Canadian Atlantic Fisheries
Scientific Advisory Committee

CAFSAC Research Document 92/ 45

Comité scientifique consultatif des pêches
canadiennes dans l'Atlantique

CSCPCA Document de recherche 92/ 45

DISCRIMINATION OF THE 4Vs/4T COD STOCKS USING OTOLITH SHAPE ANALYSIS

by

Steven E. Campana
Marine Fish Division
Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, Nova Scotia
B2Y 4A2

and

John M. Casselman
Ontario Ministry of Natural Resources
Fisheries Research, Glenora Fisheries Station
R.R. #4
Picton, Ontario
K0K 2T0

Abstract

Otolith shape has long been known to be species-specific, but recent reports have pointed to its value as an indicator of stock identity. As a test of this hypothesis, all three pairs of otoliths were sampled from 2349 spawning Atlantic cod (*Gadus morhua*) collected throughout the northwest Atlantic. Otolith shape was determined with Fourier analysis, and combined with measures of otolith area and perimeter. There were highly significant differences in otolith shape among most of the cod samples, but shape also differed among ages, sexes, and yearclasses. The first discriminant function was highly correlated with both fish and otolith growth rate, indicating that stock discrimination improved as the difference in stock-specific growth rate increased. Much of the stock discrimination power appeared to be associated with environmental, rather than genetic, sources. Reevaluation of published studies on other species indicated that growth rate variation better explains regional differences in otolith shape than does stock-specific shape. Differences among ages, sexes and yearclasses were also attributable to growth rate differences. In the case of the 4Vs and 4T cod stocks, classification of test (unknown) samples resulted in 70-75% classification success. Given the consistent difference in growth rates between the two stocks, otolith shape analysis may be capable of providing real-time discrimination of mixed samples with moderate accuracy levels. However, as is the case with the age-length key method of distinguishing between the stocks, stock discrimination based on otolith shape analysis will likely misclassify slow-growing fish in 4Vs as 4T fish, and vice versa.

Résumé

On savait déjà que la forme des otolithes varie selon l'espèce, mais il ressort de rapports récents que c'est là un indicateur utile de l'identité d'un stock. Pour en faire la preuve, on a prélevé les trois paires d'otolithes sur 2 349 morues de l'Atlantique (*Gadus morhua*) en frai provenant de l'Atlantique nord-ouest. On a établi la forme des otolithes au moyen de l'analyse de Fourier et combiné les mesures de leur surface et de leur périmètre. On a constaté des différences importantes de forme des otolithes parmi les échantillons de morues, mais également des variations selon les âges, les sexes et les classes d'âge. La première fonction de discrimination a fait l'objet d'une étroite corrélation avec le taux de croissance du poisson et avec celui des otolithes, la discrimination parmi le stock s'améliorant en même temps que l'écart du taux de croissance propre au stock augmentait. Une bonne partie de la capacité de discrimination semblait être associée à des causes environnementales plutôt que génétiques. Le réexamen d'études réalisées à propos d'autres espèces a révélé que les variations du taux de croissance expliquent mieux les différences dans la forme des otolithes selon les régions que celles qui sont propres au stock. Les différences selon l'âge, le sexe et la classe d'âge ont aussi été reliées à des différences dans le taux de croissance. Dans le cas des stocks de morue de 4V et de 4T, la classification d'échantillons d'essai (inconnus) a abouti à un taux de réussite de 70 à 75 p. 100. Compte tenu de la différence constante dans les taux de croissance des deux stocks, l'analyse de la forme des otolithes peut se révéler apte à établir une discrimination en temps réel parmi des échantillons mélangés, cela avec un taux d'exactitude moyen. Toutefois, comme pour ce qui est de la méthode de distinction des stocks selon l'âge et la longueur, la discrimination fondée sur l'analyse de la forme des otolithes entraînera vraisemblablement l'identification de poisson à croissance lente de 4Vs comme des poissons de 4T et vice-versa.

Introduction

The shape of the otolith would appear to be an ideal natural tag of fish populations. Otolith shape is markedly species-specific (Morrow 1976; Gaemers 1984; L'Abée-Lund 1988) and is less variable in growth than is fish growth, presumably due to the dual function of the otolith as an organ of equilibrium and hearing. Otoliths grow throughout the life of the fish, and unlike scales and bone, are metabolically inert; once deposited, otolith material is unlikely to be resorbed or reworked (Campana and Neilson 1985; Casselman 1987). Therefore, otoliths remain unaffected by the short-term changes in fish condition (eg-starvation) which can confound body morphometrics. Since otoliths are composed of calcium carbonate, otolith shape is unaffected by the mode of fish preservation (excluding acidic preservatives which promote dissolution). As is the case with morphometrics, meristics, and all other stock identification procedures except DNA sequencing, the use of otolith shape to infer stock identity does not distinguish between genetic and environmental differences. Nevertheless, as long as such differences exist, otolith shape should vary among populations as long as there are also differences in the mean home range of the populations.

Otolith appearance and shape often vary geographically within a species, although there are mixed reports concerning the potential for stock discrimination. In some cases, reports of stock identification based on otoliths appear to reflect environmental differences among regions; environmental effects are almost certainly the cause of geographic variations in the shape or appearance of the otolith nucleus (Messieh 1972; Godo 1984; Neilson et al. 1985; Dawson 1991), otolith annuli (Rollefsen 1933, cited in Reisagg and Jorstad 1984; Rauck 1974; Godo 1984), and variations in the ratio of otolith size to fish size (Templeman and Squires 1956; Rojo 1977). However, measures of otolith shape based on ratios of otolith radii (Maceina and Murphy 1989) and in particular Fourier analysis (Williams 1980; Casselman et al. 1981; Bird et al. 1986; Castonguay et al. 1991) have had some success in distinguishing among stocks, and have been assumed to be based, at least in part, on genetic differences. Nevertheless there have been consistent reports of otolith shape variations among ages, sexes and yearclasses within a stock (Casselman et al. 1981; Bird et al. 1986; L'Abée-Lund 1988; Castonguay et al. 1991), leaving some doubt as to the robustness of the technique.

The objectives of this study were to provide an in-depth appraisal of the value of otolith shape analysis for stock identification. Fourier analysis was selected as the most objective and powerful of the shape analysis techniques. The study was designed to examine a large number of cod, spread over a broad geographic area and a large number of putative stocks. Additional factors evaluated in this study were age, sex, and yearclass effects, and an assessment of the relative contributions of genetic and environmental effects to otolith shape variation. We also examined the additional stock structure information present in the shape of two otolith pairs not normally examined, and gave careful attention to the influence of fish length and other variables on the Fourier analysis. The results of this study have been presented elsewhere (Campana and Casselman 1992), and will not be repeated here. Rather, the current manuscript reports those results which are relevant to the 4Vs/4T stock discrimination problem. Note that the intent of the current manuscript is considerably more focused than was the original study. Further work is underway to assess the value of otolith shape analysis in the context of the 4Vs/4T stock mixing problem.

Materials and Methods

Sampling

The sampling program was designed to catch fish of known stock, through capture on the spawning ground in spawning condition. It is generally assumed that stock mixing is minimal at the time of spawning, and that seasonal feeding migrations begin long after spawning has been completed (Templeman 1962). While not all of the cod used in this study were in spawning condition, most were preparing to spawn, or had just spawned. Accordingly, we believe that our samples are reasonable representations of a number of discrete stocks.

Cod were either collected at sea aboard research vessels, or sampled from commercial catches where the fishing location was unambiguous. Where possible, each tow was considered as a separate sample, to be combined with other tows in the region only if comprised of less than 50 fish. Most samples comprise fish from two or more tows. Samples were restricted to fish in the size range of 45-85 cm fork length, so as to restrict the analysis to sexually mature individuals and to reduce variability due to size-related effects. A total of 2349 fish were collected from 19 sites along the eastern coast of Canada, the northeastern coast of the U.S., and Iceland (Table 1; Fig. 1). Most of the putative cod stocks in the northwestern Atlantic Ocean were sampled. All samples were collected in 1986, with the exception of two sites (Browns Bank and Western Bank) which were re-sampled in 1988 to test for yearclass effects within a site. Note that the sampling dates varied across several months, due to the tendency for cold-water stocks to spawn later than warm-water stocks.

Immediately after capture, fork length, head length (from the tip of the snout to the posterior end of the preoperculum), sex, and state of sexual maturity were recorded. Fork length measurements were not available for sample #1 (Banq1). The head was then severed, labelled and frozen for subsequent otolith removal in the laboratory. All three otolith pairs (sagittae, lapilli, and asterisci) were removed from each fish, cleansed of adhering tissue, and stored dry in vials until they could be examined further. Broken and crystalline otoliths were discarded (< 1% of the sample).

Since the lapillar and asteriscal otolith pairs are not normally collected from fish, it is worth noting their location relative to the brain and the sagittae. Cod lapilli were located well anterior and dorsal to the sagittae, lateral to the forebrain. This small pair of otoliths was generally removed while still within the endolymphatic canals (inner ear system), which lie in a lateral cranial invagination at the location of the lapilli. Asterisci were also often removed in association with endolymphatic canals, but in a location just posterior and immediately adjacent to the sagittae. A sagitta and asteriscus could often be removed together with forceps if the endolymph around the sagitta was gripped at the same time as the sagitta.

Otolith Data

The shape of each of the six otoliths from each fish was analyzed as a two-dimensional projection (outline), as is common practice. After differentiating the left and right otoliths of each otolith pair, otolith shape was quantified in a two-step procedure, consisting of: 1) digitization of the otolith shape, and 2) conversion of the shape coordinates to Fourier coefficients.

