



114270

Scientific Excellence • Resource Protection & Conservation • Benefits for Canadians
Excellence scientifique • Protection et conservation des ressources • Bénéfices aux Canadiens

**Separation of Atlantic Herring (*Clupea harengus*)
Stocks in the Southern Gulf of St. Lawrence
Using Digitized Otolith Morphometrics and
Discriminant Function Analysis**

S.N. Messieh,¹ C. MacDougall,² and R. Claytor²

¹Biological Sciences Branch
Scotia-Fundy Region
Department of Fisheries and Oceans
Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, Nova Scotia B2Y 4A2

²Science Branch
Gulf Region
Department of Fisheries and Oceans
Moncton, New Brunswick E1C 9B6

April 1989

**Canadian Technical Report of
Fisheries and Aquatic Sciences
No. 1647**



Fisheries
and Oceans

Pêches
et Océans

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annuel des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences 1647

April 1989

SEPARATION OF ATLANTIC HERRING (CLUPEA HARENGUS) STOCKS IN
THE SOUTHERN GULF OF ST. LAWRENCE USING DIGITIZED OTOLITH
MORPHOMETRICS AND DISCRIMINANT FUNCTION ANALYSIS

by

S.N. Messieh,¹ C. MacDougall,² and R. Claytor²

¹Biological Sciences Branch

Scotia-Fundy Region

Department of Fisheries and Oceans

Bedford Institute of Oceanography

Dartmouth, Nova Scotia B2Y 4A2

²Science Branch

Gulf Region

Department of Fisheries and Oceans

Moncton, New Brunswick E1C 9B6

(c) Minister of Supply and Services Canada 1989

Cat. No. FS 97-6/1647E

ISSN 0706-6457

Correct citation for this publication:

Messieh, S.N., C. MacDougall, and R. Claytor. 1989. Separation of Atlantic herring (Clupea harengus) stocks in the southern Gulf of St. Lawrence using digitized otolith morphometrics and discriminant function analysis. Can. Tech. Rep. 1647: iv + 22 p.

CONTENTS

Abstract/Résumé	iv
Introduction	1
Materials and methods	1
Methods of examination	1
Methods of data analyses	2
Results	3
Description of otolith types	3
Back-calculated fish length	3
Discriminant function analysis	3
Validity test of spawning group assignments	4
Discussion	5
References	7
Tables	9
Figures	17

ABSTRACT

Messieh, S.N., C. MacDougall, and R. Claytor. 1989. Separation of Atlantic herring (Clupea harengus) stocks in the southern Gulf of St. Lawrence using digitized otolith morphometrics and discriminant function analysis. Can. Tech. Rep. Fish. Aquat. Sci. 1649: iv + 22 p.

A computer-based method for separating herring spawning groups using digitized otolith morphometrics is described. This method utilizes a binocular microscope and a camera lucida to reflect otolith images onto a HIPAD digitizing pad, a moving cursor as the input device, and an IBM personal computer for data storage. Several variables describing otolith morphometrics were tested for their ability in separating the spawning groups, and discriminant functions based on these variables were calculated.

Three otolith types representing spring-, summer-, and autumn-spawning groups were identified. Comparison of the original assignment by the age reader and results of the discriminant functions and maturation stages were in good agreement. Percentage agreements ranged between 77% and 96.4%. It is concluded that otolith morphometrics and discriminant function analysis can be used successfully in separating herring spawning groups in the southern Gulf of St. Lawrence.

RÉSUMÉ

Messieh, S.N., C. MacDougall, and R. Claytor. 1989. Separation of Atlantic herring (Clupea harengus) stocks in the southern Gulf of St. Lawrence using digitized otolith morphometrics and discriminant function analysis. Can. Tech. Rep. Fish. Aquat. Sci. 1649: iv + 22 p.

Le présent rapport décrit une méthode informatique utilisée pour distinguer différents groupes de frayeurs chez le hareng, à l'aide de l'otolithométrie sur enregistrement numérique. Cette méthode nécessite un microscope monoculaire et une chambre claire afin de refléter les images des otolithes sur une table traçante numérique HIPAD, un curseur mobile comme organe d'entrée et un ordinateur individuel IBM pour la mémorisation des données. On a fait l'essai de plusieurs variables qui décrivent la morphométrie des otolithes et on a calculé les fonctions discriminantes en se fondant sur ces variables.

On a identifié trois types d'otolithes, représentant les géniteurs de printemps, d'été et d'automne respectivement. Les résultats de l'examen original effectué par le préposé à la détermination de l'âge du hareng correspondent aux résultats fondés sur les fonctions discriminantes et les stades de maturation. Le degré de concordance varie entre 77% and 96,4%. On peut donc conclure que l'otolithométrie et l'analyse des fonctions discriminantes peuvent être utilisées avec succès dans la distinction des groupes de géniteurs du hareng vivant dans la zone sud du golfe du Sainte-Laurent.

INTRODUCTION

The identification of Atlantic herring (*Clupea harengus*) stocks inhabiting both sides of the northern Atlantic has been the subject of numerous investigations for many years (Cushing 1981). Since the first Canadian Fisheries Expedition in the Gulf of St. Lawrence in 1914-15, Lea (1919) concluded, "several local tribes of herring exist, each having its own particular area of distribution." In more recent years the discreteness of the spring and autumn herring populations in the Gulf of St. Lawrence has been established. Moreover, there is general agreement that each of the two spawning groups comprises a complex stock though they are less identifiable and their genetic isolation not established (Messieh and Tibbo 1971; Parsons 1973; Kornfield et al. 1982; Kornfield and Bogdanowicz 1987).

The separation of herring stocks is important for delineating proper fishery management units. Several methods employing univariate and multivariate techniques have been used to analyze meristic characters (e.g. Parsons 1973; Messieh 1975), morphometric characters (Parsons 1975), otolith characteristics (Messieh 1972; Bird et al. 1986), and electrophoresis (Kornfield et al. 1982). Separation of stocks was successful at the group level but was difficult on an individual basis due to overlapping characteristics.

Otolith characteristics, such as their shape and first year's growth (Messieh 1972), have been employed in both the Department of Fisheries and Oceans' Gulf and Newfoundland Regions for separating spring- and autumn-spawned groups. This method requires well-trained technicians who have considerable experience in herring otolith examination. The assignment of the spawning groups by these technicians was sometimes criticized because of its subjectivity.

