Stock Discrimination Using Otolith Shape Analysis

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Otolith shape has long been known to be species specific, but recent reports have pointed to its value as an indicator of stock identity. To test this hypothesis, all three pairs of otoliths were sampled from 2349 Atlantic cod (*Gadus morhua*) collected on spawning grounds 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 year-classes. The sagittal otoliths (largest pair) provided slightly better stock discrimination than did the lapillar or asteriscal otoliths. 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. Reevaluation of published studies on other species indicated that growth rate contributes more variation to regional differences in otolith shape than does stock origin. Differences in otolith shape among ages, sexes, and year-classes were also attributable to growth rate differences. To the extent that growth rates vary more between than within stocks, otolith shape analysis can provide an easily determined measure of stock identity.

On sait depuis longtemps que la forme des otolithes est particulière à une espèce, mais des travaux récents ont souligné sa valeur comme indicateur de l'identité d'un stock. Pour tester cette hypothèse, on a prélevé les trois paires d'otolithes chez 2 349 morues franches (*Gadus morhua*) capturées sur les frayères dans tout l'Atlantique nord-ouest. La forme des otolithes a été déterminée par une analyse de Fourier, et combinée à des mesures de la superficie et du périmètre des otolithes. On a noté des différences hautement significatives dans la forme parmi la plupart des échantillons de morue, mais la forme différait aussi entre les âges, les sexes et les classes annuelles. Les sagitta (la paire la plus grosse) permettaient une discrimination des stocks légèrement meilleure que les lapillus ou les asteriscus. La première fonction discriminante était fortement corrélée au taux de croissance du poisson et de l'otolithe, ce qui indique que la discrimination des stocks s'améliorait à mesure qu'augmentait la différence dans le taux de croissance de chaque stock. Le réexamen des travaux publiés sur d'autres espèces indique que le taux de croissance fait varier davantage les différences régionales dans la forme des otolithes que l'origine du stock. Les différences dans la forme des otolithes entre les âges, les sexes et les classes annuelles étaient aussi attribuables à des différences dans le taux de croissance. Dans la mesure où les taux de croissance varient davantage entre les stocks qu'à l'intérieur d'un stock, l'analyse de la forme des otolithes peut constituer une méthode facile pour déterminer l'identité des stocks.

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Stock discrimination forms the basis for much of fisheries management in the northwest Atlantic. Measures of growth, survival, and reproductive success all assume that a single population is being monitored. Where such measures are confounded by population mixing, studies of fish biology, population dynamics, and most estimates of yield can be invalidated. In principle, genetic differentiation should form the basis for any inferences concerning population distinction. However, analyses based on protein electrophoresis (Cross and Payne 1978; Mort et al. 1985; Grant et al. 1987; Mulligan et al. 1988) and mitochondrial DNA (Smith et al. 1989; Carr and Marshall 1991; Dahle 1991) have, in general, been unsuccessful in differentiating among marine populations. Under the assumption that stocks loosely represent populations, tagging (Wise 1963), morphometrics (Thorpe 1976), meristics (Blouw et al. 1988), parasite loads (Scott and Martin 1957), ichthyoplankton surveys (O'Boyle et al. 1984), immunological characteristics (Schill and Dorazio 1990), and other approaches have all been used to confirm the presence of multiple stocks, but none has provided a reliable indicator of stock identity. Indeed, there appear to be few, if any, markers which can be used to differentiate among all populations of any marine fish species.

The shape of the otolith would appear to be an ideal natural marker for fish populations. Otolith shape is markedly species specific (Morrow 1976; Gaemers 1984; L'Abée-Lund 1988) and less variable in growth than 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 altered (Campana and Neilson 1985; Casselman 1987). Therefore, otoliths remain unaffected by the short-term changes in fish condition (e.g., starvation) which can confound body morphometrics. Since otoliths are composed of calcium carbonate, otolith shape is

TABLE 1. Sample collection.

Sample No.	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

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 they remain at least partially segregated.

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; Godø 1984; Neilson et al. 1985; Dawson 1991), otolith annuli (Rollefsen 1933, cited in Reisagg and Jørstad 1984; Rauck 1974; Godø 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 year-classes 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 overall utility 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. Since the previous studies had examined relatively few samples, the current study was designed to examine a large number of fish, spread over a broad geographic area and a large number of putative stocks. The Atlantic cod (*Gadus morhua*) stock complex in the northwest Atlantic Ocean was selected as the test species, in part due to its distribution and number of resident stocks, and in part because of the background knowledge of stock structure already available for this species (McKenzie 1934, 1956; Templeman 1962; Wise 1963; Cross and Payne 1978; Lear and Wells 1984; Mork et al. 1985; Lear 1986; Dahle 1991). Additional factors evaluated in this study were age, sex, year-class effects, and the relative contributions of genetic and environmental effects to otolith shape variation. Since all previous studies examined only the sagittal (largest) otolith pair, we also examined the additional stock structure information present in the shape of the other two otolith pairs. Fourier analysis was selected as the most objective and powerful of the shape analysis techniques. However, careful attention was also given to the apparent shape differences that arise through selection of the nucleus (rather than the centroid) as the centre of the otolith, and the influence of fish length on the Fourier variables.

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 spawning stocks.

Cod were either collected at sea aboard research vessels using otter trawl gear or sampled from commercial catches where the fishing location was unambiguous. Most samples consisted of 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 caused by size-related effects. A total of 2349 fish were collected from 19 sites along the eastern coast of Canada, the northeastern coast of the United States, and several miles off of the western coast of 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

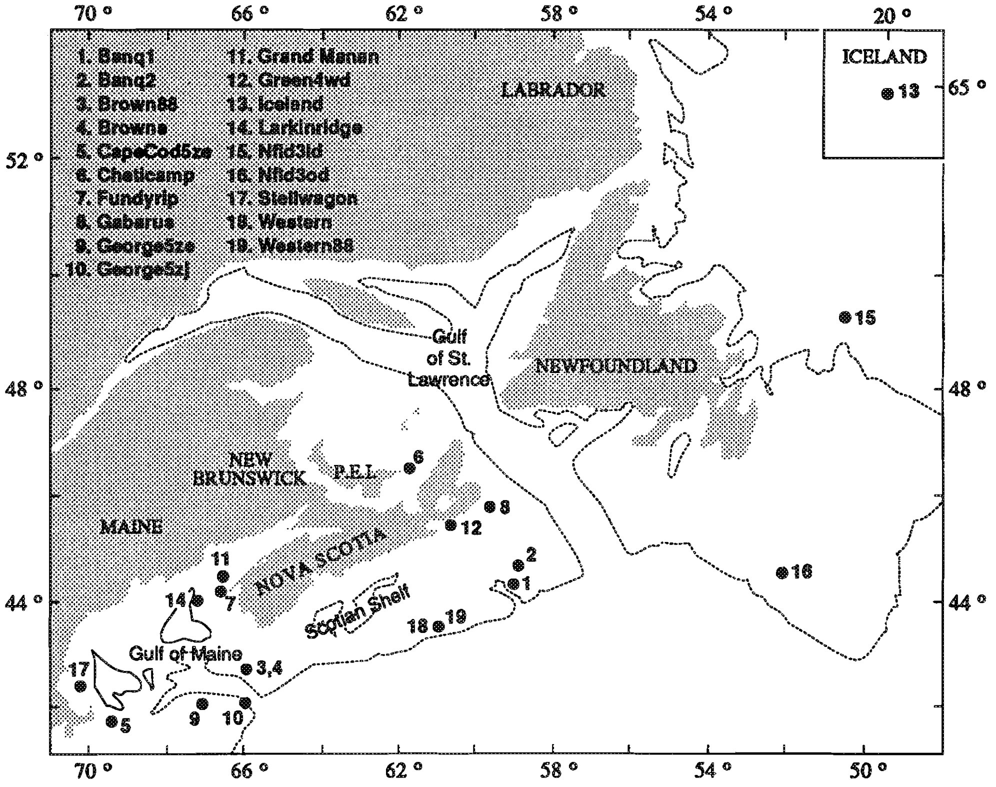

FIG. 1. Map of the study area and sampling sites. The sample from Iceland was collected several miles off the western coast of Iceland. The 200-m contour is shown.

of two sites (Browns Bank and Western Bank), which were also sampled in 1988 to test for year effects within a site. Sampling dates varied across several months because of 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. 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, their location relative to the brain and the sagittae is reported here. 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 components.

In the first step of the procedure, each otolith was oriented in a consistent manner on a dissecting microscope stage (Fig. 2); 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 3.2× (sagittae) or 8× (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 converted to a binary image 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. 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 (Fig. 2). 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 as follows: sagittae, tip of rostrum; lapilli, acute vertex at the confluence of the 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.

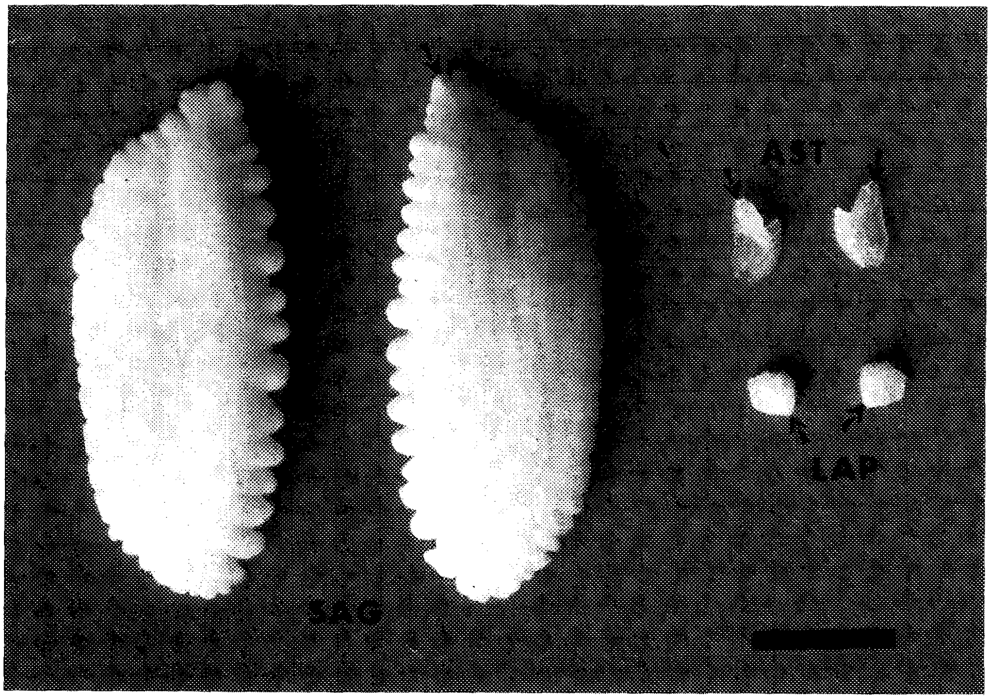

FIG. 2. Photograph of the three otolith pairs (SAG = sagittae; LAP = lapilli; AST = asterisci) from an 85-cm cod. Each otolith pair is presented with the left-hand otolith on the left side. The landmark used to begin the edge-following procedure is indicated by an arrow. Bar = 5 mm.