In the first step of the procedure, each otolith was oriented in a consistent manner on a dissecting microscope stage; sagittae were oriented with the sulcus up, the pyramid-shaped lapilli were placed with the large, rough surface down, and the asterisci were oriented with the concave side down. All otolith measurements and examinations were carried out at microscopic magnifications of 3.2X (sagittae) or 8X (lapilli and asterisci) via an image analysis system (Campana 1987). Using external otolith morphology for guidance, the coordinates of the otolith nucleus were digitized. The image was then binarized and the area and perimeter of the otolith calculated using standard image analysis procedures. The X-Y coordinates of the otolith edge were determined using an edge-following subroutine; as a result, errors due to tracing on a digitizer pad were avoided. In order to provide a common starting point for the otolith edge coordinates, the position of a standard landmark was digitized on each of the otolith images by the operator before the edge detection procedure was started. While the selection of a given landmark was arbitrary, use of standard landmarks ensured that the phase angles of the subsequent Fourier analysis were interpretable in the same way across all otoliths of a given type. The landmarks used were: sagittae - tip of rostrum; lapilli - acute vertex at confluence of rough and smooth surfaces; asterisci - tip of the largest lobe. Typically, this stage of the shape analysis procedure resulted in 700-1000 X-Y coordinate pairs for the sagittae, 300-400 for the asterisci, and 200-300 for the lapilli. The length of the long axis of the otolith was also calculated from these data.

The second stage of the shape analysis procedure involved the interpretation of the otolith contour data in terms of Fourier coefficients. Fourier analysis has been described in detail elsewhere (Younker and Erlich 1977; Full and Erlich 1982; Bird et al. 1986), and will not be repeated here. Mathematically, the length of the otolith radius R at angle Θ is described by

$$R(\theta)=A_0+\sum_{n=1}^{\infty}A_n\cos(n\theta-\phi_n) \tag{1}$$

where Θ is the polar angle measured from the landmark on the contour, A_0 is the mean radius length (the amplitude of the 0th harmonic), A_n is the amplitude of the nth harmonic, and ϕ_n is the phase angle of the nth harmonic (Bird et al. 1986).

Conceptually, Fourier analysis can be considered as a means of describing a shape in terms of additive cosine waves. Using some central location within the contour, a series of radii are drawn at equal angular intervals from the central location to the corresponding coordinates along the contour. The shape is then opened, or unrolled, from a specified landmark, leaving the radii as a sequence of lines of variable length. A single cosine wave is fitted to the data, so as to mimic as closely as possible the undulation of the top of the unrolled radii. This cosine wave can be described as an amplitude (height) and phase angle (position along the unrolled contour). Since cosine waves are orthogonal, and thus additive, a second cosine wave of different amplitude and phase angle can then be added to the first, and thus explain more of the observed shape variation. Similarly, subsequent cosine waves (also termed harmonics) can be added to the first two, until the observed shape has been almost fully described. Addition of successive harmonics adds increasing detail to the description of the shape. The shape described by the *n*th harmonic represents that of a *n*-leafed clover. There is no reasonable limit to the number of harmonics which can be used to describe and/or reconstruct a shape. In practice however, it is best to describe the shape in as few terms as possible so as to facilitate the statistical analysis of the amplitude and phase variables which follows. Fig. 2 demonstrates the sequential addition of harmonic terms which would be used to describe the shape of a typical sagittal otolith.

In this study, the Fourier analysis procedure of Jarvis et al. (1978) was used to convert the otolith contour coordinates (n=72, corresponding to an angular interval of 5°) into Fourier coefficients. Fourier coefficients were calculated in two ways: otolith contours were unrolled counterclockwise around both the digitized nucleus and the calculated centroid, starting from the digitized landmark. Twenty harmonics were calculated for each otolith. However, since 95-99% of the shape variation was described by the first 10 harmonics, only the latter were included in the statistical analysis. Both the raw (unstandardized) and the standardized (amplitude divided by the mean radial length) amplitudes were available for statistical analysis, as were the corresponding phase angles.

After the shape data had been collected, the sagittae were sectioned and aged according to established procedures (J. Hunt, St. Andrews Biological Station, N.B. E0G 2X0, pers. comm.). None of the fish were of known age, so ageing accuracy could not be determined. However, the age reader (R. Robicheau) was one of the most experienced cod otolith readers in the Maritimes. In order to evaluate ageing precision, a random subsample (10%) of each of the sagittal samples was blind-coded and re-read by the same person approximately 6 months after the initial reading. Ageing precision was high for all stocks, with coefficients of variation (Chang 1982) ranging from 0-9%, and a mean of 2.0%.

The mean growth rate of each fish was calculated as the fork length divided by the age. The mean otolith growth rate of each fish was the length of the otolith's long axis divided by the age.

Statistical Analysis

The intent of the statistical design was to test the classification success of a series of discriminant functions used to predict cod stock identity. Stock identity was presumed to be known based on the collection of near-spawning individuals on or near a primary spawning ground in the middle of the spawning season. Discriminant functions were developed using the Fourier coefficients (which by definition are orthogonal), otolith area, and otolith perimeter, and then tested for classification success against otoliths of known stock which were not used in the preparation of the discriminant functions. The latter will be referred to as the test sample.

All variables entered into the discriminant analysis were first examined for normality, and if necessary, ln or square root transformed. The phase angle data were, of course, circularly distributed between 0-360°, and thus were often bimodally distributed if the median value was near 0 or 360. There was no obvious method by which these data could be normalized (Batschelet 1981). We attempted to induce a unimodal distribution in each bimodal phase variable by adding 360° to all observations which appeared to be more closely associated with the lower mode than the upper mode. However, selection of the threshold value separating the two modes was based only on visual inspection of the data distribution, and in any case, often resulted in a non-normal, unimodal distribution which could not be readily transformed. Accordingly, we have given relatively little weight to the phase variables in this study.

Since all variables (except the phase angles) were correlated with otolith length and fish length, significant inter-sample differences in otolith shape could have resulted due only to the differences in length frequency among samples. Accordingly, the effect of otolith length was removed from all variables by removing the common, within-group slope. The effect of otolith length, rather than fish length, was removed from the variables since fish length measurements were not available for two of the samples, and more importantly, otolith length was unaffected by inter-sample differences in preservation, shrinkage and distortion. Bartlett's test for homogeneity of variance indicated that there was often significant heterogeneity of variance among samples in the analysis of covariance (ANOCOVA) model. However, given the large sample sizes, the conservative nature of Bartlett's test, and the random distribution of the model residuals, we do not believe that the model results were unduly influenced by the inter-sample differences in variance. Similarly, the finding that a nested ANOCOVA explained significantly more of the variance in some variables than did the common slope model was considered to be more an artifact of the large sample sizes than real differences. Discriminant analyses based on data adjusted for length using nested slopes performed poorly in classifying fish not used in the preparation of the discriminant functions. A second series of analyses was conducted using the standardized amplitudes (amplitudes divided by the mean radial length); the effect of otolith length was not explicitly removed from these variables.

Results

Age and Length Composition

Despite having restricted the sample collections to fish between the lengths of 45-85 cm, there were significant differences in age and length frequency among sample sites. Cod collected in the Gulf of Maine region averaged 2-5 years old, while those in more northerly waters averaged 6-8 years old and sometimes reached 14 years. Comparisons of growth rate among sample sites indicated that cod were fastest growing in the Gulf of Maine, of intermediate growth rate on the outer Scotian Shelf, southern Newfoundland, and Iceland, and slow growing in the Gulf of St. Lawrence and its approaches. There was more than a 3-fold difference in mean annual growth rates between cod in the Gulf of Maine and those in the Gulf of St. Lawrence.

The length and age frequencies of the cod samples collected in 4T, 4Vs and the intervening regions also differed among themselves (Fig. 3). The estimated annual growth rate of the 4Vs samples (#1, #2) was noticeably more rapid than that of the 4T sample (#6) (Fig. 4). To determine if the discrepancy in growth rates between the otolith shape samples was representative of the stocks, commercial age-length keys from the same year (1986) and quarter (Q1-4Vs; Q2-4T) were compared with those collected as part of this study. The age-length relationships of the commercial samples was very similar to those of the otolith shape samples, indicating that the latter were representative of the fishery.

Note that the growth rate of the Gabarus (#8) sample lay intermediate between that of the 4T and 4Vs samples, while that for Green Island (#12) is the slowest of the entire region.

Fourier Analysis

Visual examination of the otoliths from each region indicated that otolith shape varied to a different extent among the three otolith types. Lapilli appeared to be most uniform in shape, while asterisci were highly variable, even in a comparison of left versus right from the same fish. In general, otolith size appeared to be more consistent within a fish than otolith shape. Image analysis operators were unable to visually detect substantial and consistent shape differences among regions, suggesting that any inter-sample shape differences were either subtle or masked by individual variability.

Fourier analysis indicated that more than 99.9% of the otolith shape variability could be summarized using 20 harmonics. The first 10 harmonics explained an average of 99.0%, 97.2%, and 99.5% in the lapilli, asterisci and sagittae respectively; since the distribution of phase angles started to become random after the 10th harmonic, the higher harmonics contained relatively little systematic shape variation, and were not included in the subsequent analysis.

The harmonics which explained most of the otolith shape variance were relatively consistent across sample sites, but very different across otolith types. The choice of otolith centre (nucleus versus centroid) used in the Fourier analyis was also influential, although by design, centroid-based Fourier calculations discount the influence of the first harmonic to near zero. Using the nucleus as the centre, over 80% of the variance in lapillar shape was explained by the first harmonic, while the 3rd harmonic explained most of the remaining variance. Fourier analysis using the centroid as the otolith centre resulted in harmonics 2-5 being most important. The more variable asteriscal shapes required 6 harmonics to explain most of the variation, while the sagittae required 5. For all otolith types, each harmonic after the 8th explained less than 1% of the overall shape.

Comparison of the amount of shape variability within and among fish based on the Fourier coefficients confirmed the visual impressions of shape variability described earlier. Coefficients of variation (CV) for the Fourier amplitudes were 1.5-4.0 times higher among fish of a given sample than between left and right otoliths of the same fish. Otolith area and perimeter CV's were 4-8 times higher among fish than within otolith pairs. Thus, otolith shape, and in particular otolith size, was more consistent between the left and right otoliths of a given otolith type than among fish. Comparisons among otolith types within a given fish indicated that asteriscii were most variable and sagittae least variable. There were no significant CV differences among otolith types across fish.