However, a semi-automated microcomputer-based measuring system has been developed for otolith measurements in the Gulf Region (Messieh and MacDougall 1985) to eliminate subjectivity. This method uses a microscope with a drawing tube and a digitizer interfaced to a personal computer (PC) which analyzes the digitized otolith measurements using software packages. The purpose of this report is to present results using this system to separate herring spawning groups by otolith morphometrics.

MATERIALS AND METHODS

METHODS OF EXAMINATION

Samples of herring from commercial fisheries are routinely examined every year for biological data such as fish length, weight, maturity, age, and spawning group. Samples used in the present study involved stratified herring samples (about 2,000 fish) collected from the spring and autumn fisheries in the southern Gulf of St. Lawrence, during 1985 and 1986. Spawning groups were assigned by methods described elsewhere (Messieh and MacDougall 1984; 1985).

l₁s were back-calculated for sub-samples of fish of different otolith types. Back-calculation was based on linear relationship between fish length and otolith size at time of formation of the first annulus.

A semi-automated measuring system was used to measure otolith morphometrics. The system consists of a binocular microscope (15x) with a drawing tube, and a HIPAD digitizing tablet interfaced to an IBM PC. An otolith image from the microscope is reflected on the digitizing tablet, and data points representing measurements are entered into the computer. The HIPAD digitizing tablet is a compact digitizer with an active surface area of approximately 28 x 28 cm and a cursor or stylus as the input device. There is no external controller necessary, as all of the electronic circuitry is contained within the HIPAD case. The data rate is up to 100 coordinate pairs per second; the resolution is 0.125 mm. Precision of digitized measurements was checked by comparing input points on the digitizer with a set of pre-measured angles along different radii (Fig. 1).

Four otolith measurements (L1PROST, L1ROST, L2PROST; L2ROST) were taken (Fig. 1). L1PROST is the distance between the nucleus and postrostrum at the first annulus. L2PROST is the distance between the nucleus and postrostrum at the second annulus. L2ROST is the distance between the nucleus and rostrum at the second annulus. For samples collected in 1986, another variable (ANG1), which is the angle ABC (Fig. 2), was also measured.

METHODS OF DATA ANALYSES

Simple computer programs for data entry and a statistical analysis system (SAS) run on both a mainframe and an IBM PC were used for data analysis. Univariate analysis for estimating means and analysis of variance (ANOVA) were performed. Discriminant analysis programs were used to examine the interrelationships between the different spawning groups. Discriminant function (DISCRIM), stepwise discriminant function (STEPDISC), and canonical analysis (CANDISC) were used.

The pairwise discriminant analysis procedure tests variance/covariance matrices for homogeneity and pools if the hypothesis is rejected (Morrison 1976). The generalized distance function (Rao 1952) was used as a measure of the distance between multivariate populations in terms of the overlapping. Both the linear discriminant function (LDF) and the quadratic discriminant function (QDF) were tested as suggested by Misra (1985).

Validity of the application of the discriminant function from the classification set was tested by a "blind test" on unknown samples. The age reader was asked to assign the spawning group for each otolith by two methods. First, the assignment to spawning group was carried out by an experienced technician on the basis of his experience in herring aging. Secondly, the assignment of the same samples was done on the basis of digitized measurements and without knowledge of original assignments or maturity stages. The two results were then compared with the maturity stages of fish sampled.

RESULTS

DESCRIPTION OF OTOLITH TYPES

Three otolith types were initially identified (Fig. 3) and described as follows:

- Autumn-Spawning Group (A-type): The otolith has a nucleus with a hyaline or translucent appearance; the hyaline appearance is sometimes masked by overgrowth from outer layers. The postrostrum is more developed than is the pararostrum. The first annulus outside of the nucleus which represents the actual second winter is large, and its rostrum and antirostrum are well developed.
- Spring-Spawning Group (P-type): The otolith usually has an overgrown opaque center with no visible nucleus. The pararostrum and postrostrum are equally developed. The first annulus is smaller in size than is the A-type. This annulus shows a well developed rostrum, but the antirostrum is less developed.
- Summer-Spawning Group (E-type): The otolith has an overgrowth opaque center with no visible nucleus. The first annulus is smaller in size than is either the A-type or P-type. This annulus is oval or pear-shaped, indicating little development of the rostrum. The summer-spawning type (E-type) otoliths were found to comprise two groups: the first group has an otolith with well developed pararostra (similar to the P-type), and was denoted PE-type. The second group has otoliths with less developed pararostra (similar to the A-type) and was denoted AE-type.

BACK-CALCULATED FISH LENGTH

Back-calculated fish lengths of the otolith types (A-type, P-type, E-type) were significantly different (Table 1). Mean l_1 for the A-type was 60 ± 8 mm. This is the length of fish at the time of formation of first annulus, which is in this case the nucleus. Mean l_2 (actual first annulus outside of the nucleus) was 178 ± 17 mm. Mean l_1 s of the E-type and P-type were 121 ± 20 mm and 160 ± 18 mm, respectively.

DISCRIMINANT FUNCTION ANALYSIS

Variables describing otolith morphometrics (Fig. 2) were tested for their ability to separate the spawning groups. Analysis of variance and results of the Waller-Duncan test (Table 2) of a random sample including 900 observations (200 of each spawning group) showed that the four variables ($L1PROST$, $L1ROST$, $L2PROST$, and $L2ROST$) were contributing to the separation of the groups.

Linear and quadratic discriminant function analyses based on these characters were only slightly different. However, because of heterogeneity of within-covariance matrices, data were not pooled and the quadratic discriminant function was used only in subsequent analysis. Results of

classification of herring spawning groups by quadratic discriminant function analysis using four variables are presented in Table 3.

A general linear regression analysis (GLM) on the four variables showed that LlPROST and LlROST variables were contributing to most of the group separation. A discriminant function analysis using only these two variables showed results similar to those based on four variables (Table 3). Agreement for the A-type was 91.7% using two variables compared to 91.5% using four variables; for the E-type, agreement was 95.7% compared to 96.4%; for the P-type, agreement was 77.0% compared to 77.7%

Results of the classification data set using two variables were applied to a test data set comprising of 150 fish of known spawning origin (Table 4). Agreement between the classification set and test data set was 98.4% for the A-type, 96.0% for the E-type, and 77.6% for the P-type. Canonical plots of the first and second canonical variates for the three spawning groups using two variables are shown in Figure 4.

