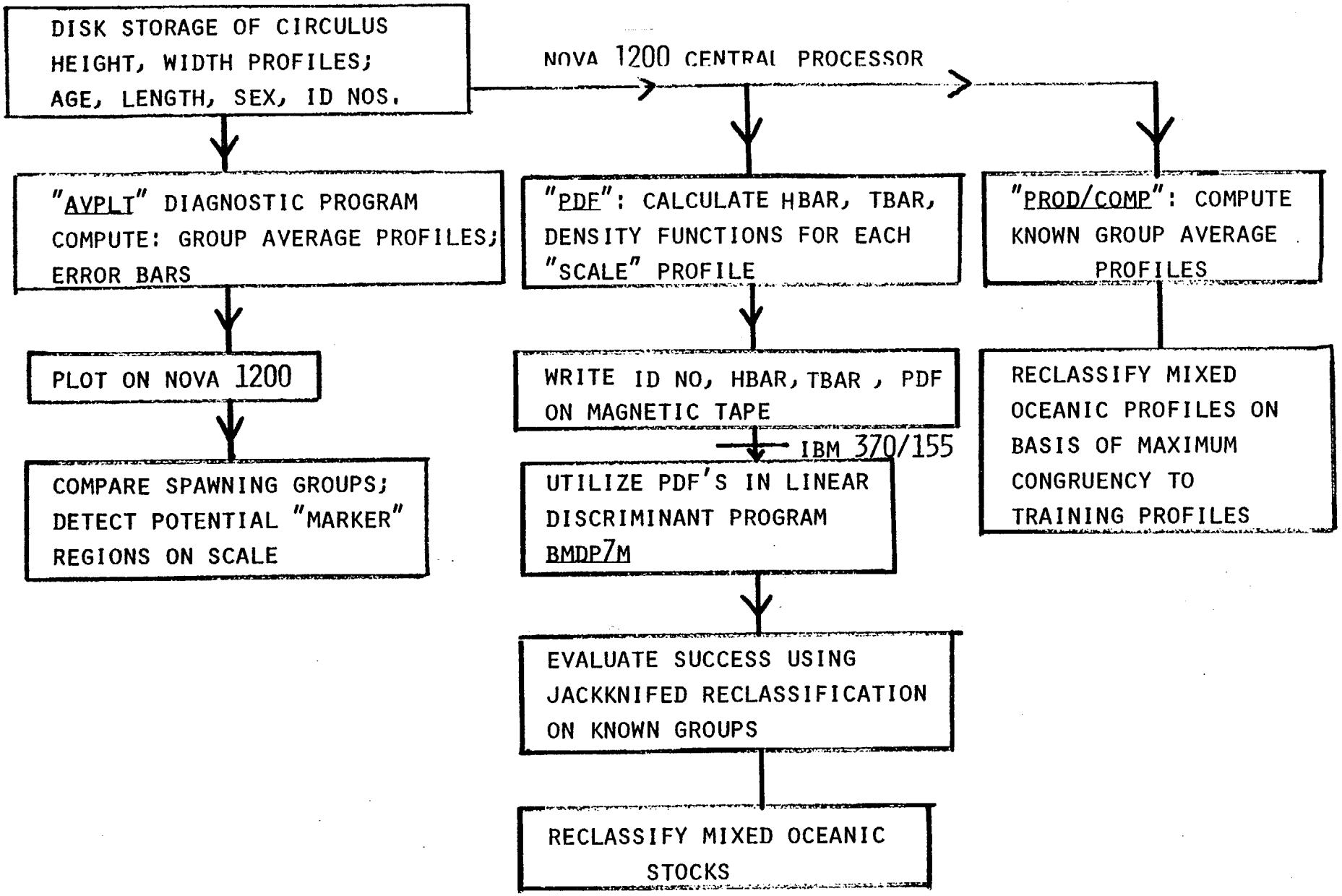


FIGURE 9. SCALE PATTERN ANALYSIS METHOD - PHASE II

as were used in Phase I, program PDF is used to compute normalized probability density functions (PDF's) for circulus height and spacing for each profile. Normalized PDF's are histograms with values in each interval proportional to relative frequency of occurrence of circuli having values within the interval. After examination of the effects of varying the interval on PDF shape, values of 1.31μ per interval for height and 3.93μ per interval for spacing were selected as optimal for computation of PDF spectra. In other words, circuli having height values between 0 and 1.3μ increment the first interval; values from 1.3 through 2.6 increment the second, and so on. Twenty intervals for height and 25 for spacing have been established. Typical PDF's for both height and spacing are illustrated in Figures 10 and 11. These PDF's are of 3X resolution used in discriminant analysis, and are given to illustrate the general appearance of the histogram. For PDF program listing, see Appendix 1.

The second branch of the analytical scheme uses the program AVPLT to produce visual displays of group averaged circulus height and width profiles. These group averaged profiles incorporate data taken from several individual profiles onto one display. Mean values are calculated at each circulus position, for circuli numbers 1-60. Circuli 1-60 represent growth within the first 4 mm of scale, where maximum regional variation would be expected to occur. Error bars ($\pm$ 1 standard error) are generated at each circulus position. This permits the assessment of variation in circulus dispersion along the surface of the scale. Superimposing group averaged profiles reveals specific areas of scale surface which differ maximally between regions. Previous methods of analysis (Fourier, Discriminant, PDF) do not preserve physical (i.e. sequential) location of circuli along the scale. A typical AVPLT group averaged profile is illustrated in Figure 12.

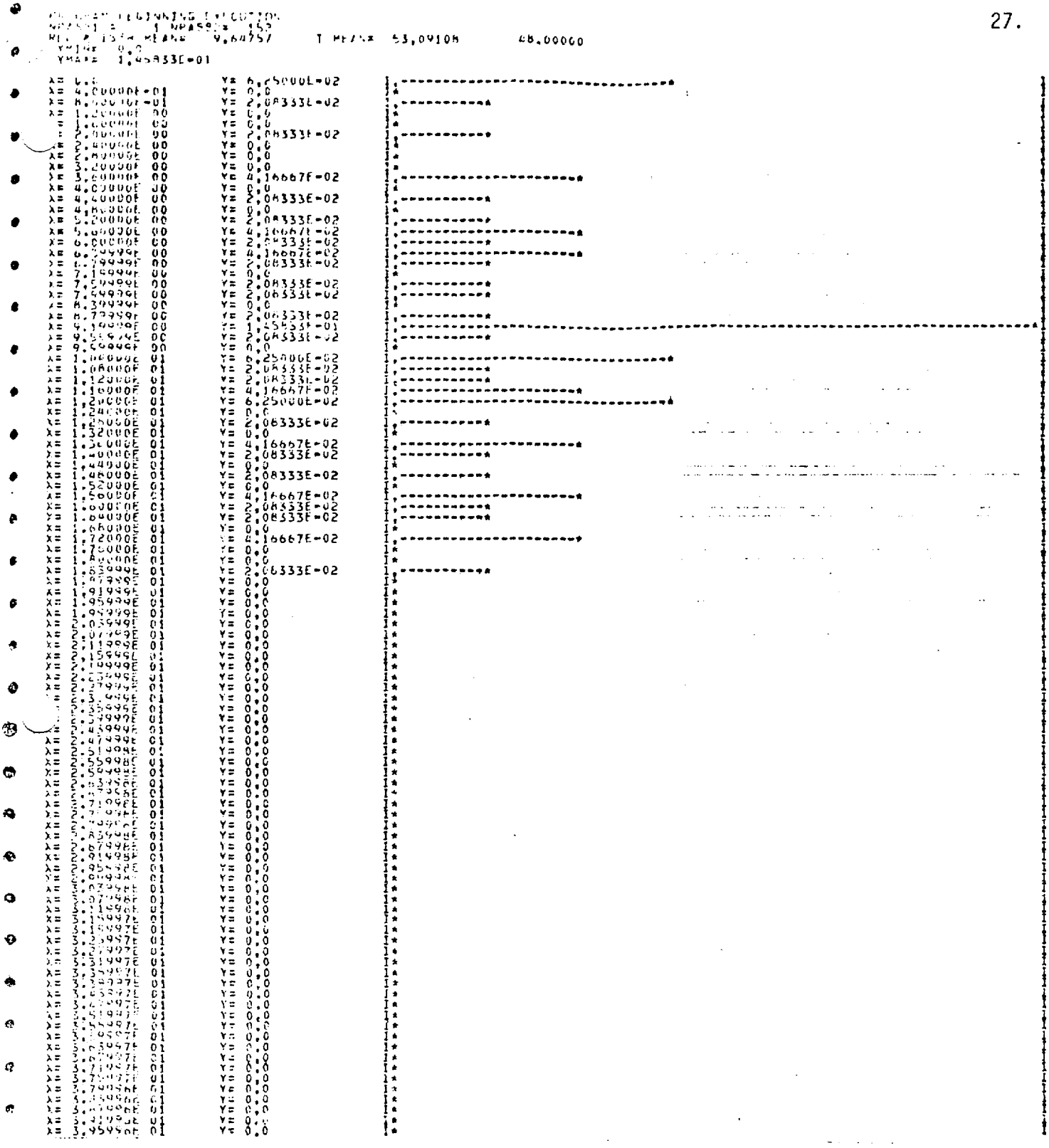


Figure 10. Computer listing of typical circulus height probability density function.

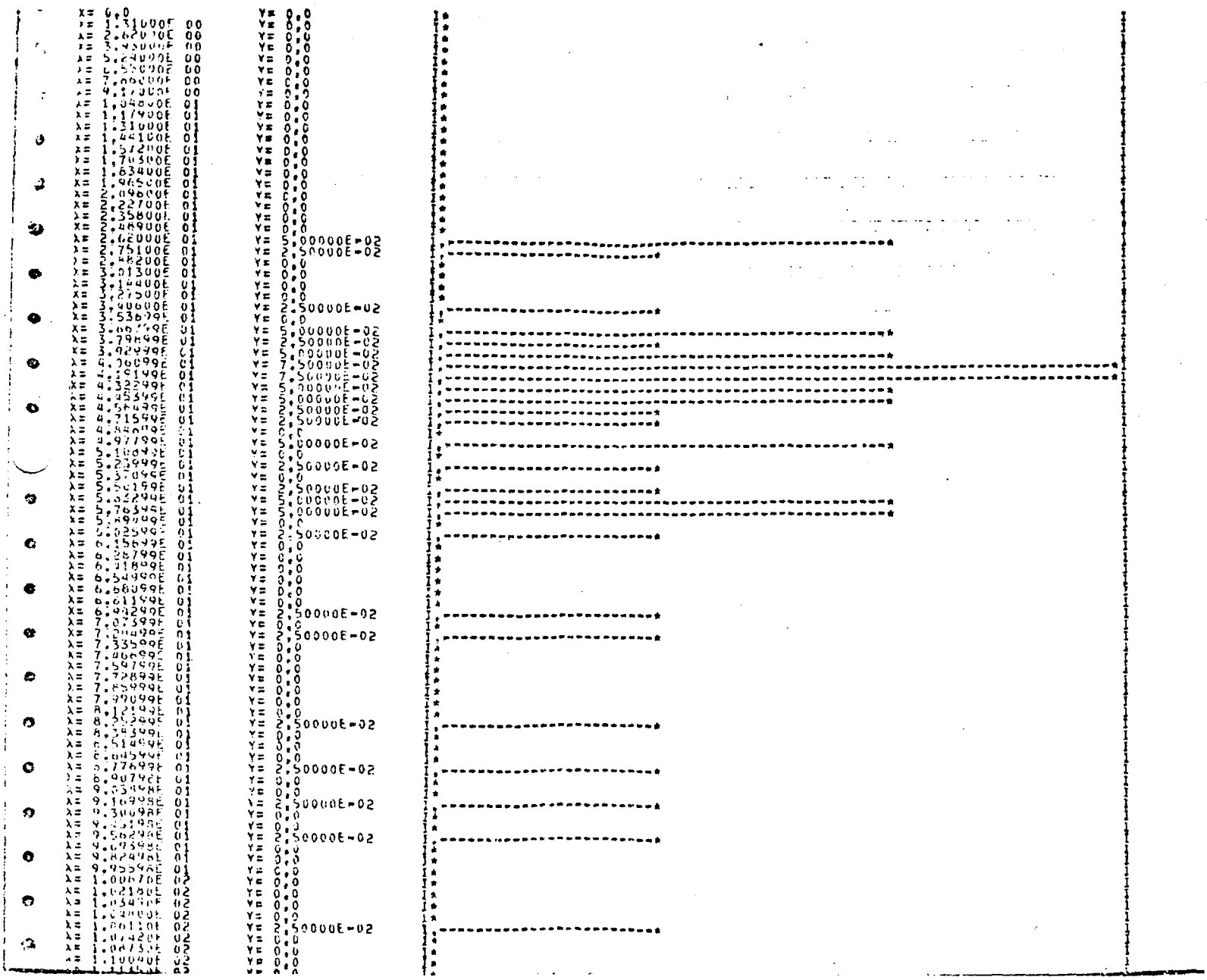


Figure 11. Computer listing of typical circulus width probability density function.

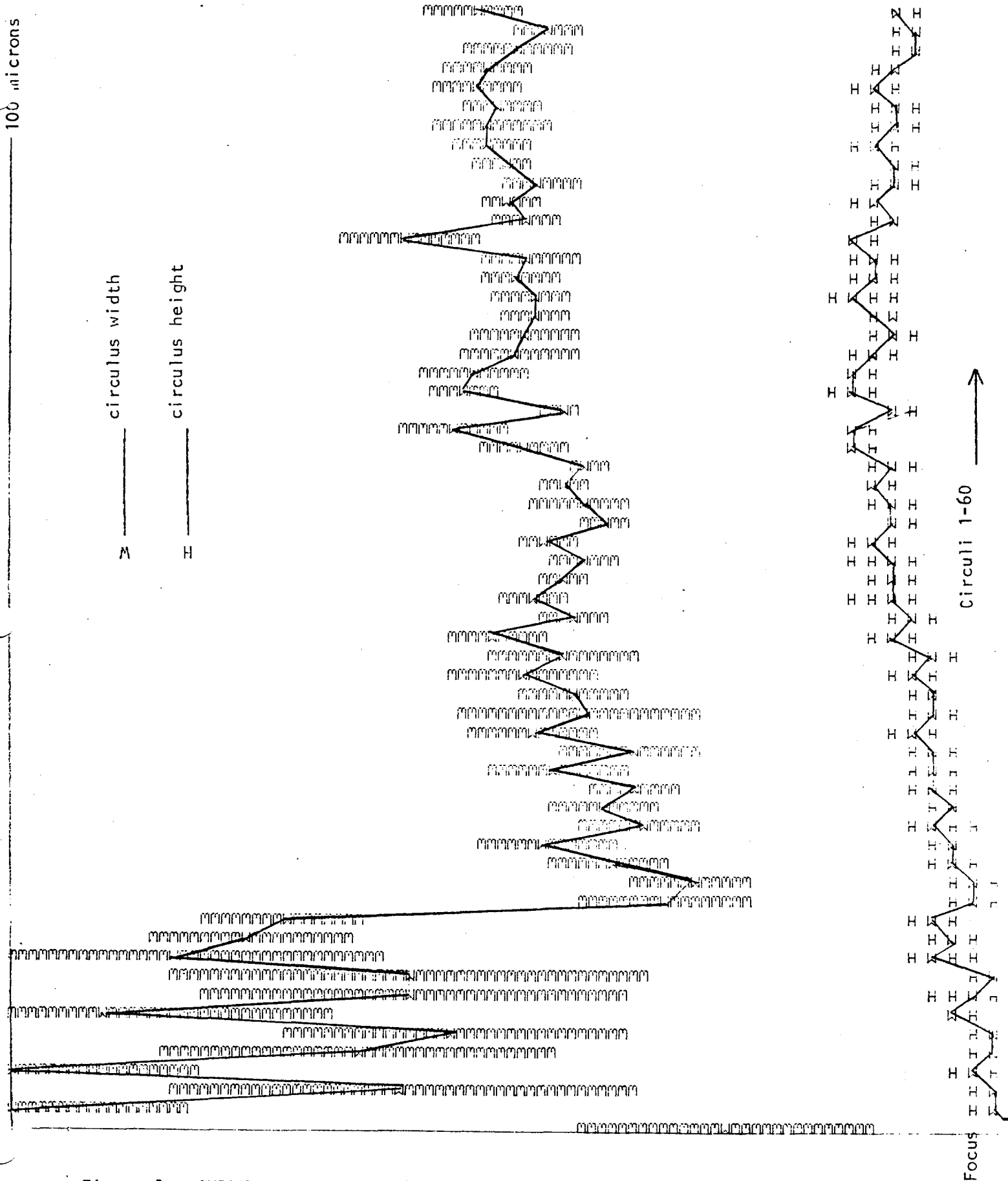


Figure 12. AVPLT group averaged profile illustrating mean circulus values (dark line) with dispersion error bars (± one standard error).

In previous studies (Anas and Murai, 1968) specific regions on the scales of sockeye had been found to be optimal for use in discriminating between Asian and North American sockeye salmon. It was hoped that this technique (AVPLT) would provide a simple and rapid method for the visual detection of distinctive "marker" regions, i.e. where in the profiles the groups demonstrated minimal degrees of overlap, or maximal separation potential. If strong differences were noted in particular circulus positions, these values could be selected for in preparation of PDF's for use in the linear discriminant analysis.

The final branch of the analytic scheme involved application of the program PROD/COMP. The program listing appears in Appendix 1. Results of manual circulus width curve analyses performed on salmon smolts from various principal Alaskan spawning streams (Koo, 1963) demonstrated consistent differences in the shape of the circulus width curves for fish taken in different rivers. Similar environmental conditions within the parent streams were reflected in the high degrees of similarity in shapes of growth curves found in fish from the same river.

In the PDF program, although all circulus magnitude information is preserved, the actual sequential ordering of circuli is destroyed. Thus, a profile whose values increase monotonically from 50 to 70μ is indistinguishable from one whose values decrease monotonically from 70 to 50μ. No record is preserved of where along the profile the given values occur. PROD/COMP does preserve circulus ordering. This should augment the ability to detect recurring regional patterns for scales arising from a given spawning region.

The first step (PROD) in this analysis is to generate for each spawning river, a surface profile consisting of an overall average circulus profile from each spawning river. A separate group averaged profile is generated for

each training set of fish from each region. In the analyses, to reduce effects on variation in growth between the sexes, separate training profiles were computed for male and female fish from each region. These training profiles were next stored in a training library.

Upon initiation of the COMP portion of the routine, the desired oceanic profile is brought out of storage. The computer next requests a "test river" profile from the training library. The desired source river profile is next compared, on a circulus by circulus basis, with the unknown scale profile. Values occurring on the known source river and the oceanic individual profiles are multiplied together and stored. Two numbers, one expressing the relative similarity of the heights, the other expressing the relative similarity of widths to each regional average are generated. A larger score indicates a better match at all circulus positions between the known region's profile and the oceanic scale. In practice, perfect matches never occur, and what is looked for is a maximum value between known and unknown. Results of analyses performed using PROD/COMP are presented below. This program represents a potentially significant addition to techniques for evaluating relative similarity based on regional scale growth variations.

In addition to the above computer assisted data analysis techniques the pattern recognition capacity of the human eye was brought into the analytical process. Scanning electron microscopy of scale circulus structures has been used as an adjunctive aid in speciation of various fish groups (Tilapia; Paralichthys spp.) (DeLamater and Courtenay, 1973). Several scales from known spawning regions were selected for SEM analysis and subsequent photomicrograph interpretation. Distinctive patterns which might be missed by our particular surface profile analytic approach were sought during this interpretation process. Results of analyses of a subset of known source region scales are described below.

RESULTS

Probability Density Function and Linear Discriminant Analysis

Following development of the PDF analysis program, the new technique was given a preliminary trial in order to assess the utility of the new method vs. the older summary statistic approach. The new analysis was first applied to previously recorded 1974 surface profiles originally used under Phase I of the Critical Scale Analysis Study. To simulate the full Phase II analysis scheme, the former WHGHT program was modified to incorporate the moving baseline feature. This led to a general reduction in TBAR (average circulus width) to values between 50 and 55 microns. Using these values of circulus height and width, PDF spectra were generated for the Hudson and Chesapeake profiles. Using the 45 variables generated by the PDF program, classification functions resulted in complete separation of the two training sets. This result arose from two factors. The raw data was of higher quality, stemming from incorporation of the moving baseline technique. The PDF method, although it does destroy sequential ordering, appears to function better in preserving the details of scale structure than does the former summary statistic method which destroyed sequential order and only provided two numbers to characterize the entire scale. Classification accuracies for the two test sets were: Hudson test, 60% (9/13 correct); Chesapeake test, 61% (14-23 correct). Only these limited numbers of scales were available from the 1974 data tape. The stepwise feature in the discriminant analysis program indicates X(1) followed by X(15) as the variables contributing the greatest amount to intergroup separability. In this case, these correspond to height variables, the first in the interval 0-1.3μ, the second in the interval 18.2-19.5μ. Using a subset of only 5 out of the

original 45 variables, reclassification accuracy was still possible at 90% for Hudson and 83% for Chesapeake training sets, with an intergroup $F(6,73)$ value of 16.3, significant at the $p < .001$ level.

Fork length differences were present among fish collected in 1974 from the Chesapeake and Hudson regions. The average fork length for fish used in the 1974 Hudson training set was 723 mm, while average length for fish in the 1974 Chesapeake set was 424 mm. Additional 1974 scales were run to determine effects of varying fork length on average circulus height for fish from the two regions. Results of regression and correlation analyses (Table 4) suggest that Chesapeake fish demonstrate a significant effect of fork length on circulus height ($F = 19.67$, $p < .001$). The effect of fork length on circulus height for Hudson fish tested was not significant ($F = 2.87$). Since circulus width was not a significant classificatory variable, regression of circulus widths vs. fork lengths were not performed. Regression analyses indicated that the best regional separation among similar sized fish lay in use of fish in the size range 400-600 mm (Fig. 13).

Stock Analysis Using Homogeneous Length Fish (400-600 mm)

Scales collected during the 1975 spawning season were selected using the 400-600 mm criterion for size range. Scales were mounted and subjected to surface profile analysis. Scales used in this portion of the analysis are listed by ID number in Table 6. Data were collected for male fish from the Hudson River, Roanoke River, and Chesapeake regions.

Upon completion of surface profile analysis of the above listed groups, program PDF was used to compute normalized probability density functions (Data Set 2, Appendix 2). These spectra were then submitted to linear discriminant analysis using the jackknifed feature of the BMDP7M stepwise linear discriminant analysis program. Classification accuracies are

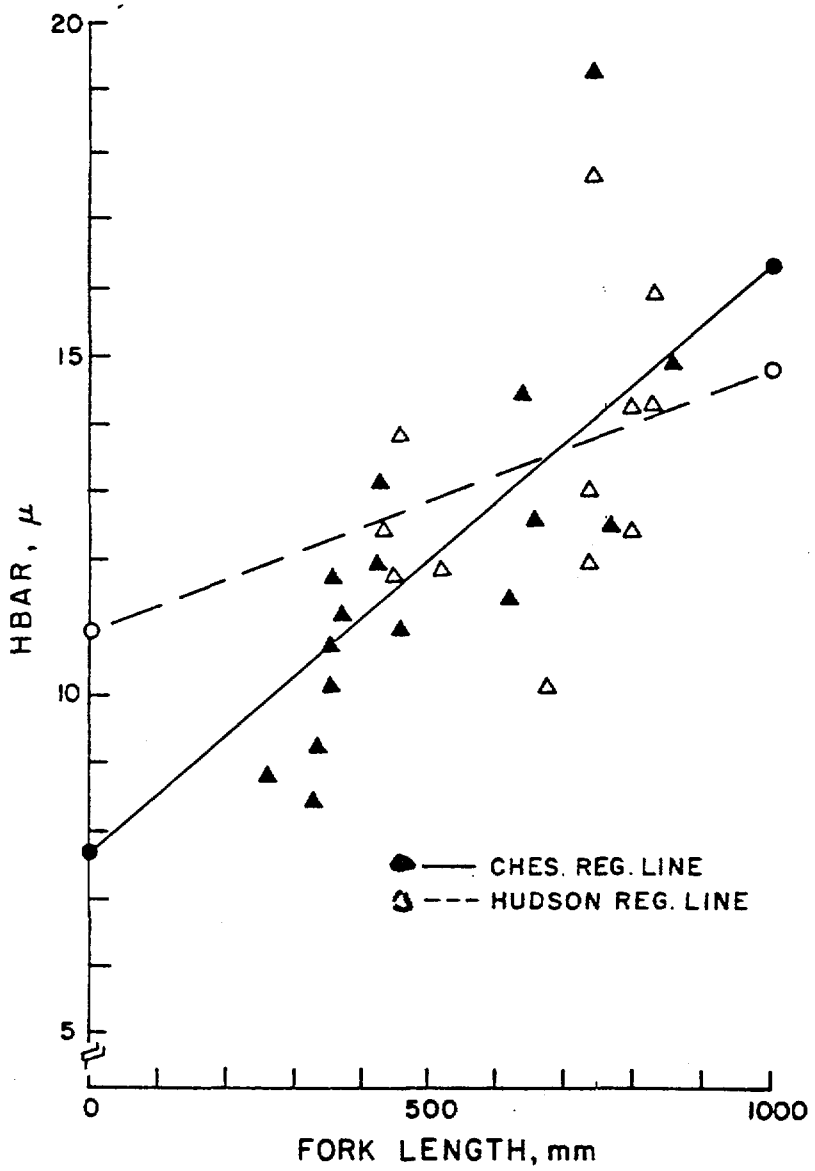


Figure 13. Regression line for HBAR (average circulus height, microns) vs. fork length. Chesapeake vs. Hudson groups.

Table 4. Correlation study summary: circulus height vs. fork length.

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

Table 5. Scales used in homogeneous size range regional training sets.

Chesapeake Bay Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
16	92	454.003	5.375	47.733
[illegible]	92	429.000	4.758	49.326
[illegible]	92	621.003	6.658	65.621
51	92	14.000	6.342	55.494
[illegible]	92	505.002	4.307	55.006
58	92	403.000	5.635	57.212
[illegible]	92	376.003	4.267	52.050
[illegible]	92	449.000	5.594	45.346
29	92	414.000	5.028	45.009
34	92	476.000	5.652	55.071
38	92	347.000	5.141	52.393
56	92	393.000	5.075	48.816
57	92	410.000	5.094	50.970

Roanoke River Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
[illegible]	99	595.003	7.135	[illegible]
[illegible]	99	541.000	5.686	[illegible]
[illegible]	99	554.000	6.329	[illegible]
[illegible]	99	444.000	5.387	[illegible]
[illegible]	99	497.000	6.449	[illegible]
[illegible]	99	[illegible]	8.016	[illegible]
[illegible]	99	[illegible]	6.514	[illegible]

Hudson River Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
345	99	485.000	6.157	56.211
344	99	492.000	5.774	54.465
1283	199	496.000	5.624	51.277
1281	199	533.000	6.185	54.121
1305	199	587.000	7.916	54.135
351	99	591.000	7.600	51.000
398	99	504.000	6.043	51.[illegible]
1292	199	560.000	8.008	51.[illegible]
1287	199	439.000	5.244	51.[illegible]
1298	199	572.000	5.505	52.[illegible]
1284	199	583.000	5.191	47.[illegible]
1312	199	489.000	5.705	50.[illegible]
1311	199	418.000	4.613	51.[illegible]
1310	199	458.000	4.866	50.[illegible]
1309	199	443.000	4.397	51.[illegible]
1308	199	502.000	4.511	44.[illegible]
1307	199	572.000	4.688	46.[illegible]
1329	199	494.000	6.225	55.[illegible]
1369	199	489.000	4.726	51.067
1337	199	591.000	2.506	41.[illegible]
1363	199	439.000	4.245	47.[illegible]
1361	199	418.000	4.628	47.[illegible]
1306	199	451.000	5.[illegible]	47.[illegible]
1365	199	464.000	5.189	60.[illegible]
1639	99	545.000	6.321	52.887
1496	99	644.000	6.515	50.[illegible]
1497	99	478.000	6.512	53.[illegible]
1656	99	560.000	7.312	55.[illegible]

Notes

1. HBAR: Average circulus height in microns (.001 mm).
2. TBAR: Average circulus width in microns.

illustrated in Table 6. Comparison of classification by PDF vs. classification via summary statistics is illustrated in Table 7. This table shows results obtained when analysis is limited to the use of only the two summary statistics, HBAR and TBAR. Jackknifed reclassification accuracy for the Hudson group drops to 35.7%. A 50% reduction in accuracy (relative to Table 6) arising from use of summary statistics for fish labelled Hudson may be noted. An F-value of 4.59 (d.f. - 18,74) is obtained using the PDF spectra (Table 6). This value indicates a highly significant intergroup difference ($p < .001$). Using only HBAR and TBAR (Table 7), the F-value (d.f. - 2,44) drops to 2.87 (N.S.).