Reconstruction of the mean otolith shape from the centroid-based Fourier variables for five representative samples demonstrated that regional shape differences were subtle but discernable. Lapillar and asteriscal shapes were faithfully replicated by the reconstruction. The overall outline of the sagittae was also well reconstructed; however, the lateral scalloping so characteristic of cod sagittae was absent, since it represented fine detail not fully described by the first 10 harmonics (Fig. 2).

Age, Length and Sex Effects on the Shape Variables

With the exception of the phase angles, all of the shape variables were significantly correlated ($p<0.01$) with both fish length and otolith length. Given the differences in length frequencies among samples, failure to remove this length effect could have introduced apparent (but unreal) shape differences among samples. A significant length effect ($P<0.01$) was also evident when the amplitude variables were standardized to mean otolith radius, as per common practice. All standardized amplitudes were so affected. Since there was no advantage to using the standardized variables, we elected to remove the length effect from each of the unstandardized variables through an analysis of covariance (ANOCOVA) approach. Otolith length, rather than fish length, was treated as the covariate, since the otolith could be measured without significant measurement error and in the absence of distortion due to shrinkage or preservation.

Both age and sex were significant modifiers of several of the otolith shape variables. Discussion of these results is presented elsewhere (Campana and Casselman 1992).

Discriminant Analyses

The predictive power of the discriminant functions based on the otolith shape variables varied considerably with the otolith type, otolith centre, and shape variables which were used. All of the discriminant analyses of the otolith shape data were highly significant ($p<0.001$). While the assumption of homogeneity of the variance-covariance matrices was not met, there was probably no substantive effect on the results, given the low F values, the conservative nature of the test used (Box's M), and the large sample sizes. This conclusion was confirmed by the classifications of the test samples, which reflected the patterns in classification evident in the known samples.

Comparisons of various discriminant analyses of the left sagitta demonstrated that otolith area (LNSQA) and perimeter (LNP) contributed most of the explanatory power to the analysis (Table 2). Analyses using only the amplitude (AMP) variables were slightly less successful than were those which used only area and perimeter. Use of all variables, including the phase angle (PHA) variables, provided the most accurate classification rates. There was no obvious difference in classification success between covariate-removed AMP's and those standardized to mean radial length. Results obtained using either the centroid or the nucleus as the otolith centre were comparable. The sample from the Gulf of St. Lawrence was usually the most accurately classified individual sample (up to 58% in the test sample), while that from Iceland was poorly differentiated from the other samples (less than 20% accurate classification). In general, samples from the Gulf of Maine region were accurately classified as to region (up to 80% correct), but poorly differentiated within the region. Classification results for the samples in the 4T/4Vs area are presented in Fig. 5.

Discriminant functions based on 1986 collections classified both 1986 and 1988 samples as to geographic origin with similar levels of accuracy. In the case of the centroid-based sagitta variables, 6% of the 1988 sample from Browns Bank (#3) was correctly identified as being from Browns Bank, as compared to 13% of the 1986 test sample (#4). However, 67% of the 1988 sample was correctly assigned to the Gulf of Maine region (versus 48% of the 1986 sample). In the case of the Western Bank sample, 7% of the 1988 sample (#19) and 17% of the test 1986 sample (#18) were correctly classified. However, 48% of the 1988 sample and 45% of the test 1986 sample were assigned correctly to the eastern Scotian Shelf. For reasons discussed later, neither the Browns Bank nor the Western Bank samples were particularly accurately classified. However, misclassification errors were similar across sampling years.

Comparison of discriminant analyses among the three otolith types indicated that the sagitta tended to give the most accurate classifications. There were no consistent differences between the two otolith centres (nucleus vs centroid) within a given otolith type, nor between covariate-removed and standardized AMP's. Given the significant relationship between the latter and otolith size, the effect of otolith length was probably both weak and incompletely removed by both the ANOCOVA and the standardized variable methods. There were consistent differences in classification rates among the otolith types. For instance, lapillar shape was a much better indicator of Icelandic origin than was asteriscal shape, while the converse was true for the Gulf of St. Lawrence. All otolith types performed well in identifying cod from the Gulf of Maine.

The results of the discriminant analysis of the left sagitta indicated that otolith area, perimeter and the lower order amplitudes were among the most influential variables in the analysis. While the first 6 discriminant functions were all statistically significant, the first 2 functions explained almost 84% of the variance.

To determine if the probability of classification increased with proximity to the source sample, a discriminant analysis of the centroid-based sagitta shape data was carried out in which the discriminant functions were based on the aggregated samples listed in the footnote of Table 2. The rates of incorrect classification were then plotted against the distances to the other regions. No relationship was evident, suggesting that regions were not simply misclassified to the nearest alternate region. On the other hand, if the Iceland sample was excluded from the analysis, misclassification rate was significantly and inversely correlated with the distance to the misclassified region.

Classification success increased markedly as the number of groups entered into the discriminant analysis was reduced. While this is a phenomenon common to most discriminant analyses, it appeared to be particularly marked in this study. For instance, analysis of three widely-spaced samples (Bay of Fundy-#7; Gulf of St. Lawrence-#6; Nfld3Ld-#15) resulted in an overall classification success of 78% for the test sample. There was greater than 89% differentiation between the Gulf of St. Lawrence (#6) and the Bay of Fundy (#7) samples. On the other hand, analysis of the 7 Gulf of Maine samples resulted in 30% classification accuracy by sample, which is not much greater than the overall classification success with all 19 samples. Therefore, sample number by itself did not explain apparently-high misclassification rates among some samples.

In the context of the 4T/4Vs stock mixing problem, cod samples outside of the area of interest are not required, and indeed, lessen stock discrimination power. Accordingly, discriminant functions of the sagittal shape variables (centroid as centre) were prepared based only on a 50% subsample of the 4T (#6) and 4Vs (#1-2) samples. The functions were then used to classify the remainder of the 4T and 4Vs samples, as well as those from 4Vn (#8) and 4Wd (#12). The 4Vs and 4T test samples were classified with 70-74% accuracy (Table 3). Classification results for the 4Vn and 4Wd samples were consistent with a mixed and 4T origin respectively. However, the true stock identity of the 4Vn and 4Wd samples is unknown.

Since the classification success of a given sample varied with the otolith type that was used, and since no one otolith type provided the best classifications for all samples, use of all three otolith types could provide more information about sample origin than any one otolith alone. Accordingly, the centroid-based shape variables (without PHA's) from each of the three left-hand otoliths of each fish were treated as independent variables in a discriminant analysis. Overall classification success of the test sample (25.4%) was higher than that of any individual otolith type, and appeared to accurately identify regional aggregations of samples (Fig. 6). While not used to construct the discriminant functions, the 1988 samples were also reasonably well classified on the basis of the corresponding 1986 samples. Of the 24 shape variables which entered the discriminant analysis, only 4 consisted of higher order harmonics (eg-harmonics > 6). Otolith area and perimeter from all of the otolith types were influential variables; sagittal and lapillar area and perimeter made up 4 of the first 7 variables entered into the analysis. Overall, the sagittal shape variables were more influential than the shape variables from the other otolith types. As was the case with the analysis of the sagittae alone, both the Fourier amplitudes and otolith area/perimeter variables by themselves were moderately successful in classifying samples, although considerably less so than was the case with all non-PHA variables combined.

Otolith Shape as a Function of Growth Rate

Examination of Figs. 4 and 5 (and a more detailed figure presented in Campana and Casselman 1992) indicates that, in general, samples from a given region were classified into the same region. However, misclassification rates among certain samples did not follow this pattern. For instance, samples from Newfoundland (#15 and #16) were often misclassified onto the eastern Scotian Shelf (#1, #2, #8, #12, #18, #19) and vice versa. As will be discussed later, large-scale dispersion between these regions appears unlikely, but cannot be ruled out. However, classification errors between Iceland and the other regions cannot realistically be assigned to fish movements. Reexamination of Fig. 6 indicates that all samples were most often misclassified to samples with similar growth rates. This is most evident in the Iceland sample, which has growth rates which are comparable to those of the eastern Scotian Shelf and Newfoundland.

Classifications mistakenly assigned to samples of similar growth rates suggest that otolith shape is correlated with growth rate. To test this hypothesis, the discriminant function values (from the combined-otolith type, centroid-based discriminant analysis) corresponding to each of the sample means (sample centroids) were regressed against mean annual growth rate (Fig. 7). There was a significant relationship between growth rate and the values of the first discriminant function corresponding to the sample centroids ($p<0.01$, $R^2=0.78$), indicating that growth and otolith shape were highly correlated. The residuals were curvilinear, suggesting that the relationship was not strictly linear. However, the second discriminant function was not significantly correlated with the residuals. Both somatic growth rate and otolith growth rate were related to the first discriminant function in a similar manner, indicating that otolith shape was related to growth rate in general.

The relationship between growth rate and otolith shape was both persistent and independent of variable type. Significant regressions between growth rate and the first discriminant function values at the sample centroids were observed when each of the otolith types was analyzed separately. The relationship was also significant if only the AMP's were entered into the discriminant analysis. Similar results were obtained with or without the PHA variables, if only otolith area/perimeter were analyzed, or using only the standardized AMP's. Analysis of covariance indicated that growth rate was a significant covariate of most (but not all) shape variables.

Given the strong correlation between growth rate and the first discriminant function, and in light of the substantial contribution of the first discriminant function to explained variance (~65%), it is possible that much of the observed classification success of the various discriminant analyses was due to environmentally-induced differences in growth rate among samples, rather than genetic differences in otolith shape. In order to test this hypothesis, the sagittal shape data were re-analyzed after having removed otolith growth rate as a covariate. Classification success declined substantially, although some test samples were still classified with 30-40% success. Similar results were obtained if otolith area was removed as a covariate from the shape variables. However, the sample which would be expected to have the greatest genetic differentiation from the other samples (Iceland) was still classified poorly (0-20% success). Accordingly, the genetic component of the observed differences in otolith shape did not appear to be strong.