The affinity of the E-type (summer spawners) otolith to either spring- or autumn-spawning group was examined by comparing the morphology of their pararostra. Measurement of the angle ABC (Fig. 1), which represents the developmental rate of the pararostra, showed that the summer spawners comprise two groups of fish. The first group (denoted AE-type) has a postrostrum angle of a mean value of 70.97 ± 6.69 , which is similar to the postrostrum angle of the autumn-spawning group (A-type). The second group (denoted PE-type) has a postrostrum angle of a mean value of 80.98 ± 8.22 , which is similar to the postrostrum angle of the spring-spawning group (P-type) (see Table 5).

Means and standard deviations of the three variables (LlPROST, LlROST, and ANGl) used in the discriminant function to separate the four groups are presented in Table 5. Analysis of variance (Table 6) showed that all three variables contributed to the separation of the spawning groups ($R^2 = 0.88$, 0.67 , and 0.44). In contrast, correlations between areas and between ages were weak ($R^2 = 0.24$ to 0.41 between areas, and $R^2 = 0.11$ to 0.22 between ages). There were no differences between sexes (Table 6).

The Waller-Duncan k-ratio t-test (Table 7) indicated significant differences in the spawning groups. Slight differences were observed in only two areas and one age group.

Results of the classification set by the discriminant function (Table 8) showed a good agreement with the original assignments, ranging between 82.9% and 100%. Mahalanobis distances (Table 9) showed the largest distance (7.99) between the A-type and the PE-type. The smallest separation (3.07) was between the AE-type and the PE-type.

Plots of the canonical variates using three variables (Fig. 5) show the distribution of the observations of the four spawning groups on the first and second canonical variates.

VALIDITY TEST OF SPAWNING GROUP ASSIGNMENTS

Comparison of the original assignment of spawning groups by the age reader and by the discriminant function for the 1985 and 1986 classification data set showed a good agreement. In 1985, percent correct classification ranged from 77.0% for the spring-spawning group to 91.7% for the autumn-spawning group (Table 10). In 1986, correct classification ranged from 82.9% to 100% for the two groups respectively (Table 11).

Validity of the classification into spawning groups by the discriminant function was further examined by comparison with the maturity stages. Results of assignment of a test data set including 1,572 fish showed 75.0% agreement between the discriminant function and maturity stages results (Table 12). Of the 25.0% disagreement, 13.5% were reclassified from original assignment by maturity stages as an autumn-spawning group, to a spring-spawning group assignment by the discriminant function. The remainder (11.5%) were reclassified the other way around.

Results of the blind test of spawning group assignments of samples of known origin were comparable to results obtained from the larger data set (Table 12). To achieve objectivity of the test, two anonymous investigators picked three otolith trays without knowledge of the age reader and changed their labels with different codes to mask the identity and origin of these samples. The first tray included 26 pairs of otoliths; the second, 50 pairs; the third, 49 pairs.

The first sample showed 76.9% agreement between spawning assignments by the discriminant function and the age reader. This sample (26 fish) was taken from a purse-seine catch in Miscou Flats, off the Bay of Chaleur on August 18, 1986. All of the fish were ripe (Maturity Stage 6). Results of the discriminant function analysis showed that 76.9% of these fish belong to the autumn-spawning group. The second sample (50 fish) was taken from a gill net catch near Lismore, N.S., on August 29, 1986. Ninety-two percent of these fish were in Maturity Stage 6; 6%, in Maturity Stage 5; and 2%, in Maturity Stage 4. Results of the discriminant function analysis showed that 96.0% of this sample belonged to the autumn-spawning group. The third sample (49 fish) was taken from a gill net catch near Blue Cove, Bay of Chaleur, on May 9, 1986. Seventy-nine percent of these fish were ripe (Stage 6), 4% were near ripe (Stage 5), 2% were spent (Stage 7), and 15% were recovering or maturing (Stage 8). Results of the discriminant function analysis showed that 75.5% of this sample belonged to the spring-spawning group.

DISCUSSION

The computer-based measuring system presented here provides a rapid and quantitative method for examining otolith morphometrics. The advantages of the HIPAD digitizing measuring system are its simplicity, the increased efficiency and precision of measurements, and the elimination of transcribing and editing errors. Measurements can be easily made by moving the digitizing cursor, and data are simultaneously entered into the computer. Consequently, a sample of 100 fish can be measured for four to five variables in approximately 2 h with the data formatted for immediate statistical analysis.

Traditionally, the identification of otolith types and spawning groups have been carried out by technicians who have been involved in herring aging for a long time. This is a tedious job and requires much experience. The use of the digitizing measuring system has overcome this problem.

The difference in the general shape of otolith types is explained in terms of morphological differences among herring spawning groups. Genetic basis for the separation between spring-spawning and autumn-spawning groups has been established (Kornfield et al. 1982). Results of genetic polymorphism by electrophoresis showed significant differences, indicating genetic isolation of the two spawning populations. Within both spring- and autumn-spawning populations, significant spatial heterogeneity was noted but was not temporally stable. Messieh and Tibbo (1971) and Messieh (1972) provided evidence for the discreteness of the spring- and autumn-spawning populations, based on meristic differences and otolith types.

The most prominent differences in otolith shape between spring- and autumn-spawning populations is the relative development of the pararostrum and the adjacent excisura minor. In the case of a spring-spawning population, the pararostrum is more developed, thus forming a wider angle (Angle ABC, Fig. 2 and 3). Another difference is the radius of the first annulus (L1PROST and L1ROST) which reflects the first summer growth period. For spring-spawned fish, sufficient growth occurs before winter, allowing the formation of the first annulus. For autumn-spawned fish, no growth would be expected until the following summer. Thus, the first annulus in this case represents approximately a 15-mo growth period (Table 1). The summer-spawned fish (E-type otolith) would have a first annulus similar to that of spring-spawned fish, though smaller in size because of a missing part of the summer growth season.

Discriminant functions based on three variables of otolith morphometrics (L1PROST, L1ROST, and ANGL) were found adequate in separating the spawning groups. The high level of agreement between the assignments by the discriminant function analysis, the original assignment by experienced age reader, and assignments based on maturity stages provide supporting evidence for the validity of results of the discriminant functions.