The second stage of the shape analysis procedure involved the interpretation of the otolith contour data in terms of Fourier components. Fourier analysis has been described in detail elsewhere (Younker and Ehrlich 1977; Full and Ehrlich 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

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

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 to be a means of describing a shape in terms of cosine waves. A series of radii are drawn at equal angular intervals from some central location within the contour 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 additive, a second cosine wave of different amplitude and phase angle can then be added to the first, thus explaining 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 nth harmonic represents that of a n-leafed clover. There is no limit to the number of harmonics that 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. Figure 3 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), modified by J.M. Casselman and K. Scott (unpublished), 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, St. Andrews, N.B. E0G 2X0, personal communication). 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 reread by the same person approximately 6 mo after the initial reading. Ageing precision was good for all stocks, with coefficients of variation (Chang 1982) ranging from 0 to 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.

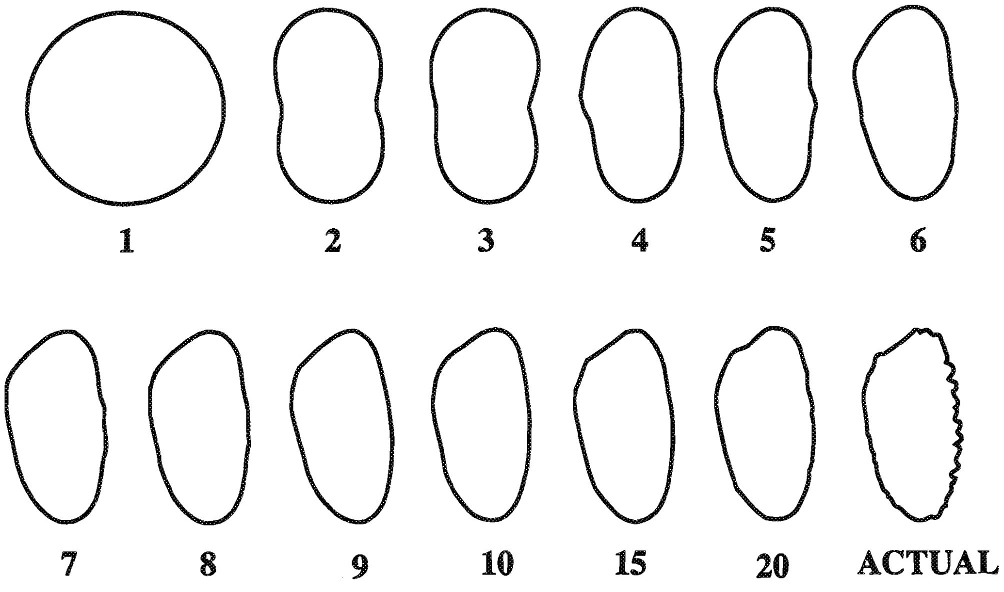

FIG. 3. Reconstruction of a typical sagitta from its Fourier variables, using the centroid as the otolith centre. The number within each shape represents the number of harmonics used to prepare the shape. The actual digitized shape is presented last. Note that the rough outline is reconstructed relatively accurately with only the first six harmonics, but that the scalloped edges require more than 20 harmonics.

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 sampling site 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, natural log (ln) or square root transformed. The phase angle data were, of course, circularly distributed between 0 and 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 that 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 nonnormal, unimodal distribution that 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 intersample differences in otolith shape could have resulted artifactually from differences in length frequency among samples. Accordingly, all otoliths were standardized to a common size by removing the common, within-group slope of otolith length on all variables. 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 intersample 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 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 the model results were unduly influenced by the intersample 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 otolith 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 obvious differences in length frequency among sample sites (Fig. 4). Modal lengths for the southern samples were less than 60 cm, while those for the Newfoundland and Iceland samples were up to 20 cm longer. The distribution of age frequencies among samples also differed substantially (Fig. 5), although not necessarily in parallel with the length frequencies. Cod collected in the Gulf of Maine region averaged 2–5 yr old, while those in more northerly waters averaged 6–8 yr old and some reached 14 yr. 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 threefold difference in mean annual growth rates between cod in the Gulf of Maine and those in the Gulf of St. Lawrence.

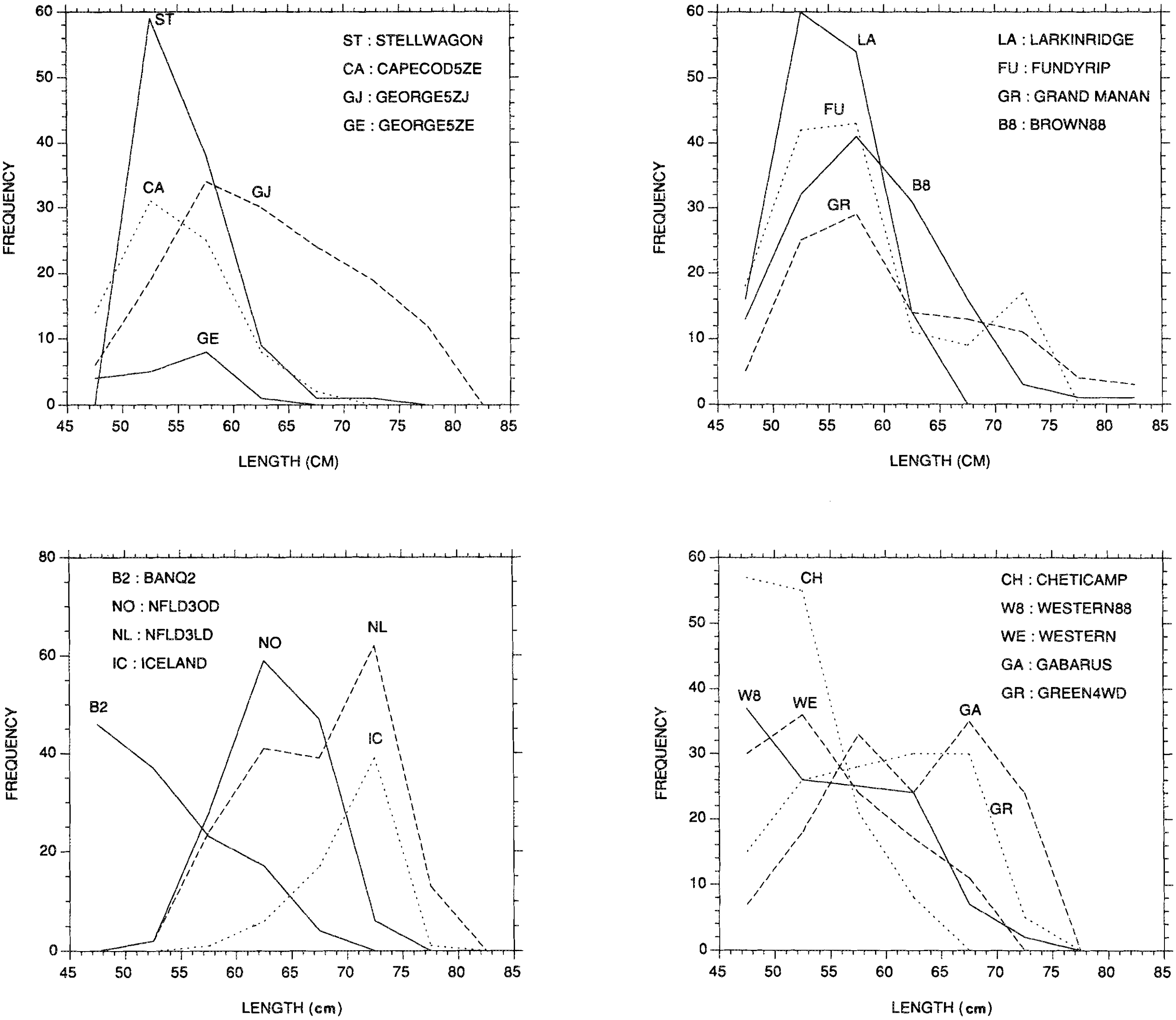

FIG. 4. Length frequencies of cod by sample collection site. Samples were restricted to a length range of 45–85 cm. All fish lengths are presented, with the exception of Browns and Banq1, for which only heads were collected.

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 consistent 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.

