A COMPUTER BASED METHOD FOR SEPARATING HERRING SPAWNING GROUPS USING DIGITIZED OTOLITH MORPHOMETRICS

by

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ABSTRACT

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-PC computer for data storage.

Nine variables for otolith dimensions were taken. A graphic software (AUTOCAD) with a Bernoulli disc drive was used for plotting. SAS statistical software packages DISCRIM, STEPDISC and CANDISC were used for analyses. Three spawning groups of herring: spring, summer and autumn, were separated. Percentage agreement of the original assignment by otolith characteristics and the results of discriminant function analysis were 77.7%, 93.9% and 85.4% for the three spawning groups respectively.

The new technique is recommended for herring ageing and the assignment of their spawning groups. It is faster and more accurate than the current method since it reduces human error, and requires little experience by the operator.

RESUME

Une méthode informatique de séparation des groupes de géniteurs chez le hareng à partir de données morphométriques sur les otolithes est décrite. Dans cette méthode, on utilise un microscope binoculaire et une chambre claire pour réfléchir des images d'otolithes sur un digitaliseur HIPAD, un curseur mobile comme dispositif d'entrée et un ordinateur IBM-PC pour stocker les données.

Neuf variables de dimension des otolithes ont été mesurées. Le traçage a été réalisé au moyen d'un logiciel graphique (AUTOCAD) et d'une unité de disques. Les modules DISCRIM, STEPDISC et CANDISC du progiciel statistique SAS ont été utilisés pour les analyses. Trois groupes de géniteurs (printemps, été et automne) ont été séparés. Les pourcentages de correspondance entre le classement original selon les caractéristiques des otolithes et les résultats d'analyse de fonctions discriminantes ont été de 77,7, 93,9 et 85,4 % pour les trois groupes respectivement.

La nouvelle technique est recommandée pour déterminer l'âge des harengs et classer leurs groupes de géniteurs. Elle est plus rapide et plus précise que la méthode actuelle car elle diminue l'erreur humaine et exige de l'opérateur peu d'expérience.

INTRODUCTION

The structure of herring stocks in the Gulf of St. Lawrence is complex (Messieh 1985). Separation of the spawning groups has been the subject of considerable research because of its importance in delineating proper management-units. Several methods have been used for stock separation, including meristics (e.g. Messieh and Tibbo 1971; Parsons 1972), morphometrics (e.g. Messieh 1972), electrophoresis (e.g. Kornfield et al 1982) and tagging (e.g. Winters and Beckett 1978).

Results of these studies were in general agreement that there are spring and autumn spawning groups, with each comprising a less identifiable stock. In 1984, Messieh and MacDougall (1984) identified a summer spawning group which differed in otolith characteristcs and growth from the spring and autumn groups.

The above methods of separating the spawning groups were successful on a group basis, but separation on an individual basis was difficult and sometimes impossible because of the overlap between their characteristics. Maturation stages and gonadosomatic indices were suggested for identifying the spawning groups but in recent years difficulties were encountered in the application of these methods (Hunt 1983). Cleary et al (1982) proposed histological analysis of gonads to improve the assignment of maturity stages, but this method was not appropriate for routine application and use in day-to-day laboratory operation.

Recently, the technical staff of the Gulf Region, Moncton adopted a method for separating the spawning groups based on the characteristics of herring otoliths in addition to their maturity stages. Similar methods, although they may vary in minor details, are conducted by the St. John's, Newfoundland laboratory. Both the Moncton and St. John's methods require technicians who have considerable experience in herring otolith examination. Although the ability of these experienced technicians has been demonstrated, the results were occasionally subject to criticism because of the subjectivity involved. Moreover, maturity stages examined in recent years indicated problems arising from freezer storage of herring samples for varying periods prior to examination (Hunt, person. com.; Martin and Chouinard, person com.).

In the present report, a new method developed in the Gulf Region is described. This method utilizes a digitizer, a binocular microscope and computer software for digitizing otolith morphometrics and storing data on diskettes for subsequent analysis. This method proved to be relatively fast, requires little experience by the operator and can be used for routine application in assigning individual herring specimens to their proper spawning groups.