A further test for genetic differences in sagittal shape among samples was made by analyzing 5 samples (#1, 8, 13, 16 and 19), carefully matched for growth rate so as to control for the growth effect. Only ages 6 and 7, subsampled to produce the same age ratio in each sample, were analyzed so as to control for any age effect. The resulting classifications of the test sample were usually highest for the correct area, but were not particularly high (20-50%) given the low numbers of samples in the analysis. The Iceland sample was only classified correctly in 20% of the cases. As a result, the environmental component of otolith shape would appear to be considerably stronger than the genetic component, but the latter may still have contributed to the significantly better than random classifications of the test sample.

A final test of the influence of growth rate on otolith shape was made by comparing the classification of the 1988 samples (which were not used in the preparation of the discriminant functions) with that predicted on the basis of the growth rates. As predicted, the 1988 Browns Bank sample, which grew at about the same rate as the 1986 sample, was classified similarly in both samples. The 1988 Western Bank sample, which grew more slowly than its 1986 counterpart, was classified into other groups with more similar growth rates. These results suggest that the utility of otolith shape for stock identification may depend upon relative year-to-year constancy in the growth rate of a given stock.

Discussion

Can otolith shape be used as a tool for differentiating among cod stocks? The answer would appear to be "sometimes". Under Ihssen et al.'s (1981) definition of a stock as an "intraspecific group of randomly mating individuals with temporal or spatial integrity", stocks are defineable using population characteristics such as growth rate, among other things. The results of our study indicate that cod from stocks with clearly different growth rates can be reasonably well differentiated on the basis of otolith shape alone. Both genetic and environmental influences undoubtedly contributed to the observed stock differences in otolith shape; however, the latter appeared to be most influential. With the influence of the environment being paramount, the utility of otolith shape for stock identification would depend on the relative constancy of the environment in a given stock area, integrated over the lifetime of the fish. For all but short-lived species, this would be a reasonable assumption, since year to year differences in the environment would be smoothed out over the lifetime of the fish.

Those cod samples which could be classified into discrete aggregations using otolith shape analysis largely conformed to stock boundaries determined using other techniques. Tagging (McKenzie 1956; Templeman 1962; Wise 1963), meristics and morphometrics (Templeman 1962; Lear and Wells 1984), ichthyoplankton surveys (O'Boyle et al. 1984; Hurley and Campana 1989), and parasite loads (Scott and Martin 1957) are all consistent with the view that cod from around Iceland, Newfoundland, the southern Gulf of St. Lawrence, the Scotian Shelf and the Gulf of Maine are largely discrete aggregations. Within each of these aggregations, extensive mixing is believed to occur, resulting in complex stock mixtures. This is particularly true of the Gulf of Maine, where tagging studies have demonstrated extensive mixing throughout the Gulf, and at the entrance to the Gulf of St. Lawrence, where large-scale seasonal migrations from the Gulf to the Scotian Shelf have been shown to occur (Wise 1963; Halliday 1973; W.T. Stobo, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, pers. comm.). With the exception of the Iceland sample, otolith shape analysis distinguished among the above five regions with reasonable accuracy. However, sites within the Gulf of Maine could not be clearly differentiated, nor could those in and around the entrance to the Gulf of St. Lawrence. As a result, we could not determine whether our within-region results were due to the innate imprecision of otolith shape analysis or to true mixing within the region. Note that protein electrophoresis (Cross and Payne 1978; Mork et al. 1985) and mitochondrial DNA studies (Smith et al. 1989; Carr and Marshall 1991; Dahle 1991) have been largely unsuccessful in distinguishing among cod populations.

The finding that otolith shape changed largely in response to differences in growth rate is consistent with numerous studies which have documented growth-related changes in the size of the otolith relative to that of the fish (Templeman and Squires 1956; Boehlert 1985; Mosegaard et al. 1988; Secor and Dean 1989; Campana 1990; Casselman 1990). In the above studies, slow-growing fish formed larger otoliths than fast-growing fish of the same size. While changes in size do not necessarily confer changes in shape, the ubiquity of the response of relative otolith size to an environmentally-mediated growth change indicates that the otolith growth process is highly susceptible to environmental effects. While we have no experimental evidence that otolith shape changed in response to environmental versus genetic growth differences, the former seems more likely. The large-scale gradients in cod size-at-age observed in this study are, at least superficially, correlated with similar gradients in water temperature (Drinkwater and Trites 1987). Temperature is known to be a primary modifier of growth rate in fish (Brett 1979). A growth effect on otolith shape is also evident with respect to the age, sex and yearclass differences observed in this study; in each instance, the presence of significant otolith shape differences among groups could be linked to differences in growth rate.

The finding that otolith shape was highly correlated with growth rate would appear to explain most, if not all, of the significant otolith shape differences observed within other species. Otolith shape accurately discriminated among a number of whitefish (*Coregonus clupeaformis*) stocks in Lake Huron (Casselman et al. 1981). However, subsequent reexamination of the data revealed that stocks were discriminated only where mean stock growth rates were substantially different, and failed to do so when growth rates were similar. Growth rate effects also appeared to account for the observed differences among age groups. In a study of herring (*Clupea harengus*) otoliths, Bird et al. (1986) reported relatively few significant differences among the first 10 harmonics when comparing between Alaskan stocks, or between widely-spaced Atlantic stocks (Georges Bank and Gulf of St. Lawrence herring). On the other hand, most of the lower order harmonics differed significantly in a comparison of the Alaskan stocks with the Gulf of St. Lawrence stock, and even more so with the Georges Bank stock. This pattern of similarities and differences accurately reflects corresponding changes in growth rates. While growth rates were not reported in Bird et al.'s (1986) paper, other sources indicate that the two Alaskan stocks had similar lengths at age 5 (245-255 mm) (Lebida 1987), while the Georges Bank and Gulf of St. Lawrence herring were considerably larger (280-310 mm, with Georges Bank being the largest) (Winters 1976; Anthony and Waring 1980). The size at age discrepancy was largest between the Alaskan fish and the Georges Bank fish. Since there is no reason to expect the Georges Bank - Alaskan herring distinction to be any greater than the Gulf of St. Lawrence - Alaskan herring distinction, growth-related otolith shapes appear to better account for Bird et al.'s (1986) results than stock-specific differences. Smith (1992) came to a similar conclusion in a recently-published study on red snapper (*Etelis carbunculus*).

A final test of the hypothesis linking otolith shape with growth rate was available in Castonguay et al.'s (1991) detailed study of mackerel (*Scomber scombrus*) otolith shapes. Castonguay et al. (1991) reported relatively few significant differences in lower order harmonics between northern and southern contingents on the Atlantic coast, but numerous differences between either contingent and the North Sea stock. Classification of test samples reflected these differences, in that discrimination between northern and southern fish was relatively poor (36-68%), but discrimination between either northwest Atlantic contingent and the North Sea stock was much better (60-91%). Comparison of growth rates among the samples indicates relative similarity between the northern and southern contingent (eg- 29.1 cm at age 3 for the southern contingent; 29.5 cm at age 3 for the northern contingent) (Anderson and Paciorkowski 1980), and greater differences with the North Sea stock (32.9 cm at age 3) (Hamre 1980). Therefore, either stock effects or growth rate differences could be used to explain the otolith shape differences between the North Sea and the northwest Atlantic stocks. However, Castonguay et al.'s (1991) greatest discrimination success (87-91%) occurred in a comparison of southern contingent otoliths with those from the North Sea. There is little genetic basis for expecting southern contingent mackerel to be any more distinct from North Sea mackerel than northern contingent fish. However, growth rate differences are maximal between the southern contingent and North Sea fish; therefore, on the basis of the growth rate hypothesis, one would expect the best discrimination between these two groups of fish. Castonguay et al's (1991) observations of numerous otolith shape differences among age groups and yearclasses are also consistent with mackerel's propensity for changes in growth rate with yearclass strength (Anderson and Paciorkowski 1980; Hamre 1980).

The conclusion that the observed otolith shape differences were more a function of the environment than genetics is largely based on the analysis of the Icelandic cod samples. There is no evidence of mixing of Icelandic cod with those from the eastern coast of Canada (Templeman 1962), indicating that the former should be genetically distinct, at least relative to the other samples. However, the Icelandic cod sample tended to be among the least accurately discriminated by otolith shape analysis. Since the growth rate of the Icelandic cod was very similar to that of the Scotian Shelf cod, such would appear to explain the consistent misclassification of Icelandic cod as Scotian Shelf cod. It would also point to an environmental, rather than a genetic basis, for the growth rate effect on otolith shape. However, in the context of a stock identification study, the environmental effect on otolith shape is probably no more severe than that on any other stock discrimination technique. Both meristics and morphometrics, the traditional mainstays of stock identification, are now known to be heavily influenced by environmental (particularly temperature) effects (Ihssen et al. 1981; Blouw et al. 1988; Swain et al. 1991). Population characteristics are also so affected. As long as the environmental effect is recognized, and as long as year-to-year shifts in environmental conditions can be incorporated into the conclusions, stock identification using non-genetic protocols should not be compromised. Indeed, after a comparison of a broad suite of morphometric, meristic and genetic stock indices of lake whitefish, Casselman et al. (1981) concluded that characters influenced by environmental differences could be just as reliable in indicating stock discreteness as genetically-based characters.

Given that otolith shape analysis has some utility as a stock identification tool, a number of recommendations can be made concerning the most appropriate otolith type, otolith centre used in the Fourier analysis, and analysis of the Fourier components. These are discussed in full in Campana and Casselman (1992).

While otolith shape analysis appears to be a useful stock identification tool in many situations, its use would be inappropriate under some conditions. Obviously, otolith shape will not differentiate well among populations with similar growth rates. It is also not suited to the identification of individual fish, since slow-growing fish from the fast-growing stock will be classified with the slow-growing stock, and vice versa. Finally, shape analysis (and most other stock identification techniques) are not suited for addressing the site affinity problem; that is, the technique does not have sufficient power to determine if individual fish are apparently misclassified because of the imprecision of the methodology, or because the fish is actually a stray from another area. Otolith shape will also not indicate where a fish was hatched, although it will point to the area where it grew up.