The AVPLT routine was implemented in order to produce average regional circulus height-width profiles. The goal in this phase of the analysis was to generate plots with which to compare the three groups. Discrimination accuracy would be maximized by selecting for PDF analysis those segments along the scale which demonstrate significant intergroup variability. Figures 14, 15 and 16 illustrate results of AVPLT analysis applied to each of the three groups. Note the enhanced circulus widths in the early portion of the Roanoke profiles. This agrees with results of Texas Instruments scale measurements performed earlier on fish from this region (personal communication, T. Berggren). Texas Instruments studies independently demonstrated increased inner circulus widths for scales from Roanoke River fish. This type of feature could be most easily detected through the use of PROD/COMP. Although occasional individual circuli do deviate significantly when the profiles are overlapped in a pairwise fashion, no single major segment of scale surface appeared preferable on the basis of AVPLT diagrams. It was decided to retain circulus information, and use multivariate stepwise techniques to select out those values contributing most significantly to intergroup separation (e.g. in this data set, circuli in interval X(6)

Table 6. Classification functions, classification accuracies for homogeneous test group (Data Set 2), using PDF spectra.

Classification Functions

Variable	Chesapeake	Roanoke	Hudson
6 X (6)	55.87701	21.44775	18.04816
30 X (30)	6.58762	96.00290	85.19183
31 X (31)	304.43115	239.39740	174.29678
32 X (32)	31.38863	70.92770	84.30550
34 X (34)	176.60321	185.19652	153.13908
36 X (36)	74.05748	95.15736	130.44667
39 X (39)	344.76636	336.47046	252.19168
40 X (40)	125.66248	157.31322	84.71785
48 X (48)	460.00244	414.22607	347.80713
Constant	-52.84898	-50.32281	-39.17108

Classification Matrix

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	84.6	11	2	0
Roanoke	71.4	0	5	2
Hudson	85.7	1	3	24
TOTAL	83.3	12	10	26

Jackknifed Classification

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	61.5	8	4	1
Roanoke	28.6	3	2	2
Hudson	78.6	3	3	22
TOTAL	66.7	14	9	25

Table 7. Classification functions, classification accuracies for homogeneous test group (Data Set 2), using HBAR, TBAR.

Classification Functions

Variable	Chesapeake	Roanoke	Hudson
2 HBAR	-2.90460	-1.40314	-1.96420
3 TBAR	2.76378	2.62212	2.56388
Constant	-65.91135	-67.14557	-60.68608

Classification Matrix

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	69.2	9	1	3
Roanoke	71.4	0	5	2
Hudson	35.7	8	10	10
TOTAL	50.0	17	16	15

Jackknifed Classification

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	61.5	8	2	3
Roanoke	71.4	0	5	2
Hudson	35.7	8	10	10
TOTAL	47.9	16	17	15

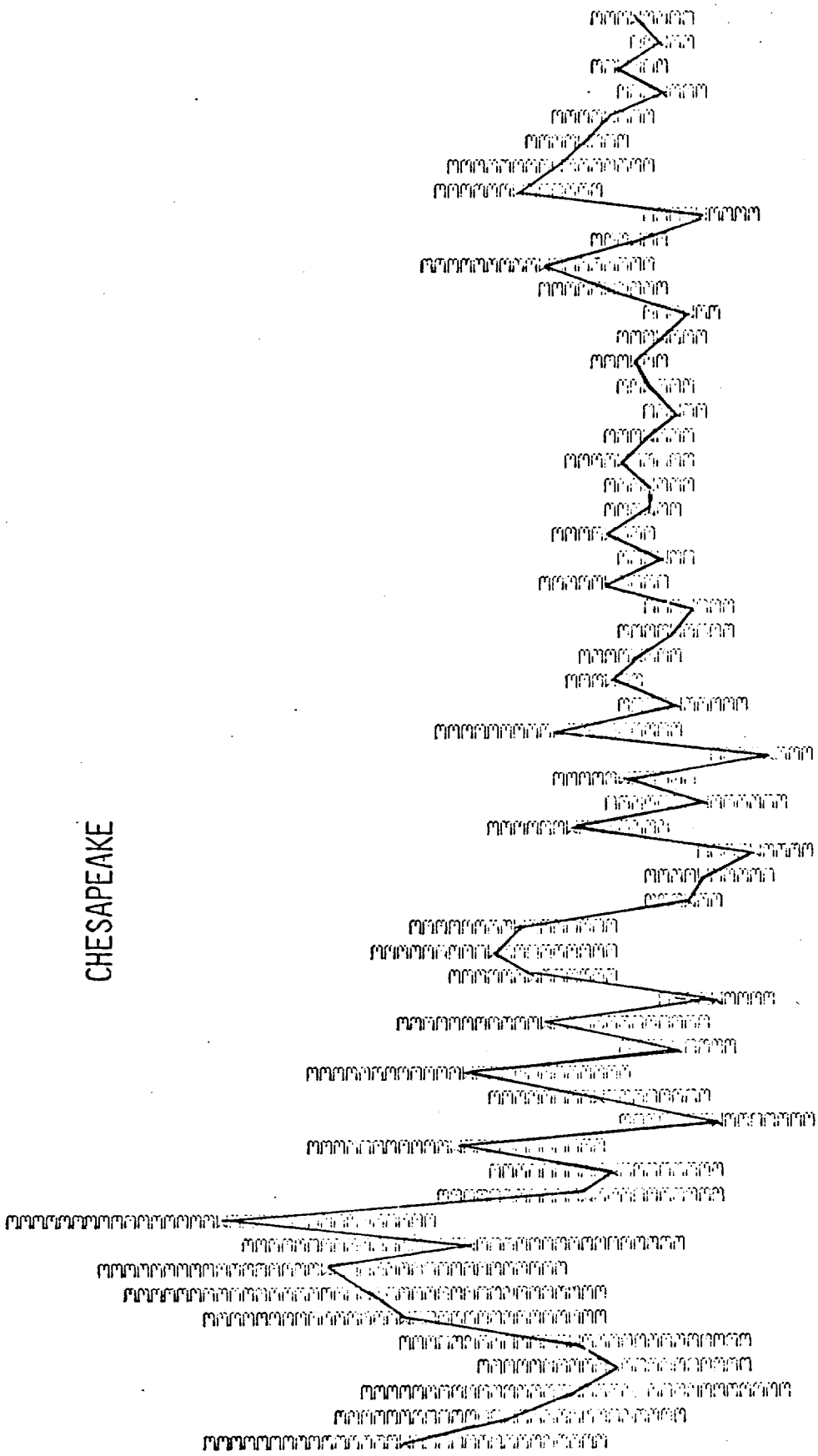


Figure 14. AVPLT group averaged profile, Chesapeake region

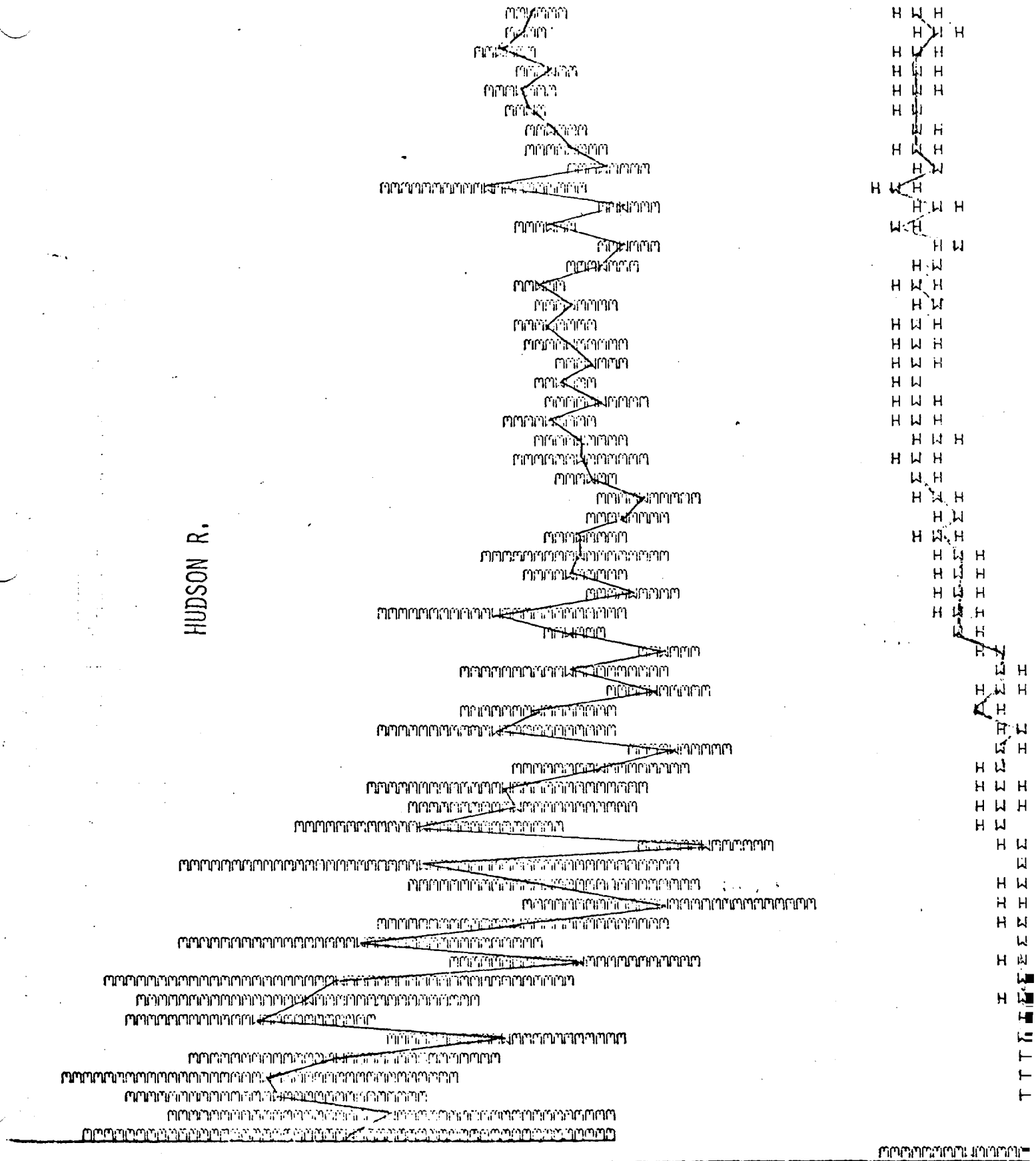


Figure 15. AVPLT group averaged profile, Hudson region

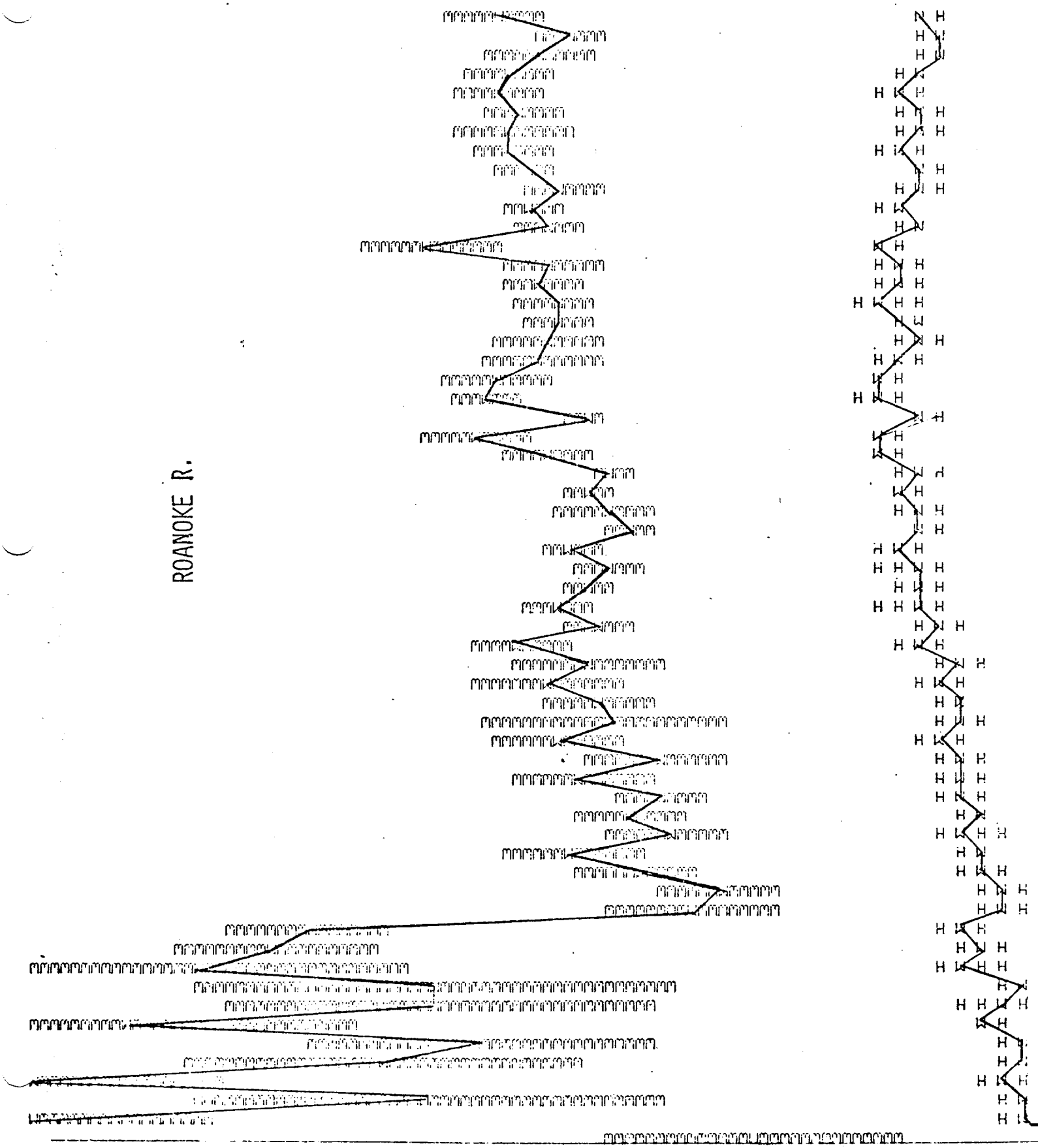


Figure 16. AVPLT group averaged profile, Roanoke region

(6.5-7.8μ circulus height) contributed most significantly to discrimination between the regions).

Stock Analysis Using Extended Size Range Fish (400-1000 mm)

Scale impressions used in this portion of the analysis were selected from the three major natal regions: Hudson River, Roanoke River, and Chesapeake Bay. Selection was based on deriving comparable fork length distributions from each region. Table 8 indicates ID numbers and lengths of fish used in this section of the analysis. A total of 197 fish were selected from the three regions. PDF spectra were derived (Data Set 3, Appendix 2) for all profiles, and these spectra were submitted to linear discriminant analysis using BMDP7M. Jackknifed classification accuracies for the three training groups are displayed in Table 10, and length distributions are shown in Table 9.

Investigation into classification of fish in the Texas Instruments oceanic sample was undertaken. Fish were selected according to the following criteria. First, the fish had to fall within the limits of size range imposed by lengths present within the training sets. Second, in order to adequately cover the entire yearly cycle, fish were taken from each of the six time periods. Fish from geographic strata 5,7,8,9, and 10 were submitted to profile analysis. ID numbers and lengths of these fish appear in Table 11. Since these fish are of unknown geographic origin, they were classified using the previously computed training group discriminant functions.

PROD/COMP Analyses

A profile training library was assembled for the impressed data set listed in Table 8. Separate group average profiles were computed for each sex within each region.

Table 8. Scales used in extended size range regional training sets.

Chesapeake Bay Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
1578	82	521.000 mm	7.169	63.322
1579	82	561.000	6.909	60.453
1586	82	643.000	11.018	66.258
1589	82	345.000	5.669	62.306
1720	82	484.000	8.525	63.610
1736	82	612.000	6.922	69.727
5055	82	815.000	9.464	56.976
1800	82	486.000	7.289	61.127
5059	82	535.000	6.540	56.590
1582	82	576.000	9.067	59.055
1734	82	586.000	8.333	61.203
1561	83	537.000	3.980	57.249
1572	83	615.000	6.781	62.870
1553	83	445.000	6.043	59.272
4872	83	621.000	7.130	61.035
4839	83	568.000	4.857	58.095
4837	83	504.000	7.038	72.762
4829	83	415.000	4.941	58.199
1198	83	653.000	7.548	58.170
1174	83	418.000	5.794	62.618
1558	83	529.000	3.591	59.824
1176	83	572.000	11.606	56.865
1562	83	544.000	8.929	70.750
4874	83	603.000	9.128	59.923
1799	83	533.000	7.780	57.712
1188	83	480.000	8.868	58.382
4843	83	542.000	8.383	53.448
5076	83	818.000	10.875	64.094

Chesapeake Bay Female

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
1581	82	635.000 mm	6.891	58.43[illegible]
1584	82	635.000	6.580	61.16[illegible]
1587	82	543.000	8.913	64.85[illegible]
1588	82	600.000	7.008	58.84[illegible]
1591	82	664.000	8.604	58.81[illegible]
1715	82	824.000	10.385	65.10[illegible]
1716	82	884.000	9.070	65.23[illegible]
1727	82	633.000	7.654	59.78[illegible]
1730	82	560.000	8.084	69.91[illegible]
1717	82	623.000	8.438	71.26[illegible]
1725	82	872.000	11.501	60.29[illegible]
5056	82	700.000	7.943	68.91[illegible]
1712	82	886.000	10.375	59.30[illegible]
1739	82	591.000	8.220	55.35[illegible]
5072	83	705.000	7.931	61.75[illegible]
5075	83	579.000	7.428	62.45[illegible]
1598	83	590.000	6.335	75.56[illegible]
1595	83	566.000	6.684	67.36[illegible]
4854	83	864.000	4.914	59.88[illegible]
4819	83	861.000	8.501	63.04[illegible]
1178	83	866.000	6.862	56.96[illegible]
1169	83	738.000	9.195	67.19[illegible]
5070	83	883.000	9.482	59.05[illegible]
* 5074	83	608.000	8.038	59.43[illegible]
* 1593	83	613.000	5.853	56.35[illegible]
* 5073	83	856.000	10.241	64.75[illegible]
* 4823	83	889.000	10.811	62.74[illegible]
* 1171	83	719.000	10.769	64.58[illegible]
* 1596	83	573.000	9.251	67.95[illegible]
* 4856	83	838.000	9.271	59.43[illegible]
* 5077	83	828.000	9.991	72.57[illegible]
* 1173	83	680.000	11.147	69.32[illegible]
* 1179	83	875.000	11.464	61.76[illegible]
* 1200	83	702.000	8.295	55.33[illegible]
* 1168	83	710.000	10.789	56.54[illegible]
* 4811	83	716.000	11.646	65.59[illegible]
* 4816	83	886.000	10.813	55.73[illegible]
* 1732	82	594.000	9.824	60.78[illegible]
* 5058	82	517.000	7.603	59.43[illegible]
* 4841	83	866.000	7.604	57.65[illegible]
* 4867	83	542.000	8.776	58.40[illegible]
* 4878	83	706.000	10.021	63.67[illegible]

Table 8. (continued)

Hudson River Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
[illegible]	[illegible]	533.0 mm	7.552	[illegible]
[illegible]	[illegible]	569.0	6.292	50.345
[illegible]	[illegible]	496.0	4.050	51.491
[illegible]	[illegible]	499.0	5.962	50.568
[illegible]	[illegible]	573.0	5.913	70.697
[illegible]	[illegible]	583.0	7.583	51.463
[illegible]	[illegible]	409.0	4.944	63.632
[illegible]	[illegible]	465.000	6.376	50.615
[illegible]	[illegible]	459.000	5.290	67.307
[illegible]	[illegible]	473.000	7.496	58.909
[illegible]	[illegible]	503.000	6.202	54.774
[illegible]	[illegible]	572.000	8.559	56.854
[illegible]	[illegible]	554.000	6.495	63.585
[illegible]	[illegible]	482.000	4.941	51.763
[illegible]	[illegible]	489.000	7.003	57.764
[illegible]	[illegible]	550.000	4.894	64.094
[illegible]	[illegible]	[illegible]	10.240	68.114
[illegible]	[illegible]	481.000	4.864	58.569
[illegible]	[illegible]	508.000	7.710	78.669
* 265	[illegible]	495.000	7.595	61.388
* [illegible]	[illegible]	475.000	11.890	57.615
* [illegible]	[illegible]	494.000	9.363	58.994
* [illegible]	[illegible]	514.000	8.472	61.515
* [illegible]	[illegible]	706.000	9.052	64.530
* [illegible]	[illegible]	547.000	11.586	59.001

Hudson River Female

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
[illegible]	[illegible]	[illegible] mm	7.682	[illegible]
[illegible]	[illegible]	681.000	5.316	[illegible]
[illegible]	[illegible]	865.000	8.920	[illegible]
[illegible]	[illegible]	725.000	10.795	[illegible]
[illegible]	[illegible]	772.000	10.013	[illegible]
[illegible]	[illegible]	675.000	8.927	[illegible]
[illegible]	[illegible]	764.000	5.913	[illegible]
[illegible]	[illegible]	693.000	6.919	[illegible]
[illegible]	[illegible]	758.000	9.810	[illegible]
[illegible]	[illegible]	808.000	6.843	[illegible]
1270	199	768.000	8.292	[illegible]
1303	199	729.000	8.014	[illegible]
1319	199	848.000	8.670	[illegible]
1306	199	653.000	8.351	[illegible]
1328	199	861.000	10.862	[illegible]
1318	199	867.000	9.575	[illegible]
1225	199	914.000	5.777	[illegible]
1237	199	836.000	8.316	[illegible]
1233	199	842.000	7.374	[illegible]
1323	199	632.000	6.316	[illegible]
1258	199	946.000	7.805	[illegible]
* 1295	199	563.000	5.447	[illegible]
* 1227	199	862.000	9.871	[illegible]
* 1327	199	881.000	10.879	[illegible]
* 1313	199	722.000	9.281	[illegible]
* 1266	199	[illegible]	11.849	[illegible]
* 1252	199	[illegible]	8.970	[illegible]
* 1329	199	[illegible]	7.1[illegible]	[illegible]
* 1297	199	853.000	10.882	[illegible]
* 1267	199	961.000	10.776	[illegible]
* 1238	199	780.000	10.312	[illegible]
* [illegible]	199	899.000	9.467	[illegible]
* [illegible]	199	768.000	8.798	[illegible]
* [illegible]	199	819.000	10.917	[illegible]
* 1271	199	963.000	10.957	[illegible]
* 1275	199	[illegible]	9.291	[illegible]
* 1225	199	868.000	6.744	[illegible]
* 1268	199	873.000	11.861	[illegible]
* 1314	199	722.000	11.091	[illegible]
* 1234	199	909.000	9.341	[illegible]

Notes

1. HBAR: Average Circulus Height, microns

2. TBAR: Average Circulus Width, microns

Table 8. (continued)

Roanoke River   Male

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
1753	90	445.000 mm	6.862	62.000
1755	90	456.000	7.572	65.366
1756	90	512.000	5.497	61.504
1765	90	551.000	7.585	57.027
5005	90	476.000	7.627	[illegible]
5011	90	446.000	7.203	63.524
[illegible]	90	453.000	6.200	63.474
5039	90	558.000	8.474	57.393
5041	90	671.000	8.320	63.577
1759	90	411.000	7.348	61.209
5024	90	558.000	8.990	60.398
[illegible]	90	554.000	7.917	60.040
[illegible]	90	535.000	7.166	60.555
[illegible]	90	477.000	8.576	60.879
5082	90	570.000	8.199	62.316
[illegible]	90	771.000	13.487	58.450
*1772	90	516.000	7.917	55.856
[illegible]14	90	618.000	9.236	59.122
*1786	90	593.000	6.713	60.167
*5022	90	[illegible]	10.961	56.774

Roanoke River   Female

ID No.	River	Fork Length	HBAR[1]	TBAR[2]
1777	90	557.000 mm	2.417	59.030
1778	90	540.000	5.878	63.007
1771	90	529.000	7.490	57.759
5010	90	525.000	7.994	58.060
5020	90	674.000	9.658	58.022
5021	90	[illegible]	7.293	58.196
5042	90	[illegible]	9.186	[illegible]
5046	90	655.000	8.243	[illegible]
5043	90	[illegible]	9.008	61.411
5048	90	[illegible]	9.110	[illegible]
5019	90	753.000	9.408	[illegible]
5025	90	660.000	9.512	73.822
5054	90	588.000	9.365	[illegible]
5044	90	649.000	8.530	59.241
5080	90	754.000	10.409	[illegible]
1752	90	687.000	6.783	62.659
5023	90	624.000	8.968	58.751
5029	90	545.000	8.541	[illegible]
5035	90	714.000	10.792	55.557
5028	90	632.000	10.124	[illegible]
*5079	90	[illegible]	10.232	[illegible]
*1781	90	506.000	7.974	[illegible]
*5026	90	597.000	10.660	[illegible]
*1757	90	549.000	7.430	59.020
*1787	90	[illegible]	9.751	[illegible]
*1784	90	[illegible]	9.9[illegible]	[illegible]
*5005	90	514.000	8.447	[illegible]
*5034	90	588.000	8.246	[illegible]
*2004	90	714.000	1[illegible].232	[illegible]
*5007	90	509.000	9.246	[illegible]
*1774	90	508.000	8.543	58.167
*5012	90	533.000	8.911	[illegible]
*5017	90	551.000	8.059	[illegible]
*1786	90	503.000	8.885	[illegible]
*5033	90	570.000	9.916	56.701
*5036	90	637.000	9.607	62.935
*5001	90	549.000	9.353	57.621
*1751	90	516.000	8.094	54.777
*1763	90	510.000	8.364	51.058
*5018	90	656.000	10.457	[illegible]
*1790	90	500.000	8.463	53.545
*5032	90	757.000	12.699	62.158

Table 9. Length distribution of fish used in URI discriminant analysis training set.

Region	Sex	Number in Group	Average Fork Length (mm)	Average Circulus Height (HBAR)	Average Circulus Width (TBAR)
Chesapeake	Male	28	558.606	7.507	61.139
Chesapeake	Female	42	719.856	8.791	63.748
Roanoke	Male	20	527.649	8.090	60.860
Roanoke	Female	42	609.071	8.890	59.623
Hudson	Male	25	579.04	7.244	60.957
Hudson	Female	40	809.899	8.864	60.254
		N = 197			

Table 10. Classification functions, classification accuracies for extended size test group (Data Set 3), using PDF spectra.

Classification Functions

Variable	Chesapeake	Roanoke	Hudson
10 X (10)	39.04692	45.36171	34.44536
19 X (19)	105.57477	46.99236	118.01414
26 X (26)	96.70950	32.95345	40.29999
43 X (43)	56.27234	34.27371	26.19862
48 X (48)	91.95665	73.77776	86.20134
Constant	-8.27973	-7.07970	-6.54607

Classification Matrix

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	47.1	33	19	18
Roanoke	61.3	7	38	17
Hudson	47.7	17	17	31
Total	51.8	112	137	139

Jackknifed Classification

Group	Percent Correct	Number of Cases Classified into Group		
		Chesapeake	Roanoke	Hudson
Chesapeake	45.7	32	19	19
Roanoke	59.7	7	37	18
Hudson	46.2	18	17	30
Total	50.3	112	136	140

Table 11. Scales used in oceanic (Strata 5,7,8,9,10) profile test sets.