Fourier analysis indicated that more than 99.9% of the otolith shape variability could be summarized by 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 (Table 2). The choice of otolith centre (nucleus versus centroid) used in the Fourier analysis was also influential, although by design, centroid-based Fourier calculations discount the influence of the first harmonic to near zero. When the nucleus was used as the centre, over 80% of the variance in lapillar shape was explained by the first harmonic, while the third harmonic explained most of the remaining variance. Fourier analysis with the centroid as the otolith centre resulted in harmonics 2–5 being most important. The more variable asteriscal shapes required six harmonics to explain most of the variation, while the sagittae required five. For all otolith types, each harmonic after the eighth 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 visual impressions that shape was quite variable. Coefficients of variation (CV) for the Fourier amplitudes were 1.5–4.0 times

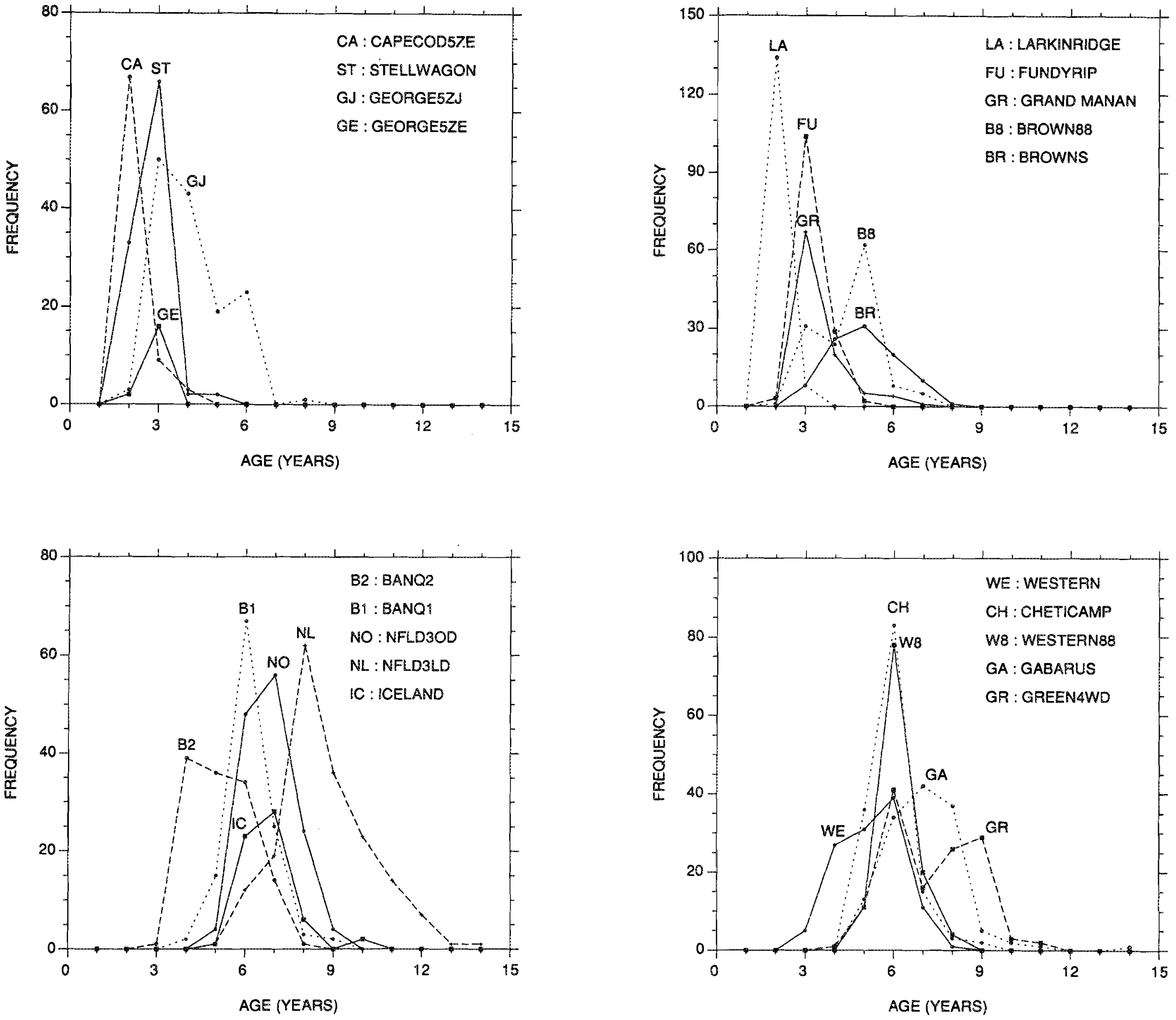

FIG. 5. Age frequencies of cod by sample collection site.

higher among fish of a given sample than between left and right otoliths of the same fish. Otolith area and perimeter CVs were 4–8 times higher among fish than within otolith pairs. Thus, otolith shapes and in particular otolith size were 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 (using the mean of the within-sample, centroid-based Fourier variables) for five representative samples demonstrated that regional shape differences were subtle but discernable (Fig. 6). 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, or even 20, harmonics (Fig. 3).

Age, Length, and Sex Effects on the Shape Variables

Correlation tables prepared for each of the otolith types demonstrated that few of the shape variables were significantly correlated with each other. With respect to the sagittae, only the otolith perimeter (ln transformed) and otolith area (ln-transformed square root of the area) were highly correlated in either the centroid-as-centre or nucleus-as-centre analysis ($r = 0.93$ in both analyses). Most of this correlation was due to a fish length effect which, when removed, eliminated much of the correlation. Hence, both variables were left in the analysis. The distance from the nucleus to the centroid (CFOCUS) was highly correlated with the first amplitude ($r = 0.86$); therefore, it was removed from the analysis. While there was some tendency for the first amplitude to be correlated with the next one to four amplitudes, this was not considered to be a serious problem. None of the amplitudes were significantly correlated with the corresponding phase angle. Correlation tables for the other two otolith types revealed similar patterns, but of lesser magnitude.

TABLE 2. Fourier components from each of the three otolith types for five representative sampling sites. Amplitude (AMP), phase angle (PHA), and percent explained variance (EXV) by sampling site are presented for harmonics 1–10. Phase angles are presented in terms of the median value. N = nucleus as center for Fourier calculations; C = centroid as center for Fourier calculations; ASTL = left asteriscus; SAGL = left sagitta; LAPL = left lapillus.

			Harmonic 1				Harmonic 2				Harmonic 3				Harmonic 4				Harmonic 5			
			AMP				AMP				AMP				AMP				AMP			
	Site		Mean	SD	PHA med.	EXV	Mean	SD	PHA med.	EXV	Mean	SD	PHA med.	EXV	Mean	SD	PHA med.	EXV	Mean	SD	PHA med.	EXV
ASTL	5	N	0.179	0.088	305.2	29.3	0.118	0.044	31.8	13.6	0.149	0.033	307.3	21.1	0.124	0.036	302.3	15.0	0.099	0.023	316.8	9.2
		C	0.050	0.021	166.2	2.8	0.170	0.044	329.2	33.2	0.164	0.039	302.5	30.6	0.112	0.032	319.9	14.9	0.088	0.028	326.7	9.3
	6	N	0.215	0.094	303.4	40.8	0.111	0.045	34.5	13.0	0.119	0.035	323.0	13.9	0.109	0.030	314.6	12.3	0.083	0.027	324.7	7.2
		C	0.038	0.015	161.2	2.1	0.157	0.048	25.5	36.0	0.124	0.040	318.2	22.7	0.098	0.030	323.1	15.4	0.088	0.030	34.8	11.5
	13	N	0.223	0.110	306.4	35.2	0.131	0.051	36.4	14.1	0.151	0.035	318.7	17.0	0.125	0.039	309.6	12.8	0.097	0.026	330.9	7.7
		C	0.051	0.019	159.8	2.6	0.192	0.052	25.9	36.7	0.160	0.040	311.2	26.0	0.111	0.031	325.8	13.4	0.103	0.029	49.0	11.0
	16	N	0.233	0.097	303.4	41.9	0.109	0.056	45.7	11.4	0.132	0.039	320.9	14.6	0.116	0.035	313.2	12.1	0.086	0.029	322.9	7.1
		C	0.039	0.020	163.4	2.0	0.164	0.057	33.6	34.7	0.133	0.046	318.5	23.9	0.098	0.034	329.9	14.1	0.092	0.037	37.9	11.4
	18	N	0.208	0.097	312.6	36.2	0.104	0.049	38.7	11.0	0.136	0.033	318.1	17.1	0.126	0.032	308.8	15.1	0.084	0.027	319.2	7.0
		C	0.044	0.017	168.7	2.4	0.159	0.049	325.2	31.8	0.153	0.038	313.0	29.6	0.103	0.029	322.8	14.3	0.089	0.028	42.0	10.1
SAGL	5	N	1.248	0.266	93.7	33.1	1.573	0.149	324.1	52.7	0.631	0.108	68.7	8.4	0.350	0.081	294.1	2.7	0.305	0.043	40.7	2.0
		C	0.079	0.023	290.4	0.2	1.731	0.130	343.2	89.5	0.145	0.046	112.2	0.7	0.477	0.062	334.8	6.8	0.164	0.038	62.9	0.9
	6	N	1.245	0.256	102.2	30.6	1.654	0.153	325.7	54.4	0.684	0.115	67.8	9.2	0.356	0.078	306.9	2.6	0.329	0.057	43.2	2.1
		C	0.088	0.026	261.5	0.2	1.794	0.151	343.9	88.9	0.142	0.058	77.4	0.7	0.503	0.073	342.0	7.0	0.189	0.044	53.0	1.0
	13	N	1.487	0.273	98.5	36.1	1.722	0.182	322.3	48.9	0.766	0.126	65.9	9.6	0.346	0.099	291.3	2.1	0.358	0.059	40.3	2.1
		C	0.094	0.026	281.2	0.3	1.909	0.175	343.7	88.9	0.178	0.057	94.0	0.9	0.519	0.083	338.8	6.6	0.217	0.043	60.1	1.2
	16	N	1.490	0.265	101.0	38.0	1.638	0.162	322.0	46.5	0.765	0.128	65.9	10.0	0.331	0.090	298.7	2.0	0.361	0.062	39.6	2.2
		C	0.086	0.030	277.4	0.2	1.825	0.161	342.6	88.4	0.175	0.058	94.8	0.9	0.511	0.078	343.3	6.9	0.204	0.049	58.9	1.2
	18	N	1.346	0.271	94.7	33.6	1.653	0.160	323.8	51.3	0.719	0.122	64.5	9.6	0.339	0.081	301.0	2.3	0.337	0.057	42.0	2.1
		C	0.094	0.030	270.6	0.3	1.829	0.163	344.1	88.8	0.157	0.065	89.0	0.8	0.516	0.070	340.3	7.1	0.186	0.047	60.5	1.0
LAPL	5	N	0.222	0.053	317.6	81.3	0.032	0.016	261.3	2.5	0.081	0.015	316.0	12.3	0.024	0.012	214.3	1.3	0.018	0.009	227.9	0.8
		C	0.003	0.002	104.5	0.1	0.048	0.018	332.9	26.0	0.064	0.015	287.1	44.4	0.036	0.012	61.9	15.6	0.021	0.011	289.3	5.4
	6	N	0.269	0.052	321.3	88.1	0.033	0.017	276.3	1.6	0.077	0.014	335.1	8.0	0.026	0.012	257.0	1.0	0.017	0.009	82.5	0.5
		C	0.003	0.002	86.8	0.2	0.046	0.018	34.9	27.2	0.058	0.015	305.2	43.3	0.037	0.011	91.8	18.8	0.019	0.009	299.3	5.4
	13	N	0.244	0.058	324.6	82.6	0.029	0.015	264.4	1.6	0.086	0.019	333.9	11.8	0.032	0.013	250.6	1.8	0.018	0.011	70.7	0.7
		C	0.004	0.002	63.7	0.2	0.042	0.019	46.9	18.4	0.075	0.017	305.5	54.3	0.036	0.011	110.0	13.7	0.026	0.009	322.7	7.0
	16	N	0.237	0.063	320.0	82.0	0.036	0.016	297.1	2.8	0.084	0.018	333.9	11.8	0.023	0.012	243.1	1.1	0.019	0.009	64.7	0.9
		C	0.004	0.002	103.4	0.1	0.056	0.022	331.6	31.6	0.063	0.016	306.1	40.0	0.039	0.012	84.1	16.4	0.023	0.009	307.0	6.1
	18	N	0.293	0.053	319.9	89.2	0.036	0.019	261.1	1.5	0.076	0.015	331.8	6.6	0.035	0.014	263.7	1.4	0.018	0.008	106.7	0.5
		C	0.004	0.002	54.7	0.2	0.040	0.018	259.9	19.7	0.065	0.015	290.8	49.7	0.039	0.011	84.5	19.6	0.020	0.008	302.2	5.1