The separation of the summer-spawning population into two groups (AE-type and PE-type) based on shape and development of otolith pararostrum indicated that this group comprises a mixture of populations of spring-spawning and autumn-spawning origin. This is in agreement with previous studies which showed that the herring spawning season in the Gulf of St. Lawrence extends from late April through to the end of June for spring spawners, and from late July through to the end of September for autumn spawners. Hence, it is conceivable that the summer-spawning group (E-type) described in the present study comprises two components: a late-spawning component of the spring-spawning populations, and an early-spawning component of the autumn-spawning population. A hypothetical model showing the relationships among the spawning groups is presented in Figure 6.

In conclusion, otolith morphometrics and discriminant function analysis can be used successfully in separating herring spawning groups in the southern Gulf of St. Lawrence. Three variables: the angle of the pararostrum, the

diameter of the first annulus toward the rostrum, and the diameter of the first annulus toward the postrostrum, were successful in separating four spawning groups. The use of a microcomputer-based digitizing system provides rapid, precise, and objective means for measuring otolith morphometrics and stock separation.

REFERENCES

- Bird, J.L., D.T. Eppler, and D.M. Checkley. 1986. Comparisons of herring otoliths using Fourier series shape analysis. *Can. J. Fish. Aquat. Sci.* 43: 1228-1234.
- Cushing, D.H. 1981. Fisheries Biology. Univ. Wisconsin Press: 295 p.
- Kornfield, I. and S.M. Bogdanowicz. 1987. Differentiation of mitochondrial DNA in Atlantic herring, Clupea harengus. *U.S. Fish. Bull.* 85(3): 561-568.
- Kornfield, I., B.D. Sidell, and P.S. Gagnon. 1982. Stock definition in Atlantic herring (Clupea harengus harengus): Genetic evidence for discrete fall and spring spawning populations. *Can. J. Fish. Aquat. Sci.* 39: 1610-1621.
- Lea, E. 1919. Age and growth of the herring in Canadian waters. *Can. Fish. Exped.* 1919: 75-164.
- Messieh, S.N. 1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. *J. Fish. Res. Board Can.* 29: 1113-1118.
- 1975. Delineating spring and autumn herring populations in the southern Gulf of St. Lawrence by discriminating function analysis. *J. Fish. Res. Board Can.* 32: 471-477.
- Messieh, S.N. and C. MacDougall. 1984. Spawning groups of Atlantic herring in the southern Gulf of St. Lawrence. *Can. Atl. Fish. Sci. Adv. Comm. Res. Doc.* 84/74: 28 p.
- 1985. A computer based method for separating herring spawning groups using digitized otolith morphometrics. *Can. Atl. Fish. Sci. Adv. Comm. Res. Doc.* 85/106: 13 p.
- Messieh, S.N. and S.N. Tibbo. 1971. Discreteness of herring populations in spring and autumn fisheries in the southern Gulf of St. Lawrence. *J. Fish. Res. Board Can.* 28: 1009-1014.
- Misra, R.K. 1985. Quadratic discriminant analysis with covariance for stock delineation and population differentiation: A study of beaked redfishes (Sebastes mentella and S. fasciatus). *Can. J. Fish. Aquat. Sci.* 42: 1672-1676.
- Morrison, D.F. 1976. Multivariate statistical methods. McGraw-Hill Book Co. (New York, NY): 415 p.

Parsons, L.S. 1973. Meristic characteristics of Atlantic herring (Clupea harengus harengus L.) stocks in Newfoundland and adjacent waters. Res. Bull. Int. Comm. Northw. Atl. Fish. 10: 37-52.

Rao, R.C. 1952. Advanced statistical methods in biometric research. John Wiley and Sons, Inc. (New York, NY): 390 p.

Table 1. Back-calculated herring length-at-age (mm) of different otolith types.

Otolith type	Spawning Group	Number of Fish	l ₁		l ₂	
			\bar{X}	SD	\bar{X}	SD
A-type	Autumn-spawned	98	60	8	178*	17
E-type	Summer-spawned	100	121	20	209	16
P-type	Spring-spawned	100	160	18	232	14

* For autumn-spawned fish this is the first annulus outside the nucleus.

Table 2. Analysis of variance procedure (ANOVA) and results of Waller-Duncan test on 4 dependant variables tested for separating herring spawning groups.

Dependant Variables	F-Value	R-Square	Waller-Duncan Grouping		
			Autumn,	Spring,	Summer
L1PROST	1828.3*	0.803	1.130	0.909	0.647
L1ROST	807.6*	0.643	1.510	1.291	0.923
L2PROST	729.8*	0.619	1.644	1.485	1.234
L2ROST	622.2*	0.581	2.240	2.078	1.729

* P > 0.001

Table 3. Classification of herring spawning groups by quadratic discriminant function analyses using different sets of variables.

=====
 Percent of Fish Classified into Spawning Group
 (four variables used)

From Spawning Group	A	E	P	Number of Fish
A	91.5	0	8.5	586
E	0	96.4	3.6	332
P	14.7	7.6	77.7	1035

Percent of Fish Classified into Spawning Group
 (two variables used)

From Spawning Group	A	E	P	Number of Fish
A	91.7	0	8.3	586
E	0	95.7	4.3	332
P	15.0	8.0	77.0	1035

Table 4. Classification of test data set of known spawning origin by quadratic discriminant function, using 2 variables, for samples collected in 1985.

=====				
Number of observations and Percents Classified into spawning group				
From Spawning Group	A	E	P	Total
A	55 94.8	0 0	3 5.2	58 100
E	0 0	24 96.0	1 4.0	25 100
P	5 7.5	10 14.9	52 77.6	67 100
Total	60 40.0	34 22.7	56 37.3	150 100

Table 5. Mean(x), Standard deviation (SD) and Coefficient of Variability (CV) of 3 variables used in separating the spawning groups by discriminant function analysis.

=====										
Otolith Type	N	L1PROST			L1ROST			ANG1		
		\bar{x}	SD	CV	\bar{x}	SD	CV	\bar{x}	SD	CV
	99	1.15	0.12	10.18	1.64	0.12	7.55	69.51	7.09	10.19
AE	101	0.68	0.08	12.47	1.12	0.18	16.45	70.97	6.69	9.43
P	100	0.92	0.11	11.93	1.03	0.45	43.92	77.87	6.78	8.71
PE	100	0.55	0.08	15.46	0.59	0.23	39.54	80.98	8.22	10.15

Table 6. Results of analysis of variance (ANOVA) of 3 variables used in discriminant function analysis.