Strata 5,7 Female

ID No.	Stratum[1]	Fork Len.	HBAR[2]	TBAR[3]
5501	530	480.000	6.357	61.486
5537	720	536.000	7.865	54.878
5758	520	458.000	6.387	59.248
5627	530	485.000	7.181	51.879
5107	720	718.000	8.260	59.647
5715	520	408.000	5.581	57.884
5103	740	581.000	8.706	65.613
5767	740	694.000	3.893	54.175
5797	740	434.000	5.100	59.317
4372	520	325.000	6.105	63.537
5926	530	642.000	8.811	61.463
4621	520	357.000	6.382	58.037
5929	530	634.000	5.000	64.270
5931	530	706.000	9.122	60.624
5934	530	672.000	7.636	63.900
4202	720	650.000	6.790	61.396
4257	720	621.000	6.631	66.092
4275	740	528.000	5.713	57.995
5248	740	587.000	6.001	63.336
4414	530	611.000	7.174	64.920
1007	520	636.000	5.571	55.612
4428	530	653.000	5.825	69.644
4080	720	803.000	8.535	56.467
4624	720	773.000	7.403	58.448
4787	740	661.000	8.597	61.176
4775	740	649.000	8.196	68.548
3401	720	594.000	6.545	59.545
3496	720	590.000	8.352	68.695
4275	740	528.000	9.460	59.063
4079	720	996.000	13.321	59.999
4163	520	384.000	12.404	56.820
4768	740	662.000	11.333	60.339
5924	530	686.000	9.784	60.710
5928	530	660.000	7.693	72.669
5933	530	971.000	12.338	58.552
4898	530	514.000	8.249	58.523
5223	520	937.000	13.722	55.632
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	830	654.000	9.675	59.949
3211	420	810.000	10.145	52.404
2153	920	702.000	9.998	62.033
1116	430	797.000	7.999	62.191

Strata 5,7 Female

ID No.	Stratum	Fork Len.	HBAR	TBAR
4404	530	566.000	7.550	54.9[illegible]
4627	520	494.000	8.139	61.8[illegible]
5999	720	700.000	9.117	61.24[illegible]
4658	520	471.000	6.474	47.55[illegible]
5623	520	550.000	8.076	58.9[illegible]
5707	530	615.000	8.478	54.5[illegible]
5933	530	744.000	11.359	60.61[illegible]
5927	530	650.000	8.957	66.79[illegible]
5760	520	457.000	7.491	60.6[illegible]
3500	720	528.000	11.315	54.9[illegible]
5245	740	577.000	10.502	65.5[illegible]
4091	720	818.000	9.519	56.21[illegible]
5238	740	497.000	7.547	57.57[illegible]
6000	720	629.000	10.457	6[illegible]
5768	740	758.000	5.357	63.53[illegible]
5800	740	554.000	6.465	55.11[illegible]
4770	740	626.000	8.647	52.05[illegible]
5108	720	676.000	8.322	67.17[illegible]
5103	740	608.000	9.479	53.63[illegible]
5000	740	666.000	9.582	56.787
5104	720	675.000	6.735	60.79[illegible]
1901	530	633.000	8.753	58.11[illegible]
4412	530	653.000	8.873	57.22[illegible]
5514	530	467.000	8.830	55.48[illegible]
4772	740	632.000	9.625	66.31[illegible]
42[illegible]	720	733.000	9.451	60.87[illegible]
4730	720	903.000	10.037	64.80[illegible]
5109	740	591.000	7.675	59.97[illegible]
4277	740	586.000	8.813	60.62[illegible]
3745	720	640.000	7.716	58.5[illegible]
1907	530	453.000	8.395	54.47[illegible]
4305	530	619.000	8.595	54.21[illegible]
5517	530	790.000	9.807	55.30[illegible]
4775	740	558.000	8.517	52.31[illegible]
5215	530	501.000	7.263	58.84[illegible]
[illegible]	[illegible]	914.000	10.393	61.845
[illegible]	530	563.000	7.810	54.915
4277	740	586.000	8.672	59.206
4268	720	508.000	7.935	53.330
1902	530	451.000	7.813	56.416

Table 11. (continued)

Strata 8,9,10 Female

ID No.	Stratum[1]	Fork Len.	HBAR[2]	TBAR[3]
5081	92[illegible]	557.000	11.064	61.321
4131	940	624.000	11.061	60.609
4939	830	743.000	8.703	68.937
4903	830	755.000	10.451	53.857
4910	830	737.000	11.992	56.665
4150	940	552.000	3.638	51.177
4137	940	785.000	10.692	53.954
4917	830	506.000	8.518	56.360
1115	103	806.000	13.476	68.959
1105	103	973.000	11.523	59.073
2216	102	869.000	9.638	54.678
5299	82[illegible]	588.000	11.315	61.616
1110	103	877.000	9.717	59.817
2221	102	813.000	10.600	67.352
1109	103	884.000	8.197	51.816
[illegible]02	103	859.000	13.219	62.288
[illegible]85	820	686.000	11.375	65.180
2209	102	879.000	8.820	61.119
1123	103	973.000	8.054	71.049
4913	830	571.000	9.249	60.782
2222	102	894.000	14.660	68.386
1112	103	889.000	11.086	55.386
4151	940	521.000	6.894	55.713
4125	940	725.000	8.648	64.113
1516	920	678.000	7.629	59.765
4902	830	611.000	9.368	55.574
4899	830	573.000	10.476	68.608
4132	940	751.000	10.238	52.933
3422	830	699.000	11.163	59.038
3405	830	720.000	8.850	51.840
4895	830	583.000	7.759	56.971
3407	830	724.000	9.264	60.303
1106	480	855.000	10.632	59.707
5145	910	981.000	11.940	61.794
1120	103	898.000	10.147	58.030

Strata 8,9,10 Female

ID No.	Stratum	Fork Len.	HBAR	TBAR
1104	103	896.000	10.952	58.945
1107	103	865.000	9.786	65.717
1111	103	948.000	7.689	60.975
1122	103	968.000	9.692	62.583
1126	103	945.000	8.737	60.715
1504	920	504.000	6.361	62.439
1508	920	504.000	6.989	55.839
1512	920	551.000	6.828	57.196
1519	920	599.000	6.813	67.509
4892	830	526.000	6.233	58.219
4903	830	581.000	5.594	60.045
4914	830	614.000	9.621	65.509
4912	830	573.000	6.333	56.019
5083	920	544.000	6.774	59.764
4134	940	531.000	6.927	67.170
4143	940	620.000	5.486	58.189
4152	940	596.000	7.011	67.451
5282	820	621.000	6.465	62.617
5287	820	629.000	7.565	56.067
5341	910	685.000	3.841	52.269
5346	910	871.000	10.437	62.297
5344	910	868.000	7.026	61.599
2202	102	734.000	7.481	59.313
2207	102	818.000	9.817	65.297
2217	102	849.000	7.981	71.475
2224	102	919.000	9.457	54.919
2219	102	883.000	9.366	74.944
5283	820	781.000	7.965	58.177
4147	940	576.000	9.638	59.018
4139	940	757.000	9.525	61.531
2218	102	867.000	12.478	57.029
2205	102	899.000	12.137	57.635
1121	103	912.000	11.021	59.524
1127	103	945.000	10.438	56.457
1510	920	515.000	8.477	53.039
1520	920	617.000	9.077	51.259
1511	920	553.000	4.865	52.673

Table 11. (continued)

Strata 5,7 Male

ID No.	Stratum[1]	Fork Len.	HBAR[2]	TBAR[3]
1906	530	464.000	7.874	53.899
5516	530	473.000	6.790	61.244
5547	720	415.000	6.538	68.106
5551	720	518.000	8.398	63.815
5716	520	450.000	6.435	60.844
5239	740	455.000	6.649	65.023
4497	530	572.000	6.206	63.453
4459	520	563.000	7.962	63.506
3481	720	537.000	6.516	59.463
3495	720	565.000	8.952	59.020
5236	740	590.000	8.909	61.888
3449	730	496.000	7.223	55.904
4431	530	674.000	10.786	61.955
4434	530	514.000	9.439	54.998
1010	520	407.000	7.461	61.429
4425	530	660.000	8.797	67.779
1011	520	474.000	6.134	57.365
366	530	796.000	9.255	59.604
3 [illegible]	720	562.000	9.927	55.325
5549	720	488.000	9.927	54.616
4629	520	545.000	9.295	54.639
5597	720	514.000	7.381	57.379
4274		5.000	7.877	57.054
5595	720	630.000	7.529	58.575

Strata 8,9,10 Male

ID No.	Stratum	Fork Len.	HBAR	TBAR
4159	930	560.300	4.955	55.109
4132	940	794.000	8.531	62.556
1501	920	508.000	6.981	52.457
4915	830	514.000	8.979	59.690
4124	940	659.000	10.882	66.341
4153	930	505.000	8.259	58.551
5647	910	380.000	8.832	54.762
5755	920	676.000	10.210	64.015

Notes

1. Stratum: URI code for Tex. Inst. designator: e.g. URI 530 = TI 5-3
2. HBAR: Average circulus height in microns (.001 mm)
3. TBAR: Average circulus width in microns

To assess the utility of PROD/COMP using fish from known spawning regions the three groups of scale profiles listed in Table 8 were used to perform a pseudo-jackknife classification study. Scales marked with an asterisk in each data set were intentionally omitted from the training library profile. These were then reclassified using the COMP program. Results of these studies, which used the sequential ordering of height and width of circuli as discriminatory variables were compared with Texas Instruments results for validity assessment (Tables 12, 13, 14, 16). The data file for the three groups was next used to compute a spawning river training library. All scales from the unknown mixed oceanic group were next selected for circulus by circulus comparison followed by subsequent classification. Table 11 gives ID numbers for fish used in this portion of the analysis.

URI-Texas Instrument Inter-Program Validation Study

Three different classification techniques (PROD/COMP height; PROD/COMP width; PDF-Linear Discriminant Analysis) were applied to the URI scale data base. This data base consisted of spawning river plus oceanic scale circulus height and width profiles, with associated ID, regional, sex, and fork length data for each fish in the data set.

All three methods result in classification of an individual into one of three natal (spawning) regions (Roanoke River, Chesapeake Bay, Hudson River). The following procedures were implemented to assess validity for each of the three URI techniques. First, pseudo-jackknife reclassification tables were generated for spawning groups using each of the URI techniques. These resulted in the assignment of individuals to specific spawning areas.

Table 12. University of Rhode Island-Texas Instruments inter-program validation study: relative natal proportions derived using URI analytic techniques vs. Texas Instruments natal proportions.

Group Name	Sex	TECHNIQUE: P/C Height R	P/C Height C	P/C Height H	P/C Width R	P/C Width C	P/C Width H	URI Discriminant R	URI Discriminant C	URI Discriminant H	Texas Instruments Discriminant R	Texas Instruments Discriminant C	Texas Instruments Discriminant H	N[A]
TRAINING SET														
Chesapeake	Male	43	57	0	14	0	86	38	50	13	0	88	13	8
Chesapeake	Female	26	21	53	11	32	58	26	32	42	9	57	35	19
Roanoke	Male	50	50	0	50	0	50	75	0	25	75	25	0	4
Roanoke	Female	18	14	68	10	29	62	73	5	23	73	23	5	22
Hudson	Male	0	100	0	50	0	50	33	16	50	0	33	67	6
Hudson	Female	12	6	82	6	38	56	32	21	47	5	37	58	19
TEST SET														
Strata 8,9,10	Male	0	80	20	40	0	60	60	40	0	0	80	20	5
Strata 8,9,10	Female	3	29	68	29	9	61	28	28	44	12	67	21	75
Strata 8,9,10	Male & Female	2.8	32	65	29.7	8.4	60.9	29.8	28.7	41.4	11.3	67.8	20.9	80
Strata 5,7	Male	8	88	4	62	0	38	42	25	33	17	39	43	24
Strata 5,7	Female	3	29	68	29	9	61	28	28	44	3	54	43	75
Strata 5,7	Male & Female	4.2	43.3	52.5	37	6.8	53.4	31.2	27.3	41.5	6.6	50.2	43	99

A: Number used in discriminant calculation

Table 13. University of Rhode Island-Texas Instruments inter-program validation study deviation of URI natal proportions from Texas Instruments derived proportions.

Group Name	Sex	TECHNIQUE											
		P/C Height			P/C Width			URI Discriminant			All Spawning URI Discriminant		
		R	C	H	R	C	H	R	C	H	R	C	H
TRAINING SET													
Chesapeake	Male	+43	-31	-13	+14	-88	+73	+38	-38	0	+18	-25	+7
Chesapeake	Female	+17	-36	+18	+2	-25	+23	+17	-25	+7	+10	-24	+14
Roanoke	Male	-25	+25	0	-25	-25	+50	0	-25	+25	-30	-5	+35
Roanoke	Female	-55	-9	+63	-63	+6	+57	0	-18	+18	-19	-3	+22
Hudson	Male	0	+67	-67	+50	-33	-17	+33	-17	-17	+20	+7	-27
Hudson	Female	+7	-31	+24	+1	+1	-2	+27	-16	-11	+22	+3	-25
TEST SET													
Strata 8,9,10	Male & Female	-8.5	-35.8	+44.2	18.4	-59.4	40	18.5	-39.1	20.5			
Strata 5,7	Male & Female	-2.4	-6.9	9.5	30.4	-43.4	12.4	24.6	-22.9	-1.5			

Table 14. University of Rhode Island-Texas Instruments inter-program validation study: relative natal proportions derived by URI linear discriminant analysis vs. Texas Instruments quadratic discriminant analysis for all scales in URI training set data base.

Group Name	Sex	TECHNIQUE						N
		URI Discriminant			Texas Instruments Quadratic Discriminant*			
		R	C	H	R	C	H	
Chesapeake	Male	32	50	18	14	75	11	28
Chesapeake	Female	24	45	31	14	69	17	42
Roanoke	Male	60	5	35	90	10	0	20
Roanoke	Female	62	14	24	81	17	2	42
Hudson	Male	20	24	56	0	17	83	24
Hudson	Female	30	28	43	8	25	68	40

* Subset selected from larger TI training set

Table 15. University of Rhode Island intra-program validation study: percentage agreement between three analytic techniques (PROD/COMP height, PROD/COMP width, PDF-linear discriminant analysis) applied to URI scale profile data base.

Group Name	Sex	Percent Agreement Level 0*	2 (Height)*	2 (Width)*	3*	Number in Group
TRAINING SET						
Chesapeake	Male	0	86	14	0	8
Chesapeake	Female	32	21	21	26	20
Roanoke	Male	25	0	25	50	4
Roanoke	Female	50	18	9	23	22
Hudson	Male	34	17	50	0	6
Hudson	Female	38	13	6	44	16
Entire Training Set (Male & Female)		34.9	23.9	16.1	25.4	76
TEST SET						
Strata 8,9,10 (south)	Male	28	28	43	0	7
Strata 8,9,10 (south)	Female	47	15	9	28	67
Strata 8,9,10	Male & Female	45.2	16.2	12.2	25.4	74
Strata 5,7 (north)	Male	30	22	39	9	23
Strata 5,2 (north)	Female	32	28	24	17	72
Strata 5,7	Male & Female	31.5	26.55	27.6	15.1	95
Strata 5,7,8, 9,10		37.5	22.03	20.9	19.6	169

* 0 = No agreement
2 = URI discriminant + given
3 = all URI techniques agree

Table 16. University of Rhode Island-Texas Instruments inter-program validation study: percent agreement of University of Rhode Island and Texas Instruments source region classifications for three analytic techniques (PROD/COMP height, PROD/COMP width, PDF-linear discriminant).

Group Name	Sex	URI-TI Agreement % P/C Height	P/C Width	URI Discriminant	Number in P/C	Number in URI Discriminant
TRAINING SET						
Chesapeake	Male	43	14	50	7	28
Chesapeake	Female	16	26	33	19	42
Roanoke	Male	25	25	55	4	20
Roanoke	Female	14	5	52	22	42
Hudson	Male	33	33	44	6	25
Hudson	Female	47	47	38	17	40
Entire Training	Male & Female	27	24	44.2	75	197
TEST SET						
Strata 8,9,10	Male	71	14	14	7	7
Strata 8,9,10	Female	30	23	21	80	82
Strata 8,9,10	Male & Female	33	22	20	87	89
Strata 5,7	Male	46	15	31	26	26
Strata 5,7	Female	44	26	40	70	70
Strata 5,7	Male & Female	45	23	38	96	96
Strata 5,7,8, 9,10	Male & Female	39	23	29	183	185

SCANNING ELECTRON MICROSCOPE ANALYSES OF SCALE SURFACE STRUCTURE

The scanning electron microscope has been used to investigate the structural morphology of fish scales. Investigations have concentrated either on the detailed histological investigation of scales of a single species (Lanzing and Higginbotham, 1974) or on detection of taxonomic scale characters useful for accurate speciation of closely related fish genera (DeLamater and Courtenay, 1974).

The scanning electron microscope has a much greater depth of field than does the transmission electron microscope device. Electrons are reflected from surface features only. This results in a more accurate depiction of surface structures. Underlying obscuring structures which would be detected by transmission electron or photon (light) microscopy are completely masked in scanning electron microscope analyses.

Scanning electron micrographs of striped bass scales collected in the Texas Instrument synoptic subpopulation survey are illustrated in Figures 17, 18, and 19. All scales were cleaned, examined and mounted on scanning electron microscope sample stubs using conductive silver colloid adhesive. Mounted scales received a vapor deposition coating of gold-palladium to enhance electron beam reflectivity.

Figure 17 at 20X magnification illustrates the lateral, rostral, and caudal fields into which striped bass scales are divided. Ctenii (spines) are visible at the edge of the caudal (lower) field. The focus, or center of circulus development represents the location of the original scale platelet. Two annuli (year marks) are visible. These appear as horizontal lines which run across the rostral (upper) field. Regular extra-focal circuli are visible in the lateral field.

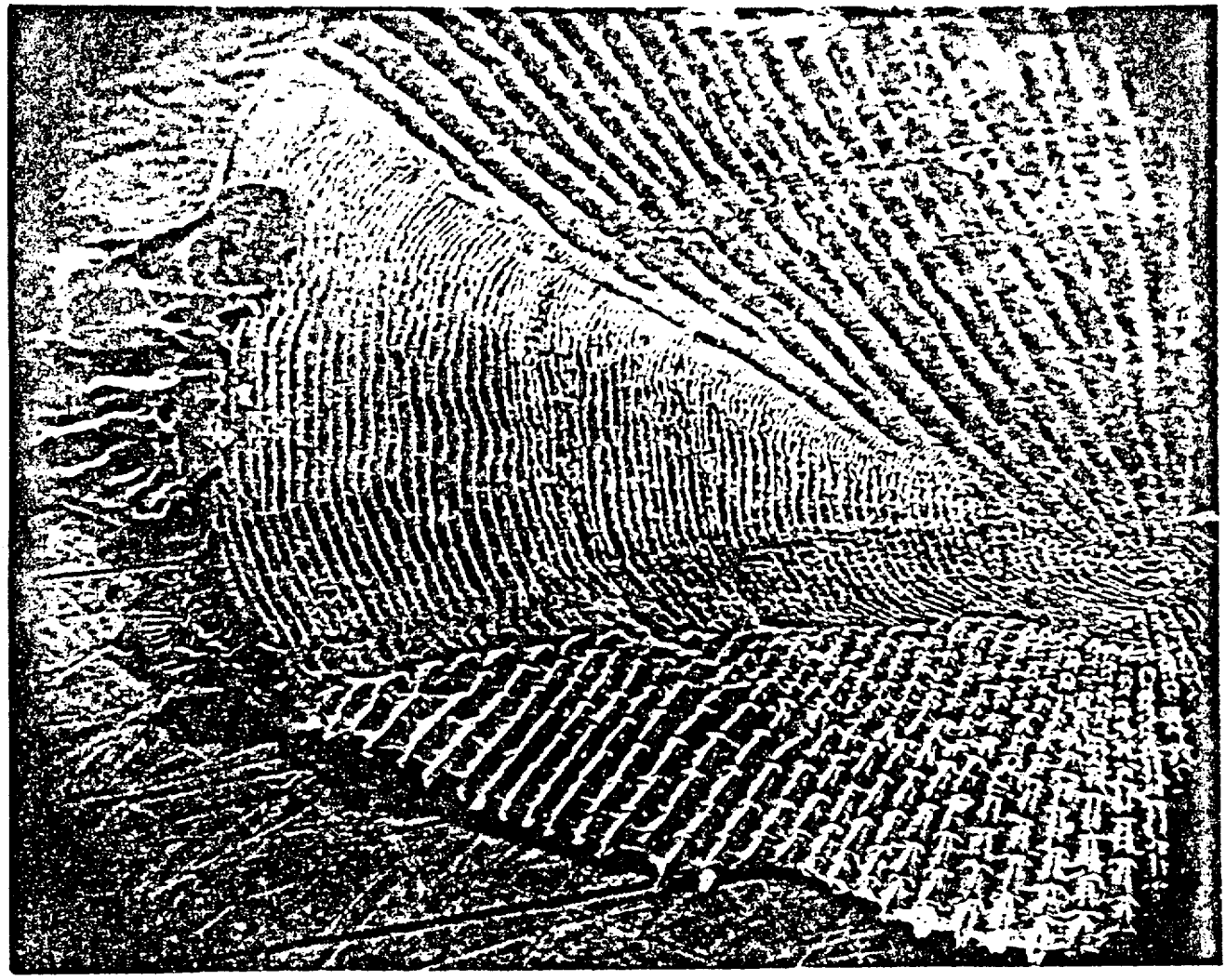


Figure 17. Scanning Electron micrograph of scale of striped bass (Morone saxatilis) illustrating lateral, rostral and caudal fields and associated circulus ridge structure. 20X Magnification.

Figure 18 illustrates the structure of circuli in the lateral field. The higher magnification (110X) demonstrates the bifurcation (splitting) of circulus ridges which occurs in this region. Since ridge structure is not constant over the entire lateral field, standardization of scale orientation is a necessary factor for inter-scale comparison. Average circulus width for this segment is equal to 54.1 μ.

Figure 19 displays four scales derived from Hudson and Chesapeake spawning regions. The three Hudson scales (H1375, H1348, H1338) demonstrate enhanced circulus growth immediately adjacent to the focal region. This is in agreement with results of computer analyses of Hudson River scale surface profiles. Fourier analyses of Hudson River scales emphasized the spectrally pure, unimodal nature of circulus width distributions.

The Chesapeake scale (Rappahannock 1580, Fig. 19B) demonstrates reduced circulus growth until considerably removed (13-15 circuli) from the focal area. URI Phase I analyses demonstrated that Chesapeake scales (especially those from the Potomac River) demonstrated significantly greater within scale variability of circulus width distributions. Scanning electron microscope analyses thus tend to confirm trends noted in surface profile analysis with regards to slight regional differences in scale circulus structure.

Scanning electron microscope micrographs are useful for developing an overall picture of scale surface morphology. Quantitative data are lacking in this method for critical regional difference analyses. Two dimensional Fourier transform procedures have been developed in military photo interpretation studies (Chen, 1975). Application of such procedures to photographs of selected areas of scale surface structure could prove useful for recognition and quantification of distinctive regional scale features.

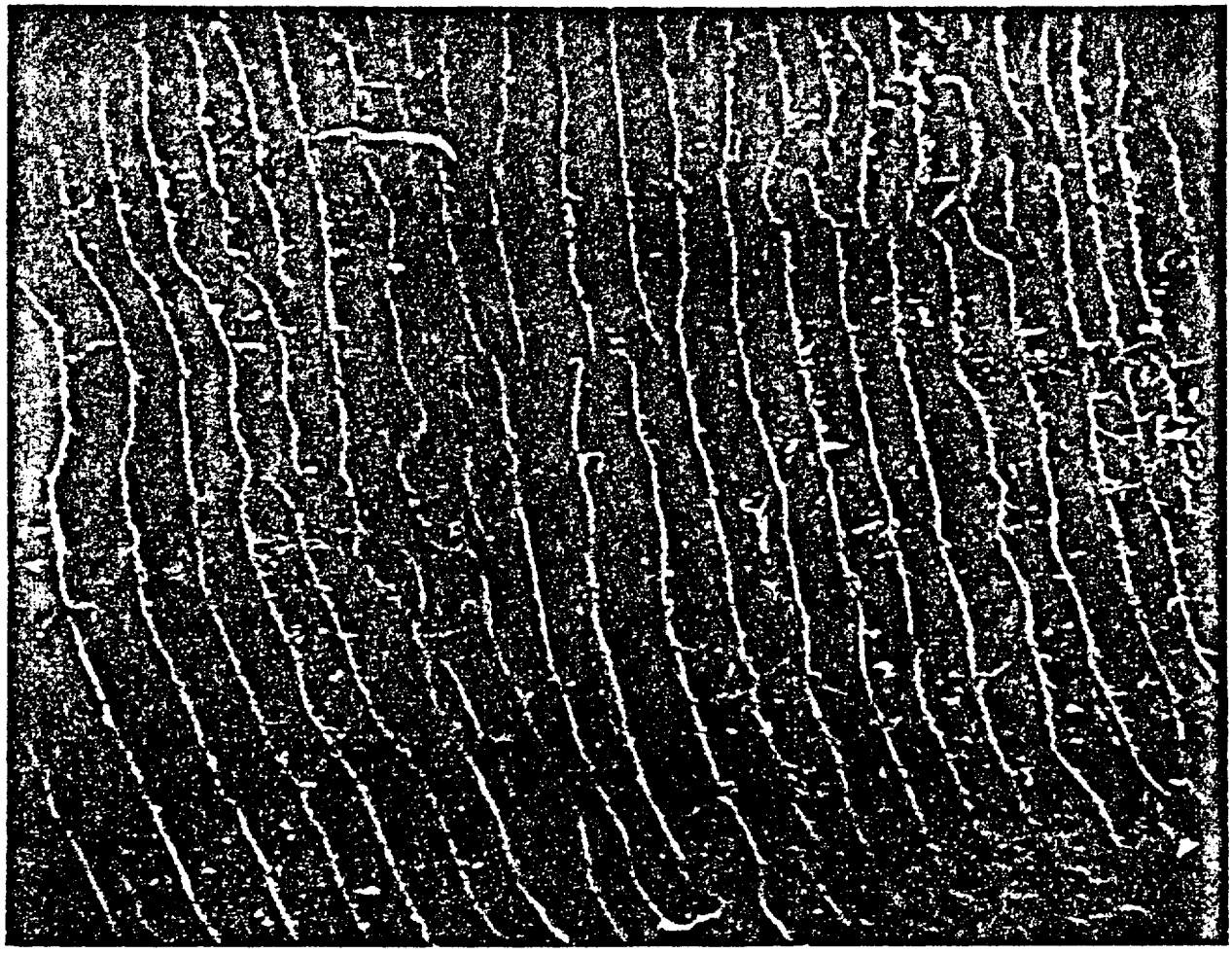
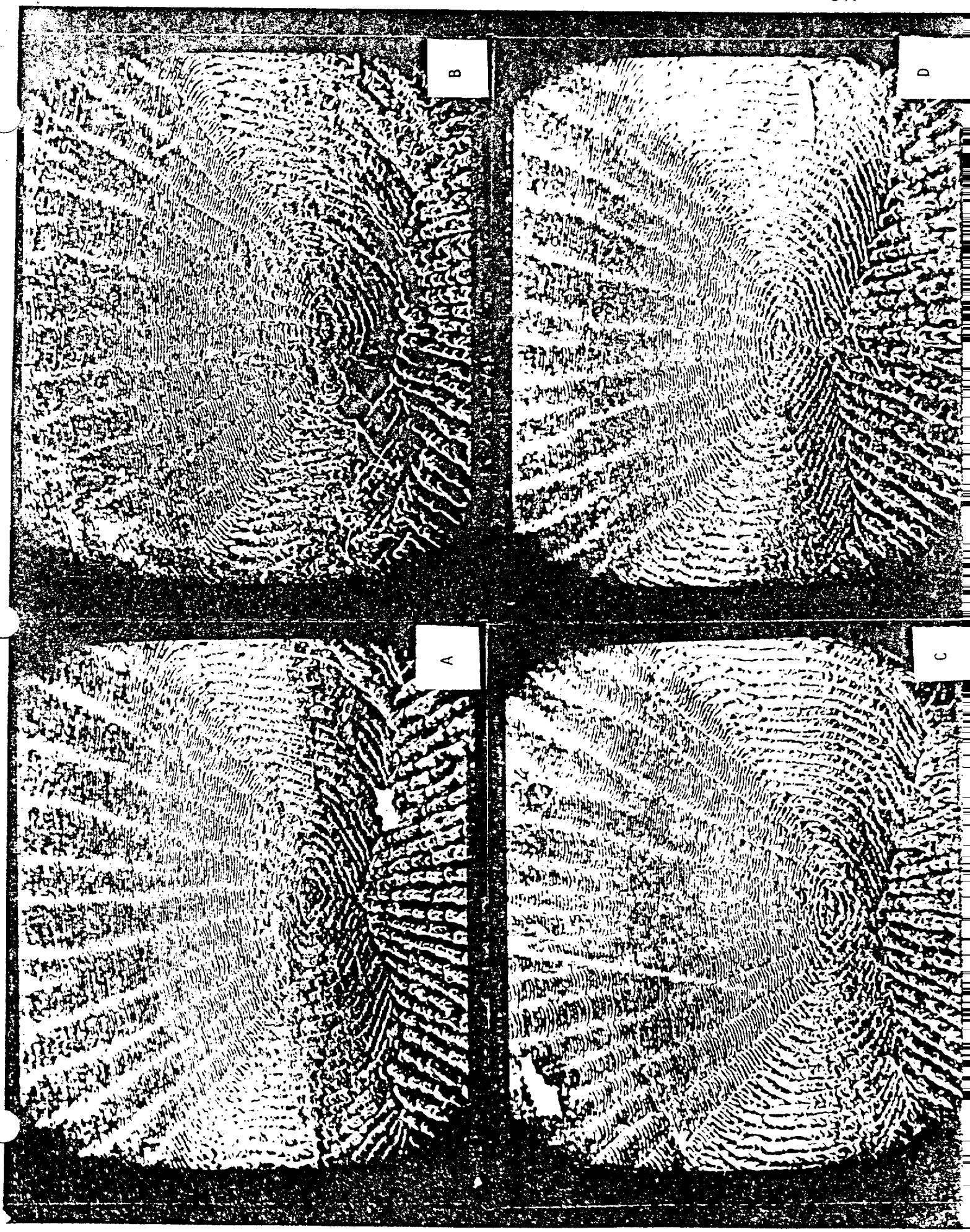


Figure 18. Enlargement of portion of lateral field illustrated in Fig. 16 demonstrating bifurcation of circulus ridge structure, dominant regularity of circulus widths. 110X magnification.