TABLE 2. (*Concluded*)

			Harmonic 6				Harmonic 7				Harmonic 8				Harmonic 9				Harmonic 10			
			AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA		AMP		PHA	
	Site		Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV	Mean	SD	med.	EXV
ASTL	5	N	0.074	0.025	318.0	5.3	0.045	0.018	313.6	2.1	0.034	0.016	236.8	1.2	0.022	0.012	171.7	0.6	0.021	0.011	160.9	0.5
		C	0.047	0.021	167.1	3.0	0.032	0.015	77.0	1.4	0.025	0.015	187.2	0.9	0.021	0.013	209.3	0.7	0.020	0.010	242.3	0.6
	6	N	0.076	0.027	315.3	5.8	0.043	0.018	82.2	2.0	0.038	0.018	64.8	1.6	0.027	0.013	118.7	0.8	0.021	0.012	112.0	0.6
		C	0.046	0.023	50.0	3.8	0.039	0.021	74.3	2.5	0.025	0.013	218.8	1.2	0.021	0.013	197.2	0.9	0.022	0.011	250.0	0.9
	13	N	0.090	0.026	334.5	6.3	0.049	0.022	295.1	2.1	0.042	0.016	42.2	1.4	0.028	0.013	129.0	0.7	0.022	0.012	126.4	0.5
		C	0.049	0.021	53.2	3.0	0.038	0.016	71.8	1.8	0.028	0.014	256.6	1.0	0.022	0.012	257.0	0.6	0.025	0.015	259.4	0.9
	16	N	0.078	0.031	281.5	5.5	0.045	0.022	128.4	1.9	0.039	0.020	66.8	1.5	0.030	0.016	128.8	0.9	0.023	0.012	172.5	0.5
		C	0.047	0.025	71.4	3.5	0.039	0.021	90.4	2.4	0.031	0.017	248.6	1.6	0.025	0.014	255.1	1.1	0.027	0.014	283.8	1.2
	18	N	0.079	0.027	317.0	5.8	0.047	0.022	275.7	2.3	0.039	0.020	65.6	1.6	0.028	0.017	186.9	0.9	0.022	0.012	124.0	0.5
		C	0.048	0.025	83.8	3.5	0.034	0.020	98.1	1.8	0.030	0.016	234.1	1.5	0.022	0.014	243.5	0.8	0.026	0.013	255.7	1.0
SAGL	5	N	0.060	0.029	210.5	0.1	0.116	0.032	31.2	0.3	0.064	0.025	60.4	0.1	0.057	0.027	304.3	0.1	0.054	0.020	45.1	0.1
		C	0.123	0.034	331.6	0.5	0.126	0.029	47.2	0.5	0.044	0.019	285.1	0.1	0.087	0.020	38.0	0.2	0.031	0.015	246.0	0.0
	6	N	0.060	0.034	271.9	0.1	0.127	0.035	30.5	0.3	0.071	0.028	67.6	0.1	0.066	0.029	313.9	0.1	0.054	0.025	55.4	0.1
		C	0.150	0.041	337.9	0.7	0.141	0.033	42.4	0.6	0.060	0.027	317.4	0.1	0.084	0.027	45.5	0.2	0.035	0.018	296.3	0.0
	13	N	0.072	0.040	185.9	0.1	0.136	0.036	57.1	0.3	0.086	0.034	64.4	0.1	0.072	0.031	309.5	0.1	0.058	0.028	63.5	0.1
		C	0.135	0.040	325.7	0.5	0.154	0.034	50.6	0.6	0.057	0.030	304.3	0.1	0.093	0.028	46.1	0.2	0.044	0.023	280.2	0.1
	16	N	0.067	0.035	196.4	0.1	0.136	0.042	32.2	0.3	0.080	0.033	52.2	0.1	0.066	0.028	303.3	0.1	0.061	0.025	45.1	0.1
		C	0.147	0.048	335.2	0.6	0.147	0.038	40.7	0.6	0.059	0.026	300.0	0.1	0.093	0.026	40.4	0.2	0.039	0.019	290.0	0.1
	18	N	0.058	0.034	192.5	0.1	0.132	0.034	35.8	0.3	0.073	0.030	69.4	0.1	0.061	0.025	284.2	0.1	0.062	0.025	51.4	0.1
		C	0.144	0.040	329.6	0.6	0.144	0.033	50.7	0.6	0.049	0.025	295.9	0.1	0.088	0.027	47.1	0.2	0.036	0.018	268.2	0.0
LAPL	5	N	0.015	0.008	313.0	0.5	0.012	0.006	293.5	0.3	0.009	0.004	294.3	0.2	0.009	0.004	296.4	0.2	0.007	0.004	299.9	0.1
		C	0.012	0.006	306.0	2.1	0.011	0.006	309.2	1.6	0.011	0.005	294.9	1.4	0.008	0.004	287.5	0.9	0.006	0.003	299.2	0.5
	6	N	0.014	0.007	68.6	0.3	0.009	0.004	294.2	0.1	0.008	0.004	303.0	0.1	0.007	0.003	316.0	0.1	0.006	0.003	302.4	0.1
		C	0.010	0.005	236.5	1.6	0.008	0.004	300.0	0.9	0.007	0.004	301.3	0.9	0.005	0.003	67.2	0.3	0.004	0.002	103.4	0.3
	13	N	0.016	0.007	307.1	0.5	0.010	0.005	306.7	0.2	0.010	0.004	315.6	0.2	0.009	0.005	306.0	0.1	0.007	0.004	314.8	0.1
		C	0.012	0.006	208.3	1.8	0.010	0.005	282.0	1.3	0.010	0.005	298.3	1.2	0.005	0.003	230.0	0.4	0.006	0.003	127.0	0.4
	16	N	0.017	0.007	306.5	0.5	0.011	0.005	304.9	0.2	0.009	0.004	311.9	0.2	0.009	0.004	315.9	0.1	0.006	0.003	305.5	0.1
		C	0.012	0.005	314.0	1.7	0.010	0.005	291.1	1.2	0.009	0.004	305.4	1.0	0.006	0.004	83.6	0.4	0.004	0.003	64.6	0.3
	18	N	0.016	0.007	242.2	0.3	0.010	0.005	301.9	0.1	0.008	0.004	290.9	0.1	0.007	0.004	304.6	0.1	0.006	0.003	302.9	0.1
		C	0.010	0.005	274.6	1.4	0.008	0.004	308.3	0.9	0.008	0.004	295.7	1.0	0.006	0.003	163.9	0.5	0.005	0.002	274.7	0.3