MATERIAL AND METHOD

Samples of herring collected from inshore fisheries in the southern Gulf of St. Lawrence throughout the 1984 fishing season were examined for biological data such as length, weight, age, maturity and spawning groups in

a similar manner described in previous reports (e.g. Messieh and MacDougall 1984). Assignment of the spawning groups was conducted by visual examination of otoliths. The assignment of the spawning group was then used to determine the proper year-class of the age group. Age groups were determined by counting the number of annual rings, including the nucleus as 1st year in case of autumn spawning group.

Samples comprising about 300 fish were used for this study. Spawning groups were assigned in the routine manner and otolith measurements were digitized using a HIPAD digitizer connected to an IBM PC. Otolith images from a binocular microscope (15X) were reflected by a camera lucida on the digitizing pad, while in point mode, and measurements were entered in the computer. Nine sets of variables were taken for otolith dimensions (Figure 1 and Table 1). Maturity stages were assigned according to Blaxter's maturity scale (Anon. 1962). Gonadosomatic index (GI) was calculated as percentage of gonad weight to fish weight (Hunt 1983).

The HIPAD used for otolith measurements 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 since all electronic circuitry is contained within the HIPAD case. The resolution is selectable at .125 or .25 mm; the data rate up to 100 coordinate pair per second; operating mode are either point, switch or stream.

Methods of Analysis

Simple computer programs were used for data entry. AUTOCAD (IBM software) with Bernoulli disc drive was used for plotting. Statistical Analysis System (SAS) package run on main frame in UNB was used for the analysis (Rae 1982). Discriminant function (DISCRIM), stepwise discriminant function (STEPDISC) and canonical analysis (CANDISC) were used. STEPDISC sets up a discriminant function which would distinguish between the 3 spawning groups; spring, summer and autumn. The variables were entered into the discriminant function in a stepwise manner according to their relative ability to discriminate between the groups. The stepwise ends when none of the variables outside the model has a significant F statistic. CANDISC is a dimension-reduction technique related to principal components and canonical correlation. A plot of the variables on each observation as transformed by the first two canonical discriminant functions was made to give an optimal two-dimensional picture of the separation between groups.

RESULTS

Maturity Stages

Maturity stages of herring samples used in this analysis (Table 2) showed that they were all adult fish (stages III-VIII), either pre-spawning (stages III-V), spawning (stage VI) or post-spawning (stages VII-VIII). None of the fish were immature (stages I-II). The frequency distribution of herring maturity stages showed that 80.5% of fish in May were in stage VI (i.e. spring spawners). In June, the percentage of stage VI fish dropped to 15.0% and the percentage of maturity stages III and IV (pre-spawners) increased to 30.0% and 33.3%, respectively. In August, 30.1% of the fish were stage VI (i.e. Autumn spawners), but a significant proportion (46.8%)

were in stage IV. In September 64.1% of the samples were stage VI and 30.5% were in stage V. The latter are autumn spawners which will probably spawn in late fall.

The gonadosomatic index (GI) distributions in relation to maturity stages (Figure 2) show that maturity stages IV, V and VI have the highest GI. However, based on the index alone it is difficult to distinguish between these stages. The GI for maturity stages III, VII and VIII are of similar levels and again unseparable by this index.

Discriminant Function Analysis

Results of the stepwise discriminant function analysis showed that five variables of nine variables entered were significant. Of these only three variables: L1PR, L2PROST and RL1RTOPR (Table 1) have the best ability in separating the groups. F-values for these variables were 44.03, 8.87 and 6.25, respectively. In subsequent analysis, only these three variables were used.

Results of the discriminant function (DISCRIM) classification are presented in Table 3. Percentage agreements between the original assignment and the results of DISCR were 85.9%, 93.9% and 77.7% for autumn spawning (A), summer spawning (E) and spring spawning (P) fish, respectively. After application of chance-corrected procedure (Titus et al 1984), the kappa statistic test showed that these agreements are highly significant (kappa, $K=0.70$; $Z=14.29$, $p < 0.001$). A plot of the variables as transformed by the first two canonical discriminant functions (Figure 3) showed the separation between the three groups.

