Distinction between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics

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Abstract.–A method for using whole otolith morphometrics to identify silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic is described. Whole sagittal otolith morphometric variables of length, width, area, perimeter, circularity, and rectangularity were extracted by image processing and, in combination with age-specific discriminant analyses, were used to differentiate two stocks of silver hake: a northern stock from the Gulf of Maine to northern Georges Bank and a southern stock from southern Georges Bank to the Middle Atlantic. Further support for these groupings is supported by growth rate analyses: fish of the northern stock grew slower than those of the southern stock, resulting in typically larger otoliths for fish from the northern stock. This study demonstrates that whole otolith morphometrics, specific to fish age, are useful in identifying silver hake stocks and can be a useful tool in identifying other fish stocks.

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Accurate distinction between fish stocks is necessary for effective fisheries management to prevent overfishing of individual stocks, to preserve the reproductive and genetic diversity of stock complexes, and to determine optimal strategies for rebuilding stocks (Cushing, 1968; Booke, 1981; Grimes et al., 1987; Begg et al., 1999; Stephenson, 1999). Integral to attaining such management strategies and objectives, is the necessary requirement of reevaluating stock definitions when needed, particularly when the status of the resource changes, or when new technologies become available that may provide more effective stock discrimination tools than those already in use (Begg and Waldman, 1999). Because of the uncertainties surrounding the stock definitions for silver hake (*Merluccius bilinearis*) in U.S. waters of the northwest Atlantic, such a need for reevaluation of stock definitions arose. Furthermore, there was no efficient method for adequately discriminating between silver hake stocks that could also provide a fast and accurate way to determine levels of stock mixing for current fishery assessment and management. In our study, we focused on these issues by incorporating the technology and efficiency of image analysis systems, in combination with multivariate statistical analyses, to distinguish between silver hake stocks on the basis of phenotypic differences in whole otoliths.

Silver hake, (or whiting), is a principal groundfish species found along the continental shelf and slope from Newfoundland to South Carolina, inhabiting depths from shallow inshore waters to those greater than 400 m (Bigelow and Schroeder, 1953; Almeida, 1987; Helser et al., 1995). Silver hake are ecologically important both as a predator and prey in the northwest Atlantic ecosystem (Edwards and Bowman, 1979; Bowman and Michaels, 1984), and form an important part of the commercial fisheries throughout this region (Anonymous, 1998). With annual landings averaging 16,600 metric tons, silver hake are currently considered overexploited (Anonymous, 1998).

Two stocks of silver hake are currently defined in U.S. waters on the basis of analysis of research survey and commercial catch data, as well as multivariate analysis of external morphometric data: a northern stock (from the Gulf of Maine to northern Georges Bank) and a southern stock (from southern Georges Bank to the Middle Atlantic) (Almeida, 1987). Distributions of the two stocks vary seasonally and spatially, probably in response to temperature and depth (Helser et al., 1995). Silver hake that compose the northern stock overwinter in the deeper regions of the Gulf of Maine and during their peak spawning months of July and August, are found on northern Georges Bank and in coastal waters east of Cape Cod, north to Grand Manan (Bigelow and Schroeder, 1953; Almeida, 1987). In contrast, the southern stock is found predominantly in waters from Cape Cod to Montauk Point, New York, and during peak spawning, which occurs in June and July, it is found on southern Georges Bank, in the New York Bight, and as far south as Cape Hatteras (Almeida, 1987; Helser et al., 1995).

Although silver hake in U.S. waters are currently assessed according to this two-stock scheme, uncertainty exists in the stock definitions. A variety of other stock identification techniques have also been used to identify these two stocks of silver hake, including examining external morphometrics (Conover et al., 1961), growth rate comparisons (Nichy, 1969), immunological analyses (Konstantinov and Noskov, 1969), research survey distribution studies (Anderson, 1974), and genetic analyses (Schenk, 1981). However, the stock boundaries suggested in each of these studies have all tended to vary from one another. More recently, Helser (1996) found that silver hake growth rates differed both between and within the two stocks—a finding that is inconsistent with the stock boundaries currently defined (Almeida, 1987) and that confounds the delineation of the stocks and the degree to which the two stocks mix. Given this finding, we believed further investigation into the stock structure of silver hake was needed to clarify the current management units defined for U.S. waters. In addition, we aimed to introduce a more efficient and accurate method for distinguishing between silver hake stocks than the previous methods that have been used, one which would have the potential to enable rapid and accurate in-season assessment of the degree of stock mixing for silver hake.