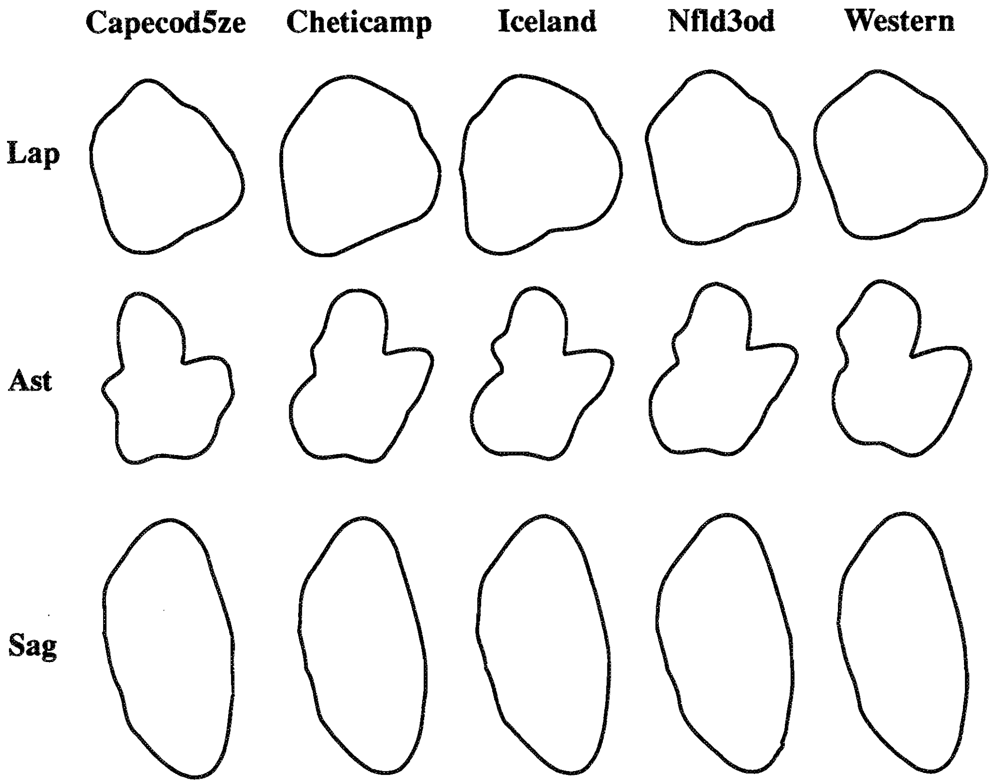

FIG. 6. Reconstruction of the mean otolith shape for five representative samples based on the mean amplitudes and median phases of the first 10 centroid-based harmonics. From top to bottom, the otolith outlines are those of lapilli (Lap), asterisci (Ast), and sagittae (Sag) for each of the five sample sites indicated. The relative sizes of the outlines are not drawn to scale.

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, which were in turn highly correlated with each other. 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 is 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 ANOCOVA (Table 3). 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.

Age was a significant modifier of several of the otolith shape variables. In light of the large disparity in age ranges between slow- and fast-growing samples, it was not possible to analyze all of the shape data for age effects in a single analysis. Accordingly, the data were arbitrarily subdivided into a slow-growing northern aggregation (Banq1, Banq2, Cheti, Gabarus, Green4Wd, Western, and Western88) and a fast-growing southern aggregation (Fundyrip, George5Zj, and Grandmanan). A two-way ANOVA (age and sample) of each of the shape variables for the left sagitta (centroid as centre), across ages common to all samples, resulted in relatively few significant age–sample interaction terms. Age was significant as a main effect for otolith area and the first six amplitudes in both the slow- and the fast-growing aggregations. In all cases, the estimated age parameters were internally conistent in that they formed a unidirectional trend. Neither the phase angle variables nor otolith perimeter varied significantly with age.

As a test of the strength of the age effect, the shape variables (with the length effect removed) from sites sampled in 1986 were entered into a discriminant analysis and used to predict the ages of the corresponding site sampled in 1988. For cod from Western Bank, ages 5–7 were predicted to within ± 1 yr with $\geq$80% accuracy. The same accuracy was achieved for Browns Bank cod of ages 3–5. Classification accuracy was considerably less for older fish, but sample sizes were also low ($<$10) for those ages.

Sex effects on otolith shape were statistically significant, but of smaller magnitude than those of age. An analysis of age and sex by sample using two-way ANOVAs indicated that there were no significant sex effects (either as an age–sex interaction or a main effect) for amplitudes 1–4. On the other hand, otolith area, perimeter, amplitudes 5 and 7, and phase angles 1–5 all had a significant interaction and main effect in several samples. Few significant sex effects were observed in any of the fast-growing samples. Of all the variables, otolith area and perimeter appeared to have the strongest and most consistent response to sex.

There was evidence of sexually dimorphic growth in several of the sample sites (ANOVA of fish length by age and sex within samples), particularly at the slower growing sites (e.g., Banq2 and Cheti). Females tended to be larger at a given age than were males. However, the response was not common to all samples.

All of the samples had sex ratios not significantly different from unity, with the exception of Grandmanan (34 males, 65 females), Iceland (42 males, 18 females) and Western88 (97 males, 16 females).

Table 3. Common, within-group slope of otolith length removed as a covariate from each of the left-hand otolith shape variables. Where indicated (superscript "s") variables were square root transformed prior to ANOCOVA. Otolith perimeter (LNP) and area (LNSQA) were first ln transformed, as were their covariates. CFOCUS = distance from the nucleus to the centroid.

	Asteriscus		Lapillus		Sagitta	
Variable	Slope	SE	Slope	SE	Slope	SE
			Centroid as centre			
AMP 1	0.0568^s	0.0034	0.0301^s	0.0023	0.0197^s	0.0008
AMP 2	0.1346	0.0034	0.0481	0.0026	0.1333	0.0016
AMP 3	0.0382	0.0035	0.0915	0.0037	0.0169	0.0011
AMP 4	0.0211	0.0027	0.0342	0.0015	0.0440	0.0011
AMP 5	0.0480	0.0024	0.0271	0.0012	0.0221	0.0008
AMP 6	0.0503^s	0.0047	0.0383^s	0.0038	0.0153	0.0007
AMP 7	0.0480^s	0.0040	0.0557^s	0.0033	0.0150	0.0006
AMP 8	0.0291^s	0.0040	0.0103^s	0.0033	0.0125^s	0.0011
AMP 9	0.0275^s	0.0037	0.0590^s	0.0029	0.0085	0.0005
AMP 10	0.0374^s	0.0034	0.0405^s	0.0026	0.0116^s	0.0009
LNP	0.8587	0.0113	0.8981	0.0077	1.0030	0.0095
LNSQA	0.6904	0.0090	0.8158	0.0075	0.8744	0.0084
			Nucleus as centre			
AMP 1	0.1226^s	0.0088	0.1326	0.0076	0.0650	0.0053
AMP 2	0.1457^s	0.0058	0.0468^s	0.0070	0.1213	0.0020
AMP 3	0.0597	0.0028	0.0672	0.0018	0.0590	0.0021
AMP 4	0.0177	0.0029	0.0603^s	0.0055	0.0284	0.0016
AMP 5	0.0346	0.0022	0.0892^s	0.0044	0.0331	0.0009
AMP 6	0.0451	0.0022	0.0208	0.0009	0.0059	0.0007
AMP 7	0.0703^s	0.0042	0.0402^s	0.0036	0.0156	0.0006
AMP 8	0.0714^s	0.0040	0.0493^s	0.0032	0.0056	0.0006
AMP 9	0.0453^s	0.0039	0.0416^s	0.0031	0.0068	0.0005
AMP 10	0.0290^s	0.0035	0.0295^s	0.0029	—	—
LNP	0.8569	0.0114	0.8984	0.0077	1.0084	0.0095
LNSQA	0.6897	0.0090	0.8160	0.0075	0.8742	0.0084
CFOCUS	0.1208^s	0.0074	0.1336	0.0077	0.0815	0.0052

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 and perimeter contributed most of the explanatory power to the analysis (Table 4). Analyses using only the amplitude variables were slightly less successful than were those which used only area and perimeter. Use of all variables, including the phase angle variables, provided the most accurate classification rates. There was no obvious difference in classification success between covariate-removed amplitudes and those standardized to mean radial length. Results obtained with 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.

The similarity in classification success between discriminant analyses based on covariate-removed versus standardized amplitudes was somewhat surprising, given the fact that the covariate-removed amplitudes should have had all otolith/fish size effects removed while there was still a significant relationship between the standardized amplitudes and otolith/fish length. The similarity in classification success could have been a result of either different amplitude covariate slopes among the samples, or a weak relationship between the standardized amplitudes and otolith length. As a test of the former, discriminant analyses of the centroid-based sagittal shape variables were repeated using fish restricted to a length range of 50–60 cm. Classification success did not change in a consistent manner, confounding the interpretation. However, since a similar result was obtained when the same analysis was conducted with the standardized amplitudes, the length effect may be relatively minor. Whatever length effect was present was probably incompletely removed by both the ANOCOVA and the standardized variable methods.

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 was cor-

Table 4. Summary of discriminant analyses of the left sagitta comparing the effects of choice of otolith centre (nucleus versus centroid), "standardized" (AMPs 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. The discriminant functions were calculated on the basis of the individual samples, not those aggregated into regions.

	Classification success (%)					
		By region[a]				
Analysis type	By sample	Gulf of Maine	Eastern Scotian Shelf	Gulf of St. Lawrence	Newfoundland	Iceland
Centroid; LNP, LNSQA only	17.6	61	37	58	58	7
Centroid; AMPs 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 AMPs	21.2	69	46	54	58	14
Centroid; standardized AMPs only	13.0	61	28	19	43	3

[a] Samples included in each region: Gulf of Maine = samples 3, 4, 5, 7, 9, 10, 11, 14, 17; Eastern Scotian Shelf = samples 1, 2, 8, 12, 18, 19; Gulf of St. Lawrence = sample 6; Newfoundland = samples 15, 16; Iceland = sample 13.

Table 5. Summary of discriminant analyses of the left otolith comparing the predictive power of otolith type (AST = asteriscus; LAP = lapillus; SAG = sagitta), choice of otolith centre (nucleus versus centroid), and "standardized" (AMPs divided by mean radial length) versus covariate-removed data. All discriminant analyses used the Fourier amplitudes (AMPs), LNP (perimeter), and LNSQA (area), without the PHA variables. Classification success refers to classification of samples not used in the discriminant analysis. The discriminant functions were calculated on the basis of the individual samples, not those aggregated into regions.