Discrimination of 4T versus 4Vs cod based on otolith shape analysis appears to be possible with moderate (70-75%) levels of success. In addition, the approach does offer some advantages over other techniques. Primary among these is logistical ease and speed. Sagittal otoliths are quickly, easily and routinely removed from cod samples. The shape analysis procedure is non-destructive and leaves the otolith intact for subsequent ageing. However, ageing is **not** required for stock discrimination based on shape analysis. Nor is the body of the fish or any measurements thereof. Given the absence of any requirements for otolith sectioning or ageing, otolith shape analysis can be carried out quickly and semi-automatically on samples collected as part of routine commercial sampling procedures. Indeed, the shape analysis procedure can be streamlined to produce a stock identification result for a given otolith in only 2 minutes from the time that the otolith arrives in the laboratory. Thus it can be considered a real-time stock identification procedure.

The assumptions and disadvantages of otolith shape analysis are similar to those for the age-length key method of distinguishing between 4T and 4Vs cod. The major assumption is that the wave of slow-growing fish which appears in the winter 4Vs cod fishery originates in 4T. Tagging results support this assumption, at least in part (Stobo and Lambert, pers. comm.). However, the spatial gradient in size at age is such that inshore cod in 4Vn (and perhaps even 4Wd) may be as slow growing as those in the Gulf of St. Lawrence. (Note that the age composition of the 4Wd sample (#12) from the current study had an apparent growth rate which was equal to or less than that from 4T.) If the inshore fish migrate offshore prior to spawning (a reasonable assumption, given observations elsewhere), the pulse of slow-growing fish observed in the 4Vs winter fishery may originate in inshore 4VW as well as 4T. Both the otolith shape and the age-length key methods would mistakenly identify these inshore fish as being from 4T.

A second possible source of error is associated with the high correlation between growth rate discrepancy and discriminatory power. Otolith shape analysis of a mixed 4T-4Vs sample would tend to misclassify the slower-growing 4Vs fish as being from 4T, and the faster-growing 4T fish as 4Vs. Once again, this problem is common to the age-length key method.

Comparison of the age composition of the samples collected in this study with those collected as part of routine commercial sampling indicate that the former is representative of the fishery. Nevertheless, further samples are required to insure that all possible spawning aggregations in the 4TVW area are sampled and can be accurately identified in test mixtures. This work is now underway, as is a comparison of the stock discrimination capabilities of the otolith shape and the age-length key methods based on the same set of otoliths.

Acknowledgements

We are very grateful to the many people who assisted us with the collection of the cod samples: Terry Decker, Gilbert Donaldson, Bill Dougherty, Jim Fennell, Daryl Lyon, Jim Simon, Bill Smith, Björn Steinarsson, Spencer Stevenson, and Kees Zwanenburg. Joan Nix and Kristin Zakariasen conducted much of the image analysis work. Joe Hunt and Reg Robicheau kindly provided the age data. Joanne Hamel and Jim Simon provided technical support. Bev Charlton, Jim McMillan and Ken Scott provided data handling and computer expertise. Bob Mohn made helpful comments on the manuscript.

Literature Cited

ANDERSON, E.D. and A.J. PACIORKOWSKI. 1980. A review of the northwest Atlantic mackerel fishery. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 177:175-211.

ANTHONY, V.C. and G. WARING. 1980. The assessment and management of the Georges Bank herring fishery. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 177:72-111.

BATSCHELET, E. 1981. Circular statistics in biology. Academic Press. London. 371 pp.

BIRD, J. L., D.T. EPPLER and D.M. CHECKLEY JR. 1986. Comparisons of herring otoliths using Fourier series shape analysis. Can. J. Fish. Aquat. Sci. 43:1228-1234.

BLOUW, D. M., S.D. SAXON and E.M.P. CHADWICK. 1988. Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock, and implications for stock identification. Can. J. Fish. Aquat. Sci. 45:1330-1339.

BOEHLERT, G. W. 1985. Using objective criteria and multiple regression models for age determination in fishes. Fish. Bull. 83:103-117.

BRETT, J. R. 1979. Environmental factors and growth. p. 599-675. In: W.S. Hoar, D.J. Randall and J.R. Brett [ed.]. Fish Physiology, Vol.8. Academic Press. New York.

CAMPANA, S. E. 1990. How reliable are growth backcalculations based on otoliths? Can. J. Fish. Aquat. Sci. 47:2219-2227.

CAMPANA, S. E. 1987. Image analysis for microscope-based observations: an inexpensive configuration.

Can. Tech. Rep. Fish. Aquat. Sci. 1569:iv+20 pp.

CAMPANA, S. E. and J.M. CASSELMAN. 1992. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. In press.

CAMPANA, S. E. and J.D. NEILSON. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014-1032.

CARR, S. M. and H.D. MARSHALL. 1991. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome b gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Can. J. Fish. Aquat. Sci. 48:48-52.

CASSELMAN, J. M. 1987. Determination of age and growth. P.209-242. In: A.H. Weatherley and H.S. Gill [eds]. The Biology of Fish Growth. Chap. 7. Academic Press. N.Y.

CASSELMAN, J.M. 1990. Growth and relative size of calcified structures of fish. Trans. Am. Fish. Soc. 119:673-688.

CASSELMAN, J. M., J.J. COLLINS, E.J. CROSSMAN, P.E. IHSSEN and G.R. SPANGLER. 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci. 38:1772-1789.

CASTONGUAY, M., P. SIMARD and P. GAGNON. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. Can. J. Fish. Aquat. Sci. 48:296-302.

CHANG, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39:1208-1210.

CROSS, T. F. and R.H. PAYNE. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach. J. Fish. Res. Board Can. 35:117-123.

DAHLE, G. 1991. Cod, *Gadus morhua L.*, populations identified by mitochondrial DNA. J. Fish Biol. 38:295-303.

DAWSON, W. A. 1991. Otolith measurement as a method of identifying factors affecting first-year growth and stock separation of mackerel (*Scomber scombrus*). J. Cons. Int. Explor. Mer 47:303-317.

DRINKWATER, K. F. and R.W. TRITES. 1987. Monthly means of temperature and salinity in the Scotian Shelf region. Can. Tech. Rep. Fish. Aquat. Sci. 1539: iv + 101 p.

FULL, W. E. and R. EHRLICH. 1982. Some approaches for location of centroids of quartz grain outlines to increase homology between Fourier amplitude spectra. Int. Assoc. Math. Geol. 14:43-55.

GAEMERS, P. A. M. 1984. Taxonomic position of the Cichlidae (Pisces, Perciformes) as demonstrated by the morphology of their otoliths. Neth. J. Zool. 34:566-595.

GODφ, O. R. 1984. Cod (*Gadus morhua*) off Mφre - composition and migration. P. 591-608. In: E.Dahl, D.S.Danielssen, E.Moksness and P.Solemdal [eds]. The Propagation of Cod Gadus morhua L. Flodevigen rapportser 1.

GRANT, W. S., C.I. ZHANG, T. KOBAYASHI and G. STAHL. 1987. Lack of genetic stock discretion in Pacific cod (*Gadus macrocephalus*). Can. J. Fish. Aquat. Sci. 44:490-498.

HALLIDAY, R. G. 1973. Notes on the status of cod and haddock stocks of the Scotian Shelf. ICNAF Res. Doc. 73/7. Ser. 2909.

HAMRE, J. 1980. Biology, exploitation, and management of the northeast Atlantic mackerel. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 177:212-242.

HURLEY, P. C. F. and S.E. CAMPANA. 1989. Distribution and abundance of haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) eggs and larvae in the waters off southwest Nova Scotia. Can. J. Fish. Aquat. Sci. 46(Suppl. 1):103-112.

IHSSEN, P. E., H.E. BOOKE, J.M. CASSELMAN, J.M. MCGLADE, N.R. PAYNE and F.M. UTTER. 1981. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38:1838-1855.

JARVIS, R. S., H.F. KLODOWSKI and S.P. SHELDON. 1978. New method of quantifying scale shape and an application to stock identification in walleye (*Stizostedion vitreum vitreum*). Trans. Am. Fish. Soc. 107:528-534.

L'ABEE-LUND, J. H. 1988. Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. J. Fish Biol. 33:899-903.

LEAR, W. H. and R. WELLS. 1984. Vertebral averages of juvenile cod, *Gadus morhua*, from coastal waters of eastern Newfoundland and Labrador as indicators of stock origin. J. Northw. Atl. Fish. Sci. 5:23-31.

LEBIDA, R.C. 1987. Age, sex and size composition of Pacific herring (*Clupea harengus*) from eastern Bering Sea coastal spawning sites, Alaska 1986. Alaska Dept. Fish Game Tech. Data Rep. 216:1-64.

MACEINA, M. J. and B.R. MURPHY. 1989. Differences in otolith morphology among the two subspecies of largemouth bass and their F1 hybrid. Trans. Am. Fish. Soc. 118:573-575.

MCKENZIE, R. A. 1956. Atlantic cod tagging off the Southern Canadian mainland. Bull. Fish. Res. Bd. Canada 105:1-93.

MESSIEH, S. N. 1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Bd. Canada 29:1113-1118.

MORK, J., N. RYMAN, G. STAHL, F. UTTER and G. SUNDNES. 1985. Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. Can. J. Fish. Aquat. Sci. 42:1580-1587.

MORROW, J. E. 1976. Preliminary keys to otoliths of some adult fishes of the Gulf of Alaska, Bering Sea,and Beaufort Sea. NOAA Tech. Rep. NMFS Circ. 420:1-32.

MOSEGAARD, H., H. SVEDANG and K. TABERMAN. 1988. Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. Can. J. Fish. Aquat. Sci. 45:1514-1524.

NEILSON, J. D., G.H. GEEN and B. CHAN. 1985. Variability in dimensions of salmonid otolith nuclei: implications for stock identification and microstructure interpretation. Fish. Bull. 83:81-89.