Comparison of the results of the DISCRIM classification with the maturity stage assignment (Figure 4) showed that 84.4% of the spring spawning group in May were in maturity stages VI and VII. The rest (15.6%) were in stages III, IV and V. In June, 69.6% of the summer spawning group were at stage IV, 10.9% at stage V, and 13.0% at stage VI. In August, 36.5% of the autumn spawning group were at maturity stage IV, 8.8% at stage V, 46.3% at stage VI,and 4.9% at stage VII. In September, the autumn spawning group comprised 30.5% at stage V, 64.1% at stage VI and 5.3% at stage VII.

DISCUSSION

Results presented in this study have shown that otolith morphometrics are useful in classifying the herring spawning groups. These results are particularly important for 4T herring because of the complexity of their stock structure and the difficulty of proper classification of individual fish into the spawning groups by their maturity stages.

The assignment of spawning groups by maturity stages is subject to error, especially when herring samples are collected outside the spawning season. Maturity stages III and VIII are most problematic and difficult to use in separating the spawning groups. Similar problems, involving misclassification of maturity stages were recently observed in previously frozen fish. Hunt (person. com.) found a consistent trend for maturity stage to change from predominantly stages V and VI to stages IV and V after 8

months freezer storage. This problem was clearly demonstrated in the autumn spawning group caught in August, where 36.5% were assigned maturity stage IV (Figure 4). Other problems are related to stress-induced spawning, where fish of maturity stage V are misclassified to stage VI.

The gonad index (GI) cannot be used in maturity stage assignment. Hunt (1983) found between-stage variations in mean GI by length are significant for stage V being positively correlated and stage IV negatively correlated. He suggested that misclassification of stages III and IV could be a factor for this anomaly.

Results of the discriminant function analysis showed good agreement between the assignment of the spawning groups by their otolith characteristics and the classification by DISCR. The lowest level of agreement was 77.7%, and the highest was 93.9%. The original assignments were done without reference to maturity stages or fish length to achieve objectivity. The plot of the canonical discriminant analysis showed little overlap between the three spawning groups.

The differences in otolith characteristcs of the three spawning groups, as shown by the discriminant function analysis, can be explained on the basis of differences in growth characteristics and development rates for the different spawning groups. In spring spawning, the fish are hatched during the spring plankton bloom, taking advantage of full feeding season; their growth and development rates are therefore relatively fast. By the completion of their first year of life, the fish would attain a larger size otolith and a larger l_1 (back-calculated length at 1st year). In summer spawning, the fish would have a chance to engage in active feeding, only on the later part of plankton bloom, attaining some growth by year end. However, their otolith size and 1st year growth would be smaller than those of the spring group. In autumn spawning, the larvae have only a short time for development between time of hatching and the end of year. Otolith size would be small, appearing as a nucleus, closely encircled by the 1st annulus. First year growth (l_1) of autumn spawned fish would be smaller than that of spring and summer spawned fish.

The use of the HIPAD digitizer saves the time spent in fish otolith examination. It was estimated that 100 fish with 5 measurements each can be processed in less than one hour. The digitizer is relatively fast, more accurate since it reduces human error, and requires little experience for operation. It is recommended that this technique be adopted for herring ageing and the assignment of these spawning groups.

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Table 1. Otolith measurements and variables used in DISCR analysis for separating herring spawning groups

Code	Measurement
L1ROST	OG
L2ROST	OH
L2PROST	OF
RL1RTOPR	OG/OE
LDOC	Angle DOC
LAOB	Angle DOA
L1R	OG/OD*FL
L1PR	OE/OA*FL
L2PR	OF/OA*FL

[1] See Figure 1.

Table 2. Percent frequency distrubition of maturity stages of herring samples by month

	% Maturity Stage					
Month	III	IV	V	VI	VII	VIII
May	4.1	3.3	8.2	80.5	3.8	-
June	30.0	33.3	8.3	15.0	-	13.3
August	5.6	46.8	12.1	30.1	3.9	1.5
September	-	-	30.5	64.1	5.3	-

Table 3. Comparison of classification of herring spawning groups (autumn, A; summer, E; spring, P) by the discriminant function analysis and original assignment by the otolith characteristics.