	Classification success (%)					
		By region[a]				
Analysis type	By sample	Gulf of Maine	Eastern Scotian Shelf	Gulf of St. Lawrence	Newfoundland	Iceland
AST centroid	12.6	69	28	42	25	14
AST centroid; standardized AMPs	12.4	67	37	49	14	6
AST nucleus	10.9	73	31	18	22	4
AST nucleus; standardized AMPs	11.5	72	33	32	16	4
LAP centroid	11.4	76	41	3	25	12
LAP centroid; standardized AMPs	15.1	75	37	15	37	12
LAP nucleus	13.9	71	33	6	40	13
LAP nucleus; standardized AMPs	14.4	69	35	12	32	22
SAG centroid	18.3	70	45	40	54	10
SAG centroid; standardized AMPs	21.2	69	46	54	58	14
SAG nucleus	21.4	73	48	39	49	13
SAG nucleus; standardized AMPs	23.0	75	49	33	52	13

[a] Samples included in each region: Gulf of Maine = samples 3, 4, 5, 7, 9, 10, 11, 14, 17; Eastern Scotian Shelf = samples 1, 2, 8, 12, 18, 19; Gulf of St. Lawrence = sample 6; Newfoundland = samples 15, 16; Iceland = sample 13.

rectly identified as being from Browns Bank, as compared with 13% of the 1986 test sample. 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 and 17% of the test 1986 sample 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 (Table 5). There were no consistent differences between the two otolith centres (nucleus versus centroid) within a given otolith type, nor between covariate-removed and standardized amplitudes. On the other hand, 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 (Table 6). While the first six discriminant functions were all

TABLE 6. Statistics and unstandardized function coefficients from the discriminant analysis of the centroid-based, left sagitta shape variables based on covariate-removed data. AMP = Fourier amplitude; LNP = ln-transformed otolith perimeter; LNSQA = ln-transformed otolith area; superscript "s" = square root transformed.

	Discriminant function						
Shape variable	1	2	3	4	5	6	7
$AMP1^s$	−2.688	0.710	15.991	−15.181	−32.433	8.742	20.279
AMP2	3.568	7.783	4.859	8.005	9.575	−0.926	6.001
AMP3	3.491	3.331	−0.417	13.368	12.742	−3.076	−19.312
AMP5	−8.089	−0.387	−10.678	14.149	16.687	15.746	0.732
AMP6	−5.399	−1.664	−4.423	33.392	−7.760	−5.594	17.828
$AMP8^s$	−4.065	4.285	−3.987	−2.536	12.025	4.426	5.734
AMP9	14.395	−6.152	19.240	10.861	12.468	−20.054	−6.841
LNP	33.457	−9.712	−15.573	8.984	−0.097	10.914	3.549
LNSQA	−27.624	−8.365	34.693	28.839	12.859	−11.604	52.445
Constant	−38.753	9.849	22.176	5.237	7.681	−12.844	7.241
Eigenvalue	0.929	0.319	0.095	0.057	0.027	0.022	0.018
Percent of variance	62.47	21.45	6.43	3.88	1.83	1.53	1.23
Cumulative percent	62.47	83.91	90.34	94.22	96 05	97.58	98.81
Significance	0.000	0.000	0.000	0.000	0.016	0.069	0.209

statistically significant, the first two 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 to Table 4. The rates of incorrect classification were then plotted against the distances to the other regions (Fig. 7). No relationship was evident, suggesting that regions were not simply misclassified to the nearest alternative region. However, 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 (Fundyrip, Cheticamp, and Nfld3Ld) resulted in an overall classification success of 78% for the test sample. There was greater than 89% differentiation between the Cheticamp and Fundyrip samples. Conversely, analysis of the seven 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.

Since the classification success of a given sample varied with the otolith type that was used (Table 5), 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 type alone. Accordingly, the centroid-based shape variables (without phase angles) 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 (Table 5) and appeared to accurately identify regional aggregations of samples (Fig. 8). 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 that entered the discriminant analysis, only four consisted of higher order harmonics (e.g., harmonics >6). Otolith area and perimeter from all of the otolith types were influential variables; sagittal and lapillar area and perimeter made up four of the first seven variables entered into the analysis. Overall, the shape variables derived from sagittae 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-phase-angle variables combined.

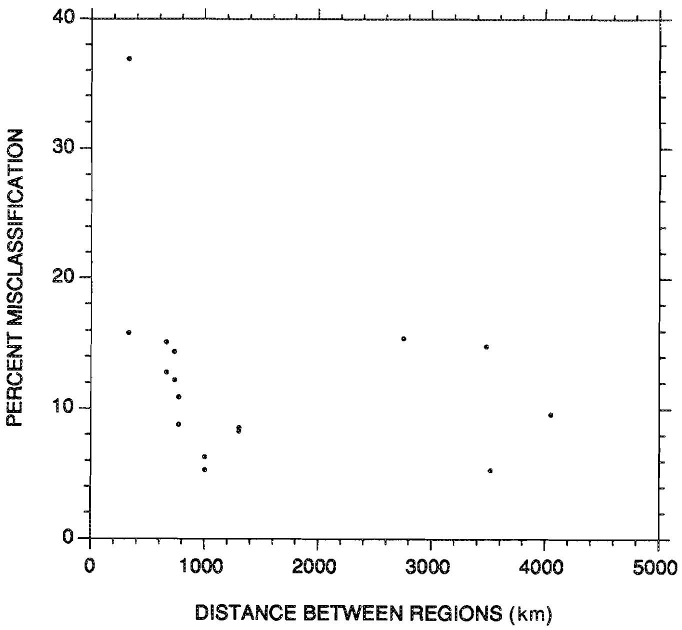

FIG. 7. Misclassification by region as a function of the distance to the approximate centre of the samples comprising that region. Samples included in each region are identified in the footnote to Table 4.

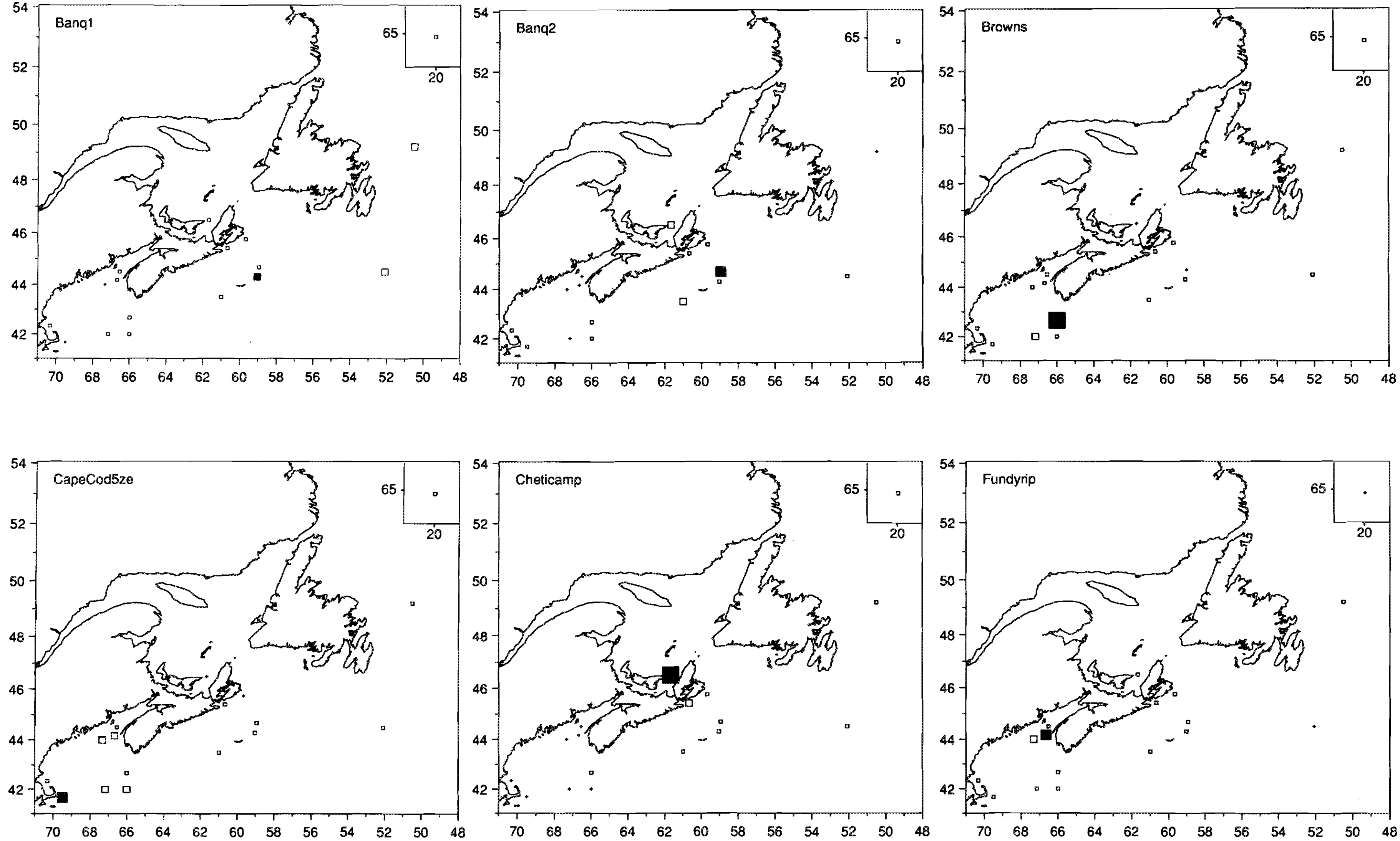

FIG. 8. Expanding symbol plots of percent classification success based on the discriminant analysis of the 1986 samples using all three otolith types (centroid as origin). The actual sample collection site is shown as a solid square. The sample from Iceland is shown as an insert in the top right-hand corner. The scale for the expanding symbols is presented to the right of the last panel. (*Fig. 8 continued next page*)