Figure 19. (facing page). Scanning electron micrographs of scales derived from Hudson and Chesapeake spawning regions and used in inter-region surface feature comparison. 57X Magnification. Fig. 19A;Hudson 1375. Fig. 19B;Rappahannock 1580 Fig. 19C; Hudson 1348. Fig. 19D; Hudson 1338.


B
D
A
C

DISCUSSION

The URI Critical Scale Analysis Investigation has followed an orderly sequence of steps. The goal of this investigation has been to detect, analyze, and record scale surface microstructure, and to assess the utility of this data in source stock assessment of the striped bass (M. saxatilis).

Initially, Fourier (harmonic frequency) analysis was employed. This technique alone would have sufficed for racial analysis had major differences in modal circulus width occurred between regions. No such major shifts were discovered, over the range covered by this investigation.

Subsequently, more sensitive techniques have been employed. HBAR and TBAR (average circulus height and width) were calculated for each profile. These represent summary statistics, which reduce each profile to only two numbers. These were calculated automatically by program WHGHT, and submitted to linear discriminant analysis. Initial separation between fish collected in 1974 from Chesapeake and Hudson River spawning areas was high (82.5%) but this was due to the following extraneous (non-racial) factors. Most significant was the large (70%) difference in mean fork length between the two training sets. This alone (given the significant correlation present between fork length and circulus height) could have provided an unrealistically high separation. Further, since only two regions were used, random accuracy was 50%, as opposed to the 33% used in the later (three regions) analyses. Finally, in Phase I analyses, jackknifed classification tables were unavailable. This would tend to artificially inflate separation accuracies.

Phase II analyses concentrated on increasing analytic sensitivity of methods to intergroup differences. Normalized probability density function (PDF) techniques were developed. These demonstrated significant enhancement of intergroup separation when applied to Phase I data values.

The PROD/COMP method, and the AVPLT technique were developed to supplement

the PDF-linear discriminant analysis technique. These two techniques are sensitive to sequential pattern along the circulus profile, while the PDF technique is more sensitive to intergroup differences in distribution of circulus height and width values. Since there was no a priori method of determining which character (sequential vs. distributional difference) would be dominant, all three methods were applied to the data sets.

Initial results using PDF techniques on a small number of 1975 scales in the 400-600 mm fork length range demonstrated significant inter-region circulus distribution differences. The actual task, however, was to assess utility of scale growth data for classification of oceanic fish, whose size range was 407-1000 mm. Therefore, a new extended size range training set was assembled. Since fork length is a factor correlated with scale growth, previous studies indicates that such an extension in size range of training set would impact separation capabilities in a negative manner.

Validity Assessment: URI Scale Analytic Techniques

Table 12 displays classification proportions for each of the three techniques applied to the URI data base. The general criterion used in evaluation of the techniques may be stated thus: "Do the results indicate successful racial separation, or do they indicate response to some extraneous (non-racial) variation within each spawning group?" Using this criterion, and the results displayed in Tables 12 through 16, the relative merit of the three URI techniques is: URI PDF-Discriminant Analysis (best), PROD/COMP height (second best), PROD/COMP width (worst).

The dominant racial proportion for each of the spawning grounds is indicated in Table 12. Reference to Texas Instruments classifications demonstrates the dominant proportion of each region to correspond to the title for the given region. This is what is to be expected for an adequate racial

analytical technique. The URI discriminant analysis technique is totally successful, in terms of training set dominance (Table 14). Percentage dominance is not as high as in Texas Instruments discriminant analysis. Two factors may be responsible for this lower degree of separation. Scale growth in ctenoid-scaled fish like the striped bass is not as variable as that of cycloid scaled fishes (e.g. sockeye salmon) (Koo, 1962). Environmental differences are reflected more clearly especially in circulus width variation among cycloid scales. This would indicate that meristic features, which have been demonstrated to be environmentally plastic in the striped bass (Raney and DeSylva, 1953) are of greater utility in racial analysis than the features arising during the relatively non-plastic process of scale circulus formation. Additionally, larger training samples were processed by the Texas Instruments team of researchers. This could have led to higher separations, based on their reduced intra-group variances. Increase to the URI training sample size from 85 to 200 scales did not appear to enhance separation accuracy, indicating that further residual variance was real and would not likely be reduced merely by increasing URI's training sample size further.

The two PROD/COMP analyses do not adequately follow the distribution of source region fish in the training set. PROD/COMP width, especially appears to be assigning constant proportions of fish to each region, regardless of regional origin of fish classified (e.g. 56-62% of female fish assigned to Hudson, regardless of source region). This indicates that technique is producing a selection result based upon an extraneous (non-racial) source of variability (e.g. natural variations in growth rate) which assumed proportions relatively unrelated to racial origin of striped bass.

The PROD/COMP height method appears more useful. Racial proportions appear to reflect source region more adequately than PROD/COMP width (although Hudson reclassifications are dominant for female fish from Roanoke, Chesapeake

regions). Reference to Table 16 shows that higher levels of agreement with Texas Instruments' classifications were obtained with PROD/COMP height than with PROD/COMP width for the training set. Highest level of URI-Texas Instruments agreement were returned by the PROD/COMP height method applied to oceanic profiles. This serves to indicate that circulus height may be used more confidently than variation of circulus width for racial analysis of the striped bass.

University of Rhode Island-Texas Instruments Discriminant Classification Consistency Analysis

Table 16 indicates the percentage of individuals in each group classified identically by URI and Texas Instruments techniques. URI discriminant analysis is clearly the best of the URI techniques although inferior to Texas Instruments separation results. PROD/COMP height yields highest agreement levels for oceanic scales (39%).

To assess the significance of a given percentage of inter-method agreement it is necessary to evaluate the level of agreement which would occur by chance and then determine whether observed deviations from the mean are statistically significant.

The following formula describes the probability of a match occurring for an individual classified by the URI and Texas Instruments discriminant programs, in the presence of inherent misclassification error rates for each technique.

$$[P_U(C) \cdot P_U(C/C) + P_U(R) \cdot P_U(C/R) + P_U(H) \cdot P_U(C/H)] \quad X$$

$$[P_T(C) \cdot P_T(C/C) + P_T(R) \cdot P_T(C/R) + P_T(H) \cdot P_T(C/H)] \quad +$$

$$[P_U(C) \cdot P_U(R/C) + P_U(R) \cdot P_U(R/R) + P_U(H) \cdot P_U(R/H)] \quad X$$
$$[P_T(C) \cdot P_T(R/C) + P_T(R) \cdot P_T(R/R) + P_T(H) \cdot P_T(R/H)] \quad + \qquad (1)$$

$$[P_U(C) \cdot P_U(H/C) + P_U(R) \cdot P_U(H/R) + P_U(H) \cdot P_U(H/H)] \quad X$$
$$[P_T(C) \cdot P_T(H/C) + P_T(R) \cdot P_T(H/R) + P_T(H) \cdot P_T(H/H)] \quad = \quad p'$$

where: C = Chesapeake

R = Roanoke River

H = Hudson River

$P_U(x)$ = per cent x in sample (URI results)

$P_T(x)$ = per cent of x in sample (Texas Instruments results)

$P_U(x/y)$ = probability of misclassifying y individual as x

$P_T(x/y)$ = probability of misclassifying y individual as x (Texas Instruments)

p' = probability of successful match/trial

This equation was used to assess results of discriminant analysis. The factor p' represents the probability of a match in a single trial. Over many trials the results (match vs. non-match) are described by the binomial distribution in which:

$$P \text{ (n matches in N trials)} = \binom{N}{n} p'^{n} \; q'^{N-n}$$

where: p' = probability of successful match/trial

q' = 1-p'

$$\binom{N}{n} = \frac{N!}{n!(N-n)!}$$

For the Texas Instruments discriminant analysis, the following results have been reported (Texas Instruments, 1976).

$$P_T[H/C] = P_T[C/H] = .24$$

$$P_T\ H/R = R/H = .04$$

$$P_T\ C/R = R/C = .11$$

$$P_T\ H/H = .87$$

$$P_T\ R/R = .86$$

For the URI discriminant analysis:

$$P_U[H/C] = P_U[C/H] = .25$$

$$P_U\ H/R = R/H = .30$$

$$P_U\ C/R = R/C = .20$$

$$P_U\ H/H = .46$$

$$P_U\ C/C = .46$$

$$P_U\ R/R = .60$$

For the oceanic set derived from strata 8,9,10

$$P_U[C] = .30;\ P_U[R] = .29;\ P_U[H] = .41$$

$$P_T[C] = .68;\ P_T[R] = .11;\ P_T[H] = .21$$

When above values are inserted into Eq. (1):

$$p' = (.2985)\ (.52) + (.357)\ (.1778) + (.3506)\ (.3293) = .33414$$

$$q' = 1 - p' = .6658$$

Reference to Table 15 shows 18 observed matches (20%) in strata 8,9, and 10. For large samples, the binomial distribution closely approximates the normal with mean:

$\mu = Np'$ and variance:

$\sigma^2 = Np'q'$

For this case

$\mu = (87)\ (.334) = 29.06$ and

$\sigma^2 = (87)\ (.334)\ (.669) = 19.35$ or

$\sigma = 4.399$

Since only 18 matches occurred, we may use the standard normal deviate, Z, where

$$Z = \frac{\overline{X}_{OBS} - \mu}{\sigma}$$

$\overline{X}_{OBS} = 18$ or $\quad Z = \frac{18 - 29.06}{4.399} = -2.5$

There is significantly less agreement ($P < .01$) than would be expected if methods responded solely to similarities among fish. URI discriminant analysis provides 50-60% separation levels when applied to the training set. An individual may possess either a central or peripheral position amid the multivariate swarm of data points comprising its training group. A given fish might be central (i.e. close to the mean in its principal regional identifying character values) for one technique, and peripheral (i.e. large

Mahalanobis D^2 (Dixon, 1973) for another, and hence potentially misclassified. Texas Instruments' higher separation levels indicate a smaller amount of intra-group residual variance. The agreement between groups as classified by the two techniques is significantly smaller than would be expected to occur by chance. This may be due to high residual URI intra-group variance coupled with the fact that centrally located scales for one technique could be peripherally located in the other.

When Eq. (1) is applied to the oceanic data set from strata 5,7

$$P_U[C] = .27 \quad P_U[R] = .31; \; P_U[H] = .42 \quad P_T[C] = .50$$

$$P_T[R] = .07 \quad P_T[H] = .43$$

for these values:

$$p' = (.2912)\,(.4509) + (.366)\,(.1324) + (.3537)\,(.4539) = .3403$$

$$q' = 6597$$

$$N = 96$$

$$N_{obs} = 36 = \overline{X}$$

$$\mu = Np = 33$$

$$\sigma^2 = 21.77$$

$$\sigma = 4.66$$

$$Z = \frac{3}{4.66} = 0.64$$

This is a better performance than would occur by chance, but the deviation is not statistically significant ($p > .20$). Hudson classification percentages for the group as a whole are within 1% for fish from Strata 5 and 7 for the two methods. Peripheral vs. central factors, as well as high residual URI intra-group variances are presumed to be responsible for the lack of significant agreement between the two methods.

In spite of the lack of synchrony among results for individuals classified by the two techniques, incorporation of the values derived from scale analysis (i.e. those demonstrated to vary most strongly between regions) could, if added to a multi-character data base, increase, and would certainly not degrade overall reclassification accuracies.

Evaluation of Scale Analysis for Racial Analysis of Striped Bass

The results of this study do not indicate that scale surface micro-structure may be used to confidently assign striped bass to natal spawning regions. Two key assumptions underlie the URI scale analysis investigation. First, that fish collected while spawning in a particular spawning river were themselves native to that river during their juvenile stages of development (i.e. validity of homing hypothesis). Based on results of the Texas Instrument meristic study, this hypothesis appears valid for striped bass. The second necessary condition is that there be differences in early scale growth surface structures sufficiently marked to permit reliable reclassification based solely on these structures. At the present time, such marked differences do not appear to occur on scales of the striped bass.

Reasons for the lack of occurrence might include: alteration of early circulus growth features in subsequent life history stages; insensitivity of scale growth to environmental differences; lack of strong genetic control of scale growth.

The first possibility (reworking of early circulus features) appears probable. A significant correlation of fork length with circulus height (as shown in Table 4) implies that early circulus growth structures used in racial analysis have been recalcified. Salmon are known to resorb scales during spawning, so it is known that scales are subject to severe post-depositional alteration, at least in such cases.

Circulus width, for ctenoid-scaled fish (like the striped bass) is, according to Wallin (1957) the result of a mechanical process, produced by impact of the growing scale against the dermal scale pocket. Periods of slow growth produce fewer circuli, but these do not vary significantly in width. Our methods, being sensitive to individual circuli would not be expected to be sensitive to changes in growth rate among striped bass. Texas Instruments studies have used the annular markings on scales (Dist. from first to second Annulus/Dist. from Focus to first Annulus) as a classificatory variable. Annular markings are not present in the lateral field, and thus were not incorporated into URI analytic techniques. The final possibility (lack of genetic control) cannot be dismissed. It is difficult to define strong selection pressures which would act to produce genetically distinctive scale structures.

Of the three techniques investigated, PDF-discriminant analysis appears to provide optimal utilization of the racial information encoded into the circulus profiles. For Data Set 2 striped bass from 400-600 mm fork length an acceptable (70-80%) separation was obtained. The addition of selected PDF values (those demonstrated to vary most significantly between regions) could be incorporated as supplemental data values for future combined meristic, morphometric and/or biochemical analyses. The supplemental data could enhance (and would certainly not degrade) classification accuracies in such studies.

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APPENDIX 1: COMPUTER PROGRAM DOCUMENTATION

P1: SCALE

P2: PDF

P3: AVPLT

P4: MOVAV

P5: PROD

P6: COMP

```
C       SCALE CIRCULI PROGRAM
        DIMENSION H(200),CIRC(200),IPLT(100)
        INTEGER RUN(-50:50),INAM(10)
        TYPE 'OUTPUT FILE NAME? '
        READ(11,101) INAM(1)
101     FORMAT(S20)
        CALL FOPEN(3,INAM)
        TYPE 'ID TO PRINT?YES(0),NO(1)'
        ACCEPT IDOC
400     READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K,(H(I),CIRC(I),I=1,K)
        WRITE(10,240) ID1,ISX,IRIV
        IF(IDOC.EQ.0)WRITE(12,240)ID1,ISX,IRIV
        GO TO 400
420     TYPE 'ENTER TRAVERSE SPEED'
        ACCEPT SPEED
        SPEED=100.0*SPEED/6.0
        CALL FOPEN(4,'$ADC')
300     PAUSE 'POSITION AT 10 ON RECORDER'
        REWIND 4
        XMIN=0.0
        DO 20 I=1,20
        READ BINARY(4) L,ICHN1
20      XMIN=XMIN+0.05*L
        PAUSE 'POSITION AT 0 ON RECORDER'
        REWIND 4
        XMAX=0.0
        DO 25 I=1,20
        READ BINARY(4) L,ICHN1
25      XMAX=XMAX+0.05*L
        LMIN=XMIN
        LMAX=XMAX
        HSCL=100.0/ABS(XMIN-XMAX)
        ACCEPT 'ENTER ID#,SEX,RIVER,AGE,LENGTH ',ID1,ISX,IRIV,IAGE,FLEN
        IF(ID1.LT.1) GO TO 500
        DOLD=0.
        Z=0.
        XMAX=-1.E10
        XMIN=1.E10
        C1=1.0/101.0
        K=0
C       CALL IN SCALE INFO
        REWIND 4
1       READ BINARY(4)ICHN0,ICHN1
        DO 70 J=-50,49
70      RUN(J)=RUN(J+1)
        RUN(50)=ICHN0
        IF(ICHN1.LT.256) GO TO 1
        TYPE '<007><15><007>'
        I=0
        SUM=0.
        DO 80 J=-50,50
80      SUM=SUM+RUN(J)
30      ZOLD=Z
```

```
        I=I+1
        READ BINARY(4) L,ICHN1
        IF(L.LT.LMIN.OR.L.GT.LMAX) GO TO 36
        IF(ICHN1.GT.256.AND.I.GT.50) GO TO 35
        SUM=SUM-RUN(-50)+L
        DO 60 J=-50,49
60      RUN(J)=RUN(J+1)
        RUN(50)=L
        Z=RUN(0)-SUM*C1
        IF (Z.GT.XMAX) XMAX=Z
        IF (Z.LT.XMIN) XMIN=Z
        IF (Z.LT.ZOLD) GO TO 30
        IF (Z*ZOLD.GT.0.) GO TO 30
        K=K+1
        IF(K.GT.200) K=200
        H(K)=XMAX-XMIN
        D=(I-1.0)-ZOLD*(Z-ZOLD)
        CIRC(K)=D-DOLD
        DOLD=D
        XMAX=-1.E10
        XMIN=1.E10
        GO TO 30
36      TYPE '<7>SCALE EXCEEDED CALIBRATION LIMITS.<7><15>'
35      DO 55 I=1,K
        H(I)=H(I)*HSCL
55      CIRC(I)=CIRC(I)*SPEED
        WRITE(12,210) ID1
        DO 100 I=1,K
        DO 40 J=1,100
40      IPLT(J)='  '
        L=2.0*H(I)
        IF(L.LT.1) L=1
        IF(L.GT.100) L=100
        IPLT(L)='H '
        L=CIRC(I)
        IF(L.LT.1) L=1
        IF(L.GT.100) L=100
        IPLT(L)='W '
        WRITE(12,200) I,H(I),CIRC(I),(IPLT(J),J=1,100)
100     CONTINUE
        WRITE(12,230)
        ACCEPT 'WRITE ON DISK? YES(0) NO(1). ',I
        IF(I.NE.0) GO TO 90
        WRITE BINARY(3)ID1,ISX,IRIV,IAGE,FLEN,K,(H(I),CIRC(I),I=1,K)
90      ACCEPT 'END OF RUN? YES(0) NO(1). ',I
        IF(I.NE.0) GO TO 300
        CALL FCLOS(3)
        STOP
200     FORMAT(' ',I3,2X,F7.2,2X,F6.2,T30,100A1)
210     FORMAT('1',' #   H UM.    W UM.',3X,'SCALE ID #=',I5)
230     FORMAT('1')
240     FORMAT(' ','SCALE ',I4,' SEX ',I2,' RIVER ',I2)
500     CALL FCLOS(3)
        STOP
        END
```

```
      DIMENSION H(200),CIRC(200),INAM(10),PDFH(20),PDFC(25)
      CALL FOPEN(7,'$MT0800W')
1     TYPE 'INPUT FILE NAME'
      READ(11,101)INAM(1)
101   FORMAT(S18)
      CALL FOPEN(3,INAM)
      TYPE 'RECORD TO SKIP TO?'
      ACCEPT NSKIP
      TYPE 'ENTER STARTING CIRCULUS NO. '
      ACCEPT N1
300   READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K1,(H(I),CIRC(I)
     *,I=1,K1)
      IF(ID1.EQ.NSKIP)GO TO 320
      GO TO 300
305   READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K1,(H(I),CIRC(I)
     *,I=1,K1)
320   DO 10 I=1,25
10    PDFC(I)=0.
      DO 15 I2=1,20
15    PDFH(I2)=0.
      DO 20 J=N1,K1
      INDH=IFIX(H(J)/1.31)
      INDC=IFIX(CIRC(J)/3.93)
      IF(INDH.LT.1)INDH=1
      IF(INDH.GT.20)INDH=20
      IF(INDC.LT.1)INDC=1
      IF(INDC.GT.25)INDC=25
      PDFH(INDH)=PDFH(INDH)+1.
20    PDFC(INDC)=PDFC(INDC)+1.
      WHT=0.
      WTT=0.
      DO 25 JJ=N1,K1
      WHT=WHT+H(JJ)
25    WTT=WTT+CIRC(JJ)
      HBAR=WHT/(K1-N1+1)
      TBAR=WTT/(K1-N1+1)
C     NORMALIZE PDF'S
      DO 30 K=1,20
30    PDFH(K)=PDFH(K)/(K1-N1+1)
      DO 31 K=1,25
31    PDFC(K)=PDFC(K)/(K1-N1+1)
      ILEN=FLEN
      WRITE(12,111)ID1,ISX,IRIV,IAGE,ILEN,HBAR,TBAR,PDFH,PDFC
111   FORMAT(' ',/,' PDF SPECTRUM FOR SCALE',I4,/,4I3,/,2F10.6,(10F1
     *0.7,/))
      WRITE BINARY (7) ID1,ISX,IRIV,IAGE,ILEN,HBAR,TBAR,N1,PDFH,PDFC
      GO TO 305
420   TYPE 'END? YES(0). NO(1)'
      CALL FCLOS(3)
      ACCEPT ITAG
      IF(ITAG.GE.1)GO TO 1
      CALL FCLOS(7)
      PAUSE REWIND TAPE
      TYPE 'RECORD TO SKIP TO?'
      ACCEPT NSKIP2
      CALL FOPEN(8,'$MTI800W')
222   READ BINARY(8,END=644)ID1,ISX,IRIV,IAGE,ILEN,HBAR,TBAR,N1,PDFH,PDFC
      TYPE ID1
      IF(ID1.EQ.NSKIP2)GO TO 221
      GO TO 222
221   READ BINARY(8,END=644)ID1,ISX,IRIV,IAGE,ILEN,HBAR,TBAR,N1,PDFH,PDFC
333   WRITE(12,444)ID1,ISX,IRIV,IAGE,ILEN,HBAR,TBAR,N1,PDFH,PDFC
444   FORMAT(' ',5I4,2F6.2,I2,(10F10.7,/))
      GO TO 333
644   CALL FCLOS(8)
      STOP
      END
```

```
      DIMENSION SUM(2,70),SUMSQ(2,70),AVSUM(2,70),STDV(2,70),
     *  STERR(2,70),UPLIM(2,70),LOWLIM(2,70),H(200),CIRC(100)
     *  ,STO(2,70)
      INTEGER INAM(10),UL,IARRAY(100)
1     DO 99 J=1,2
      DO 199 K=1,60
      SUM(J,K)=0.
      SUMSQ(J,K)=0.
199   CONTINUE
99    CONTINUE
      TYPE 'INPUT FILE NAME?'
      READ(11,101) INAM(1)
101   FORMAT(S18)
      TYPE 'RIVERS FOR AVERAGING?'
      ACCEPT NRIV1,NRIV2
      WRITE(12,309)NRIV1,NRIV2
309   FORMAT(' ','RIVER AVERAGE FOR RIVER',I4,'AND RIVER',I4)
      CALL FOPEN(3,INAM)
      N=0
300   READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K,
     *  (H(I),CIRC(I),I=1,K)
      IF(K.LT.60)GO TO 300
      IF(IRIV.NE.NRIV1.AND.IRIV.NE.NRIV2)GO TO 300
      DO 10 I=1,60
      STO(1,I)=H(I)
10    STO(2,I)=CIRC(I)
      DO 20 J=1,2
      DO 30 K=1,60
      SUM(J,K)=SUM(J,K)+STO(J,K)
30    SUMSQ(J,K)=SUMSQ(J,K)+STO(J,K)**2
20    CONTINUE
      N=N+1
      GO TO 300
420   DO 40 J=1,2
      DO 50 K=1,60
      X=FLOAT(N)
      AVSUM(J,K)=SUM(J,K)/X
      CORRFAC=(SUM(J,K)**2)/X
      STDV(J,K)=SQRT((SUMSQ(J,K)-CORRFAC)/(X-1))
      STERR(J,K)=STDV(J,K)/SQRT(X)
      UPLIM(J,K)=AVSUM(J,K)+STERR(J,K)
50    LOWLIM(J,K)=IFIX(AVSUM(J,K)-STERR(J,K))
40    CONTINUE
      L=1
500   DO 60 I=1,100
60    IARRAY(I)=' '
      DO 70 J=1,2
      LL=LOWLIM(J,L)
      UL=IFIX(UPLIM(J,L))
      IF(LL.LT.1)LL=1
      IF(UL.LT.1)UL=1
      DO 80 I=LL,UL
      I2=2*I
      I1=I
      IF(I2.GT.100)I2=100
      IF(I1.GT.100)I1=100
      IF(J.EQ.1)IARRAY(I2)='H'
80    IF(J.EQ.2)IARRAY(I1)='W'
      IVSUM1=AVSUM(J,L)
      IF(IVSUM1.LT.1)IVSUM1=1
      IF(J.EQ.1)IVSUM1=2*IVSUM1
      IF(IVSUM1.GT.100)IVSUM1=100
70    IARRAY(IVSUM1)='M'
      WRITE(12,200)L,AVSUM(1,L),AVSUM(2,L),(IARRAY(J),J=1,100)
      L=L+1
      IF(L.GT.60)GO TO 666
      GO TO 500
200   FORMAT(' ',I3,2X,F7.2,2X,F6.2,T30,100A1)
666   CALL FCLOS(3,INAM)
      TYPE ' END? YES(0), NO(1)'
      ACCEPT ITAG
      IF(ITAG. GE. 1) GO TO 1
      STOP
      END
```

```
      DIMENSION TEMPC(150),TEMPH(150),H(150),CIRC(150),IARRAY(100),
     *  INAM(10),ID(50)
 1    TYPE 'INPUT FILE NAME?'
      READ(11,101)INAM(1)
101   FORMAT(S18)
      CALL FOPEN(3,INAM)
      TYPE 'NUMBER OF SCALES? ID NOS?'
      ACCEPT K1,(ID(I),I=1,K1)
      L=1
300   READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K2,(H(I),CIRC(I
     *  ),I=1,K2)
      DO 20 I=1,K1
      IF(ID1.NE.ID(I))GO TO 20
      GO TO 23
20    CONTINUE
      GO TO 300
23    IF(L.GT.K1)GO TO 420
      L=L+1
      KM1=K2-1
      KM2=K2-2
      DO 10 J=2,KM1
      JM1=J-1
      JP1=J+1
      TEMPH(J)=(H(J)+H(JP1)+H(JM1))/3.
10    TEMPC(J)=(CIRC(J)+CIRC(JP1)+CIRC(JM1))/3.
      TEMPH(1)=TEMPH(2)
      TEMPC(1)=TEMPC(2)
      TEMPH(K2)=TEMPH(KM1)
      TEMPC(K2)=TEMPC(KM1)
      M=1
      CUMW=0.
      WRITE(12,103)ID1
90    DO 60 I=1,100
60    IARRAY(I)=' '
      INDH=TEMPH(M)
      INDH=2*INDH
      INDC=TEMPC(M)
      IF(INDH.LT.1)INDH=1
      IF(INDH.GT.100)INDH=100
      IF(INDC.LT.1)INDC=1
      IF(INDC.GT.100)INDC=100
      IARRAY(INDH)='H'
      IARRAY(INDC)='U'
      CUMW=CUMW+CIRC(M)
      WRITE(12,104)M,TEMPH(M),TEMPC(M),CUMW,IARRAY
103   FORMAT(//' SALMON SCALE',I4,'MOVING AVERAGED BY THREES')
104   FORMAT(' ',I2,2X,F6.2,2X,F6.2,2X,F7.2,T30,100A1)
      M=M+1
      IF(M.GT.K2)GO TO 300
      GO TO 90
420   TYPE 'END? YES(0), NO(1)'
      CALL FCLOS(3,INAM)
      ACCEPT ITAG
      IF(ITAG.GE.1) GO TO 1
      STOP
      END
```

```
C       PROFILE AVERAGE PRODUCER
C       ED TAUB, PROGRAMMER, 13 MAY 1976
        DIMENSION H(200),CIRC(200),STO(2,60),SUM(2,60),AVSUM(2,60),BVSUM(2,60)
        INTEGER INAM(10),UL,IARRAY(100)
 111    TYPE 'INPUT FILE NAME?'
        READ(11,101)INAM(1)
        DO 99 J=1,2
        DO 199 K=1,60
        SUM(J,K)=0.
 199    CONTINUE
 99     CONTINUE
 101    FORMAT(S18)
        CALL FOPEN(3,INAM)
        REWIND 3
        TYPE 'ENTER RIVER CODE, SEX'
        ACCEPT IRCODE,ISXT
        TYPE 'ENTER NMAX,IDR'
        ACCEPT NMAX,IDR
        N=0
 300    READ BINARY(3,END=420)ID1,ISX,IRIV,IAGE,FLEN,K,
     *  (H(I),CIRC(I),I=1,K)
        IF(K.LT.60)GO TO 300
        IF(ISX.NE.ISXT)GO TO 300
        IF(IRCODE.NE.IRIV)GO TO 300
        IF(IDR.EQ.ID1)GO TO 300
        WRITE(12,102)ID1
 102    FORMAT(' ',I4)
        DO 10 I=1,60
        STO(1,I)=H(I)
 10     STO(2,I)=CIRC(I)
        DO 20 J=1,2
        DO 30 K=1,60
 30     SUM(J,K)=SUM(J,K)+STO(J,K)
 20     CONTINUE
        N=N+1
        IF(N.GE.NMAX)GO TO 420
        GO TO 300
 420    DO 40 J=1,2
        DO 50 K=1,60
        X=FLOAT(N)
 50     AVSUM(J,K)=SUM(J,K)/X
 40     CONTINUE
        L=1
 500    DO 60 I=1,100
 60     IARRAY(I)=' '
        DO 70 J=1,2
        IVSUM1=AVSUM(J,L)
        IF(IVSUM1.LT.1)IVSUM1=1
        IF(J.EQ.1)IVSUM1=2*IVSUM1
        IF(IVSUM1.GT.100)IVSUM1=100
        IF(J.EQ.1)IARRAY(IVSUM1)='H'
        IF(J.EQ.2)IARRAY(IVSUM1)='W'
 70     CONTINUE
        WRITE(12,200)L,AVSUM(1,L),AVSUM(2,L),(IARRAY(J),J=1,100)
 200    FORMAT(' ',I4,F8.3,2X,F8.3,2X,T30,100A1)
        L=L+1
        IF(L.GT.60)GO TO 666
```

```
      GO TO 500
666   CALL FCLOS(3)
      TYPE 'ENTER LIBRARY FILE NAME'
      READ(11,101)INAM(1)
      CALL FOPEN(4,INAM)
310   READ BINARY(4,END=620)IRCODB,ISXB,AMEDH,AMEDC,((BVSUM(J,K)
     *,K=1,60),J=1,2)
      WRITE(12,210)IRCODE,ISXT
      WRITE(10,210)IRCODE,ISXT
210   FORMAT(' ',I4,2X,I4)
      GO TO 310
620   DO 80 J=1,2
      DO 90 K=1,60
90    BVSUM(J,K)=AVSUM(J,K)
80    CONTINUE
      DO 110 J=1,2
      DO 909 L=1,59
      LP1=L+1
      DO 120 K=LP1,60
      IF(BVSUM(J,L).LT.BVSUM(J,K))GO TO 120
      TEMP=BVSUM(J,L)
      BVSUM(J,L)=BVSUM(J,K)
      BVSUM(J,K)=TEMP
120   CONTINUE
909   CONTINUE
110   CONTINUE
      AMEDH=BVSUM(1,30)
      AMEDC=BVSUM(2,30)
      WRITE(12,103)AMEDH,AMEDC
103   FORMAT(' ',2X,'AMEDH',2X,F8.3,' AMEDC',F8.3)
      WRITE BINARY (4) IRCODE,ISXT,AMEDH,AMEDC,((AVSUM(J,K),K=1,60),J=1,2)
      CALL FCLOS (4)
      TYPE ' END? YES(0), NO(1)'
      ACCEPT ITAG
      IF(ITAG. GE. 1) GO TO 111
      STOP
      END
```

```
C       PROFILE AVERAGE COMPARATOR
C       ED TAUB PROGRAMMER,* 21 MAY 1976
        DIMENSION AVSUM(2,60),H(200),CIRC(200),H1(60),CIRC1(60),DELTH(60)
     *  ,DELTC(60),INAM(10),SMSUM(2,60)
        INTEGER IARRAY(100)
 112    TYPE 'ENTER UNKNOWN FILE NAME'
        READ(11,101)INAM(1)
 101    FORMAT(S1S)
        TYPE 'AVERAGE UNKNOWN? YES(1), NO(0)'
        ACCEPT MORE
        CALL FOPEN(3,INAM)
        TYPE 'ENTER UNKNOWN ID NO.'
        ACCEPT IDT
 300    READ BINARY (3,END=666)ID1,ISX,IRIV,IAGE,FLEN,K,(H(I),CIRC(I),I=1,K)
        IF(IDT.NE.ID1)GO TO 300
        IF(K.LT.60)GO TO 656
        CALL FCLOS(3)
        TYPE 'ENTER LIBRARY FILE NAME'
        READ(11,101)INAM(1)
        CALL FOPEN(4,INAM)
 106    TYPE 'ENTER TEST RIVER'
        ACCEPT IRCOD1
        REWIND 4
 310    READ BINARY (4,END=657)IRCODE,ISXT,AMEDH,AMEDC,((AVSUM(J,K),K=1,60),
     *  J=1,2)
        IF(IRCOD1.NE.IRCODE)GO TO 310
        IF(ISX.NE.ISXT)GO TO 310
        WRITE(12,698)IRCODE,ISXT
 698    FORMAT(' ','IRCODE = ',I4,'ISXT =',I4)
        DO 10 K=1,60
        H1(K)=AVSUM(1,K)
 10     CIRC1(K)=AVSUM(2,K)
        IF(MORE.EQ.0)GO TO 903
        DO 30 K=1,60
        AVSUM(1,K)=H(K)
 30     AVSUM(2,K)=CIRC(K)
        DO 40 J=1,2
        DO 45 L=1,59
        LP1=L+1
        DO 50 K=LP1,60
        IF(AVSUM(J,L).LT.AVSUM(J,K))GO TO 50
        TEMP=AVSUM(J,L)
        AVSUM(J,L)=AVSUM(J,K)
        AVSUM(J,K)=TEMP
 50     CONTINUE
 45     CONTINUE
 40     CONTINUE
        UMEDH=AVSUM(1,30)
        UMEDC=AVSUM(2,30)
 903    CONTINUE
        RATH=AMEDH/UMEDH
        RATC=AMEDC/UMEDC
        SUMH=0.
        SUMC=0.
```

```
      DO 60 K=1,60
      H(K)=H(K)*PATH
      CIRC(K)=CIRC(K)*PATC
      DELTH(K)=H(K)-H1(K)
      DELTC(K)=CIRC(K)-CIRC1(K)
      SUMH=SUMH+DELTH(K)**2
      SUMC=SUMC+DELTC(K)**2
60    CONTINUE
      DO 69 I=1,60
      H(I)=H(I)/PATH
69    CIRC(I)=CIRC(I)/PATC
      ABHVAL=SQRT(SUMH)
      ABCVAL=SQRT(SUMC)
      WRITE(12,133)ID1,IRCOD1,ABHVAL,ABCVAL
133   FORMAT(' ',I4,2X,I4,2X,F10.4,2X,F10.4)
      TYPE 'PLOT DEVIATIONS 1-60? YES(0),NO(1)'
      ACCEPT ISKIP
      IF(ISKIP.EQ.1)GO TO 111
      L=1
500   DO 70 I=1,100
70    IARRAY(I)=' '
      INDH=50+DELTH(L)
      INDC=50+DELTC(L)
      IF(INDH.LT.1)INDH=1
      IF(INDC.LT.1)INDC=1
      IF(INDH.GT.100)INDH=100
      IF(INDC.GT.100)INDC=100
      IARRAY(50)='X'
      IARRAY(INDH)='H'
      IARRAY(INDC)='W'
      WRITE(12,200)L,DELTH(L),DELTC(L),(IARRAY(J),J=1,100)
200   FORMAT(' ',I4,2X,F10.4,2X,F10.4,T30,100A1)
      L=L+1
      IF(L.LE.60)GO TO 500
111   TYPE 'MORE RIVERS? YES(0),NO(1)'
      ACCEPT MORE
      IF(MORE.LT.1)GO TO 106
      CALL FCLOS (4)
      TYPE 'MORE UNKNOWNS?YES(0),NO(1)'
      ACCEPT MORU
      IF(MORU.EQ.0)GO TO 112
      GO TO 6666
666   WRITE(12,1)
1     FORMAT(' UNK. NOT ON DISK')
      GO TO 6666
656   WRITE(12,2)ID1
2     FORMAT(' SHORT UNKNOWN',I4)
      GO TO 300
657   WRITE(12,3)
3     FORMAT(' RIV. NOT ON LIB.')
6666  STOP
      END
```