ANOVA Source	Variable	Mean	F Value	R ²
Between Spawning Groups	L1PROST	0.82	679.9	0.88
	L1ROST	1.09	186.3	0.67
	ANG1	75.18	71.9	0.44
Between Areas	L1PROST	0.82	17.4	0.24
	L1ROST	1.09	38.6	0.41
	ANG1	75.18	17.2	0.24
Between Ages	L1PROST	0.82	8.3	0.22
	L1ROST	1.09	4.2	0.12
	ANG1	75.18	3.8	0.11
Between Sexes	L1PROST	0.82	0.1	0.00
	L1ROST	1.09	0.2	0.00
	ANG1	75.18	1.2	0.00

Table 7. Results of Waller-Duncan K-ratio for 3 variables used in discriminant function analysis.

Source	Group	Waller Grouping		
		L1PROST	L1ROST	ANG1
Spawning	A	1.17	1.65	82.74
Group	P	0.92	1.11	78.84
	AE	0.65	1.03	69.80
	PE	0.55	0.57	69.33
	Minimum Significant Diff.	0.03	0.08	1.95
Area	431	0.59	0.74	83.42
	432	1.05	1.56	70.12
	433	0.91	1.30	72.48
	436	0.80	0.66	78.08
	437	0.73	0.91	78.65
	438	0.81	1.23	72.54
	Minimum Significant Diff.	0.10	0.15	3.26
Age Groups	3	1.03	1.38	72.94
	4	0.93	1.17	75.96
	5	0.77	0.89	78.27
	6	0.71	1.00	75.68
	7	0.77	1.17	71.29
	8	0.64	1.00	73.93
	9	0.75	1.10	74.56
Minimum Significant Diff.	0.35	0.74	14.11	

Table 8. Classification data set of 4 spawning groups by quadratic discriminant function analysis using 3 variables.

=====

Number of Observations and Percents Classified into

From Spawning Group	A	AE	P	PE	Total
A	70 100.0	0 0	0 0	0 0	70 100.0
AE	0 0	66 94.3	0 0	4 5.7	70 100.0
P	4 5.7	6 8.6	58 82.9	2 2.9	70 100.0
PE	0 0	5 7.1	0 0	65 92.9	70 100.0
Total	74 26.4	77 27.5	58 20.7	71 25.4	280 100.0

=====

Table 9. Mahalanobis distances between the spawning groups using 3 variables.

Spawning Group	A	AE	P	PE
A	0	-	-	-
AE	5.97	0	-	-
P	3.80	3.43	0	-
PE	7.99	3.07	4.37	0

Table 10 Results of agreement between original assignments and discriminant function analysis of the classification data set in 1985.

Percent of fish classified into spawning group				
From Spawning group	A	E	P	Total
A	91.7	0	8.3	300
E	0	95.7	4.3	300
P	15.0	8.0	77.0	300

Table 11 Results of agreement between original assignments and discriminant function analysis of the classification data set in 1986.

=====

Percent of fish classified into spawning group

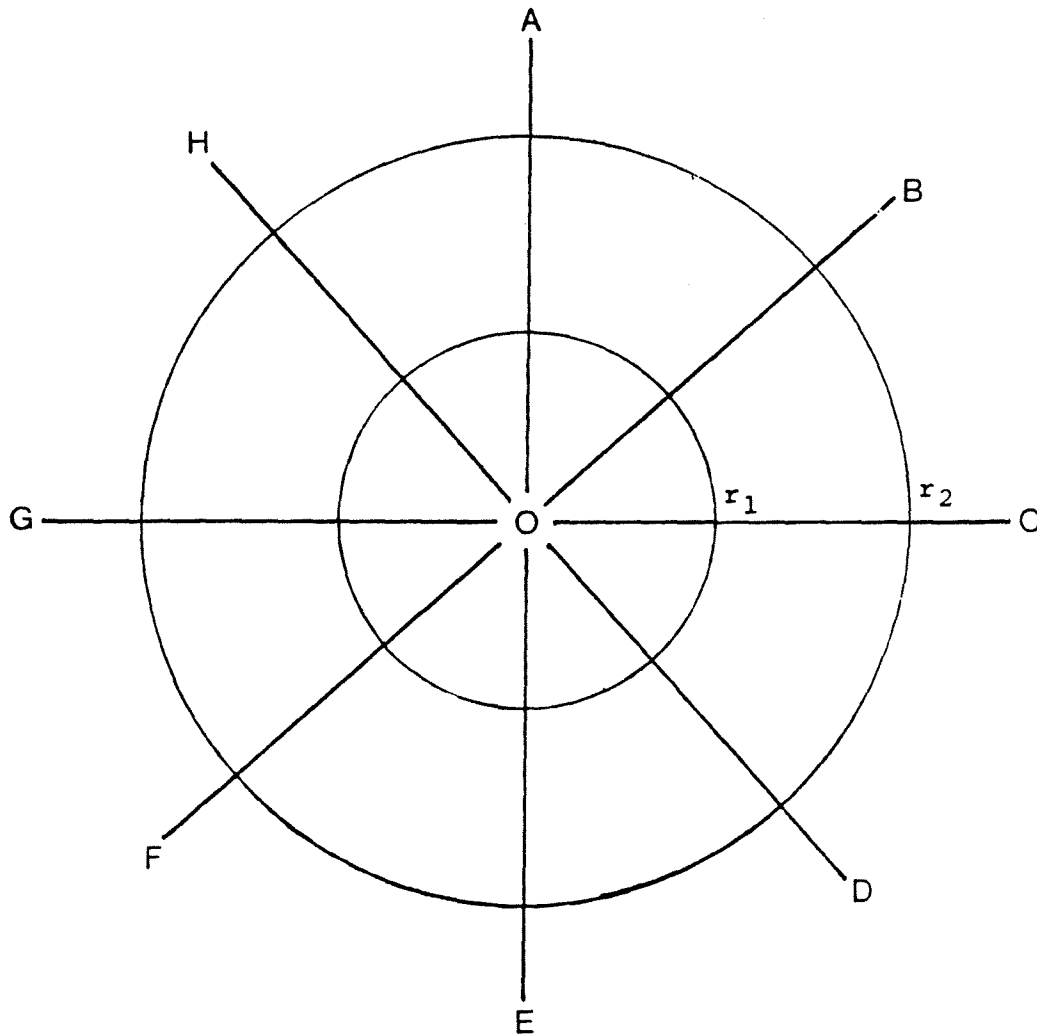
From Spawning group	A	AE	P	PE	Total
A	100.0	0	0	0	70
AE	0	94.3	0	5.7	70
P	5.7	8.6	82.9	2.8	70
PE	0	7.1	0	92.9	70

=====

Table 12 Percent agreement of spawning group assignments between different methods: (A) samples of known origin (blind test); (B) test data set.