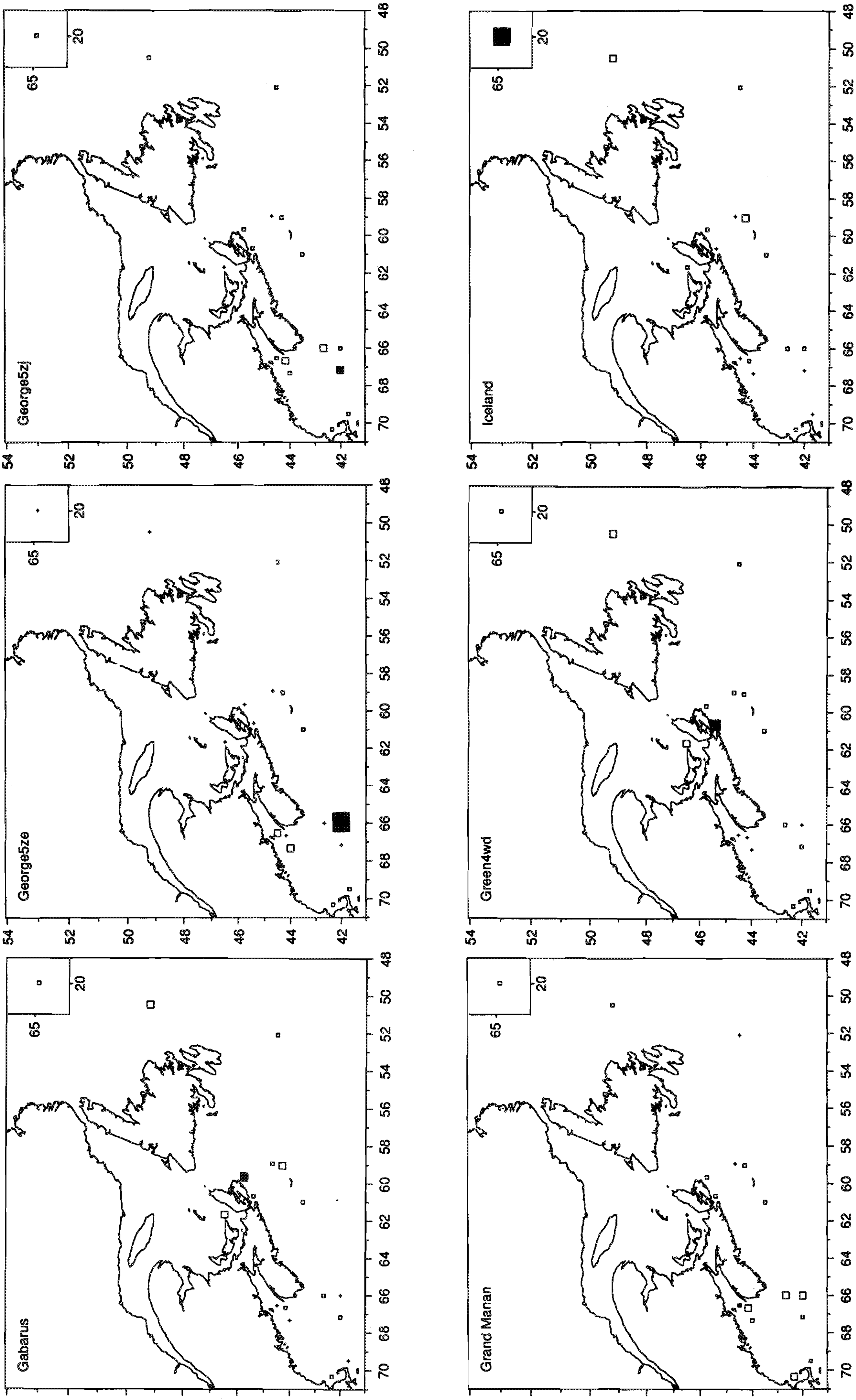

FIG. 8. (*Continued next page*)

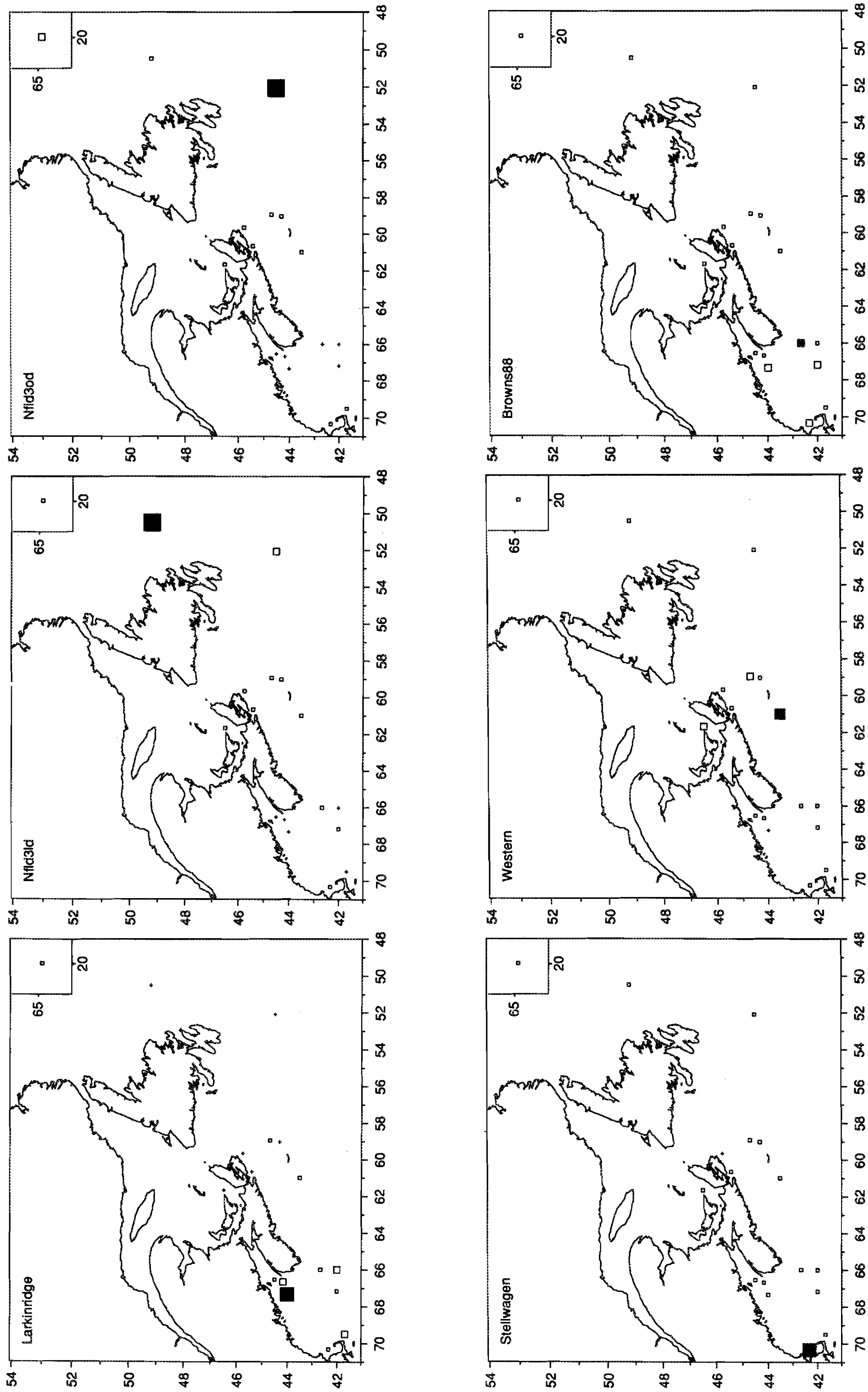

FIG. 8. *(Concluded next page)*

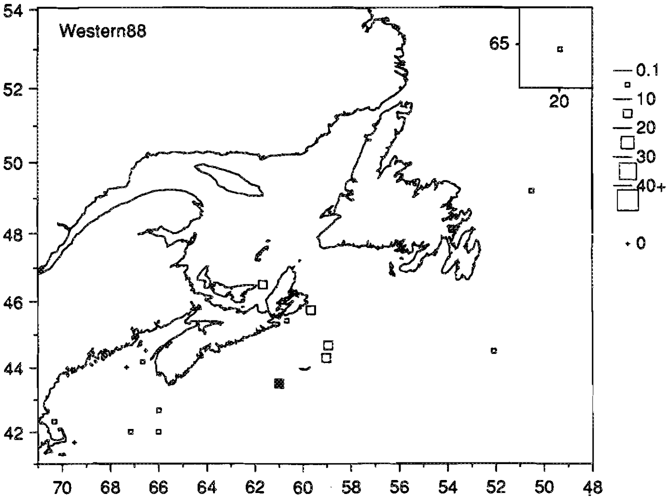

FIG. 8. (*Concluded*)

Otolith Shape as a Function of Growth Rate

Examination of Fig. 8 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 (Nfld3ld and Nfld3od) were often misclassified onto the eastern Scotian Shelf (Banq1, Banq2, Gabarus, Green4WD, Western, and Western88) 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. 8 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 that are comparable with 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 somatic growth rate (Fig. 9). 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 amplitudes were entered into the discriminant analysis. Similar results were obtained with or without the phase angle variables, if only otolith area/perimeter were analyzed, or using only the standardized amplitudes. ANOCOVA indicated that growth rate was a significant covariate of most (but not all) shape variables.

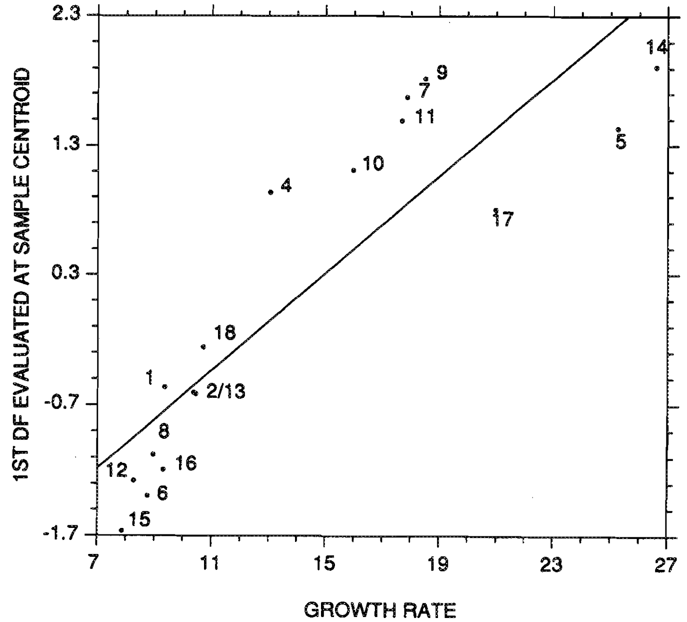

FIG. 9. Relationship between mean annual growth rate (cm/yr) of the fish and otolith shape (as indicated by the value of the first discriminant function for all three centroid-based otolith shapes, evaluated at each sample centroid). Sample numbers are shown on the plot. The regression line is described by $Y = -2.493 + 0.187X$ ($R^2 = 0.78$).