HERRING SPAWNING GROUP SEPERATION

DISCRIMINANT ANALYSIS CLASSIFICATION SUMMARY FOR CALIBRATION DATA: WORK.CURRENT

GENERALIZED SQUARED DISTANCE FUNCTION:

$$D^2_J(X) = (X-\bar{X}_J)' \, COV_J^{-1} \, (X-\bar{X}_J) + LN\,|COV_J|$$

POSTERIOR PROBABILITY OF MEMBERSHIP IN EACH TYPE:

$$PR(J|X) = EXP(-.5\, D^2_J(X)) \,/\, SUM_K\, EXP(-.5\, D^2_K(X))$$

NUMBER OF OBSERVATIONS AND PERCENTS CLASSIFIED INTO TYPE:

FROM TYPE	A	E	P	TOTAL
A	79	1	12	92
	85.87	1.09	13.04	100.00
E	0	31	2	33
	0.00	93.94	6.06	100.00
P	22	13	122	157
	14.01	8.28	77.71	100.00
TOTAL	101	45	136	282
PERCENT	35.82	15.96	48.23	100.00

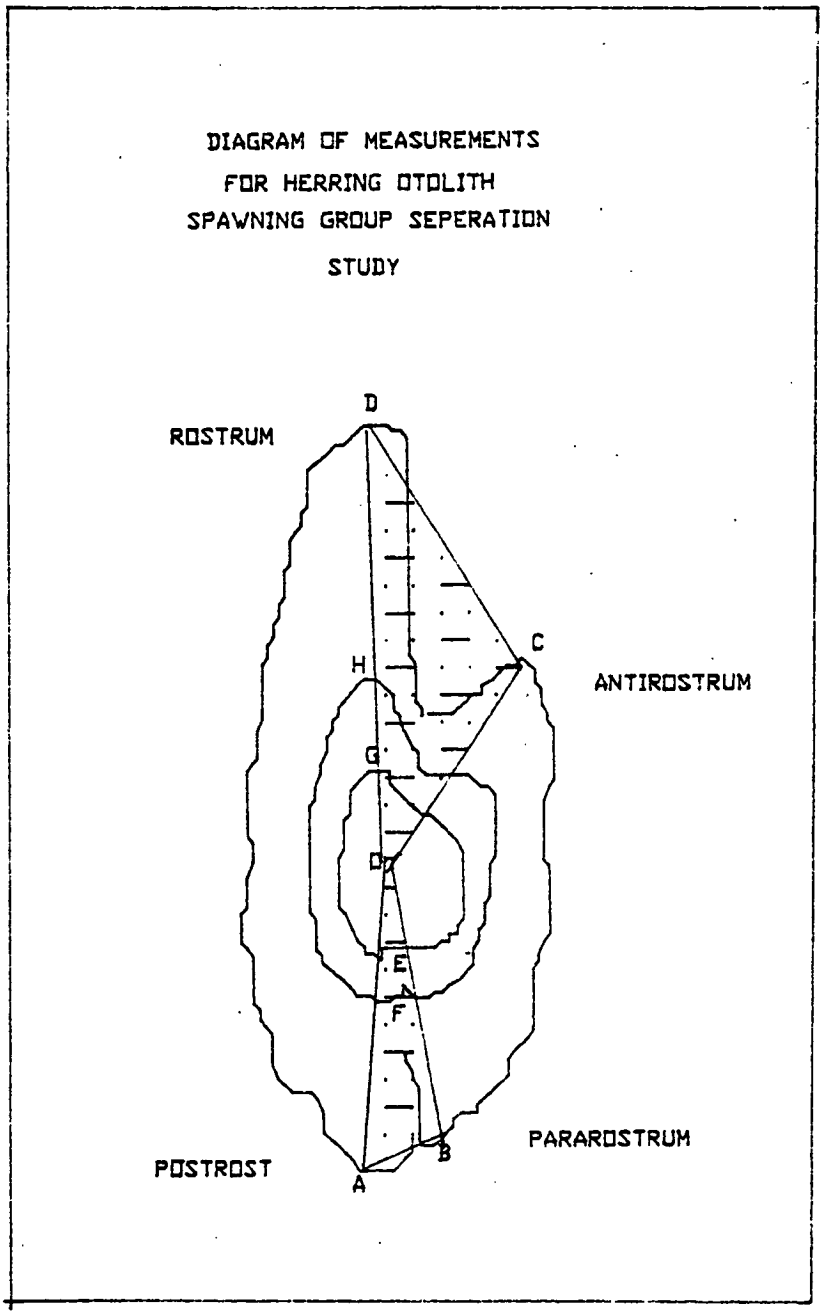

Figure 1. Plot of herring otolith showing points entered on the HIPAD digitizer.