Otolith morphometric data have recently been used to identify stocks of marine fish, including Atlantic mackerel, *Scomber scombrus* (Dawson, 1991; Hopkins[1]), Atlantic herring, *Clupea harengus* (Messieh et al., 1989), and Atlantic salmon, *Salmo salar* (Friedland and Reddin, 1994). In contrast, otolith morphometric data have not been used to differentiate between stocks of silver hake, but because silver hake have been found to differ phenotypically (Almeida, 1987), and possibly genetically (Schenck, 1981), it was assumed that otolith morphometric characteristics may also vary. Dery (1988) observed that silver hake otoliths from the northern stock were narrower and thicker in cross-section than those of the southern stock, although no statistical analyses were used to test her observations. Accordingly, it was thought that otolith morphometrics could differ between the two silver hake stocks and could prove to be useful characters by which to differentiate stocks in the future. The collection of such data by means of image processing techniques has proven to be useful in stock identification, providing accurate and efficient measures that traditional morphometric methods have not been able to provide (Jearld, 1995; Cadrin and Friedland, 1999). Given these benefits and Dery's (1988) observations, our study aimed to identify whole otolith morphometric characteristics unique to silver hake stocks in U.S. waters of the northwest Atlantic by using image processing techniques. We address the advantages and limitations encountered when using this method to distinguish fish stocks, and reevaluate the current silver hake stock scheme given the recent within-stock growth differences observed by Helser (1996).

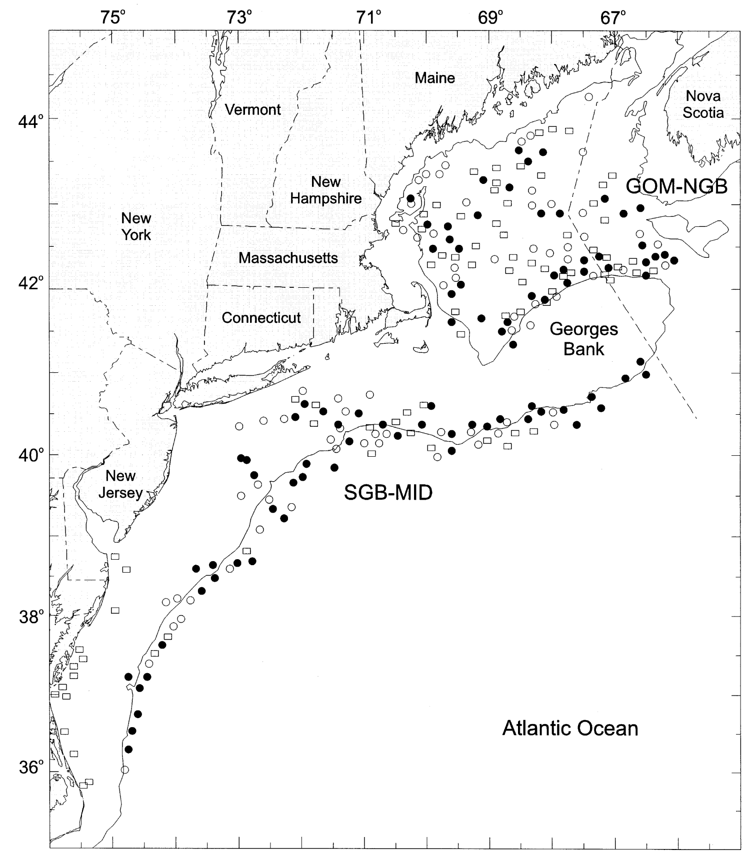

Figure 1

NEFSC spring survey stations where silver hake samples were collected for otolith morphometric-based stock discrimination analysis in 1992 (squares), 1994 (closed circles), and 1996 (open circles). GOM-NGB: Gulf of Maine to northern Georges Bank; SGB-MID: southern Georges Bank to the Middle Atlantic.

Materials and methods

Sample collection

Silver hake were collected in 1992, 1994, and 1996 during the spring Northeast Fisheries Science Center (NEFSC) stratified random bottom-trawl surveys when the fish were assumed to be on their spawning grounds. Silver hake samples were obtained from the Gulf of Maine to northern Georges Bank (GOM–NGB), and from southern Georges Bank to the Middle Atlantic regions (SGB–MID) (Fig. 1, Table 1). Silver hake were measured (fork length [FL], cm),

[1] Hopkins, P. J. 1986. Mackerel stock discrimination using otolith morphometrics. ICES Council Meeting 1986, 17 p.

sex was determined by macroscopic examination of the gonads, and sagittal otolith pairs were removed from each fish. One otolith from each pair was sectioned and assigned an age following methods in Dery (1988). The other otolith of the pair, either left or right, was then used for morphometric analysis. Samples were restricted to fish of ages 1 to 3 years, because older fish were infrequently collected in the surveys. This restriction also enabled both size- and age-related variation, which could have confounded discrimination analyses, to be minimized.