APPENDIX 2: PDF SOURCE DATA SETS

DS 2: Scale Spectra

DS 3: Impression Spectra

DS 4: Oceanic Spectra

DS2.1

```
ID =     16. RIVER =     82. HBAR =   5.375243 THAR =  47.733292
0.118812  0.099010  0.188119  0.267327  0.247525  0.069307  0.009901  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.009901  0.0       0.009901  0.029703  0.009901  0.019802  0.049505  0.089109  0.059406  0.108911
0.118812  0.118812  0.108911  0.069307  0.069307  0.059406  0.009901  0.029703  0.0       0.0
0.009901  0.0       0.0       0.0       0.019802
ID =     62. RIVER =     82. HBAR =   4.760504 THAR =  49.536075
0.137500  0.050000  0.337500  0.337500  0.125000  0.012500  0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.025000  0.0       0.0       0.025000  0.012500  0.037500  0.050000  0.137500  0.112500
0.200000  0.137500  0.087500  0.062500  0.037500  0.025000  0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.050000
ID =     24. RIVER =     82. HBAR =   6.068425 THAR =  65.600004
0.087912  0.054945  0.109890  0.120879  0.296703  0.197802  0.098901  0.032967  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.010989  0.0       0.0       0.0       0.0       0.0       0.021978  0.0       0.010989  0.032967
0.021978  0.076923  0.109890  0.142857  0.186813  0.087912  0.032967  0.043956  0.065934  0.021978
0.010989  0.021978  0.010989  0.010989  0.076923
ID =     61. RIVER =     82. HBAR =   5.342965 THAR =  55.494156
0.061728  0.049383  0.098765  0.358025  0.209877  0.172839  0.024691  0.024691  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.012346  0.0       0.012346  0.012346  0.0       0.0       0.0       0.049383  0.024691  0.111111
0.160494  0.135802  0.098765  0.061728  0.074074  0.037037  0.061728  0.037037  0.024691  0.0
0.012346  0.0       0.012346  0.0       0.061728
ID =     55. RIVER =     82. HBAR =   4.307981 THAR =  55.000812
0.206349  0.222222  0.301587  0.158730  0.047619  0.047619  0.015873  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.015873  0.031746  0.0       0.031746  0.0       0.047619  0.031746  0.015873  0.031746  0.111111
0.206349  0.111111  0.063492  0.031746  0.031746  0.0       0.015873  0.0       0.031746  0.031746
0.031746  0.0       0.031746  0.0       0.095238
ID =     59. RIVER =     82. HBAR =   5.635027 THAR =  57.212921
0.048780  0.170732  0.182927  0.268293  0.146341  0.121951  0.060976  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.012195  0.0       0.012195  0.0       0.0       0.012195  0.024390  0.036585  0.060976  0.121951
0.060976  0.134146  0.060976  0.134146  0.060976  0.085366  0.036585  0.036585  0.024390  0.0
0.012195  0.0       0.012195  0.0       0.060976
ID =     60. RIVER =     82. HBAR =   4.337931 THAR =  52.090790
0.212121  0.121212  0.393939  0.196970  0.030303  0.015152  0.030303  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.015152  0.015152  0.0       0.030303  0.015152  0.030303  0.015152  0.060606  0.045455  0.136364
0.106061  0.060606  0.151515  0.060606  0.075758  0.075758  0.0       0.045455  0.0       0.015152
0.0       0.0       0.0       0.0       0.045455
ID =      4. RIVER =     82. HBAR =   5.594984 THAR =  45.396683
0.173469  0.051020  0.122449  0.244898  0.224490  0.112245  0.051020  0.010204  0.0       0.010204
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.112245  0.030612  0.010204  0.0       0.0       0.020408  0.0       0.081633  0.051020  0.132653
0.102041  0.142857  0.091837  0.102041  0.030612  0.020408  0.0       0.0       0.0       0.0
0.0       0.0       0.010204  0.010204  0.051020
ID =     22. RIVER =     82. HBAR =   3.028290 THAR =  45.090256
0.387500  0.237500  0.225000  0.112500  0.037500  0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.225000  0.012500  0.025000  0.0       0.012500  0.012500  0.025000  0.037500  0.050000  0.050000
0.050000  0.062500  0.125000  0.087500  0.025000  0.050000  0.0       0.012500  0.0       0.025000
0.012500  0.025000  0.012500  0.0       0.062500
ID =     54. RIVER =     82. HBAR =   5.682878 THAR =  55.071152
0.141304  0.043478  0.130435  0.304348  0.206522  0.152174  0.0       0.010870  0.010870  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.043478  0.010870  0.010870  0.0       0.010870  0.010870  0.010870  0.021739  0.054348  0.032609
0.108696  0.097826  0.086957  0.152174  0.119565  0.054348  0.054348  0.021739  0.021739  0.0

0.0       0.0       0.0       0.0       0.076087
ID =     53. RIVER =     82. HBAR =   5.141831 THAR =  52.333313
0.117021  0.159574  0.191489  0.255319  0.191489  0.063830  0.021277  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.021277  0.010638  0.0       0.0       0.010638  0.010638  0.010638  0.063830  0.031915  0.127660
0.159574  0.117021  0.095745  0.074468  0.085106  0.063830  0.031915  0.010638  0.021277  0.0
0.021277  0.0       0.0       0.0       0.031915
ID =     56. RIVER =     82. HBAR =   5.075035 THAR =  48.816727
0.148936  0.148936  0.234043  0.202128  0.138298  0.074468  0.042553  0.010638  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.042553  0.010638  0.0       0.0       0.0       0.0       0.021277  0.095745  0.127660  0.085106
0.117021  0.170213  0.106383  0.031915  0.042553  0.042553  0.031915  0.0       0.010638  0.0
0.0       0.0       0.0       0.021277  0.042553
ID =     57. RIVER =     82. HBAR =   5.064999 THAR =  50.970215
0.197368  0.065789  0.250000  0.197368  0.157895  0.065789  0.052632  0.0       0.013158  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.065789  0.013158  0.013158  0.0       0.013158  0.013158  0.0       0.039474  0.026316  0.105263
0.105263  0.065789  0.157895  0.092105  0.052632  0.078947  0.052632  0.013158  0.026316  0.0
0.0       0.0       0.026316  0.0       0.039474
ID =   1766. RIVER =     90. HBAR =   7.135035 THAR =  54.945099
0.076923  0.038462  0.076923  0.128205  0.217949  0.243590  0.141026  0.076923  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.038462  0.0       0.0       0.0       0.038462  0.0       0.012821  0.025641  0.051282  0.076923
0.166667  0.102564  0.115385  0.102564  0.064103  0.064103  0.012821  0.0       0.025641  0.0
0.012821  0.0       0.0       0.0       0.089744
ID =   1756. RIVER =     90. HBAR =   5.605688 THAR =  48.840271
0.090909  0.125000  0.204545  0.238636  0.159091  0.113636  0.056818  0.0       0.0       0.011364
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.034091  0.0       0.011364  0.0       0.011364  0.011364  0.056818  0.068182  0.102273  0.238636
0.125000  0.102273  0.056818  0.045455  0.0       0.011364  0.045455  0.0       0.0       0.0
0.0       0.0       0.022727  0.0       0.056818
ID =   1755. RIVER =     90. HBAR =   6.229841 THAR =  55.230759
0.068493  0.068493  0.123288  0.273973  0.232877  0.136986  0.082192  0.013699  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.027397  0.0       0.013699  0.0       0.0       0.013699  0.013699  0.013699  0.041096  0.054795
0.136986  0.095890  0.109589  0.164384  0.123288  0.054795  0.027397  0.0       0.041096  0.0
0.0       0.0       0.0       0.027397  0.041096
ID =   1753. RIVER =     90. HBAR =   5.347358 THAR =  49.974869
0.164835  0.109890  0.131868  0.318681  0.065934  0.131868  0.054945  0.021978  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.032967  0.021978  0.010989  0.0       0.0       0.010989  0.021978  0.054945  0.043956  0.098901
0.098901  0.142857  0.120879  0.087912  0.098901  0.054945  0.021978  0.010989  0.021978  0.0
0.010989  0.0       0.0       0.0       0.032967
ID =   5009. RIVER =     90. HBAR =   6.088341 THAR =  57.574402
0.064103  0.038462  0.102564  0.307692  0.256410  0.179487  0.051282  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.012821  0.012821  0.0       0.0       0.0       0.038462  0.051282  0.076923
0.051282  0.179487  0.115385  0.102564  0.076923  0.102564  0.064103  0.038462  0.012821  0.012821
0.0       0.0       0.012821  0.0       0.038462
ID =   5022. RIVER =     90. HBAR =   8.078205 THAR =  57.120102
0.043011  0.064516  0.064516  0.107527  0.139785  0.193548  0.150538  0.118280  0.086021  0.032258
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.021505  0.021505  0.0       0.0       0.0       0.021505  0.053763  0.032258  0.053763  0.032258
0.064516  0.075269  0.150538  0.053763  0.086021  0.075269  0.118280  0.032258  0.021505  0.010753
0.0       0.0       0.010753  0.0       0.064516
ID =   5030. RIVER =     90. HBAR =   6.514188 THAR =  53.326838
0.076190  0.085714  0.076190  0.123810  0.352381  0.180952  0.057143  0.047619  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.019048  0.019048  0.009524  0.009524  0.0       0.009524  0.019048  0.009524  0.047619  0.066667
0.142857  0.123810  0.133333  0.085714  0.007619  0.085714  0.057143  0.028571  0.009524  0.019048
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0.019048  0.0       0.009524  0.009524  0.019048
ID =  305,RIVER =    99,HBAR =  6.157308 TBAR =  56.271307
0.011494  0.160919  0.137931  0.229885  0.252874  0.160919  0.011494  0.022989  0.011494  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.011494  0.0       0.0       0.0       0.0       0.0       0.034483  0.045977  0.057471  0.080460
0.091954  0.137931  0.126437  0.137931  0.103448  0.045977  0.0       0.034483  0.011494  0.0
0.0       0.0       0.0       0.022989  0.057471
ID =  344,RIVER =    99,HBAR =  5.774227 TBAR =  54.468079
0.106667  0.106667  0.066667  0.240000  0.240000  0.080000  0.080000  0.013333  0.013333  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.013333  0.013333  0.0       0.0       0.0       0.066667  0.0       0.026667  0.066667  0.120000
0.093333  0.093333  0.066667  0.146667  0.133333  0.026667  0.013333  0.0       0.013333  0.013333
0.013333  0.0       0.0       0.026667  0.053333
ID = 1283,RIVER =   199,HBAR =  5.624850 TBAR =  51.277649
0.224719  0.033708  0.056180  0.269663  0.202247  0.134831  0.067416  0.011236  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.033708  0.011236  0.011236  0.011236  0.022472  0.0       0.0       0.022472  0.056180  0.089888
0.078652  0.112360  0.191011  0.089888  0.089888  0.101124  0.0       0.0       0.033708  0.022472
0.0       0.0       0.0       0.0       0.022472
ID = 1281,RIVER =   199,HBAR =  6.105106 TBAR =  54.121521
0.083333  0.041667  0.156250  0.270833  0.281250  0.135417  0.020833  0.0       0.0       0.010417
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.020833  0.0       0.010417  0.0       0.0       0.0       0.020833  0.031250  0.093750  0.104167
0.072917  0.135417  0.187500  0.093750  0.052083  0.041667  0.041667  0.010417  0.0       0.0
0.0       0.020833  0.0       0.0       0.062500
ID = 1305,RIVER =   199,HBAR =  7.516794 TBAR =  54.198639
0.051546  0.020619  0.030928  0.175258  0.288660  0.206186  0.154639  0.061856  0.010309  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.010309  0.0       0.010309  0.010309  0.041237  0.113402
0.195876  0.123711  0.144330  0.123711  0.092783  0.072165  0.0       0.010309  0.0       0.0
0.0       0.0       0.0       0.020619  0.030928
ID =  351,RIVER =    99,HBAR =  7.609241 TBAR =  51.660889
0.090909  0.041322  0.099174  0.099174  0.173554  0.157025  0.173554  0.090909  0.057851  0.016529
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.008264  0.008264  0.0       0.0       0.024793  0.024793  0.024793  0.049587  0.074380  0.099174
0.132231  0.115702  0.165289  0.074380  0.033058  0.016529  0.024793  0.016529  0.016529  0.016529
0.008264  0.008264  0.008264  0.0       0.049587
ID =  390,RIVER =    99,HBAR =  6.033611 TBAR =  51.615036
0.067961  0.067961  0.155340  0.271845  0.233010  0.184466  0.009709  0.009709  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.019417  0.0       0.0       0.0       0.009709  0.029126  0.009709  0.048544  0.058252  0.126214
0.203883  0.135922  0.126214  0.087379  0.038835  0.009709  0.009709  0.0       0.009709  0.009709
0.009709  0.0       0.0       0.009709  0.048544
ID = 1282,RIVER =   199,HBAR =  8.096189 TBAR =  51.416611
0.091954  0.022989  0.068965  0.103448  0.126437  0.183908  0.172414  0.137931  0.057471  0.022989
0.011494  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.034483  0.0       0.0       0.011494  0.0       0.022989  0.0       0.034483  0.057471  0.091954
0.103448  0.172414  0.149425  0.126437  0.057471  0.034483  0.022989  0.022989  0.011494  0.011494
0.0       0.011494  0.0       0.0       0.022989
ID = 1297,RIVER =   199,HBAR =  5.292193 TBAR =  51.743729
0.136364  0.125000  0.181818  0.295455  0.113636  0.090909  0.056818  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.034091  0.011364  0.034091  0.0       0.0       0.0       0.045455  0.022727  0.056818  0.079545
0.136364  0.090909  0.125000  0.159091  0.056818  0.022727  0.034091  0.0       0.022727  0.0
0.011364  0.0       0.0       0.0       0.056818
ID = 1299,RIVER =   199,HBAR =  5.555858 TBAR =  56.932541
0.102041  0.132653  0.234694  0.173469  0.183673  0.122449  0.040816  0.010204  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.020408  0.0       0.020408  0.010204  0.020408  0.0       0.010204  0.010204  0.071429  0.051020
0.061224  0.030612  0.153061  0.112245  0.071429  0.091837  0.081633  0.071429  0.040816  0.010204

0.0       0.020408  0.010204  0.010204  0.020408
ID = 1304,RIVER =   199,HBAR =  6.161680 TBAR =  47.859482
0.148760  0.082645  0.082645  0.214876  0.231405  0.082645  0.066116  0.057851  0.033058  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.024793  0.024793  0.0       0.016529  0.0       0.0       0.024793  0.033058  0.090909  0.099174
0.123967  0.148760  0.123967  0.140496  0.049587  0.033058  0.024793  0.008264  0.008264  0.0
0.008264  0.008264  0.0       0.0       0.008264
ID = 1312,RIVER =   199,HBAR =  5.785920 TBAR =  50.339737
0.148649  0.135135  0.081081  0.202703  0.162162  0.175676  0.067568  0.027027  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.027027  0.040541  0.0       0.0       0.027027  0.027027  0.013514  0.027027  0.094595  0.175676
0.135135  0.121622  0.094595  0.040541  0.013514  0.0       0.027027  0.0       0.027027  0.0
0.0       0.013514  0.013514  0.013514  0.067568
ID = 1311,RIVER =   199,HBAR =  4.622965 TBAR =  44.253525
0.279570  0.107527  0.182796  0.172043  0.118280  0.064516  0.064516  0.010753  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.096774  0.021505  0.032258  0.032258  0.032258  0.021505  0.043011  0.043011  0.032258  0.096774
0.118280  0.107527  0.075269  0.096774  0.021505  0.010753  0.032258  0.010753  0.0       0.0
0.0       0.010753  0.0       0.021505  0.043011
ID = 1310,RIVER =   199,HBAR =  4.858158 TBAR =  50.027115
0.238636  0.090909  0.068182  0.272727  0.250000  0.079545  0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.079545  0.011364  0.011364  0.022727  0.022727  0.011364  0.034091  0.034091  0.034091  0.079545
0.068182  0.147727  0.181818  0.045455  0.056818  0.056818  0.0       0.0       0.0       0.0
0.011364  0.011364  0.0       0.0       0.079545
ID = 1309,RIVER =   199,HBAR =  4.397492 TBAR =  42.262207
0.312500  0.145833  0.135417  0.125000  0.156250  0.062500  0.052083  0.010417  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.125000  0.041667  0.031250  0.010417  0.052083  0.020833  0.052083  0.010417  0.031250  0.062500
0.083333  0.156250  0.125000  0.072917  0.010417  0.010417  0.010417  0.020833  0.010417  0.0
0.010417  0.0       0.0       0.0       0.052083
ID = 1308,RIVER =   199,HBAR =  4.597380 TBAR =  44.572281
0.287037  0.092593  0.111111  0.203704  0.185185  0.101852  0.018519  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.083333  0.064815  0.009259  0.046296  0.0       0.009259  0.027778  0.027778  0.009259  0.074074
0.101852  0.111111  0.157407  0.101852  0.074074  0.055556  0.009259  0.0       0.0       0.0
0.009259  0.0       0.009259  0.0       0.018519
ID = 1307,RIVER =   199,HBAR =  4.688380 TBAR =  46.760315
0.287037  0.092593  0.148148  0.212963  0.111111  0.083333  0.055556  0.0       0.009259  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.101852  0.018519  0.018519  0.018519  0.037037  0.064815  0.018519  0.018519  0.046296  0.055556
0.101852  0.064815  0.101852  0.092593  0.064815  0.074074  0.0       0.009259  0.009259  0.009259
0.0       0.0       0.018519  0.0       0.055556
ID = 1329,RIVER =   199,HBAR =  6.225403 TBAR =  55.012791
0.073529  0.117647  0.132353  0.205882  0.220588  0.058824  0.117647  0.073529  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.044118  0.0       0.0       0.0       0.0       0.0       0.029412  0.014706  0.0       0.088235
0.088235  0.205882  0.235294  0.088235  0.058824  0.014706  0.014706  0.0       0.0       0.0
0.0       0.0       0.014706  0.029412  0.073529
ID = 1359,RIVER =   199,HBAR =  4.720368 TBAR =  51.037003
0.268817  0.096774  0.150538  0.215054  0.150538  0.053763  0.053763  0.010753  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.064516  0.032258  0.0       0.021505  0.010753  0.010753  0.021505  0.0       0.043011  0.064516
0.129032  0.150538  0.118280  0.118280  0.075269  0.032258  0.0       0.010753  0.0       0.0
0.021505  0.021505  0.0       0.010753  0.043011
ID = 1337,RIVER =   199,HBAR =  2.590000 TBAR =  41.522278
0.534091  0.170455  0.181818  0.068182  0.034091  0.011364  0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.136364  0.068182  0.022727  0.0       0.045455  0.045455  0.045455  0.045455  0.079545  0.034091
0.079545  0.068182  0.079545  0.079545  0.034091  0.045455  0.011364  0.0       0.0       0.0
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 0.034091  0.0       0.0       0.011364  0.034091
ID=  1363,RIVER =   199,MBAR =    4.245846 TBAR =   47.427567
 0.235294  0.141176  0.258824  0.270588  0.082353  0.0       0.011765  0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.094118  0.023529  0.011765  0.0       0.0       0.011765  0.047059  0.047059  0.070588  0.117647
 0.117647  0.023529  0.164706  0.094118  0.011765  0.047059  0.0       0.023529  0.0       0.023529
 0.0       0.011765  0.023529  0.0       0.035294
ID=  1361,RIVER =   199,MBAR =    4.608871 TBAR =   47.169907
 0.231707  0.048780  0.207317  0.317073  0.146341  0.036585  0.012195  0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.121951  0.012195  0.012195  0.012195  0.012195  0.012195  0.012195  0.024390  0.060976  0.036585
 0.109756  0.182927  0.158146  0.097561  0.073171  0.0       0.012195  0.012195  0.0       0.0
 0.0       0.0       0.0       0.0       0.060976
ID=  1360,RIVER =   199,MBAR =  260.787354 TBAR =   48.336090
 0.032258  0.043011  0.107527  0.193548  0.225806  0.139785  0.118280  0.032258  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.107527
 0.064516  0.032258  0.0       0.010753  0.010753  0.0       0.0       0.010753  0.0       0.096774
 0.096774  0.215054  0.161290  0.096774  0.053763  0.043011  0.043011  0.0       0.032258  0.0
 0.0       0.010753  0.0       0.0       0.021505
ID=  1366,RIVER =   199,MBAR =    5.143804 TBAR =   47.542618
 0.161290  0.086021  0.193548  0.268817  0.204301  0.064516  0.021505  0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.021505  0.021505  0.010753  0.010753  0.032258  0.0       0.043011  0.010753  0.032258  0.118280
 0.247312  0.150538  0.129032  0.043011  0.032258  0.0       0.010753  0.0       0.0       0.0
 0.010753  0.010753  0.010753  0.010753  0.021505  0.021505  0.010753  0.0       0.0       0.0
ID=  1365,RIVER =   199,MBAR =    5.198280 TBAR =   60.934143
 0.168406  0.140928  0.072464  0.275362  0.168406  0.072464  0.057971  0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.028986  0.0       0.0       0.014493  0.0       0.0       0.043478  0.028986  0.028986  0.043478
 0.028986  0.086957  0.159420  0.115942  0.130435  0.072464  0.043478  0.0       0.028986  0.043478
 0.0       0.0       0.028986  0.0       0.072464
ID=  1499,RIVER =   99,MBAR =    6.321285 TBAR =   52.887482
 0.102804  0.102804  0.084112  0.205607  0.233645  0.121495  0.102804  0.046729  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.009346  0.009346  0.018692  0.0       0.0       0.0       0.018692  0.046729  0.065421  0.065421
 0.112149  0.084112  0.130841  0.168224  0.121495  0.084112  0.009346  0.009346  0.0       0.0
 0.0       0.009346  0.0       0.0       0.037383
ID=  1496,RIVER =   99,MBAR =    6.555329 TBAR =   50.424973
 0.190305  0.068702  0.091603  0.099237  0.129771  0.206107  0.160305  0.061069  0.022901  0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.045802  0.053435  0.0       0.007634  0.007634  0.022901  0.007634  0.053435  0.038168  0.061069
 0.068702  0.129771  0.152672  0.091603  0.091603  0.045802  0.053435  0.015267  0.015267  0.0
 0.0       0.0       0.0       0.0       0.038168
ID=  1497,RIVER =   99,MBAR =    6.542912 TBAR =   53.827759
 0.064103  0.089744  0.115385  0.166667  0.294872  0.115385  0.141026  0.012821  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.012821  0.0       0.0       0.012821  0.012821  0.0       0.0       0.012821  0.064103  0.089744
 0.076923  0.128205  0.166667  0.192308  0.089744  0.064103  0.012821  0.0       0.0       0.0
 0.0       0.012821  0.0       0.012821  0.038462
ID=  1656,RIVER =   99,MBAR =    7.392102 TBAR =   55.237305
 0.123595  0.022472  0.056180  0.112360  0.235955  0.168539  0.101124  0.123595  0.022472  0.022472
 0.0       0.011236  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.044944  0.0       0.011236  0.0       0.011236  0.0       0.011236  0.011236  0.022472  0.089888
 0.067416  0.123595  0.101124  0.157303  0.146067  0.056180  0.022472  0.022472  0.0       0.011236
 0.011236  0.0       0.011236  0.011236  0.056180
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```
ID = 1753      1      00      1     445  6.862556  62.090857      1
0.125000  0.103566  0.077622  0.103566  0.051837  0.206740  0.129877  0.077922  0.025974  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.012987  0.0       0.038961  0.012987  0.0
0.090909  0.116883  0.142857  0.168831  0.155570  0.064935  0.0       0.038961  0.012987  0.064935
0.0       0.025974  0.0       0.0       0.077922

ID = 1755      1      00      1     456  7.532935  65.366211      1
0.055556  0.027778  0.083444  0.138889  0.291667  0.222222  0.083333  0.027778  0.027778  0.0
0.027778  0.0       0.0       0.013889  0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.013889  0.0       0.0       0.0       0.0       0.027778  0.069444
0.041667  0.138889  0.055556  0.125001  0.111111  0.138889  0.041667  0.041667  0.055556  0.013889
0.0       0.013889  0.027778  0.0       0.083333

ID = 1756      1      00      1     502  5.497175  61.504547      1
0.105263  0.105263  0.236842  0.250000  0.144737  0.105263  0.039474  0.0       0.0       0.0
0.013158  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.013158  0.0       0.0       0.0       0.0       0.013158  0.013158  0.026316  0.026316  0.039474
0.052632  0.144737  0.131579  0.118421  0.105263  0.026316  0.092105  0.052632  0.039474  0.013158
0.0       0.0       0.0       0.013158  0.078947

ID = 1777      2      00      4     557  2.417506  55.039130      1
0.750000  0.112500  0.062500  0.037500  0.012500  0.0       0.025000  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.037500  0.025000  0.012500  0.0       0.050000  0.025000  0.012500  0.012500  0.012500  0.050000
0.075000  0.125000  0.125000  0.112500  0.062500  0.037500  0.0       0.025000  0.025000  0.0
0.025000  0.012500  0.0       0.0       0.137500

ID = 1778      2      00      5     540  5.878959  63.007595      1
0.089744  0.076923  0.102564  0.320513  0.269231  0.102564  0.025641  0.012821  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.012821  0.0       0.012821  0.0       0.0       0.012821  0.0       0.038462  0.025641  0.038462
0.076923  0.051282  0.102564  0.128205  0.115385  0.128205  0.051282  0.038462  0.025641  0.038462
0.025641  0.012821  0.0       0.0       0.064103

ID = 1781      2      00      4     529  7.490376  57.759644      1
0.109890  0.043956  0.076923  0.120879  0.098901  0.263736  0.153846  0.054945  0.043956  0.0
0.010989  0.0       0.010989  0.0       0.010989  0.0       0.0       0.0       0.0       0.0
0.032967  0.010989  0.0       0.0       0.0       0.010989  0.021978  0.021978  0.087912  0.043956
0.142857  0.098901  0.076923  0.054945  0.120879  0.032967  0.032967  0.054945  0.021978  0.010989
0.010989  0.010989  0.021978  0.0       0.076923

ID = 1795      1      00      4     504  7.585938  57.027481      1
0.106383  0.042553  0.106383  0.085106  0.148936  0.127660  0.159574  0.159574  0.063830  0.010638
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.042553  0.0       0.010638  0.0       0.0       0.0       0.021277  0.021277  0.042553  0.042553
0.063830  0.127660  0.117021  0.117021  0.138298  0.074468  0.021277  0.031915  0.021277  0.010638
0.021277  0.010638  0.021277  0.010638  0.021915

ID = 5009      1      00      3     476  7.627747  60.306168      1
0.026316  0.078947  0.065789  0.105263  0.184210  0.250000  0.236842  0.039474  0.013158  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.013158  0.0       0.0       0.0       0.0       0.0       0.013158  0.013158  0.039474  0.026316
0.078947  0.105263  0.157895  0.092105  0.184210  0.052632  0.039474  0.052632  0.013158  0.026316
0.0       0.026316  0.0       0.0       0.052632

ID = 5010      2      00      4     525  7.994615  58.060837      1
0.011494  0.022988  0.045977  0.137931  0.275862  0.264368  0.114942  0.057471  0.034483  0.022988
0.011494  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.011494  0.0       0.0       0.0       0.0       0.011494  0.0       0.022988  0.034483  0.137931
0.068965  0.137931  0.103448  0.172414  0.080460  0.091954  0.034483  0.011494  0.022988  0.0
0.0       0.0       0.011494  0.0       0.045977

ID = 5011      1      00      3     436  7.233900  63.524763      1
0.075758  0.060606  0.121212  0.075758  0.242424  0.151515  0.136364  0.121212  0.015152  0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.015152  0.0       0.0       0.0       0.0       0.015152  0.0       0.030303  0.030303  0.015152
0.045455  0.151515  0.181818  0.151515  0.106061  0.045455  0.030303  0.030303  0.015152  0.0
0.030303  0.015152  0.0       0.0       0.075758

ID = 5020      2      00      7     674  9.658765  58.622452      1
0.021739  0.021739  0.032609  0.043478  0.108696  0.184783  0.195652  0.141304  0.141304  0.065217
0.010870  0.010870  0.0       0.0       0.0       0.0       0.0       0.010870  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.010870  0.0       0.074047  0.032826
0.086957  0.119565  0.119565  0.141304  0.097826  0.043478  0.043478  0.043478  0.010870  0.021739
0.010870  0.0       0.010870  0.021739  0.043478

ID = 5021      2      00      5     655  7.293296  59.186325      1
0.079545  0.022727  0.011364  0.227273  0.193182  0.261364  0.159091  0.045455  0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.011364  0.0       0.0       0.045455  0.068182  0.0
0.125000  0.136364  0.102273  0.159091  0.113636  0.056818  0.045455  0.034091  0.022727  0.011364
0.022727  0.0       0.011364  0.0       0.034091

ID = 5028      1      00      2     403  6.206244  63.434601      1
0.030760  0.153846  0.107692  0.246154  0.230769  0.184615  0.046154  0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.015385  0.030769  0.076923
0.061538  0.092308  0.169231  0.184615  0.092308  0.030769  0.123077  0.0       0.015385  0.0
0.0       0.0       0.0       0.015385  0.092308

ID = 5039      1      00      5     559  5.474160  57.393667      1
0.011765  0.070588  0.058824  0.082353  0.141176  0.200000  0.235294  0.082353  0.094118  0.023529
0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.011765  0.0       0.0       0.0       0.0       0.023529  0.011765  0.047059  0.035294  0.070588
0.117647  0.129412  0.082353  0.094118  0.094118  0.105882  0.035294  0.023529  0.023529  0.011765
0.0       0.0       0.0       0.023529  0.058824

ID = 5040      2      00      6     703  9.186708  56.865555      1
0.080102  0.009901  0.009901  0.049505  0.148515  0.198020  0.138614  0.158416  0.069307  0.049505
0.029703  0.0       0.009901  0.0       0.0       0.0       0.0       0.019802  0.0       0.0
0.009901  0.009901  0.0       0.0       0.009901  0.009901  0.029703  0.009901  0.049505  0.019802
0.128713  0.108911  0.188119  0.148515  0.089109  0.039604  0.029703  0.019802  0.009901  0.009901
0.0       0.0       0.0       0.009901  0.049505

ID = 5041      1      00      5     621  3.220648  63.527283      1
0.025316  0.0       0.101266  0.088608  0.177215  0.164557  0.278481  0.113924  0.037975  0.0
0.012658  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.012658  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.075949  0.050633
0.063291  0.075949  0.088608  0.075949  0.126582  0.113924  0.075949  0.037975  0.050633  0.037975
0.0       0.025316  0.0       0.037975  0.050633

ID = 5046      2      00      7     655  8.293740  67.194153      1
0.025316  0.063291  0.037975  0.101266  0.177215  0.202532  0.215190  0.101266  0.037975  0.025316
0.012658  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
0.0       0.0       0.0       0.0       0.0       0.012658  0.012658  0.012658  0.037975  0.025316
0.139241  0.101266  0.177215  0.088608  0.101266  0.050633  0.050633  0.0       0.012658  0.025316
0.0       0.0       0.012658  0.0       0.139241
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ID = 5043	2	[illegible]	7	[illegible]	[illegible]	1			
0.057416	0.033708	0.056180	0.078652	0.078652	0.179775	0.146067	0.101124	0.089888	0.123595
0.0	0.044944	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.022472	0.0	0.0	0.0	0.0	0.0	0.033708	0.011236	0.022472	0.022472
0.146067	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.057416	0.011236	0.022472	0.033708
0.0	0.0	0.022472	0.0	0.078652					
ID = 5048	2	90	7	700	5.110451	68.742920	1		
0.085366	0.073171	0.048780	0.085366	0.012195	0.121951	0.048780	0.146341	0.219512	0.109756
0.012195	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.024390	0.0	0.0	0.012195	0.0	0.0	0.0	0.012195	0.012195	0.024390