Agreement between methods	Blind Test	Test Data Set
Age reader vs. maturity stage	88.2	89.9
Discriminant function vs. maturity stage	83.1	75.0
Age reader vs. discriminant function	78.1	79.1
Reclassified from autumn to spring group by discriminant function	6.7	13.5
Reclassified from spring to autumn group by discriminant function	10.1	11.5
Total number of fish	125	1572

=====



ANGLE	ACTUAL	DIGITIZED (r_1)	DIGITIZED (r_2)
AOB	45	45.6	45.4
AOC	90	91.0	91.1
AOD	135	135.8	135.0
AOH	45	44.5	44.7
AOG	90	90.0	89.1
AOF	135	134.3	134.1

Figure 1: Precision test of the HIPAD digitizing tablet at different angles and radii.

DIAGRAM OF MEASUREMENTS
FOR HERRING OTOLITH
SPAWNING GROUP SEPARATION

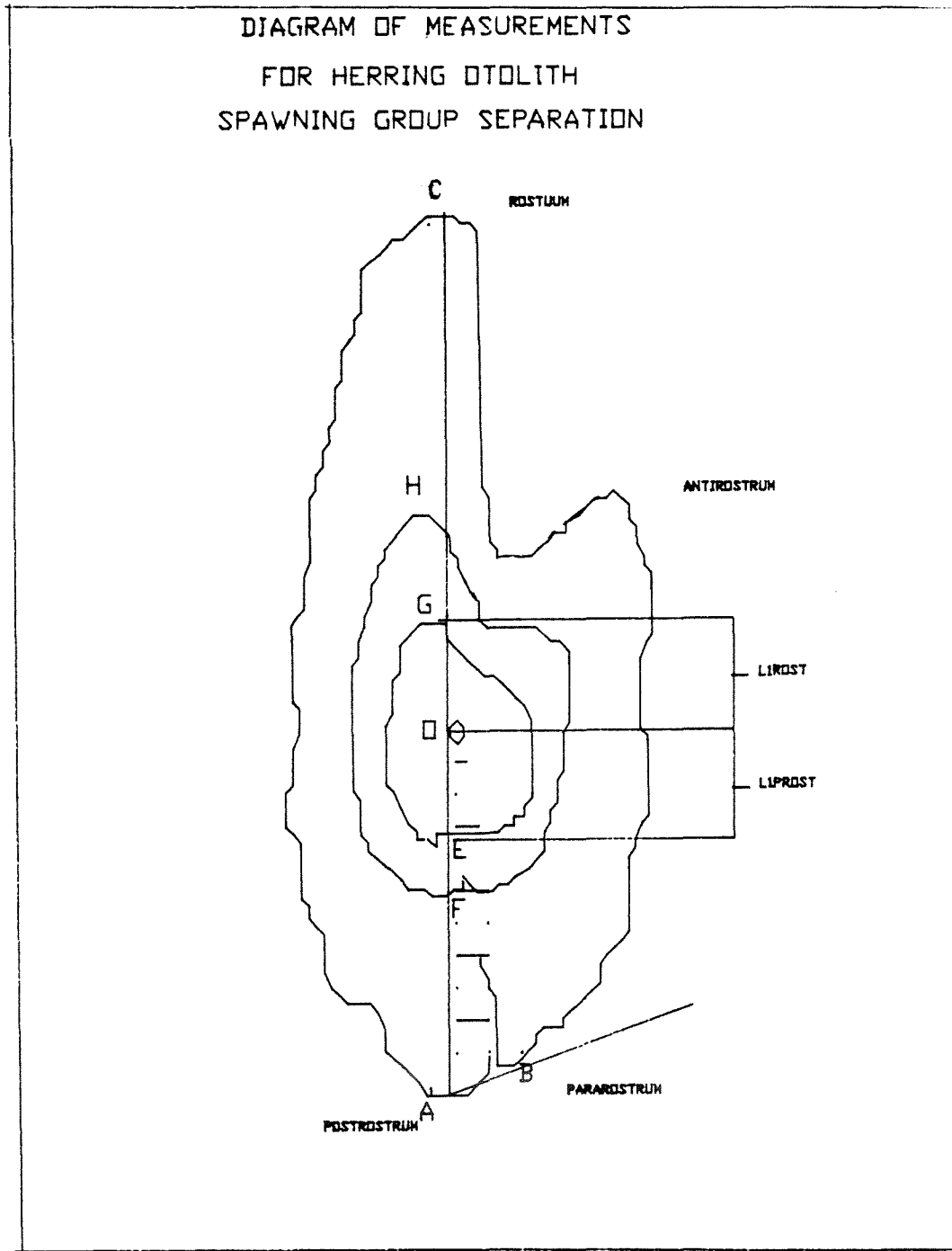


Figure 2: Plot of herring otolith showing different parts and points entered into the digitizer measuring system for input variables.