O'BOYLE, R. N., M. SINCLAIR, R.J. CONOVER, K.H. MANN and A.C. KOHLER. 1984. Temporal and spatial distribution of ichthyoplankton communities of the Scotian Shelf in relation to biological, hydrological, and physiographic features. Rapp. P.-v. Réun. Cons. int. Explor. Mer. 183:27-40.

RAUCK, G. 1974. On characteristics of plaice otoliths in the North Sea. Ber. dt. wiss. Kommn. Meeresforsch. 23:289-295.

REISAGG, J. and K.E. JφRSTAD. 1984. Stock structure of cod in the Mφre area. P. 609-624. In: E.Dahl, D.S.Danielssen, E.Moksness and P.Solemdal [eds]. The Propagation of Cod *Gadus morhua L.* Flodevigen rapportser 1.

ROJO, A. L. 1977. The otolith, discriminatory factor of codfish (*Gadus morhua*) stocks in the northwest Atlantic. Invest. Pesq. 41:239-266.

SCOTT, D. M. and W.R. MARTIN. 1957. Variation in the incidence of larval nematodes in Atlantic cod fillets along the Southern Canadian mainland. J. Fish. Res. Bd. Canada 14:975-996.

SECOR, D. H. and J.M. DEAN. 1989. Somatic growth effects on the otolith - fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 46:113-121.

SMITH, M.K. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Can. J. Fish. Aquat. Sci. 49:795-804.

SMITH, P. J., A.J. BIRLEY, A. JAMIESON and C.A. BISHOP. 1989. Mitochondrial DNA in the Atlantic cod, *Gadus morhua*: lack of genetic divergence between eastern and western populations. J. Fish Biol. 34:369-373.

SWAIN, D. P., B.E. RIDDELL and C.B. MURRAY. 1991. Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. Can. J. Fish. Aquat. Sci. 48:1783-1791.

TEMPLEMAN, W. 1962. Divisions of cod stocks in the Northwest Atlantic. ICNAF Redbook III:79-123.

TEMPLEMAN, W. and H.J. SQUIRES. 1956. Relationship of otolith lengths and weights in the haddock *Melanogrammus aeglefinus (L.)* to the rate of growth of the fish. J. Fish. Res. Bd. Canada 13:467-487.

WILLIAMS, P. J. 1980. P. 53. Use of otoliths for stock differentiation of American shad (*Alosa sapidissima* Wilson). BSc. Thesis. Dalhousie University.

WINTERS, G. H. 1976. Recruitment mechanisms of southern Gulf of St. Lawrence Atlantic herring (*Clupea harengus harengus*). J. Fish. Res. Board Can. 33:1751-1763.

WISE, J. P. 1963. Cod groups in the New England area. Fish. Bull. 63:189-203.

YOUNKER, J. L. and R. EHRLICH. 1977. Fourier biometrics: harmonic amplitudes as multivariate shape descriptors. Syst. Zool. 26:336-342.

Table 1. Sample collection.

Sample Number	Area (NAFO Division)	Site Acronym	Date	Latitude	Longitude	Sample Size
1.	Banquereau Bank (4Vs)	Banq1	Mar 7, 1986	44°18'	59°02'	122
2.	Banquereau Bank (4Vs)	Banq2	Mar 22, 1986	44 41	58 57	129
3.	Browns Bank (4X)	Brown88	Feb 29, 1988	42 45	66 10	104
4.	Browns Bank (4X)	Browns	Feb 19, 1986	42 45	66 10	141
5.	Cape Cod (5Zeg)	Capecod5Ze	Jan 30, 1986	41 40	69 30	79
6.	Cheticamp (4T)	Cheticamp	May 26, 1986	46 20	61 20	150
7.	Fundy Rip (4X)	Fundyrip	Mar 6, 1986	44 10	66 30	145
8.	Gabarus (4Vn)	Gabarus	May 27, 1986	45 40	59 45	150
9.	Georges Bank (5Ze)	George5Ze	Mar 4, 1986	42 00	66 00	20
10.	Georges Bank (5Zj)	George5Zj	Apr 16, 1986	42 10	67 05	150
11.	Grand Manan (4Xs)	Grandmanan	Mar 13, 1986	44 28	66 32	109
12.	Green Island (4Wd)	Green4Wd	Apr 29, 1986	45 25	60 45	140
13.	Iceland (-)	Iceland	May 6, 1986	65 15	23 30	65
14.	Larkinridge (5Yb,4Xq)	Larkinridge	Jan 28, 1986	43 50	67 30	145
15.	Newfoundland (3Ld)	Nfld3ld	Apr 24, 1986	49 12	50 30	199
16.	Newfoundland (3Ode)	Nfld3od	May 26-28, 1986	44 03	52 05	150
17.	Stellwagen Bank (5Ye)	Stellwagen	Jan 31, 1986	42 15	70 20	109
18.	Western Bank (4W)	Western	Mar 3, 1986	43 19 - 43 36	60 03 - 61 21	119
19.	Western Bank (4W)	Western88	Mar 23, 1988	44 00	61 35	123

Table 2. Summary of discriminant analyses of the left sagitta comparing the effects of choice of otolith centre (nucleus vs centroid), "standardized" (AMP's divided by mean radial length) versus covariate-removed data, and the various otolith size and shape variables. Classification success refers to classification of samples not used in the discriminant analysis.

Analysis Type	Classification Success (%)					
	By Sample	By Region				
		Gulf of Maine	Eastern Scotian Shelf	Gulf of St. Lawrence	NFLD	Iceland
Centroid; LNP, LNSQA Only	17.6	61	37	58	58	7
Centroid; AMP's Only	15.0	61	28	35	47	10
Centroid; No PHA Variables	18.3	70	45	40	54	10
Centroid; All Variables	18.6	77	45	47	58	17
Nucleus; No PHA Variables	21.4	73	48	39	49	13
Nucleus; All Variables	23.0	80	45	42	57	17
Centroid; No PHA, Standardized AMP's	21.2	69	46	54	58	14
Centroid; Standardized AMP's Only	13.0	61	28	19	43	3

Samples included in each region: Gulf of Maine = Samples 3, 4, 5, 7, 9, 10, 11, 14, 17
Gulf of St. Lawrence = Sample 6
NFLD = Samples 15, 16
Iceland = Sample 13

Table 3. Discrimination of otolith samples from 4T, 4Vs and the adjacent area based on Fourier analysis of the left sagitta, using the centroid as the otolith centre. The discriminant function was prepared on the basis of a 50% random subsample of the 4T (#6) and 4Vs (#1 and #2) samples, and then applied to the remaining subsamples as well as the sample from 4Vn (#8) and 4Wd (#12).

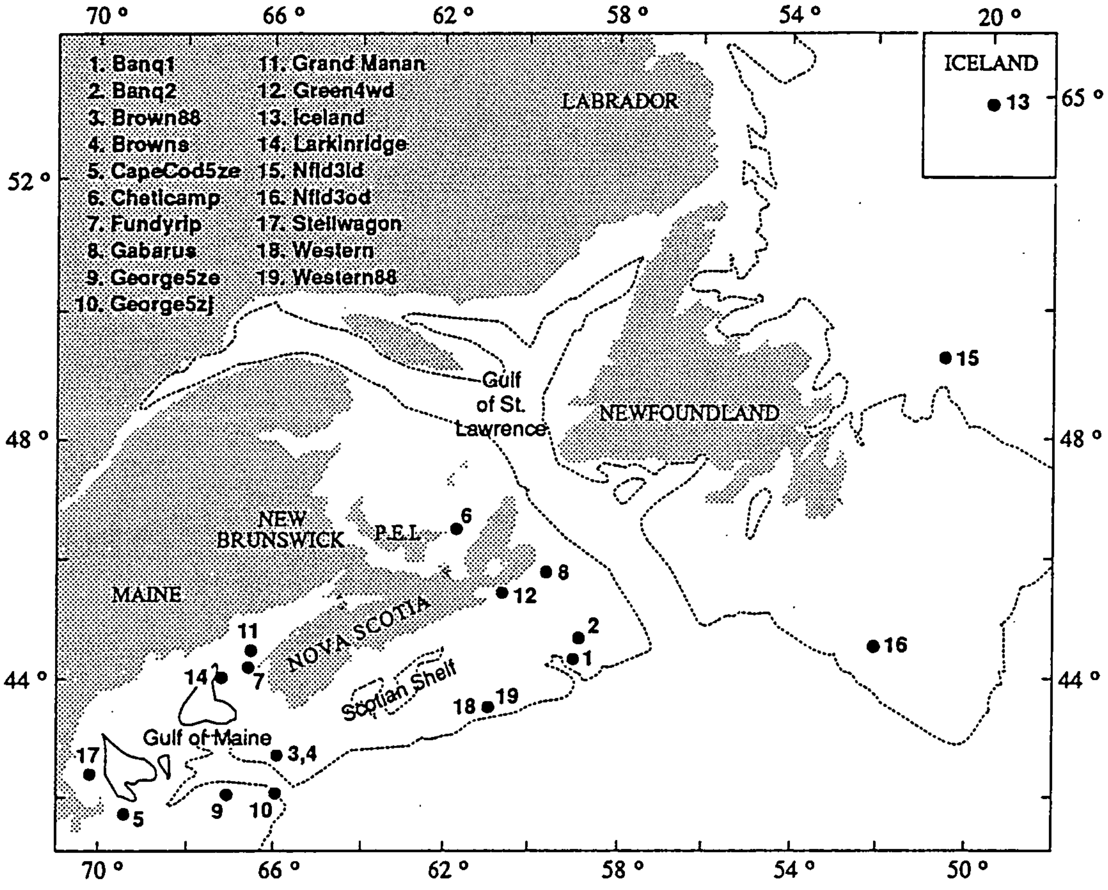
Classification Results for Cases Used in the Preparation of the Discriminant Functions

Actual Group	No. of Cases	Predicted Group Membership (%)	
		4Vs	4T
4Vs	115	67.8	32.2
4T	69	23.2	76.8

Classification Results for Cases **Not** Used in the Preparation of the Discriminant Functions

Actual Group	No. of Cases	Predicted Group Membership (%)	
		4Vs	4T
4Vs	129	69.8	30.2
4T	72	26.4	73.6
4Vn	141	44.0	56.0
4Wd	134	32.8	67.2

Fig. 1. Map of the study area and sampling sites. The sample from Iceland was collected several miles off the western coast of Iceland. The 200-m contour is shown.