0.036585	0.097561	0.073171	0.048780	0.097561	0.182927	0.121951	0.060976	0.024390	0.024390
0.036585	0.0	0.0	0.012195	0.036585					
ID = 1756	1	90	3	441	7.346556	61.289139	1		
0.025000	0.050000	0.137500	0.137500	0.212500	0.262500	0.100000	0.037500	0.025000	0.012500
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.012500	0.0	0.012500	0.037500	0.0	0.050000
0.062500	0.087500	0.112500	0.162500	0.062500	0.112500	0.100000	0.087500	0.037500	0.0
0.0	0.0	0.0	0.0	0.062500					
ID = 5019	2	90	8	753	9.468301	68.805069	1		
0.076087	0.010870	0.043478	0.021739	0.054348	0.130435	0.217391	0.173913	0.173913	0.065217
0.021739	0.010870	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.054348	0.010870	0.0	0.0	0.0	0.0	0.010870	0.010870	0.054348	0.010870
0.130435	0.097826	0.130435	0.065217	0.076087	0.054348	0.032609	0.086957	0.065217	0.021739
0.021739	0.0	0.0	0.0	0.065217					
ID = 5024	1	90	5	594	6.986941	60.348840	1		
0.058140	0.058140	0.023256	0.034884	0.127907	0.197674	0.162791	0.151163	0.093023	0.069767
0.011628	0.0	0.0	0.0	0.011628	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.011628	0.0	0.0	0.023256	0.0	0.034884	0.046512
0.151163	0.127907	0.127907	0.093023	0.058140		0.023256	0.023256	0.023256	0.0
0.011628	0.0	0.011628	0.023256	0.081395					
ID = 5030	1	90	5	594	7.917630	60.040497	1		
0.111111	0.086420	0.074074	0.037037	0.098765	0.160494	0.197531	0.111111	0.061728	0.024691
0.012346	0.012346	0.012346	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.012346	0.0	0.0	0.0	0.012346	0.012346	0.024691	0.037037	0.037037	0.012346
0.135802	0.086420	0.111111	0.148148	0.074074	0.061728	0.037037	0.037037	0.037037	0.024691
0.012346	0.0	0.012346	0.012346	0.061728					
ID = 5026	2	90	5	660	8.612486	73.422099	1		
0.011494	0.034483	0.091954	0.045977	0.126437	0.068965	0.195402	0.206897	0.114942	0.057471
0.011494	0.022989	0.0	0.011494	0.0	0.0	0.0	0.0	0.0	0.0
0.011494	0.0	0.0	0.0	0.0	0.0	0.0	0.011494	0.022989	0.057471
0.022989	0.068965	0.057471	0.045977	0.091954	0.045977	0.034483	0.149425	0.103448	0.091954
0.034483	0.011494	0.0	0.011494	0.126437					
ID = 5034	2	90	4	586	8.365812	67.098419	1		
0.093333	0.080000	0.066667	0.093333	0.093333	0.093333	0.106667	0.186667	0.106667	0.026667
0.053333	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.013333	0.0	0.013333	0.026667
0.080000	0.066667	0.146667	0.133333	0.106667	0.080000	0.093333	0.080000	0.040000	0.026667
0.0	0.0	0.0	0.0	0.093333					
ID = 5044	2	90	4	643	5.536284	55.241574	1		
0.032258	0.064516	0.075269	0.150538	0.150538	0.161290	0.096774	0.053763	0.107527	0.043011
0.0	0.053763	0.010753	0.0	0.0	0.0	0.0	0.0	0.0	0.0

0.010753	0.0	0.0	0.0	0.0	0.0	0.010753	0.032258	0.010753	0.096774
0.064516	0.118280	0.139785	0.086021	0.086021	0.096021	0.064516	0.053763	0.032258	0.032258
0.010753	0.010753	0.0	0.0	0.032258					
ID = 5080	2	90	8	754	10.408484	56.019074	1		
0.064103	0.076923	0.076923	0.051282	0.051282	0.051282	0.076923	0.089744	0.051282	0.102564
0.129205	0.038462	0.012821	0.025641	0.0	0.0	0.012821	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.025641	0.012821	0.038462	0.0	0.141026
0.115385	0.064103	0.128205	0.153846	0.089744	0.051282	0.025641	0.025641	0.051282	0.0
0.012821	0.0	0.012821	0.0	0.051282					
ID = 1752	2	90	4	607	6.723388	62.658967	1		
0.012346	0.024691	0.185185	0.259259	0.222222	0.160494	0.086420	0.024691	0.012346	0.012346
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012346	0.0	0.086420
0.111111	0.123457	0.098765	0.148148	0.098765	0.111111	0.049383	0.049383	0.037037	0.012346
0.0	0.012346	0.0	0.0	0.049383					
ID = 1760	1	90	4	495	7.168030	58.555930	1		
0.011905	0.035714	0.142857	0.214286	0.178571	0.273809	0.083333	0.035714	0.011905	0.011905
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.011905	0.023810	0.035714	0.035714	0.035714
0.095238	0.178571	0.166667	0.083333	0.095238	0.071429	0.059524	0.023810	0.0	0.0
0.011905	0.0	0.0	0.071429						
ID = 1765	1	90	3	477	5.536480	60.079041	1		
0.095890	0.095890	0.109589	0.013699	0.041096	0.068493	0.178082	0.136986	0.164384	0.082192
0.013699	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013699	0.0	0.0	0.013699	0.0	0.013699	0.013699	0.013699	0.041096	0.054795
0.041096	0.027397	0.123288	0.191781	0.123288	0.095890	0.054795	0.054795	0.027397	0.013699
0.013699	0.0	0.0	0.013699	0.041096					
ID = 5002	1	90	4	520	8.199738	62.316498	1		
0.057143	0.042857	0.014286	0.142857	0.142857	0.271429	0.100000	0.114286	0.057143	0.028571
0.028571	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.014286	0.0	0.014286	0.0	0.0	0.028571	0.0	0.057143	0.057143
0.028571	0.042857	0.057143	0.157143	0.085714	0.142857	0.100000	0.028571	0.057143	0.028571
0.014286	0.0	0.014286	0.014286	0.057143					
ID = 5022	2	90	5	624	8.563136	50.751914	1		
0.039216	0.039216	0.009804	0.029412	0.147059	0.137255	0.205882	0.127451	0.049020	0.068627
0.019608	0.019608	0.009804	0.0	0.009804	0.0	0.0	0.0	0.0	0.0
0.009804	0.0	0.0	0.0	0.0	0.009804	0.009804	0.068627	0.078431	0.176471
0.137255	0.156863	0.068627	0.068627	0.068627	0.039216	0.0	0.009804	0.0	0.0
0.019608	0.009804	0.009804	0.009804	0.029412					
ID = 5029	2	90	5	595	8.541883	62.773529	1		
0.095890	0.041096	0.041096	0.027397	0.123288	0.082192	0.219178	0.219178	0.068493	0.013699
0.0	0.0	0.013699	0.013699	0.0	0.0	0.0	0.0	0.0	0.0
0.027397	0.0	0.0	0.013699	0.013699	0.0	0.0	0.054795	0.027397	0.041096
0.027397	0.041096	0.095890	0.164384	0.123288	0.054795	0.109589	0.054795	0.0	0.0
0.013699	0.013699	0.0	0.013699	0.109589					
ID = 5025	2	90	5	414	10.792161	55.507595	1		
0.050633	0.025316	0.037975	0.0	0.088608	0.063291	0.088608	0.164557	0.202532	0.151899
0.075949	0.050633	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.012658	0.037975	0.037975	0.063291
0.164557	0.088608	0.164557	0.088608	0.101266	0.063291	0.012658	0.012658	0.0	0.0
0.0	0.0	0.0	0.0	0.063291					

ID = 2001	1	60	5	771 10.1277[illegible] 66.465821	1				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.207702	0.149857	0.126171	0.038961	0.013697	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.038961	0.012987
0.038961	0.251948	0.251948	0.077922	0.168831	0.077922	0.155844	0.090909	0.064935	0.077922
0.012987	0.025974	0.012987	0.0	0.038961					
ID = 5035	2	60	6	633 10.1244[illegible] 66.291621	1				
0.0	0.013699	0.054795	0.260493	0.164384	0.082192	0.136986	0.150685	0.150685	0.082192
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.0	0.0	0.0	0.0	0.0	0.0	0.027397	0.013699	0.054795	0.068493
0.109589	0.082192	0.123288	0.054795	0.041096	0.164384	0.068493	0.027397	0.027397	0.013699
0.027397	0.027397	0.0	0.0	0.027397					
ID = 5079	2	60	11	759 10.232142 66.665048	1				
0.075949	0.050633	0.037975	0.088608	0.265823	0.075949	0.139241	0.113924	0.088608	0.139241
0.113924	0.050633	0.0	0.0	0.025316	0.0	0.0	0.0	0.0	0.0
0.012658	0.0	0.0	0.012658	0.0	0.0	0.012658	0.025316	0.050633	0.063291
0.063291	0.075949	0.151899	0.126582	0.164557	0.050633	0.025316	0.025316	0.012658	0.012658
0.012658	0.012658	0.012658	0.012658	0.063291					
ID = 1782	2	60	3	556 7.674454 62.146048	1				
0.060606	0.030303	0.060606	0.090909	0.181818	0.272273	0.196970	0.075758	0.075758	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.015152	0.0	0.0	0.0	0.0	0.0	0.015152	0.030303	0.015152	0.045455
0.075758	0.075758	0.045455	0.075758	0.136364	0.106061	0.136364	0.075758	0.045455	0.015152
0.015152	0.0	0.030303	0.0	0.045455					
ID = 5026	2	60	5	597 10.660785 59.086319	1				
0.014085	0.028169	0.070423	0.028169	0.042254	0.112676	0.154930	0.169014	0.098592	0.126761
0.098592	0.026169	0.028169	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.014085	0.0	0.0	0.0	0.0	0.0	0.028169	0.042254	0.070423
0.070423	0.126761	0.154930	0.154930	0.070423	0.056338	0.084507	0.014085	0.028169	0.014085
0.014085	0.0	0.0	0.0	0.056338					
ID = 1772	1	60	4	514 7.817826 56.850264	1				
0.014085	0.028169	0.154930	0.126761	0.098592	0.225352	0.197183	0.084507	0.070423	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.014085	0.0	0.0	0.0	0.028169	0.0	0.028169	0.028169	0.028169	0.056338
0.098592	0.056338	0.154930	0.084507	0.169014	0.070423	0.098592	0.014085	0.014085	0.014085
0.014085	0.0	0.014085	0.0	0.014085					
ID = 1757	2	90	4	539 7.430022 50.098694	1				
0.108108	0.054054	0.067568	0.054054	0.229730	0.148649	0.148649	0.135135	0.013514	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013514	0.0	0.0	0.067568	0.027027	0.040541	0.013514	0.027027	0.081081	0.054054
0.081081	0.135135	0.040541	0.121622	0.067568	0.067568	0.081081	0.0	0.013514	0.0
0.027027	0.0	0.013514	0.027027	0.0					
ID = 1787	2	60	4	522 6.751526 66.377104	1				
0.041667	0.027778	0.027778	0.027778	0.097222	0.180556	0.152778	0.152778	0.180556	0.069444
0.013889	0.013889	0.0	0.013889	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.013889	0.013889	0.013889	0.027778	0.027778
0.069444	0.055556	0.069444	0.138889	0.152778	0.055556	0.111111	0.097222	0.027778	0.013889
0.0	0.013889	0.0	0.0	0.097222					
ID = 1784	2	60	5	559 9.999795 56.978119	1				
0.037500	0.012500	0.075000	0.062500	0.037500	0.100000	0.162500	0.225000	0.112500	0.100000
0.037500	0.025000	0.0	0.0	0.012500	0.0	0.0	0.0	0.0	0.0
0.012500	0.0	0.0	0.0	0.012500	0.0	0.025000	0.012500	0.037500	0.062500

0.087500	0.212500	0.075000	0.100000	0.037500	0.100000	0.062500	0.062500	0.012500	0.012500
0.0	0.0	0.0	0.0	0.050000					
ID = 5005	2	60	4	554 8.447366 57.557723	1				
0.028169	0.014085	0.084507	0.126761	0.140845	0.169014	0.253521	0.112676	0.028169	0.014085
[illegible]	[illegible]	[illegible]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.014085	0.0	0.0	0.014085	0.0	0.0	0.042254	0.0	0.028169	0.084507
0.112676	0.098592	0.098592	0.140845	0.056592	0.070423	0.042254	0.042254	0.014085	0.042254
0.0	0.0	0.0	0.014085	0.042254					
ID = 5034	2	90	4	588 8.246955 55.762146	1				
0.064103	0.102564	0.076923	0.064103	0.102564	0.179487	0.076923	0.102564	0.153846	0.076923
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.025641	0.0	0.0	0.0	0.025641	0.0	0.012821	0.038462	0.025641	0.051282
0.089744	0.153846	0.115385	0.089744	0.102564	0.051282	0.038462	0.025641	0.051282	0.012821
0.025641	0.012821	0.0	0.025641	0.051282					
ID = 2004	2	60	4	714 12.232776 66.760010	1				
0.056338	0.014085	0.014085	0.028169	0.028169	0.070423	0.098592	0.098592	0.140845	0.140845
0.112676	0.042254	0.042254	0.056338	0.042254	0.0	0.014085	0.0	0.0	0.0
0.014085	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.014085	0.0
0.042254	0.056338	0.056338	0.183099	0.112676	0.154014	0.070423	0.028169	0.084507	0.014085
0.014085	0.014085	0.0	0.0	0.084507					
ID = 5007	2	90	4	520 9.246072 54.319077	1				
0.086957	0.021739	0.032609	0.032609	0.032609	0.141304	0.304348	0.163043	0.065217	0.065217
0.032600	0.010870	0.0	0.010870	0.0	0.0	0.0	0.0	0.0	0.0
0.032609	0.010870	0.0	0.010870	0.0	0.021739	0.032609	0.010870	0.032609	0.043478
0.086957	0.086957	0.152174	0.141304	0.097826	0.043478	0.076087	0.043478	0.010870	0.010870
0.021739	0.0	0.010870	0.0	0.021739					
ID = 1774	2	60	4	509 8.543166 56.163300	1				
0.025974	0.064935	0.077922	0.077922	0.103896	0.324675	0.077922	0.077922	0.064935	0.051948
0.038961	0.0	0.012987	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.012987	0.0	0.0	0.0	0.012987	0.025974	0.025974	0.038961	0.064935
0.155844	0.103896	0.129870	0.077922	0.142857	0.051948	0.012987	0.012987	0.025974	0.012987
0.012987	0.0	0.0	0.0	0.077922					
ID = 5012	2	90	4	533 8.811529 61.220520	1				
0.055556	0.013889	0.055556	0.069444	0.083333	0.194444	0.250000	0.194444	0.055556	0.0
0.013889	0.013889	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013889	0.0	0.0	0.0	0.013889	0.013889	0.0	0.0	0.027778	0.013889
0.083333	0.069444	0.194444	0.138889	0.125000	0.069444	0.013889	0.041667	0.041667	0.013889
0.041667	0.013889	0.0	0.027778	0.041667					
ID = 5017	2	60	4	551 8.066656 61.216447	1				
0.041096	0.027397	0.054795	0.082192	0.123288	0.287671	0.260274	0.123288	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013699	0.0	0.013699	0.0	0.0	0.0	0.0	0.0	0.054795	0.041096
0.109589	0.082192	0.095890	0.095890	0.095890	0.123288	0.095890	0.054795	0.013699	0.027397
0.027397	0.013699	0.013699	0.0	0.041096					
ID = 5014	1	90	4	648 9.236571 59.122879	1				
0.058824	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.014706	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.014706	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.014706	0.029412	0.044118
0.014706	[illegible]	0.147059	0.191176	0.132353	0.058824	0.029412	0.014706	0.058824	0.0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]					
ID = 1788	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

0.026667 0.0 0.0 0.0 0.053333

ID = 5032 2 90 7 757 12.699487 62.158524 1
0.014286 0.014286 0.114286 0.014286 0.014286 0.057143 0.014286 0.100000 0.100000 0.114286
0.171429 0.157143 0.042857 0.028571 0.028571 0.014286 0.0 0.0 0.0 0.0
0.0 0.0 0.0 0.0 0.014286 0.0 0.0 0.014286 0.014286 0.071429
0.100000 0.128571 0.114286 0.142857 0.128571 0.100000 0.042857 0.014286 0.028571 0.0
0.0 0.0 0.0 0.014286 0.071429

ID = 5022 1 90 6 609 10.961330 64.774460 1
0.072464 0.043478 0.057971 0.014493 0.043478 0.014493 0.115942 0.173913 0.086957 0.130435
0.101449 0.086957 0.028986 0.014493 0.014493 0.0 0.0 0.0 0.0 0.0
0.0 0.0 0.0 0.014493 0.014493 0.014493 0.014493 0.014493 0.028986 0.043478
0.101449 0.159420 0.115942 0.159420 0.101449 0.101449 0.028986 0.014493 0.028986 0.0
0.0 0.0 0.0 0.0 0.043478

ID = 1578 1 82 5 521 7.169718 63.322403 1
0.070588 0.035294 0.164118 0.164706 0.200000 0.258824 0.082353 0.070588 0.035294 0.0
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
0.011765 0.011765 0.011765 0.011765 0.0 0.0 0.0 0.035294 0.011765 0.070588
0.070588 0.117647 0.058824 0.047059 0.082353 0.105882 0.070588 0.058824 0.023529 0.058824
0.035294 0.011765 0.035294 0.0 0.058824

ID = 1579 1 82 5 561 6.209525 60.458766 1
0.048780 0.060976 0.134146 0.085366 0.304878 0.219512 0.097561 0.048780 0.0 0.0
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
0.0 0.0 0.0 0.0 0.0 0.0 0.012195 0.012195 0.073171 0.036585
0.036585 0.109756 0.121951 0.158537 0.036585 0.109756 0.073171 0.024390 0.048780 0.0
0.036585 0.0 0.012195 0.024390

ID = 1581 2 82 5 635 6.891495 58.422272 1
0.114583 0.062500 0.062500 0.062500 0.208333 0.302083 0.104167 0.020833 0.031250 0.0
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
0.041667 0.0 0.010417 0.0 0.0 0.0 0.010417 0.0 0.020833 0.052083
0.062500 0.104167 0.145833 0.125000 0.135417 0.052083 0.052083 0.010417 0.010417 0.010417
0.0 0.020833 0.0 0.0 0.072917

ID = 1584 2 82 5 635 6.500142 61.188787 1
0.051282 0.064103 0.089744 0.192308 0.346154 0.179487 0.051282 0.012821 0.012821 0.0
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
0.012821 0.0 0.0 0.0 0.0 0.012821 0.012821 0.038462 0.012821 0.025641
0.012821 0.128205 0.038462 0.038462 0.089744 0.064103 0.076923 0.0 0.012821 0.025641
0.025641 0.0 0.0 0.0 0.076923

ID = 1586 1 82 5 643 11.018765 66.258545 1
0.029412 0.029412 0.088824 0.088824 0.029412 0.117647 0.147059 0.117647 0.073529 0.058824
0.147059 0.088824 0.029412 0.0 0.014706 0.029412 0.0 0.0 0.0 0.0
0.0 0.014706 0.0 0.0 0.0 0.014706 0.014706 0.029412 0.014706 0.073529
0.088235 0.088235 0.147059 0.0 0.014706 0.044118 0.058824 0.014706 0.044118 0.088235
0.044118 0.014706 0.014706 0.0 0.102941

ID = 1587 2 82 5 543 8.413450 64.864746 1
0.049383 0.012346 0.098765 0.049383 0.135802 0.148148 0.148148 0.185185 0.086420 0.061728
0.0 0.024691 0.0 0.0 0.0 0.0 0.0 0.012346 0.024691 0.061728
0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.049383 0.037037
0.148148 0.061728 0.111111 0.123457 0.037037 0.024691 0.074074 0.049383 0.049383 0.024691
0.024691 0.061728 0.0 0.0 0.074074

ID = 1588 2 82 5 [illegible] 0.0 0.0

0.0	0.0			0.0	0.0			0.0	0.0
0.0	0.0			0.0	0.0			0.0	0.0
0.075949	0.126582	0.088774	0.088774	0.088774	0.049011	0.016021	0.010753	0.032258	0.0
0.0	0.0		0.0	0.0					

ID = 1570	1	82	7	345	5.446640	55.304000	1		
0.054545	0.072727	0.218182	0.363636	0.200000	0.054545	0.018182	0.018182	0.0	0.0
0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.018182	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.036364	0.072727
0.036364	0.145455	0.181819	0.145455	0.090909	0.036364	0.054545	0.036364	0.018182	0.0
0.0	0.0	0.0	0.018182	0.036364					

ID = 1591	2	82	9	864	6.604755	56.814396	1		
0.063830	0.085106	0.021276	0.106383	0.117021	0.117021	0.106383	0.191489	0.106383	0.042553
0.063830	0.010638	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.010638	0.0	0.0	0.021277	0.042553	0.042553
0.085106	0.191489	0.117021	0.170213	0.106383	0.021277	0.042553	0.031915	0.0	0.031915
0.0	0.0	0.010638	0.0	0.053191					

ID = 1715	2	82	10	924	10.383745	65.105255	1		
0.037975	0.075949	0.025316	0.037975	0.101266	0.075949	0.126582	0.151899	0.050633	0.113924
0.075949	0.025316	0.050633	0.012658	0.0	0.012658	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.012658	0.012658	0.063291	0.037975
0.075949	0.164557	0.063291	0.101266	0.126582	0.063291	0.063291	0.037975	0.025316	0.025316
0.012658	0.0	0.0	0.025316	0.088608					

ID = 1720	1	82	3	484	8.525995	63.610764	1		
0.012346	0.049383	0.086420	0.061728	0.160494	0.148148	0.148148	0.172839	0.123457	0.061728
0.012346	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012346	0.049383
0.111111	0.086420	0.148148	0.148148	0.086765	0.074074	0.086420	0.049383	0.024691	0.0
0.012346	0.0	0.037037	0.0	0.061728					

ID = 1736	1	82	5	612	6.622341	65.727188	1		
0.082192	0.068493	0.123288	0.123288	0.164384	0.246575	0.109589	0.041096	0.041096	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013699	0.0	0.0	0.0	0.0	0.0	0.0	0.027397	0.027397	0.082192
0.013699	0.054795	0.054795	0.068493	0.082192	0.082192	0.123288	0.068493	0.054795	0.041096
0.013699	0.013699	0.0	0.0	0.136986					

ID = 5055	1	82	8	815	9.464708	56.976364	1		
0.010753	0.010753	0.043011	0.075269	0.107527	0.161290	0.268817	0.182796	0.064516	0.064516
0.0	0.0	0.010753	0.0	0.0	0.0	0.0	0.021505	0.0	0.0
0.010753	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.032256	0.032256
0.096774	0.139785	0.182796	0.236559	0.086021	0.053763	0.0	0.0	0.0	0.010753
0.0	0.0	0.0	0.0	0.064516					

ID = 1716	2	82	8	484	8.070451	65.235214	1		
0.081395	0.046512	0.058140	0.058140	0.127907	0.116279	0.116279	0.139535	0.069767	0.046512
0.046512	0.023256	0.011628	0.0	0.011628	0.0	0.0	0.0	0.0	0.011628
0.0	0.011628	0.011628	0.011628	0.011628	0.011628	0.0	0.023256	0.011628	0.011628
0.058140	0.023256	0.127907	0.093023	0.116279	0.034884	0.081395	0.058140	0.069767	0.011628
0.0	0.023256	0.011628	0.011628	0.069767					

ID = 1727	2	82	5	633	7.654402	65.780060	1		
0.058140	0.046512	0.069767	0.104651	0.081395	0.139535	0.279070	0.127907	0.023256	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.023256	0.0	0.0	0.0	0.0	0.0	0.023256	0.011628	0.058140	0.058140
0.058140	0.116279	0.151163	0.127907	0.116279	0.058140	0.046512	0.058140	0.0	0.011628
0.011628	0.023256	0.0	0.0	0.046512					

ID = 1730	2	82	6	560	8.344211	63.910431	1		
0.064935	0.077922	0.038961	0.116883	0.129870	0.207792	0.090909	0.129870	0.103896	0.012987
0.012987	0.012987	0.0	0.0	0.0	0.0	0.0	0.0	0.025974	0.038961
0.012987	0.0	0.0	0.012987	0.0	0.012987	0.012987	0.0	0.025974	0.025974
0.090909	0.077922	0.051948	0.090909	0.064935	0.064935	0.129870	0.025974	0.038961	0.025974
0.051948	0.025974	0.012987	0.0	0.129870					

ID = 1800	1	82	3	466	7.289127	61.127823	1		
0.037037	0.061728	0.086420	0.061728	0.160494	0.197531	0.197531	0.123457	0.086420	0.024691
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012346
0.012346	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012346	0.049383
0.061728	0.123457	0.197531	0.160494	0.058765	0.123457	0.024691	0.024691	0.037037	0.0
0.0	0.0	0.0	0.0	0.061728					

ID = 5060	1	82	5	535	6.540833	56.590574	1		
0.209677	0.096774	0.080645	0.112903	0.129032	0.129032	0.064516	0.048387	0.048387	0.016129
0.032258	0.032258	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.032258	0.032258	0.016129	0.0	0.016129	0.032258	0.048387	0.032258	0.032258	0.080645
0.112903	0.048387	0.064774	0.112903	0.048387	0.080645	0.0	0.0	0.0	0.0
0.016129	0.0	0.032258	0.032258	0.056774					

ID = 1582	1	82	5	576	9.067810	59.055359	1		
0.033333	0.055556	0.077778	0.111111	0.044444	0.100000	0.144444	0.244444	0.100000	0.033333
0.022222	0.011111	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.011111	0.0	0.0	0.044444	0.044444	0.066667	0.044444
0.044444	0.088889	0.122222	0.133333	0.088889	0.088889	0.055556	0.044444	0.0	0.022222
0.011111	0.033333	0.011111	0.0	0.044444					

ID = 1717	2	82	4	623	9.438550	71.266263	1		
0.037037	0.061728	0.061728	0.086420	0.160494	0.185185	0.172839	0.086420	0.074074	0.024691
0.024691	0.024691	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.012346	0.012346	0.012346	0.0	0.0	0.0	0.0	0.012346	0.024691	0.037037
0.049383	0.037037	0.111111	0.123457	0.049383	0.086423	0.024691	0.061728	0.061728	0.024691
0.061728	0.012346	0.012346	0.024691	0.148148					

ID = 1725	2	82	9	972	11.501705	60.284470	1		
0.011494	0.022989	0.034483	0.068965	0.068965	0.068965	0.091954	0.091954	0.057471	0.126437
0.114942	0.080460	0.034483	0.045977	0.011494	0.011494	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.022989	0.034483	0.045977	0.022989	0.057471
0.068965	0.057471	0.103448	0.091954	0.149425	0.045977	0.080460	0.045977	0.011494	0.022989
0.011494	0.011494	0.0	0.034483	0.057471					

ID = 5056	2	82	5	700	7.943364	68.014455	1		
0.071429	0.023810	0.023810	0.107143	0.202381	0.297619	0.071429	0.166667	0.011905	0.011905
0.011905	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.011905	0.0	0.011905	0.0	0.0	0.011905	0.0	0.011905	0.023810	0.035714
0.047619	0.035714	0.047619	0.095238	0.071429	0.154762	0.154762	0.023810	0.071429	0.071429
0.0	0.035714	0.0	0.011905	0.071429					

ID = 1734	1	82	6	585	8.239678	61.203018	1		
0.066667	0.033333	0.055556	0.100000	0.111111	0.166667	0.211111	0.155556	0.088889	0.011111
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.011111	0.0	0.011111	0.044444	0.100000
0.044444	0.044444	0.155556	0.133333	0.111111	0.077778	0.088889	0.033333	0.033333	0.033333
0.011111	0.011111	0.0	0.0	0.055556					

ID = 1713	2	82	6	554	10.375881	55.307692	1		
0.022989	0.011494	0.034483	0.034483	0.034483	0.034483	0.068966	0.230769	0.206897	0.160920
0.011494	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DS3.6

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 0.0       0.0       0.0       0.0       0.0       0.0       0.032967  0.0[illegible] 0.021978  0.021978
 0.087912  [illegible] [illegible] 0.175824 [illegible] 0.032967 [illegible] [illegible] 0.021978  0.0
 0.010989  0.010989  0.0       0.010989  0.054945

ID = 1739     2    83     5    591  8.220094 55.352249    1
 0.043011  0.043011  0.021505  0.086021  0.204301  0.247312  0.182796  0.086774  0.064516  0.010753
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.010753  0.0       0.0       0.0       0.021505  0.010753  0.010753  0.021505  0.032258  0.043011
 0.152796  0.139785  0.129032  0.107527  0.053763  0.053763  0.032258  0.021505  0.053763  0.010753
 0.010753  0.0       0.0       0.010753  0.043011

ID = 1561     1    83     5    537  3.930974 57.249527    1
 0.242424  0.257576  0.212121  0.212121  0.075758  0.0       0.0       0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.030303  0.0       0.0       0.0       0.0       0.030303  0.015152  0.045455  0.060606  0.060606
 0.060606  0.136364  0.136364  0.106061  0.090909  0.075758  0.030303  0.015152  0.015152  0.015152
 0.0       0.0       0.0       0.0       0.075758

ID = 5072     2    83     5    709  7.931430 62.759857    1
 0.034483  0.017241  0.103449  0.206897  0.137931  0.120690  0.155172  0.189655  0.017241  0.017241
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.017241  0.0       0.0       0.0       0.0       0.0       0.0       0.017241  0.034483  0.068965
 0.086207  0.155172  0.137931  0.086207  0.051724  0.068965  0.051724  0.017241  0.0       0.051724
 0.034483  0.034483  0.0       0.0       0.086207

ID = 5075     2    83     5    579  7.429721 62.458130    1
 0.031746  0.015873  0.095238  0.142857  0.285714  0.222222  0.126984  0.063492  0.015873  0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.015873  0.0       0.0       0.0       0.015873  0.0       0.0       0.031746  0.0       0.079365
 0.142857  0.126984  0.063492  0.111111  0.079365  0.095238  0.063492  0.015873  0.031746  0.0
 0.031746  0.0       0.015873  0.0       0.079365

ID = 1598     2    83     5    590  6.335896 75.563370    1
 0.145833  0.062500  0.145833  0.145833  0.187500  0.208333  0.041667  0.041667  0.0       0.0
 0.020833  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.020833  0.0       0.0       0.0       0.0       0.0       0.0       0.020833  0.0       0.020833
 0.083333  0.0       0.104167  0.125000  0.083333  0.020833  0.083333  0.062500  0.041667  0.012500
 0.041667  0.062500  0.020833  0.0       0.145833

ID = 1595     2    83     5    568  6.684086 67.365097    1
 0.105263  0.105263  0.122807  0.087719  0.175439  0.157895  0.175439  0.070175  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.017544  0.0       0.0       0.0       0.0       0.0       0.0       0.017544  0.052632  0.035088
 0.122807  0.140351  0.122807  0.157895  0.052632  0.035088  0.017544  0.035088  0.0       0.017544
 0.017544  0.035088  0.0       0.0       0.122807

ID = 1572     1    83     5    615  6.781590 62.870285    1
 0.159420  0.130435  0.057971  0.115942  0.086957  0.202899  0.072464  0.101449  0.043478  0.0
 0.028986  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.014493  0.014493  0.0       0.0       0.0       0.0       0.014493  0.043478  0.0       0.057971
 0.086957  0.115942  0.086957  0.086957  0.043478  0.028986  0.072464  0.086957  0.043478  0.0
 0.028986  0.014493  0.028986  0.0       0.101449

ID = 1553     1    83     3    445  6.042015 59.272415    1
 0.189189  0.108108  0.094595  0.162162  0.175676  0.108108  0.054054  0.054054  0.040541  0.013514
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.0       0.027027  0.013514  0.0       0.0       0.040541  0.0       0.054054  0.054054  0.013514
 0.121622  0.135135  0.135135  0.081081  0.081081  0.013514  0.027027  0.040541  0.0       0.040541
 0.013514  0.027027  0.0       0.013514  0.067568


ID = 4854     2    83     9    864  4.914579 65.886597    1
 0.121212  0.227273  0.272727  0.181818  0.090909  0.030303  0.045455  0.030303  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.015152  0.015152  0.0       0.0       0.0       0.0       0.0       0.030303  0.015152  0.060606
 0.045455  0.075758  0.075758  0.151515  0.136364  0.090909  0.015152  0.060606  0.0       0.045455
 0.0       0.0       0.015152  0.015152  0.136364

ID = 4873     1    83     6    431  7.130682 61.035355    1
 0.197368  0.078947  0.065789  0.078947  0.157895  0.078947  0.052632  0.078947  0.131579  0.052632
 0.013158  0.013158  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.0       0.052632  0.026316  0.0       0.013158  0.0       0.026316  0.0       0.039474  0.052632
 0.065789  0.105263  0.105263  0.078947  0.065789  0.092105  0.039474  0.039474  0.013158  0.013158
 0.039474  0.013158  0.013158  0.013158  0.092105

ID = 4839     1    83     5    568  4.857590 58.095825    1
 0.102941  0.117647  0.470588  0.161765  0.058824  0.058824  0.029412  0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.0       0.0       0.0       0.0       0.014706  0.0       0.058824  0.0       0.073529  0.044118
 0.117647  0.147059  0.147059  0.117647  0.102941  0.014706  0.029412  0.014706  0.0       0.029412
 0.0       0.0       0.0       0.014706  0.073529

ID = 4837     1    83     5    504  7.038127 72.762604    1
 0.138462  0.030769  0.123077  0.107692  0.230769  0.169231  0.092308  0.030769  0.030769  0.015385
 0.0       0.0       0.015385  0.0       0.0       0.015385  0.0       0.0       0.0       0.0
 0.015385  0.0       0.0       0.0       0.0       0.0       0.015385  0.0       0.015385  0.052308
 0.052308  0.030769  0.076923  0.030769  0.107692  0.138462  0.061538  0.061538  0.015385  0.030769
 0.0       0.015385  0.030769  0.015385  0.153846

ID = 4829     1    83     4    415  4.941846 58.199936    1
 0.209877  0.123457  0.160494  0.246914  0.160494  0.086420  0.0       0.012346  0.0       0.0
 0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.0       0.012346  0.012346  0.012346  0.049383  0.037037  0.012346  0.049383  0.074074  0.024691
 0.049383  0.012346  0.049383  0.074074  0.148148  0.111111  0.049383  0.037037  0.049383  0.049383
 0.012346  0.012346  0.0       0.0       0.061728

ID = 4819     2    83     9    861  3.501656 63.040649    1
 0.153846  0.012821  0.025641  0.141026  0.089744  0.115385  0.076923  0.102564  0.153846  0.051282
 0.051282  0.0       0.0       0.012821  0.012821  0.0       0.0       0.0       0.0       0.0
 0.051282  0.0       0.0       0.0       0.0       0.0       0.025641  0.012821  0.025641  0.012821
 0.025641  0.038462  0.205128  0.115385  0.089744  0.089744  0.115385  0.012821  0.064103  0.025641
 0.012821  0.0       0.012821  0.0       0.064103

ID = 1178     2    83     8    866  6.862343 66.561731    1
 0.171875  0.078125  0.093750  0.109375  0.171875  0.109375  0.109375  0.046875  0.031250  0.046875
 0.0       0.031250  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.015625  0.0       0.0       0.046875  0.031250  0.015625  0.015625  0.031250  0.015625  0.062500
 0.062500  0.046875  0.078125  0.062500  0.093750  0.046875  0.046875  0.015625  0.015625  0.046875
 0.031250  0.015625  0.015625  0.015625  0.171875

ID = [illegible]  2  [illegible]  [illegible]  733  [illegible] 67.188477    1
 0.117647  0.044119  0.058824  0.073529  0.058824  0.102941  0.117647  0.102941  0.073529  0.102941
 0.058824  0.073529  [illegible] 0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.014706  0.044119  0.044119  0.0       0.0       0.0       0.0       0.029412  0.029412  0.044118
 [illegible] [illegible] [illegible] 0.102941 [illegible] 0.044118 0.073529 0.044118  0.044118  0.044118
 0.014706  0.014706  0.029412  0.0       0.132353

ID = 1198     1    83     6    653  7.568842 58.170212    1
 0.141026  [illegible] 0.025641 0.179487 [illegible] 0.051282 0.192308 0.102564  0.051282  0.051282
 0.012821  0.012821  0.0       0.0       0.0       0.0       0.0       0.0       0.0       0.0
 0.038462  0.012821  0.051282  0.0       0.0       0.012821  0.012821  0.012821  0.0       0.025641
```