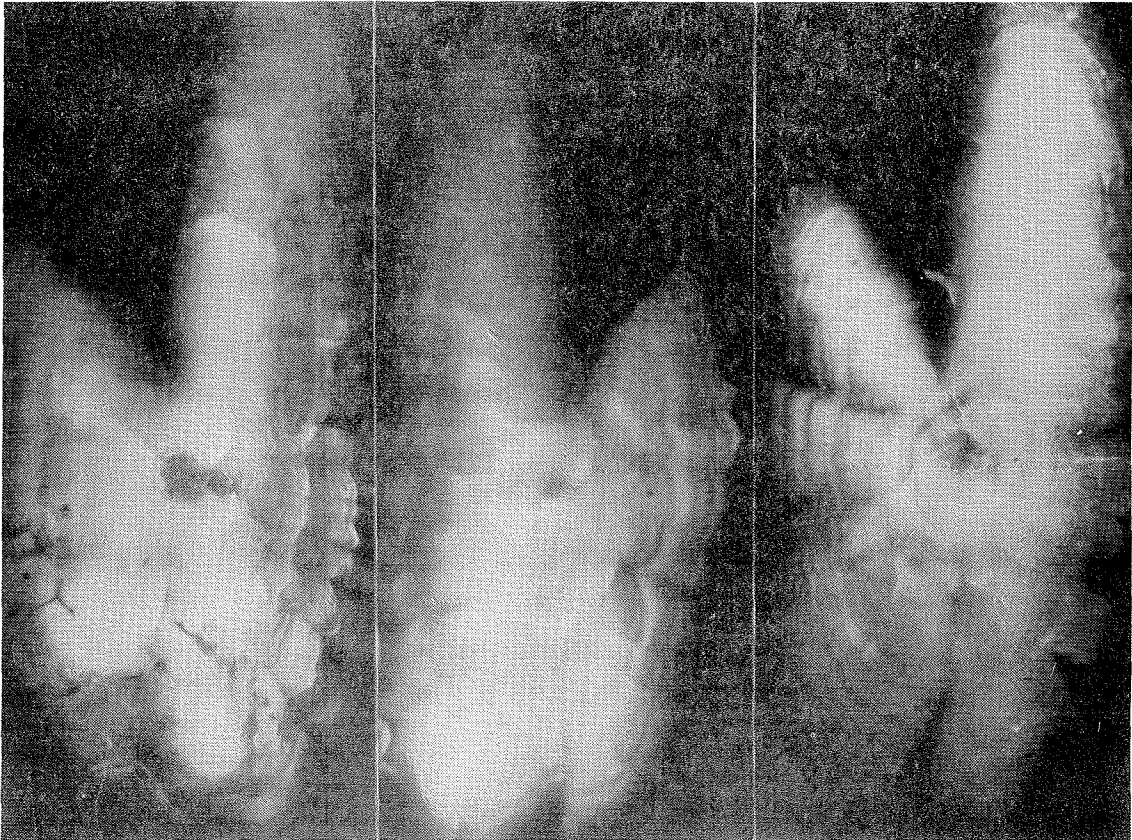
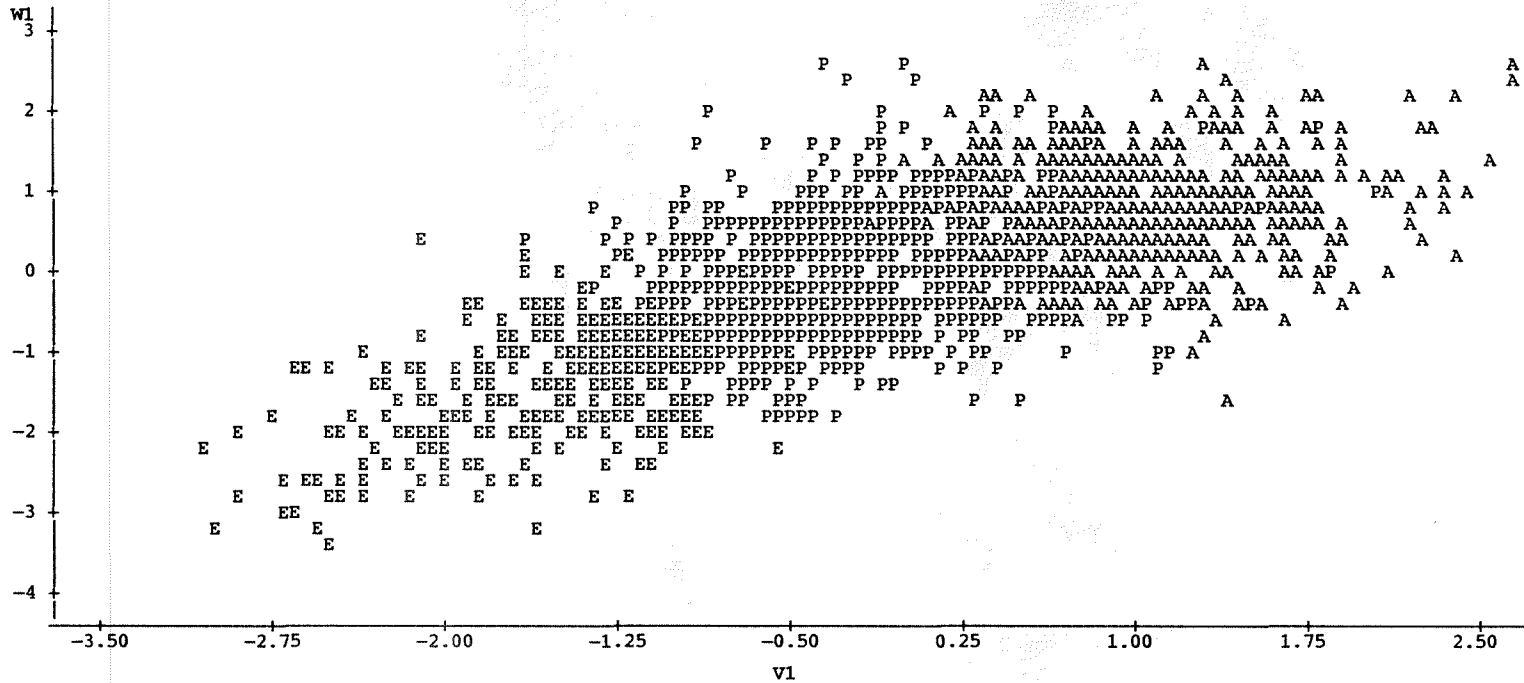


Figure 3: Types of herring otoliths:
A-type; Autumn spawning group
P-type; Spring spawning group
E-type; Summer spawning group

Classification of Spawning Herring Otolith
 PLOT OF W1*V1 SYMBOL IS VALUE OF SPGP