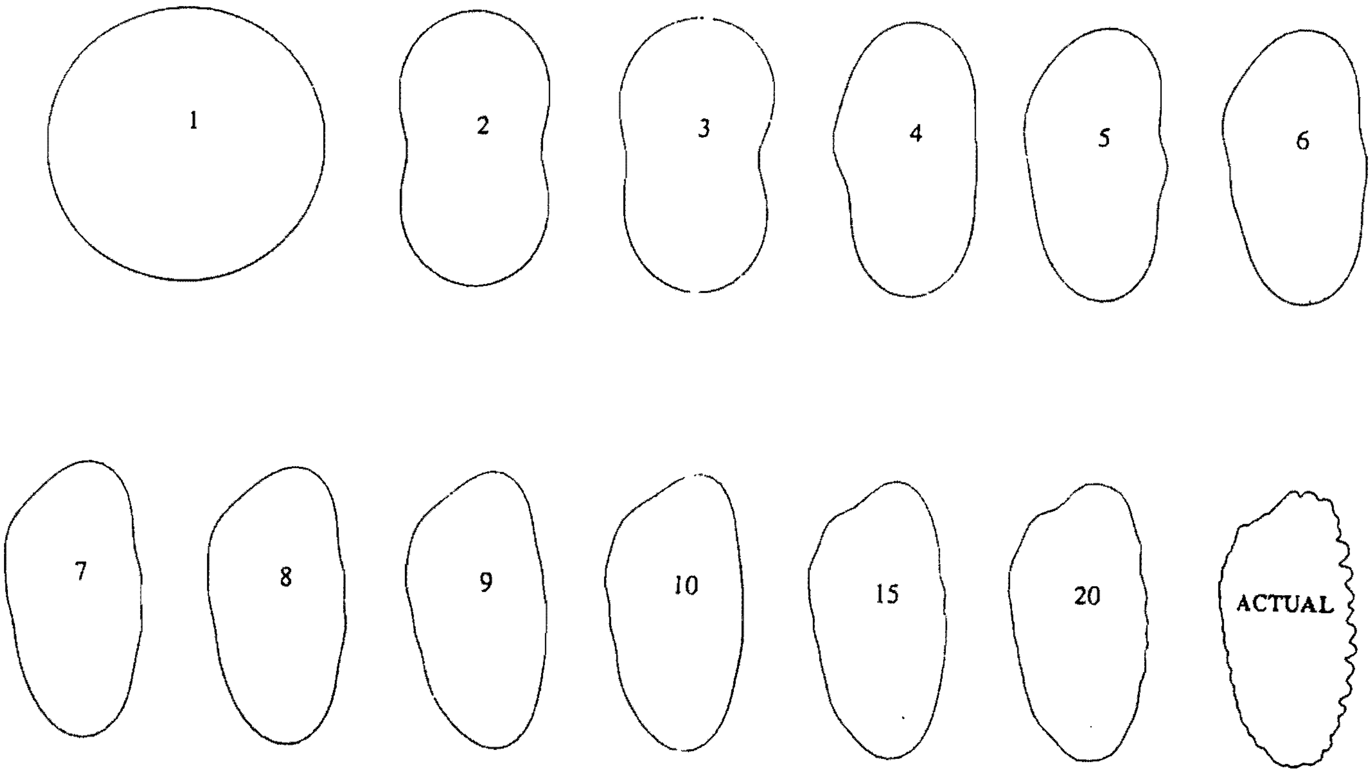


Fig. 2. Reconstruction of a typical sagitta from its Fourier variables, using the centroid as the otolith centre. The number within each shape represents the number of harmonics used to prepare the shape. The actual digitized shape is presented last. Note that the rough outline is reconstructed relatively accurately with only the first 6 harmonics, but that the scalloped edges require more than 20 harmonics.

Fig. 3. Age and length frequencies of cod samples collected in the 4T/4Vs area. Sample numbers indicated on the plots are as follows: #1 - Banquereau1; #2 - Banquereau2; #6 - Cheticamp; #8 - Gabarus (4Vn); #12 - Green Island (4Wd).

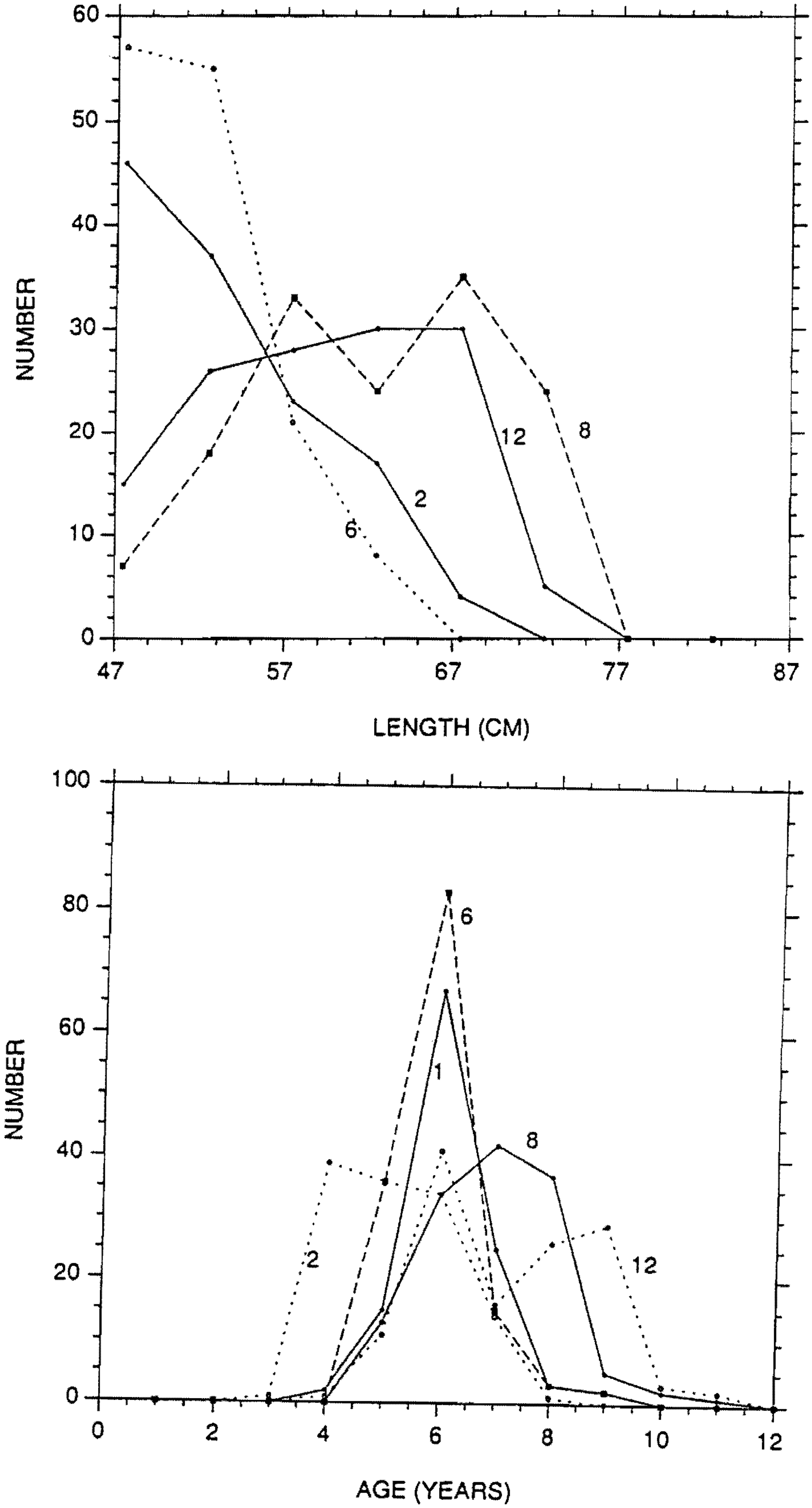


Fig. 4. Length at age of 4T and 4Vs cod in otolith shape samples. The length-age relationships from commercial samples of the corresponding subdivision, year and quarter were similar to those presented here.