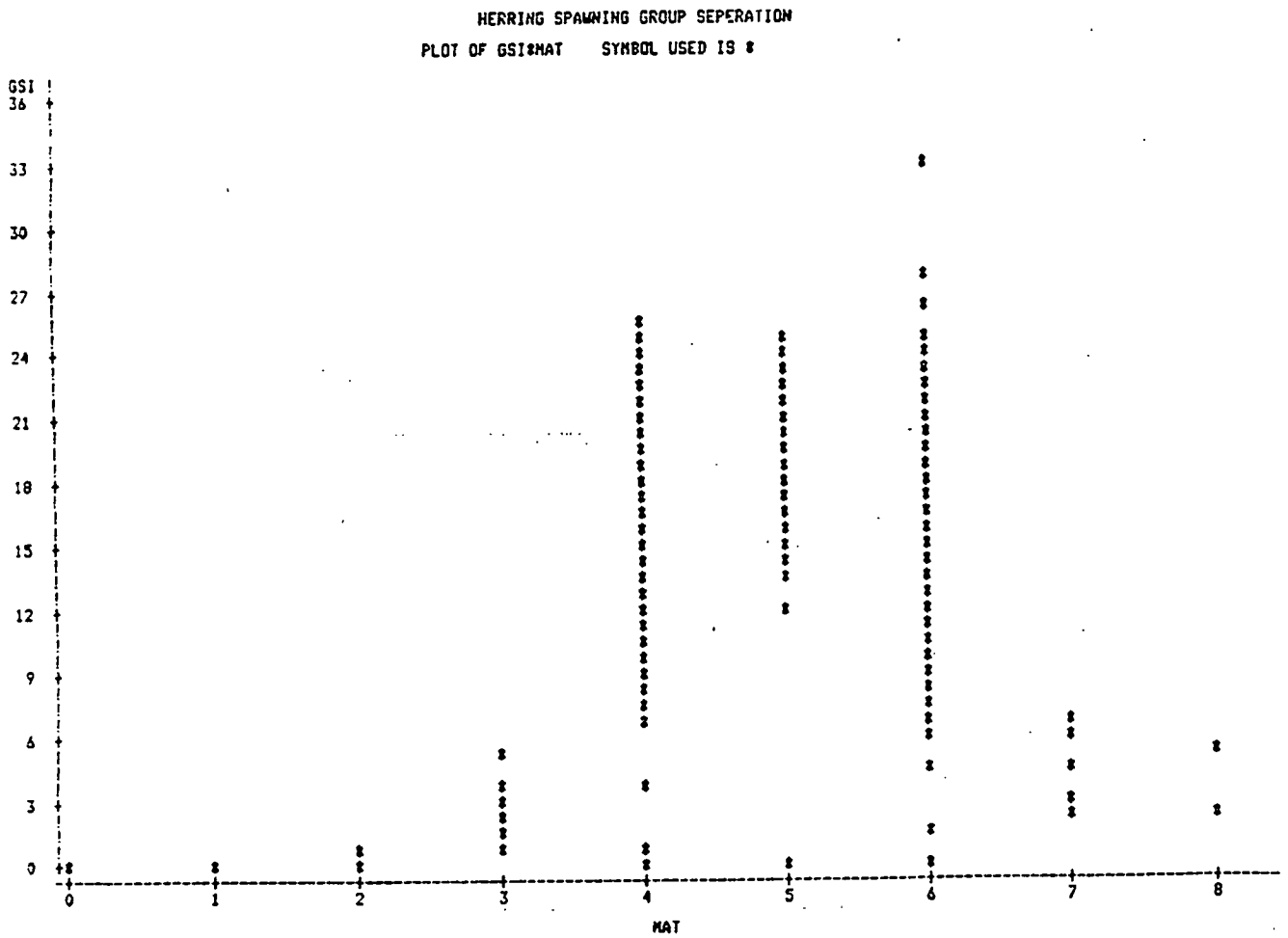

Figure 2. Relationship between maturity stages and gonadosomatic index of 4T herring.