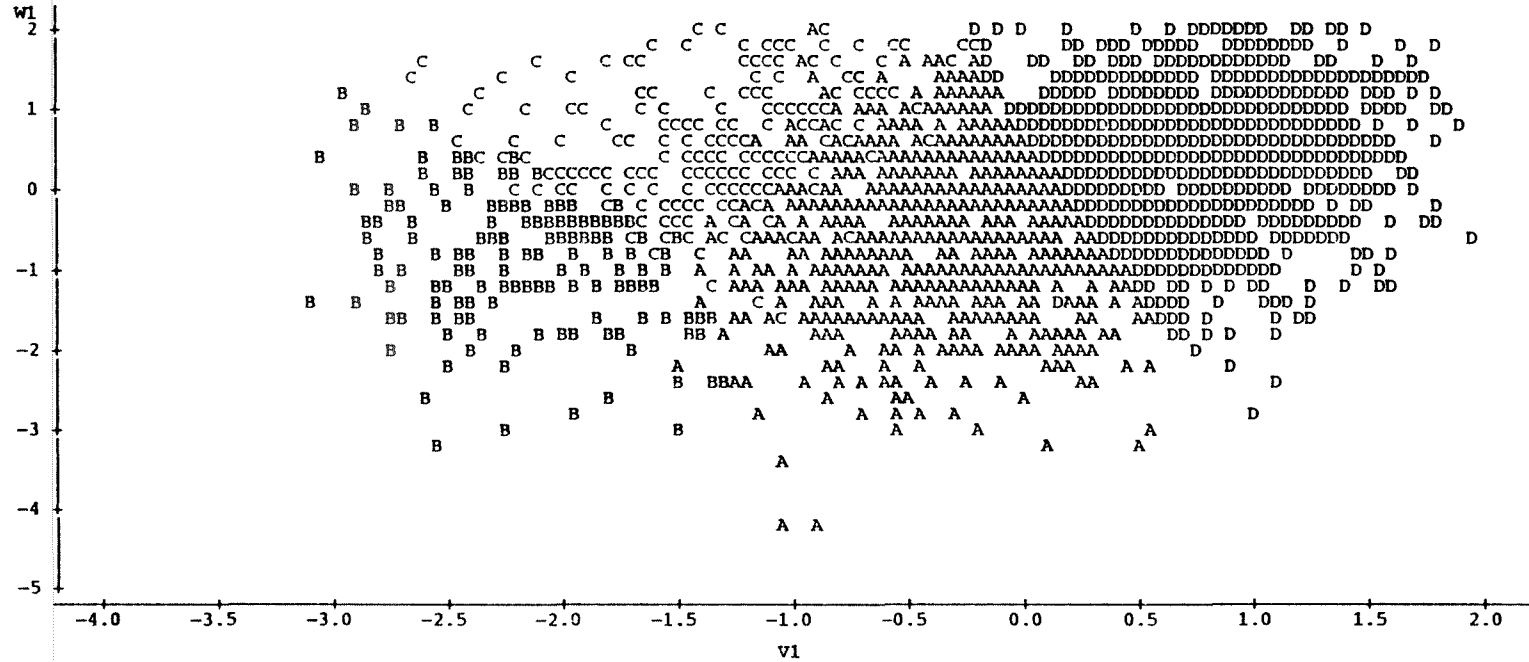


NOTE: 1059 OBS HIDDEN

Figure 4: Canonical plots showing the separation of herring spawning groups using 2 variables (LIPROST; LIROST).

Classification of Spawning Herring Otolith
 Canonical Plots of Four Spawning Components

PLOT OF W1*V1 SYMBOL IS VALUE OF TYPE



NOTE: 1374 OBS HIDDEN

Figure 5: Canonical plot showing the separation of four herring spawning groups: A= P-type; B= PE-type; C= AE-type; D= A-type.

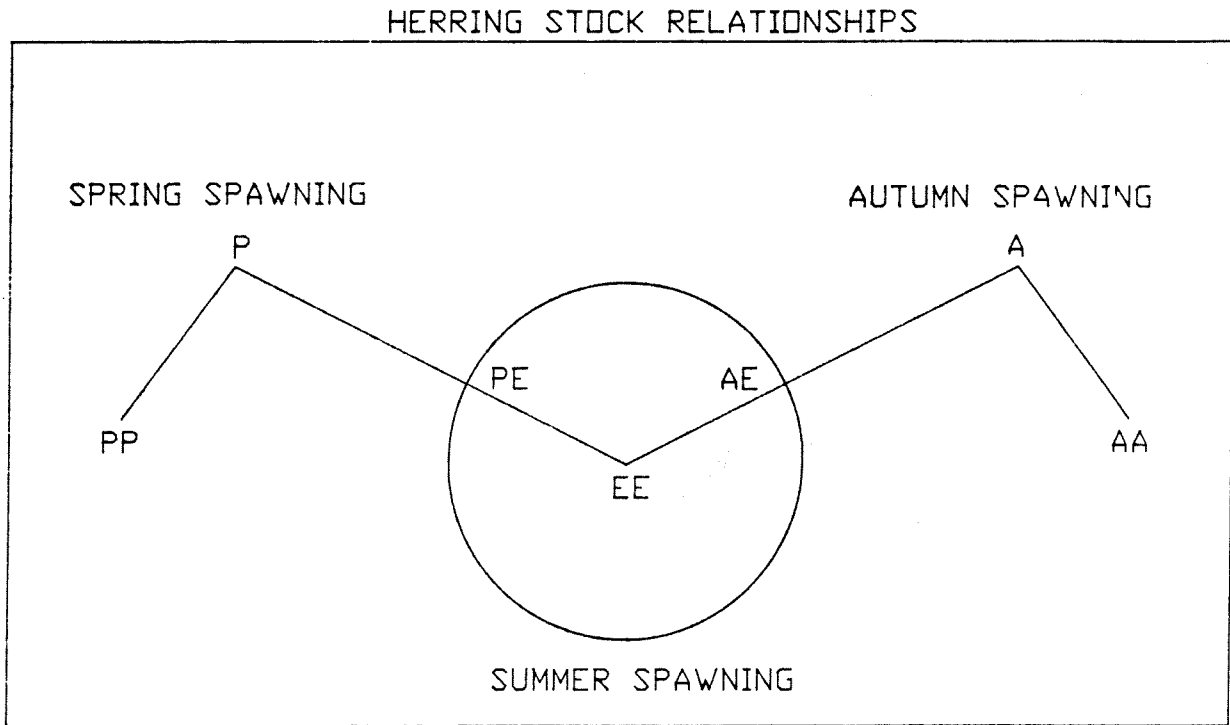


Figure 6: A model based on otolith morphometrics showing the relationship between spring, summer and autumn spawning groups in the southern Gulf of St. Lawrence.