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 reanalyzed after otolith growth rate was removed 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 as strong as that of the environmental component.

A further test for genetic differences in sagittal shape among samples was made by analyzing five samples (Banq1, Gabarus, Iceland, Nfld3od, and Western88), 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 classified correctly in only 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

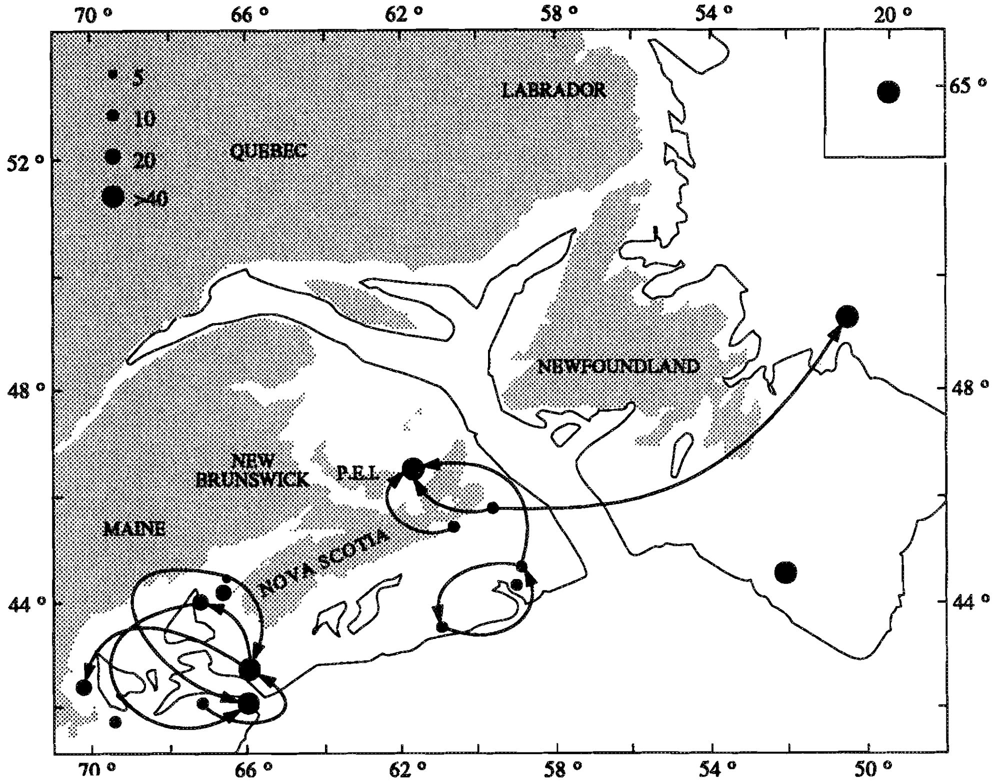

FIG. 10. Summary map depicting the classification patterns resulting from the centroid-based discriminant analysis of all three left-hand otolith types combined. The expanding symbols represent the percentage of fish entered into the discriminant functions which were correctly classified. Vectors connecting 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.

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 lie with relative year-to-year constancy in the growth rate of a given stock.

Geographic Patterns in Otolith Shape

There were consistent similarities among the classification patterns from each of the otolith types, whether based on the nucleus or centroid as the otolith centre. As summarized in Fig. 10, samples from within broad geographic regions, such as the Gulf of Maine, were well differentiated from other regions, but poorly discriminated from samples within the same region. Otoliths from Gulf of St. Lawrence (Cheticamp) cod tended to be accurately classified (40–60% accuracy), but classification errors to the northeastern Scotian Shelf were common. Classification errors in one of the Newfoundland samples were most often linked to the other sample from the same area (see also Fig. 8). The Iceland sample was the anomaly here; misclassifications from the Iceland sample were often directed to the Scotian Shelf, rather than the geographically adjacent Newfoundland samples.

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 definable 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 more 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 aggegations using otolith shape analysis largely conformed to stock boundaries determined with 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, personal communication). 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. Similarly, 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 was unexpected. Numerous studies 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 did fast-growing fish of the same size. However, the changes in relative size noted in the above studies were never associated with changes in shape, nor was there reason to expect them to be; a change in size need not induce any change in shape. The apparently common response of relative otolith size to a change in growth, especially growth changes induced by environmental conditions, 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 year-class 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 lake 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 be differentiated when growth rates were similar. Growth rate effects also appeared to account for the observed differences among age groups. In a study of herring otoliths, Bird et al. (1986) reported relatively few significant differences among the first 10 harmonics when comparing between Alaskan stocks (*Clupea pallasi*) or between widely spaced Atlantic stocks (*Clupea harengus*) (Georges Bank and Gulf of St. Lawrence herring). Conversely, 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 reflected corresponding differences 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) (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 distinction, growth-related otolith shapes appear to better account for Bird et al.'s (1986) results than do stock-specific differences. Smith (1992) came to a similar conclusion in a recently published study on Pacific deep slope 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 Atlantic 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 indicated relative similarity between the northern and southern contingent (e.g., 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 are 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 year-classes are also consistent with mackerel's propensity for changes in growth rate with year-class 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 nongenetic 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, what is the best way to go about it? In terms of the otolith type selected for use, the sagittae would appear to be as good or better than the other otolith types. Sagittae are routinely used for age determination, and because of their greater size, are easiest to collect from cod (and most other species). In addition, in this study, asteriscal shape tended to be most variable, both within and among fish, while the lapilli tended to be relatively featureless. Both of these latter otolith types resulted in weaker group discrimination than did the sagittae. These results are contrary to Radtke's (1984) observation of noticeable lapillar shape differences between inshore and offshore cod.

Selection of the otolith centre used in the Fourier analysis had little impact on the eventual classification success. Biologically, one would expect the otolith nucleus to be the most useful reference point, given its status as the origin of growth of the otolith. However, use of the biological centre did not result in any clear advantage over the mathematical centroid, and indeed, required operator intervention. In any case, the lapillar nucleus was almost impossible to view externally. There are also mathematical reasons for selecting the centroid for use in the Fourier analysis; use of the sometimes eccentrically located nucleus can potentially result in "centering error" (Full and Ehrlich 1982), although the latter does not apply to analysis of shapes which are unravelled from homologous landmarks (such as the postrostrum). For this reason, shapes should be unravelled from the same landmark on all otoliths. The presence of homologous landmarks also makes the Fourier analysis of otoliths more rigorous than that of many other objects (Younker and Ehrlich 1977; Full and Ehrlich 1982), since it reduces the variability of the Fourier amplitudes and makes the Fourier phase angles interpretable. Unfortunately, there is no straightforward way to normalize the phase angles prior to incorporation into the multivariate analysis. Most, if not all, workers have excluded Fourier phase angles from their studies (Younker and Ehrlich 1977; O'Higgins and Williams 1987).

One of the more surprising findings of this study concerned the removal of fish size effects from the otolith shape variables. Most commercial image analysis systems which incorporate Fourier analysis compute the standardized Fourier amplitudes (amplitude divided by the mean radial length). This study demonstrated that the standardized amplitudes do not completely remove the effect of fish length. However, the more statistically rigorous procedure of removing either fish or otolith length as a common, within-group covariate (Reist 1985; Claytor and MacCrimmon 1987) also failed to remove all of the length effect. Neither approach seemed to be clearly superior to the other. Since the standardized amplitudes can be calculated automatically, they are logistically easier to use. Given the widespread variation of the otolith – fish length relationship with growth rate (Templeman and Squires 1956; Boehlert 1985; Mosegaard et al. 1988; Secor and Dean 1989; Campana 1990; Casselman 1990), and given that such an effect could confound the variation in otolith shape, the Fourier amplitudes are best standardized using otolith length rather than fish length, irrespective of the standardization approach used. Note also that otolith shape varies ontogenetically independent of growth rate (Harkonen 1986), such that otoliths from young fish become increasingly difficult to differentiate among species as size decreases. Accordingly, otolith shape analysis may be of negligible value to stock identification if applied to sexually immature fish.

There is no reason to expect the principles of otolith shape analysis discussed above to differ among species; otolith growth processes appear to be ubiquitous to all species (Campana and Neilson 1985). However, the specifics may well change. Otolith shape differs substantially among species (Nolf 1985; Harkonen 1986), suggesting that the most influential shape variables may well change across species. Even the preferred otolith type may differ among species, since the sagitta is the largest otolith in many, but not all, teleosts (e.g., Cypriniformes). However, it appears unlikely that selection of the largest otolith type, whichever it is, would prove to be a bad decision.

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) is not suited for addressing the site affinity problem; that is, the technique does not have sufficient power to determine whether 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.

The potential utility of otolith shape analysis extends beyond that of stock identification. Catch misreporting by area/stock is common in some areas of the world; while otolith shape analysis would have insufficient discriminatory power to confirm the catch location of some stocks, only a handful of otoliths would have to be sampled from the catch to distinguish between, say, Gulf of Maine and Gulf of St. Lawrence cod. Studies of seal diet depend to a large extent on the species identification of otoliths collected from stomachs or scats (Murie and Lavigne 1985; Dellinger and Trillmich 1988). Otolith shape analysis could conceivably be used to help determine the area of feeding or test for size-selective prey mortality. Finally, archaeologists and paleontologists, who now use otolith shape to confirm species identity in fossil cores and archaeological digs, could extend their conclusions to include area of capture. In all of the above applications, the major advantage of otolith shape analysis is the requirement for only the otolith; neither the fish carcass nor body measurements are needed.

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