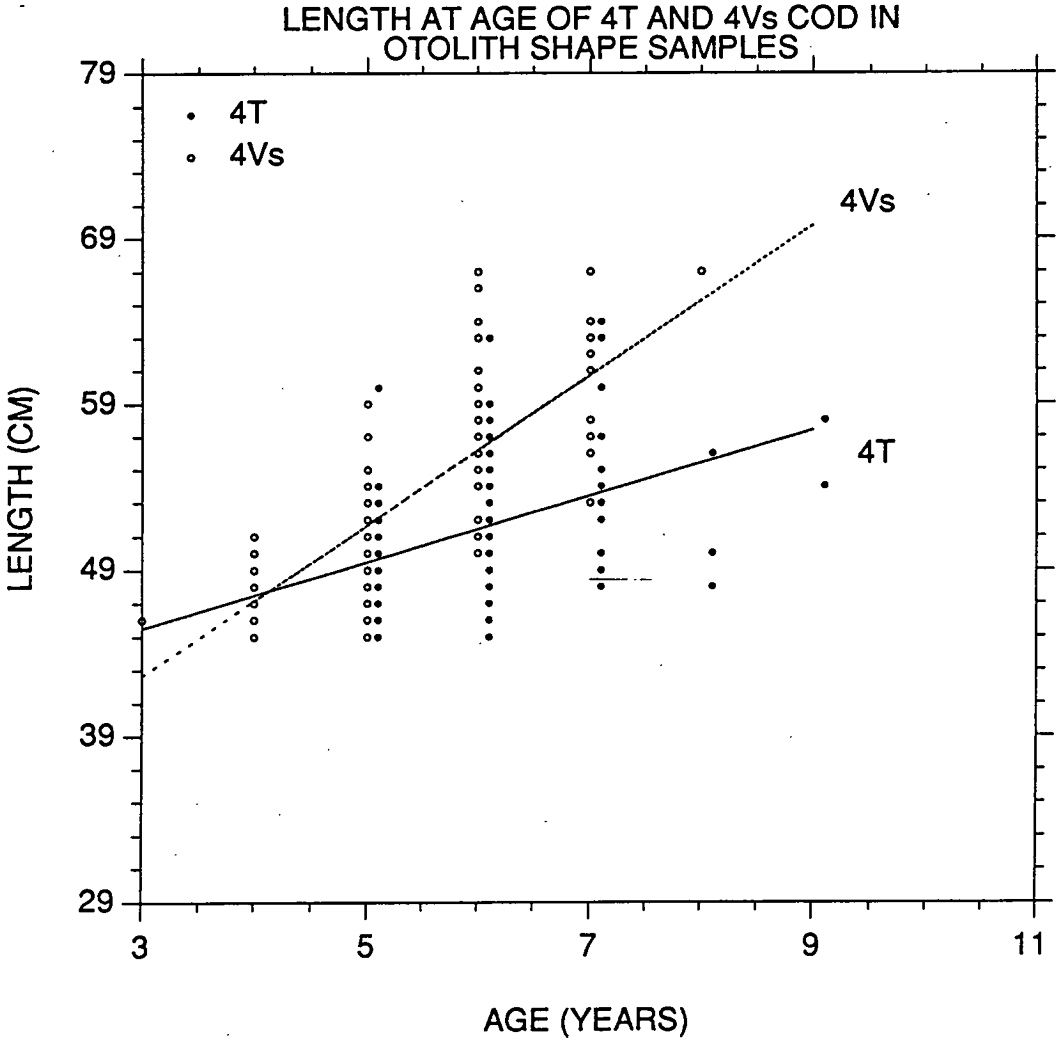


Fig. 5. Classification results of the samples in the 4T/4Vs area based on the discriminant analysis of all 1986 samples using all three otolith types (centroid as origin). The actual sample collection site is shown as a filled square. Percent classification success is as denoted by the size of the square (see scale).

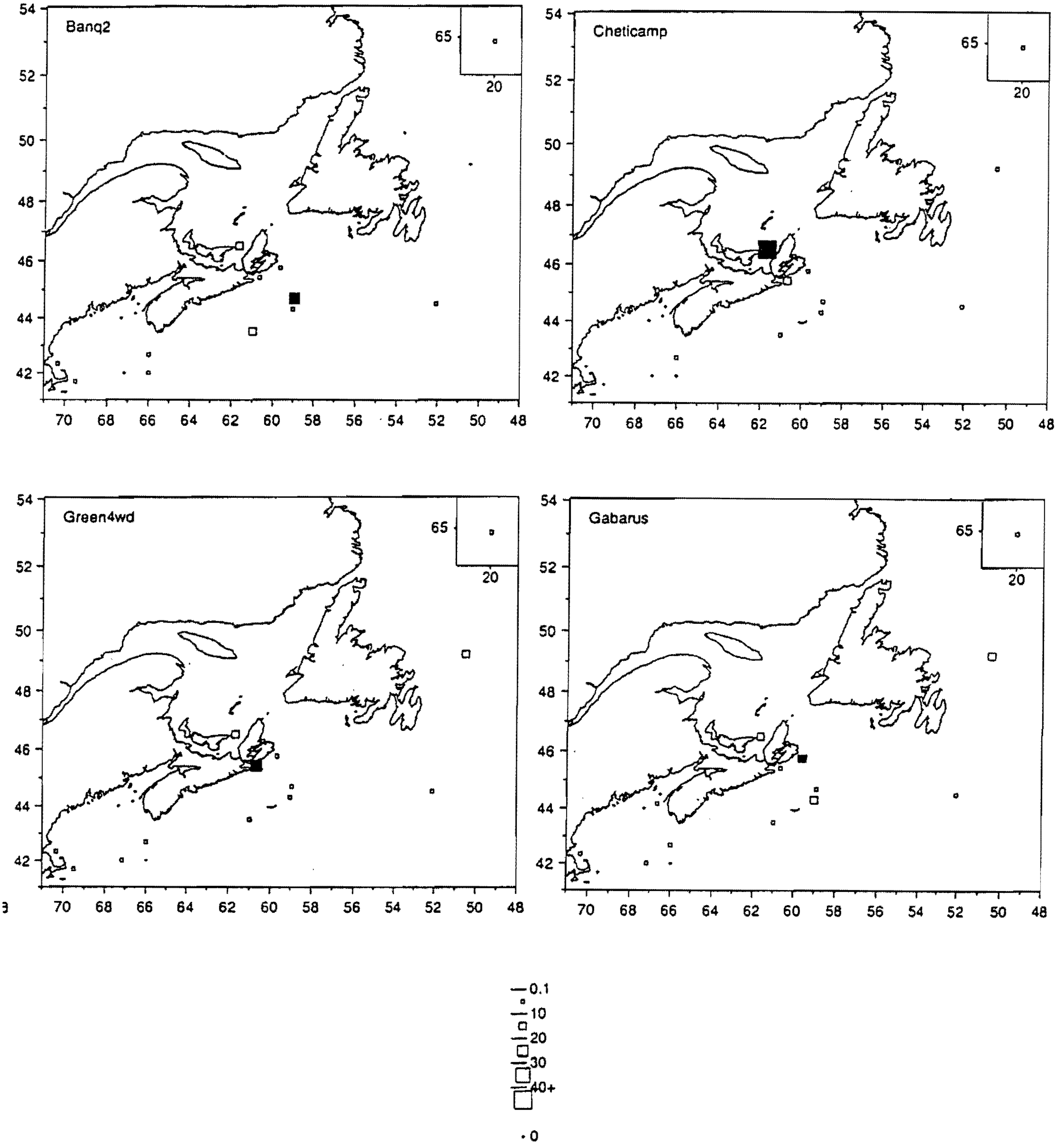


Fig. 6. Summary map depicting the classification patterns resulting from the centroid-based discriminant analysis of all three left-hand otolith types combined. The expanding symbols represent the percentage of fish entered into the discriminant functions which were correctly classified. Vectors connecting samples represent misclassification errors which exceeded 15%. None of the samples were mistakenly assigned to a single sample at a rate of more than 20%. Misclassification errors of less than 15% are not shown. The 200-m contour is shown. The sample from Iceland is shown as an insert in the top right-hand corner.

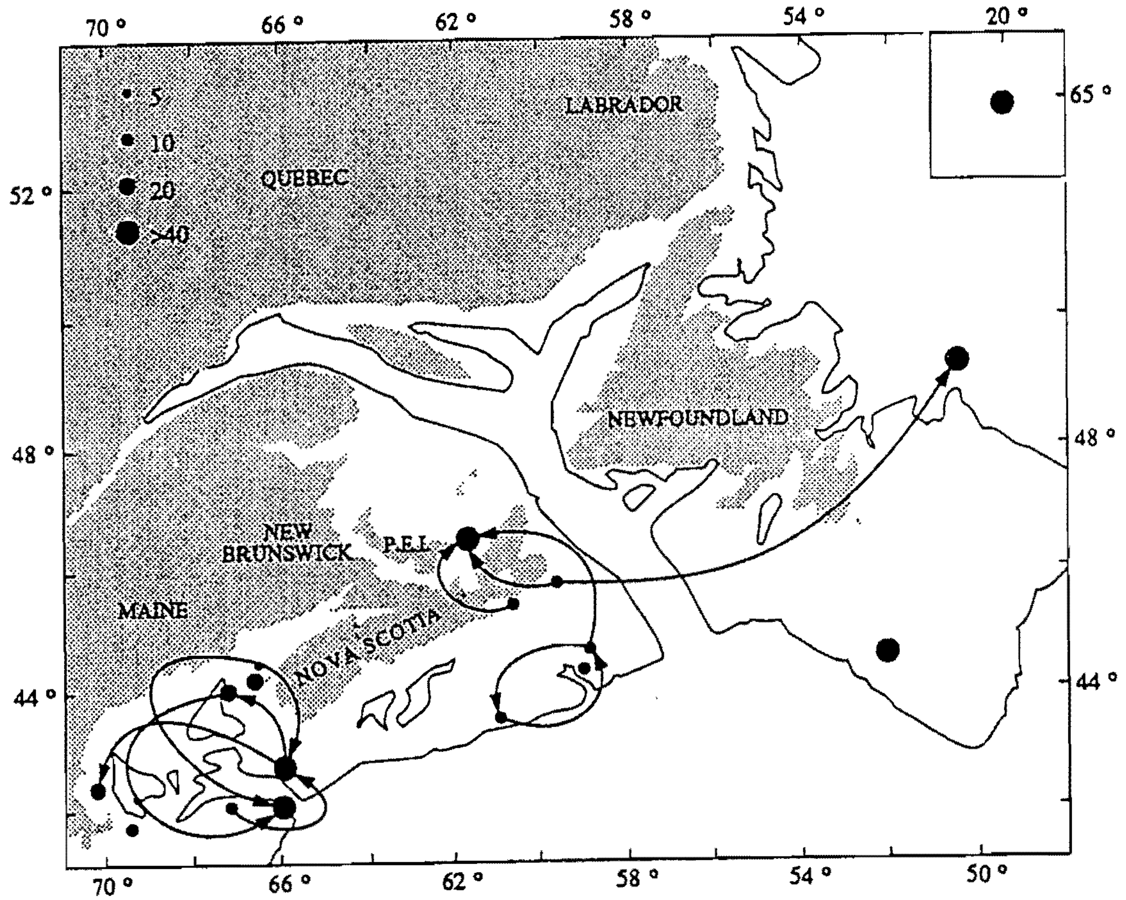


Fig. 7. Relationship between mean annual growth rate (length/age) and otolith shape (as indicated by the value of the first discriminant function for all three centroid-based otolith shapes, evaluated at each sample centroid). Sample numbers are shown on the plot. The regression line is decribed by $Y = -2.493 + 0.187X$ ($R^2 = 0.78$).