0.051760	0.121705	[illegible]	[illegible]	0.121705	0.172985	0.034482	0.017241	0.017241	0.017241
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

ID = 1174 1 83 4 413 5.784214 62.413346 1

0.036364	0.018182	0.327273	0.327273	0.200000	0.0	0.036364	0.036364	0.018182	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.018182	0.0	0.0	0.0	0.0	0.0	0.0	0.072727	0.018182
0.0	0.0	0.018182	0.018182	0.090909	0.0	0.072727	0.072727	0.0	0.018182	0.018182

ID = 5070 2 83 6 883 9.482713 59.051285 1

0.077922	0.025974	0.116883	0.051948	0.064935	0.0	0.129870	0.103896	0.129870	0.090909	0.038961
0.012987	0.025974	0.051948	0.012987	0.038961	0.0	0.0	0.0	0.0	0.0	0.0
0.012987	0.0	0.012987	0.0	0.0	0.0	0.025974	0.012987	0.077922	0.077922	0.064935
0.077922	0.051948	0.103896	0.077922	0.077922	0.0	0.051948	0.038961	0.025974	0.038961	0.025974
0.025974	0.0	0.0	0.025974	0.090909						

ID = 1555 1 83 5 520 3.531107 56.824476 1

0.268293	0.243902	0.341463	0.121951	0.012195	0.012195	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.012195	0.0	0.012195	0.0	0.0	0.0	0.036585	0.036585	0.024390	0.048780	0.0
0.073171	0.109756	0.085366	0.097561	0.097561	0.085366	0.024390	0.048780	0.0	0.0	0.036585
0.024390	0.012195	0.024390	0.0	0.073171						

ID = 5074 2 83 5 608 8.038823 59.436615 1

0.075000	0.012500	0.075000	0.100000	0.125000	0.250000	0.187500	0.100000	0.050000	0.025000	0.025000
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.012500	0.012500	0.012500	0.012500	0.0	0.0	0.0	0.0	0.0	0.012500	0.012500
0.087500	0.100000	0.187500	0.075000	0.125000	0.100000	0.050000	0.012500	0.025000	0.012500	0.0
0.0	0.0	0.012500	0.025000	0.062500						

ID = 1603 2 83 5 413 5.953126 66.354233 1

0.220779	0.025974	0.051948	0.155844	0.272727	0.194805	0.038961	0.012987	0.012987	0.0	0.0
0.012987	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.038961	0.025974	0.012987	0.012987	0.025974	0.0	0.0	0.012987	0.012987	0.012987	0.012987
0.038961	0.077922	0.051948	0.077922	0.077922	0.051948	0.090909	0.025974	0.129870	0.077922	0.0
0.038961	0.012987	0.0	0.0	0.090909						

ID = 1176 1 83 5 572 11.406528 56.865860 1

0.047619	0.0	0.035714	0.071429	0.047619	0.107143	0.035333	0.095238	0.142857	0.071429	0.0
0.071429	0.107143	0.047619	0.023810	0.035714	0.0	0.011905	0.0	0.0	0.0	0.0
0.011905	0.011905	0.011905	0.0	0.0	0.011905	0.023810	0.071429	0.011905	0.119047	0.0
0.083333	0.095238	0.095238	0.130952	0.071429	0.047619	0.047619	0.047619	0.023810	0.0	
0.0	0.0	0.0	0.0	0.059523						

ID = 1562 1 83 4 544 8.033826 70.750155 1

0.138889	0.069444	0.083333	0.083333	0.055556	0.055556	0.041667	0.125000	0.097222	0.111111	0.0
0.069444	0.055556	0.013889	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.013889	0.013889	0.027778	0.055556	0.0
0.041667	0.069444	0.041667	0.097222	0.138889	0.069444	0.069444	0.069444	0.0	0.0	0.0
0.013889	0.013889	0.041667	0.0	0.125000						

ID = 5073 2 83 9 866 10.241889 64.759262 1

0.103896	0.077922	0.038961	0.038961	0.051948	0.051948	0.051948	0.181818	0.064935	0.116883	0.0
0.103896	0.038961	0.051948	0.012987	0.025974	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.012987	0.0	0.0	0.012987	0.025974	0.025974	0.051948	0.0
0.090909	0.116883	0.090909	0.064935	0.077922	0.090909	0.051948	0.012987	0.012987	0.0	
0.012987	0.0	0.012987	0.0	0.168831						

ID = 4874 1 83 5 603 9.129866 59.923752 1

0.060241	0.072289	0.072289	0.096386	0.108434	0.060241	0.084337	0.120482	0.156626	0.096386	0.0
0.0	0.060241	0.012048	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.024096	0.012048	0.024096	0.036145	0.084337	0.0
0.072289	0.060241	0.132530	0.108434	0.108434	0.072289	0.024096	0.048193	0.060241	0.060241	0.0
0.0	0.012048	0.012048	0.0	0.060241						

ID = 4822 2 83 6 889 10.811642 62.749268 1

0.013158	0.013158	0.026316	0.026316	0.131579	0.092105	0.118421	0.131579	0.144737	0.131579	0.0
0.013158	0.026316	0.065789	0.026316	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.013158	0.013158	0.026316	0.065789	0.078947	0.0
0.026316	0.118421	0.078947	0.118421	0.105263	0.065789	0.013158	0.078947	0.026316	0.013158	0.0
0.013158	0.026316	0.013158	0.026316	0.078947						

ID = 1171 2 83 5 716 10.769744 64.591131 1

0.066667	0.066667	0.080000	0.066667	0.0	0.066667	0.066667	0.066667	0.080000	0.120000	0.0
0.066667	0.066667	0.040000	0.066667	0.0	0.013333	0.013333	0.0	0.0	0.0	0.0
0.013333	0.0	0.013333	0.0	0.040000	0.0	0.013333	0.013333	0.040000	0.013333	0.0
0.066667	0.120000	0.133333	0.146667	0.053333	0.066667	0.013333	0.026667	0.026667	0.0	0.0
0.0	0.0	0.013333	0.013333	0.133333						

ID = 1596 2 83 5 573 9.251491 63.650162 1

0.047566	0.013514	0.027027	0.040541	0.054054	0.162162	0.162162	0.162162	0.162162	0.013514	0.0
0.040541	0.013514	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.013514	0.0	0.0	0.0	0.0	0.0	0.013514	0.0	0.013514	0.094595	0.0
0.040541	0.121622	0.121622	0.067568	0.108108	0.081081	0.067568	0.013514	0.067568	0.054054	0.0
0.0	0.013514	0.013514	0.0	0.094595						