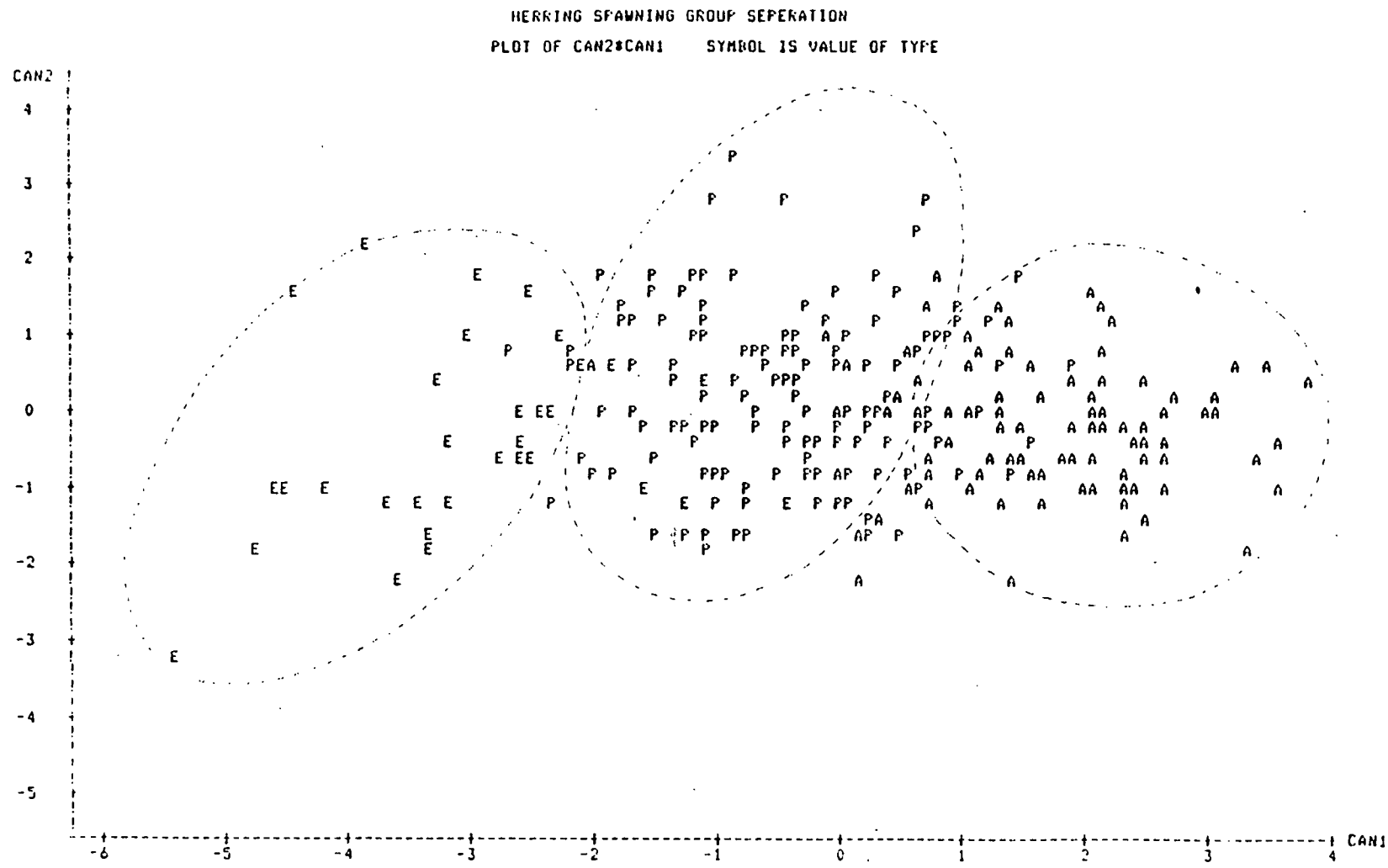

Figure 3. Canonical discriminant function plot showing the separation between the summer (E), spring (P), and autumn (A) spawning herring groups.