ID = 4856 2 83 6 636 5.271206 66.427552 1

0.038961	0.025974	0.038961	0.077922	0.103896	0.181818	0.194805	0.116883	0.129870	0.051948	0.0
0.012987	0.012987	0.012987	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.012987	0.0	0.0	0.0	0.0	0.038961	0.0	0.012987	0.051948	0.038961	0.0
0.103896	0.064004	0.155844	0.077922	0.090909	0.090909	0.090909	0.025974	0.0	0.012987	0.0
0.012987	0.012987	0.0	0.012987	0.064935						

ID = 5077 2 83 10 828 9.991731 72.578537 1

0.032258	0.064516	0.064516	0.064516	0.096774	0.129032	0.145161	0.096774	0.080645	0.0	0.0
0.048387	0.080645	0.016129	0.016129	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.016129	0.0	0.0	0.0	0.0	0.016129	0.016129	0.064516	0.0	0.0	0.0
0.032258	0.048387	0.016129	0.080645	0.064516	0.080645	0.032258	0.096774	0.096774	0.048387	0.0
0.096774	0.0	0.048387	0.0	0.145161						

ID = 1178 2 83 5 580 11.147476 66.324752 1

0.062500	0.062500	0.0	0.062500	0.0	0.078125	0.046875	0.140625	0.187500	0.140625	0.0
0.125000	0.046875	0.031250	0.015625	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.015625	0.0	0.015625	0.0	0.0	0.031250	0.015625
0.031250	0.046875	0.046875	0.046875	0.187500	0.125000	0.062500	0.031250	0.015625		
0.015625	0.031250	0.0	0.015625	0.093750						

ID = 1179 2 83 8 875 11.464547 61.765503 1

0.089552	0.059701	0.014925	0.074627	0.059701	0.104478	0.104478	0.089552	0.059701	0.179104	0.0
0.059701	0.149254	0.014925	0.014925	0.0	0.014925	0.0	0.0	0.0	0.0	0.0
0.014925	0.0	0.0	0.0	0.0	0.0	0.014925	0.0	0.044776	0.044776	0.014925
0.074627	0.104478	0.074627	0.179104	0.059701	0.044776	0.089552	0.059701	0.044776	0.014925	
0.0	0.0	0.014925	0.029851	0.059701						

0.117647	0.014706	0.029412	0.088235	0.117647	0.205882	0.205882	0.073529	0.102941	0.014706	0.0
0.014706	0.0	0.014706	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.014706	0.0	0.044118	0.117647	0.117647	0.088235	0.044118	0.014706	0.044118	0.029412	0.0
0.014706	0.029412	0.044118	0.117647	0.117647	0.088235	0.044118	0.044118	0.132353	0.029412	0.0

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ID = 1148      2      83      6      714  10.276827 57.445652      1
0.013889  0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.013889  0.069444    0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.111111  0.236111    0.138889    0.083333    0.041667    0.027778    0.027778    0.013889    0.027778    0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0

ID = 4811      2      83      6      714  11.444737 55.594737      1
0.016393  0.049180    0.049180    0.032787    0.016393    0.049180    0.147541    0.213115    0.229508    0.0
0.081967  0.049180    0.049180    0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.016393  0.0         0.0         0.0         0.0         0.016393    0.0         0.0         0.032787    0.0
0.032787  0.065574    0.081967    0.213115    0.147541    0.065574    0.032787    0.032787    0.0         0.016393
0.016393  0.049180    0.016393    0.0         0.065574    0.0         0.0         0.0         0.0         0.0

ID = 1799      1      82      6      533  7.780535 57.712662      1
0.049383  0.074074    0.049383    0.049383    0.123185    0.098742    0.172839    0.086420    0.024691    0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.012346  0.0         0.0         0.0         0.0         0.0         0.024691    0.024691    0.049383    0.086715
0.049383  0.123457    0.160494    0.135802    0.074074    0.024691    0.074074    0.012346    0.037037    0.012346
0.037037  0.012346    0.0         0.0         0.037037    0.0         0.0         0.0         0.0         0.0

ID = 4814      2      83      0      884  10.813615 55.737549      1
0.129870  0.0         0.051948    0.038961    0.051948    0.051948    0.090909    0.116883    0.103896    0.051948
0.116883  0.129870    0.012987    0.038961    0.0         0.012987    0.0         0.0         0.0         0.0
0.025974  0.0         0.0         0.0         0.0         0.0         0.012987    0.012987    0.051948    0.064935
0.142857  0.077922    0.181818    0.129870    0.103896    0.064935    0.038961    0.012987    0.012987    0.0
0.012987  0.0         0.0         0.025974    0.025974    0.0         0.0         0.0         0.0         0.0

ID = 1188      1      82      4      480  8.858418 58.382477      1
0.066667  0.053333    0.053333    0.066667    0.053333    0.200000    0.173333    0.146667    0.080000    0.066667
0.026667  0.0         0.0         0.013333    0.0         0.0         0.0         0.0         0.0         0.0
0.0       0.0         0.013333    0.213333    0.066667    0.0         0.040000    0.040000    0.013333    0.040000
0.080000  0.066667    0.080000    0.093333    0.080000    0.066667    0.066667    0.133333    0.013333    0.0
0.0       0.013333    0.013333    0.013333    0.053333    0.0         0.0         0.0         0.0         0.0

ID = 1732      2      82      6      594  9.824722 60.781128      1
0.014706  0.044118    0.044118    0.117647    0.058824    0.102941    0.161765    0.117647    0.205882    0.088824
0.029412  0.0         0.029412    0.0         0.0         0.014706    0.0         0.0         0.0         0.0
0.0       0.0         0.0         0.0         0.0         0.014706    0.014706    0.058824    0.058824    0.058824
0.044118  0.073529    0.147059    0.014706    0.132353    0.058824    0.058824    0.014706    0.029412    0.014706
0.014706  0.014706    0.014706    0.0         0.058824    0.0         0.0         0.0         0.0         0.0

ID = 5058      2      82      4      517  7.603848 58.439026      1
0.065789  0.078947    0.105263    0.065789    0.144737    0.236842    0.157895    0.052632    0.065789    0.013158
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.026316  0.0         0.0         0.013158    0.0         0.0         0.013158    0.026316    0.052632    0.013158
0.065789  0.092105    0.171053    0.065789    0.092105    0.144737    0.013158    0.039474    0.026316    0.013158
0.026316  0.0         0.0         0.0         0.065789    0.0         0.0         0.0         0.0         0.0

ID = 4841      2      83      6      866  7.604265 57.454114      1
0.088235  0.058824    0.088235    0.088235    0.161765    0.147059    0.191176    0.102941    0.044118    0.029412
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.014706  0.0         0.0         0.0         0.0         0.014706    0.014706    0.029412    0.058824    0.029412
0.014706  0.058824    0.044118    0.147059    0.132353    0.073529    0.102941    0.029412    0.058824    0.0
0.014706  0.029412    0.014706    0.0         0.117647    0.0         0.0         0.0         0.0         0.0

ID = 4843      1      82      7      642  8.383751 53.448349      1
0.041667  0.0         0.069444    0.083333    0.208333    0.263889    0.125000    0.083333    0.083333    0.027778
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0.0       0.0         0.013889    0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.013889  0.0         0.0         0.0         0.013889    0.0         0.027778    0.013889    0.069444    0.013889
0.111111  0.236111    0.138889    0.083333    0.041667    0.027778    0.027778    0.013889    0.027778    0.0
0.0       0.0         0.0         0.0         0.055556    0.0         0.0         0.0         0.0         0.0

ID = 5076      1      83      6      910  10.875793 64.054455      1
0.035714  0.035714    0.017857    0.017857    0.071429    0.035714    0.142857    0.178571    0.196429    0.089286
0.160714  0.017857    0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.0       0.0         0.017857    0.0         0.0         0.0         0.017857    0.0         0.017857    0.053571
0.053571  0.053571    0.160714    0.125000    0.035714    0.107143    0.071429    0.035714    0.017857    0.0
0.035714  0.017857    0.0         0.017857    0.125000    0.0         0.0         0.0         0.0         0.0

ID = 4867      2      83      5      542  8.776184 56.406601      1
0.054054  0.121622    0.067568    0.054054    0.054595    0.067568    0.135135    0.202703    0.135135    0.067568
0.040541  0.013514    0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.0       0.0         0.0         0.0         0.0         0.013514    0.013514    0.013514    0.054054    0.013514
0.189189  0.108108    0.148649    0.121622    0.054595    0.013514    0.027027    0.0         0.0         0.013514
0.013514  0.013514    0.0         0.0         0.067568    0.0         0.0         0.0         0.0         0.0

ID = 4876      2      83      6      706  10.021739 55.650056      1
0.104478  0.0         0.059701    0.014925    0.044776    0.119403    0.194030    0.059701    0.149254    0.134328
0.059701  0.029851    0.014925    0.014925    0.0         0.0         0.0         0.0         0.0         0.0
0.0       0.0         0.014925    0.0         0.0         0.014925    0.029851    0.029851    0.014925    0.059701
0.029851  0.119403    0.044776    0.029851    0.104478    0.029851    0.074627    0.074627    0.044776    0.014925
0.044776  0.0         0.029851    0.014925    0.074627    0.0         0.0         0.0         0.0         0.0

ID = 1281      1      100      1      1  7.552832 56.614365      1
0.342256  0.021505    0.064516    0.161290    0.236559    0.301075    0.139785    0.032258    0.010753    0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.0       0.0         0.0         0.0         0.010753    0.010753    0.010753    0.032258    0.043011    0.064516
0.053763  0.172043    0.150538    0.107527    0.064774    0.043011    0.0         0.053763    0.064516    0.010753
0.0       0.0         0.0         0.0         0.043011    0.0         0.0         0.0         0.0         0.0

ID = 1282      1      100      1      1  6.280113 60.345795      1
0.100000  0.025000    0.162500    0.262500    0.250000    0.075000    0.050000    0.037500    0.037500    0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.012500  0.0         0.0         0.0         0.0         0.012500    0.012500    0.0         0.050000    0.050000
0.050000  0.150000    0.137500    0.150000    0.100000    0.037500    0.025000    0.0         0.012500    0.037500
0.037500  0.0         0.0         0.0         0.087500    0.0         0.0         0.0         0.0         0.0

ID = 1283      1      100      1      1  4.050327 51.491501      1
0.178947  0.315789    0.263158    0.178947    0.042105    0.210526    0.010526    0.0         0.0         0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.010526  0.0         0.0         0.010526    0.021053    0.052632    0.031579    0.084211    0.073684    0.168421
0.084211  0.063158    0.105263    0.073684    0.063158    0.042105    0.021053    0.010526    0.0         0.0
0.0       0.0         0.021053    0.0         0.063158    0.0         0.0         0.0         0.0         0.0

ID = 1297      1      100      1      1  5.560157 60.549296      1
0.065789  0.131579    0.236842    0.223684    0.118421    0.105263    0.052632    0.052632    0.0         0.013158
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.013158  0.0         0.013158    0.0         0.0         0.013158    0.013158    0.039474    0.092105    0.013158
0.052632  0.105263    0.131579    0.144737    0.078947    0.052632    0.039474    0.013158    0.039474    0.0
0.026316  0.0         0.013158    0.013158    0.078947    0.0         0.0         0.0         0.0         0.0

ID = 1300      1      100      1      1  5.913618 70.653846      1
0.027778  0.013889    0.166667    0.097222    0.055556    0.013889    0.013889    0.0         0.0         0.0
0.0       0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0         0.0
0.055556  0.069444    0.069444    0.125000    0.069444    0.069444    0.097222    0.111111    0.027778    0.069444
0.055556  0.013889    0.013889    0.0         0.083333    0.0         0.0         0.0         0.0         0.0
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0.043478	0.028986	0.014493	0.0	0.028986	0.0	0.057971	0.028986	0.028986	0.057971
0.173913	0.101449	0.101449	0.057971	0.072464	0.057971	0.014493	0.0	0.014493	0.014493
0.0	0.0	0.043478	0.0	0.057971					

ID = 1363 1 199 1 439 7.083314 57.764908 1

0.085714	0.042857	0.071429	0.128571	0.285714	0.214286	0.085714	0.028571	0.042857	0.0
0.014286	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.085714	0.0	0.0	0.0	0.0	0.0	0.0	0.042857	0.042857	0.042857
0.057143	0.200000	0.128571	0.142857	0.085714	0.071429	0.028571	0.028571	0.0	0.0
0.0	0.0	0.0	0.0	0.071429					

ID = 1257 1 199 1 550 4.054362 64.064556 1

0.125000	0.250000	0.263889	0.152778	0.111111	0.055556	0.013889	0.027778	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.013889	0.0	0.0	0.0	0.0	0.013889	0.055556
0.055556	0.138889	0.152778	0.152778	0.083333	0.083333	0.027778	0.083333	0.013889	0.013889
0.027778	0.0	0.027778	0.0	0.055556					

ID = 1224 2 199 7 719 7.632475 60.671738 1

0.075960	0.053763	0.107527	0.129032	0.096774	0.172043	0.172043	0.107527	0.032258	0.032258
0.021505	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.010753	0.0	0.0	0.0	0.0	0.0	0.043011	0.0	0.032258	0.064021
0.064516	0.118280	0.118280	0.129032	0.096774	0.118280	0.053763	0.0	0.010753	0.010753
0.010753	0.0	0.010753	0.010753	0.075269					

ID = 1288 2 199 6 691 5.316458 66.184325 1

0.141176	0.129412	0.200000	0.223529	0.176471	0.117647	0.011765	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.011765	0.0	0.058824	0.058824
0.070588	0.047059	0.105882	0.117647	0.094118	0.141176	0.058824	0.070588	0.047059	0.0
0.023529	0.0	0.011765	0.0	0.082353					

ID = 1317 2 199 10 835 8.020695 54.785507 1

0.053191	0.042553	0.053191	0.106383	0.191489	0.212766	0.138298	0.127660	0.053191	0.010638
0.010638	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.010638	0.010638	0.042553	0.021277	0.010638	0.117021
0.095745	0.212766	0.117021	0.117021	0.063830	0.042553	0.021277	0.021277	0.021915	0.0
0.021277	0.010638	0.0	0.0	0.031915					

ID = 1324 2 199 8 725 10.795943 57.782837 1

0.032258	0.021505	0.064516	0.129032	0.053763	0.075269	0.043011	0.118280	0.129032	0.053763
0.118280	0.064516	0.064516	0.0	0.010753	0.0	0.0	0.0	0.0	0.0
0.010753	0.0	0.0	0.0	0.0	0.0	0.032258	0.0	0.064021	0.064021
0.075269	0.107527	0.064516	0.139785	0.064774	0.118280	0.043011	0.064516	0.010753	0.0
0.010753	0.010753	0.0	0.0	0.043011					

ID = 1326 2 199 11 962 10.313615 68.590561 1

0.032967	0.021978	0.087912	0.087912	0.109890	0.175824	0.153846	0.142857	0.076923	0.098901
0.043956	0.021978	0.0	0.032967	0.010989	0.010989	0.0	0.0	0.0	0.0
0.010989	0.0	0.0	0.0	0.0	0.0	0.010989	0.010989	0.010989	0.010989
0.021978	0.087912	0.153846	0.032967	0.131868	0.120879	0.131868	0.054945	0.043956	0.021978
0.021978	0.010989	0.010989	0.0	0.054945					

ID = 1336 2 199 6 675 5.277365 64.101855 1

0.043478	0.065217	0.094348	0.141304	0.076087	0.130435	0.086957	0.184783	0.097826	0.065217
0.032609	0.010870	0.0	0.0	0.0	0.0	0.010870	0.0	0.0	0.0
0.010870	0.0	0.0	0.0	0.021739	0.0	0.0	0.010870	0.043478	0.054348
0.097826	0.0	0.010870	0.010870	0.010870	0.076087	0.032609	0.054348	0.0	0.021739
0.0	0.0	0.0	0.010870	0.010870					

0.141026	0.012821			0.141026					0.012821	
0.012821				0.0					0.0	
0.025641	0.0		0.0	0.0	0.0		0.0	0.038462	0.025641	
0.0	0.0		0.115385	0.0	0.102564	0.076923	0.0	0.012821	0.0	
0.0	0.0	0.025641	0.012821	0.051282						

ID = 1256	2	100	6	523	5.447785	47.667145	1			
0.162393	0.114957	0.148838	0.148838	0.0	0.0	0.154359	0.041667	0.010417	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.041667	0.062033	0.031250	0.0	0.0	0.0	0.0	0.010417	0.031250	0.072917	0.114583
0.125000	0.154250	0.083750	0.072917	0.041667	0.052083	0.041667	0.020833	0.0	0.0	
0.0	0.0	0.010417	0.0	0.031250						

ID = 1246	1	100	6	435	7.556163	61.366382	1			
0.047619	0.063492	0.158730	0.111111	0.063492	0.238095	0.142857	0.142857	0.015873	0.015873	
0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	
0.015873	0.0	0.0	0.0	0.0	0.0	0.015873	0.031746	0.031746	0.031746	
0.031746	0.063492	0.047619	0.126984	0.063492	0.142857	0.095238	0.095238	0.015873	0.015873	
0.0	0.0	0.0	0.0	0.031746						

ID = 1278	1	100	10	775	11.890290	57.615219	1			
0.027027	0.0	0.0	0.013514	0.121622	0.040541	0.067568	0.189189	0.108108	0.216216	
0.121622	0.027027	0.040541	0.013514	0.013514	0.0	0.0	0.0	0.0	0.0	
0.013514	0.0	0.0	0.0	0.0	0.0	0.0	0.013514	0.027027	0.081081	
0.054054	0.162162	0.162162	0.094595	0.148649	0.081081	0.067568	0.0	0.040541	0.0	
0.013514	0.0	0.0	0.0	0.040541						

ID = 1227	2	100	11	862	8.231545	57.020813	1			
0.083333	0.041667	0.069444	0.111111	0.097222	0.069444	0.111111	0.194444	0.111111	0.069444	
0.013889	0.0	0.027778	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
0.013889	0.013889	0.0	0.0	0.0	0.055556	0.013889	0.083333	0.027778	0.083333	
0.125000	0.083333	0.083333	0.083333	0.041667	0.055556	0.013889	0.069444	0.027778	0.0	
0.013889	0.027778	0.013889	0.0	0.069444						

ID = 1277	1	100	11	794	9.363322	58.884040	1			
0.115942	0.014493	0.028986	0.028986	0.115942	0.173913	0.101449	0.173913	0.057971	0.057971	
0.086957	0.014493	0.014493	0.014493	0.0	0.0	0.0	0.0	0.0	0.0	
0.014493	0.0	0.0	0.0	0.014493	0.014493	0.028986	0.0	0.028986	0.028986	
0.115942	0.086957	0.115942	0.115942	0.101449	0.057971	0.043478	0.028986	0.014493	0.043478	
0.014493	0.014493	0.014493	0.0	0.057971						

ID = 1227	2	100	6	551	10.875386	57.687423	1			
0.064103	0.051282	0.025641	0.089744	0.025641	0.038462	0.102564	0.153846	0.115385	0.102564	
0.089744	0.064103	0.025641	0.025641	0.012821	0.0	0.012821	0.012821	0.025641	0.064103	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012821	0.012821	0.012821	
0.064103	0.102564	0.025641	0.064103	0.166667	0.051282	0.102564	0.012821	0.012821	0.0	
0.0	0.012821	0.0	0.0	0.0	0.051282					

ID = 1313	2	100	11	722	9.281676	55.233282	1			
0.095238	0.023810	0.071429	0.095238	0.071429	0.011905	0.071429	0.107143	0.083333	0.047619	
0.071429	0.059524	0.023810	0.011905	0.0	0.011905	0.0	0.0	0.047619	0.023810	
0.0	0.0	0.0	0.0	0.011905	0.011905	0.0	0.047619	0.023810	0.011905	
0.166667	0.130952	0.178571	0.047619	0.047619	0.047619	0.047619	0.023810	0.011905	0.011905	
0.0	0.011905	0.0	0.0	0.047619						

ID = 1266	2	100	6	638	14.049657	60.304138	1			
0.046154	0.030769	0.015385	0.046154	0.046154	0.0	0.061538	0.076923	0.046154	0.092308	
0.154615	0.092308	0.046154	0.046154	0.046154	0.046154	0.030769	0.015385	0.015385	0.046154	
0.015385	0.0	0.0	0.0	0.015385	0.0	0.0	0.030769	0.030769	0.046154	
0.061538	0.0	0.0	0.0	0.061538						

ID = 1252	2	100	10	911	6.930387	60.455399	1			
0.150000	0.116667	0.166667	0.083333	0.066667	0.050000	0.116667	0.100000	0.100000	0.033333	
0.016667	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.050000	
0.016667	0.0	0.016667	0.016667	0.0	0.033333	0.033333	0.033333	0.050000	0.016667	
0.050000	0.066667	0.133333	0.166667	0.083333	0.066667	0.033333	0.0	0.016667	0.016667	
0.0	0.0	0.0	0.0	0.116667						

ID = 1228	2	100	11	660	7.196258	53.423874	1			
0.012500	0.050000	0.125000	0.162500	0.287500	0.225000	0.075000	0.050000	0.0	0.012500	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
0.0	0.0	0.0	0.012500	0.0	0.0	0.075000	0.050000	0.062500	0.125000	
0.137500	0.300000	0.075000	0.025000	0.062500	0.037500	0.025000	0.012500	0.0	0.037500	
0.0	0.0	0.0	0.0	0.062500						

ID = 1302	1	100	10	814	8.478999	61.515515	1			
0.113136	0.108108	0.094595	0.067568	0.027027	0.067568	0.054054	0.121622	0.148649	0.081081	
0.054054	0.027027	0.0	0.013514	0.0	0.0	0.0	0.0	0.0	0.0	
0.0	0.013514	0.0	0.013514	0.013514	0.0	0.027027	0.067568	0.027027	0.040541	0.027027
0.081081	0.067568	0.162162	0.121622	0.027027	0.013514	0.040541	0.013514	0.040541	0.0	
0.0	0.013514	0.027027	0.027027	0.013514						

ID = 1292	2	100	12	553	12.382803	60.601160	1			
0.028571	0.0	0.014286	0.014286	0.085714	0.071429	0.128571	0.071429	0.142857	0.114286	
0.071429	0.028571	0.071429	0.071429	0.042857	0.028571	0.014286	0.0	0.0	0.0	
0.0	0.0	0.0	0.014286	0.0	0.0	0.028571	0.014286	0.014286	0.085714	
0.157143	0.085714	0.085714	0.114286	0.071429	0.057143	0.042857	0.057143	0.071429	0.014286	
0.014286	0.0	0.028571	0.014286	0.028571						

ID = 1287	2	100	10	861	10.776042	62.311035	1			
0.048387	0.048387	0.048387	0.016129	0.064516	0.032258	0.145161	0.112903	0.145161	0.145161	
0.096774	0.064516	0.0	0.016129	0.0	0.0	0.0	0.0	0.0	0.0	
0.112903	0.032258	0.112903	0.032258	0.016129	0.0	0.016129	0.0	0.016129	0.064516	
0.032258	0.0	0.0	0.096774	0.145161	0.048387	0.048387	0.112903	0.048387	0.016129	
0.0	0.0	0.0	0.032258	0.048387						

ID = 1288	2	100	7	788	10.212743	60.270691	1			
0.015625	0.015625	0.015625	0.031250	0.125000	0.125000	0.187500	0.203125	0.125000	0.062500	
0.031250	0.046875	0.0	0.0	0.015625	0.0	0.0	0.0	0.046875	0.093750	
0.015625	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.015625	0.0	
0.046875	0.109375	0.140625	0.062500	0.140625	0.093750	0.062500	0.015625	0.0	0.0	
0.0	0.0	0.031250	0.031250	0.078125						

ID = 1242	2	100	12	895	9.463328	58.322693	1			
0.081081	0.054054	0.040541	0.054054	0.108108	0.175676	0.067568	0.081081	0.108108	0.094595	
0.067568	0.013514	0.040541	0.0	0.0	0.013514	0.0	0.0	0.0	0.0	
0.040541	0.0	0.0	0.0	0.013514	0.027027	0.027027	0.0	0.027027	0.108108	
0.094595	0.135135	0.135135	0.081081	0.067568	0.027027	0.054054	0.040541	0.013514	0.013514	
0.0	0.027027	0.0	0.013514	0.054054						

ID = 1222	2	100	12	768	8.798056	58.819643	1			
0.027778	0.041667	0.027778	0.097222	0.166667	0.180556	0.152778	0.152778	0.111111	0.041667	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
0.013889	0.0	0.0	0.0	0.0	0.0	0.013889	0.055556	0.027778	0.027778	
0.069444	0.111111	0.083333	0.111111	0.097222	0.055556	0.041667	0.027778	0.013889	0.027778	
0.013889	0.0	0.013889	0.013889	0.041667						

ID = 1264	2	100	6	810	10.017251	61.782532	1			
0.074627	0.044776	0.119403	0.059701	0.014925	0.029851	0.044776	0.044776	0.074627	0.089552	

ID = 1340	2	199	7	798	8.292252	65.227707	1		
0.076923	0.064103	0.128205	0.076923	0.089744	0.051282	0.089744	0.038462	0.038462	0.025641
0.0	0.0	0.0	0.0	0.0	0.102564				
0.0	0.0	0.012821	0.012821	0.0	0.0	0.0	0.0	0.0	0.025641
0.025641	0.076923	0.128205	0.076923	0.089744	0.051282	0.089744	0.038462	0.038462	0.025641
0.025641	0.038462	0.0	0.0	0.102564					
ID = 1341	1	199	9	682	10.240023	66.114624	1		
0.013514	0.040541	0.067568	0.054054	0.027027	0.094595	0.162162	0.027027	0.135135	0.054054
0.054054	0.013514	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.027027	0.013514	0.0
0.054054	0.081081	0.067568	0.094595	0.135135	0.081081	0.094595	0.067568	0.054054	0.027027
0.013514	0.0	0.0	0.013514	0.108108					
ID = 1331	1	199	6	731	4.884607	54.888747	1		
0.261538	0.153846	0.184615	0.200000	0.030769	0.076923	0.061538	0.0	0.015385	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.015385	0.0	0.0
0.061538	0.030769	0.0	0.015385	0.0	0.015385	0.076923	0.030769	0.076923	0.030769
0.046154	0.107692	0.153846	0.092308	0.015385	0.046154	0.015385	0.0	0.0	0.0
0.015385	0.015385	0.0	0.0	0.138462					
ID = 1280	1	199	5	520	7.710469	78.669037	1		
0.0	0.021739	0.130435	0.173913	0.108696	0.304348	0.217391	0.043478	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.021739	0.0	0.021739
0.065217	0.065217	0.130435	0.195652	0.152174	0.108696	0.021739	0.0	0.0	0.0
0.0	0.0	0.021739	0.0	0.195652					
ID = 1226	2	199	10	914	5.777095	63.230774	1		
0.129032	0.193548	0.129032	0.145161	0.096774	0.177419	0.064516	0.032258	0.032258	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.016129	0.0	0.0	0.0	0.0	0.016129	0.0	0.032258	0.048387	0.080645
0.129032	0.112903	0.096774	0.112903	0.112903	0.048387	0.032258	0.0	0.016129	0.016129
0.0	0.016129	0.032258	0.0	0.080645					
ID = 1237	2	199	9	936	6.316973	63.760468	1		
0.064746	0.038668	0.116505	0.064746	0.064746	0.101456	0.200330	0.166461	0.064746	0.050447
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.016649	0.064746	0.067797
0.067797	0.116344	0.064746	0.196441	0.135593	0.067797	0.016649	0.016949	0.0	0.0
0.016949	0.016949	0.016949	0.0	0.084746					
ID = 1233	2	199	10	842	7.324716	63.349437	1		
0.171429	0.057143	0.100000	0.100000	0.142857	0.114286	0.071429	0.085714	0.042857	0.028571
0.057143	0.028571	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.051143	0.014286	0.014286	0.028571	0.042857
0.071429	0.042857	0.114286	0.114286	0.071429	0.071429	0.042857	0.100000	0.028571	0.028571
0.028571	0.0	0.0	0.028571	0.085714					
ID = 1329	2	199	6	632	6.316044	55.353531	1		
0.266667	0.133333	0.200000	0.200000	0.133333	0.200000	0.133333	0.066667	0.016667	0.033333
0.016667	0.0	0.033333	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.033333	0.0	0.0	0.016667	0.0	0.016667	0.050000	0.033333	0.033333	0.016667
0.333333	0.100000	0.066667	0.050000	0.100000	0.083333	0.016667	0.033333	0.016667	0.016667
0.016667	0.033333	0.0	0.0	0.116667					
ID = 1251	2	199	11	944	7.445179	60.621475	1