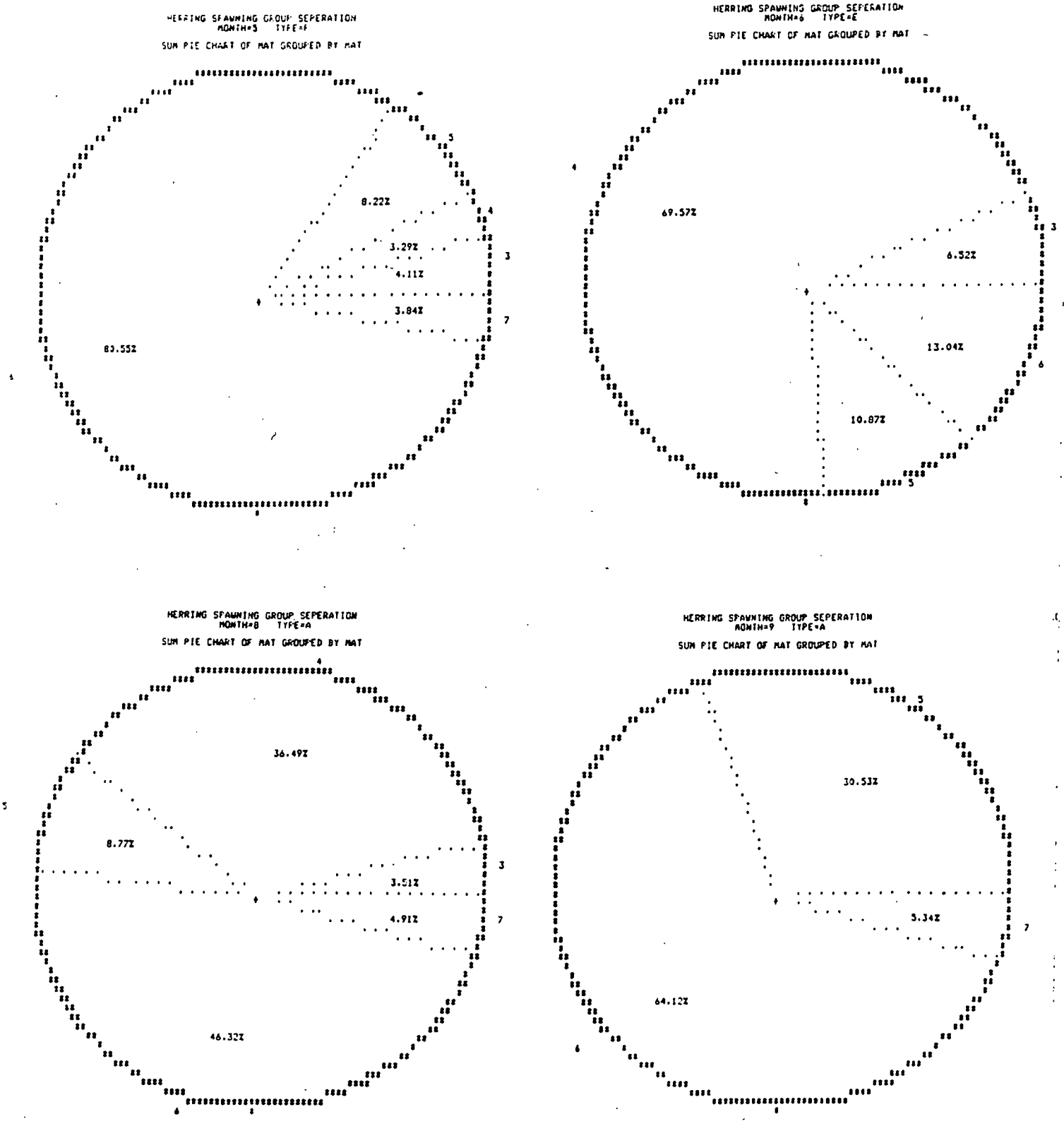

Figure 4. Frequency distribution of maturity stages in three spawning groups separated by discriminant function analysis: spring group in May; summer group in June; autumn group in August and September.