Otolith orientation was standardized by positioning the otolith with its proximal side down and the rostrum was used as a common starting point from which the perimeter was traced in a counterclockwise direction. Broken or visibly damaged otoliths were not measured. Whole otolith length, width, area and perimeter, and two shape indices—circularity and rectangularity, were collected for each otolith by using the OPTIMAS™ (version 6.2) image analysis system (OPTIMAS, 1996). Rectangularity is a measure of the otolith area divided by the area of its minimum enclosing rectangle, and circularity is the perimeter of the otolith squared divided by its area (OPTIMAS, 1996). All otolith measurements were performed at a magnification of 7×.

Table 1

Sampling information on silver hake.

Region	Age group (years)	Year class	Sex	Length range (cm)	*n*
North	1	1991	Females	—	—
			Males	12–18	45
South	1	1991	Females	11–19	22
			Males	11–20	22
North	2	1990	Females	17–28	50
			Males	16–24	31
South	2	1990	Females	18–22	6
			Males	17–26	11
North	3	1989	Females	23–32	15
			Males	22–29	12
South	3	1989	Females	26–36	9
			Males	26–28	5
North	3	1991	Females	23–31	50
			Males	23–29	26
South	3	1991	Females	23–35	50
			Males	23–32	50
North	3	1993	Females	20–32	53
			Males	22–29	24
South	3	1993	Females	21–34	53
			Males	22–33	25

Data analysis

Bottom ocean temperatures measured throughout the NEFSC spring and autumn bottom-trawl surveys from 1989 to 1996 were compared between the northern and southern regions to determine whether different thermal regimes exist between the two regions that could effect growth rates and subsequent otolith morphometric characteristics of silver hake inhabiting each of these regions. A two-way, fixed-factor, unbalanced analysis of variance (ANOVA) was used to compare bottom temperatures between the regions and time of the survey. Following a significant interaction between these factors, unpaired *t*-tests were used to compare bottom temperatures between regions for each survey, and one-way ANOVAs were used to compare bottom temperatures between surveys for each region. Significance levels were corrected for multiple testing by using the Bonferroni adjustment factor. Tukey's honestly significant difference (HSD) tests were used for *a posteriori* comparisons.

Growth rates of silver hake sampled throughout the NEFSC spring bottom-trawl surveys were calculated by using linear regressions for samples of each sex, year, and region of capture to determine if growth differences existed that might be indicative of stock separation. Analysis of covariance (ANCOVA) was used to compare differences in growth rates of silver hake between the sexes (same year and region), sampling years (same sex and region), and regions (same year and sex).

All otolith morphometric variables were first examined for normality and homogeneity of variances, and were $\log_e$-transformed prior to statistical analysis if these criteria were not satisfied. ANCOVA was then used to determine the effect of fish length on the magnitude of each otolith morphometric variable. "Region" was treated as the main factor and "length" was the covariate. Morphometric variables for which "region-length" interactions were significant were not included in any further analyses because they could not be corrected for fish length. Variables that were significantly correlated with fish length were corrected for variable fish length by using the common within-group slope (*b*).

Multivariate analysis of variance (MANOVA) was used to compare otoliths sampled for each sex (same region, year class, and age group) by using the appropriate length-corrected variables. One-way ANOVAs were then used to examine individual morphometric variables to explain any significant differences detected by the MANOVAs. Significance levels were corrected for multiple testing with the Bonferroni adjustment factor. Likewise, multi- and univariate analyses were then used to test the effects of year class (samples from the same region and age group) on otolith structure. Following these analyses, similar tests were used to investigate morphological differences in otolith samples from the northern and southern regions. Spatial comparisons were made between samples from the different regions for fish of the same age group, sex, and year class. Canonical discriminant analysis was then used to detect morphometric differences in otoliths of silver hake sampled from the northern and southern regions. Significant ($P<0.05$) canonical variates represented the optimal combination of regions and morphometric variables that provided the best overall discrimination between the groups. Standardized coefficients provided for each significant variable represented the contribution of the respective morphometric variable to the discrimination of silver hake samples between regions. Jack-knifed cross-validation procedures were used to give unbiased estimates of classification success (SYSTAT, 1997).

Results

Bottom ocean temperatures differed significantly between region and time of survey (ANOVA interaction, F=11.7, df=15, 2931, P<0.0001). Generally, temperatures were significantly lower in the northern region than in the southern region for all surveys (t-tests, P<0.0001), except during the spring of 1994 and 1996 (although consistent differences were still maintained between regions during these times, albeit not significant) (Fig. 2). Within each region, bottom temperatures have a consistent seasonal pattern, tending to be significantly lower in spring than autumn for both regions (ANOVA, P<0.0001; HSD, P<0.05), with no apparent differences between years within seasons and regions (Fig. 2).

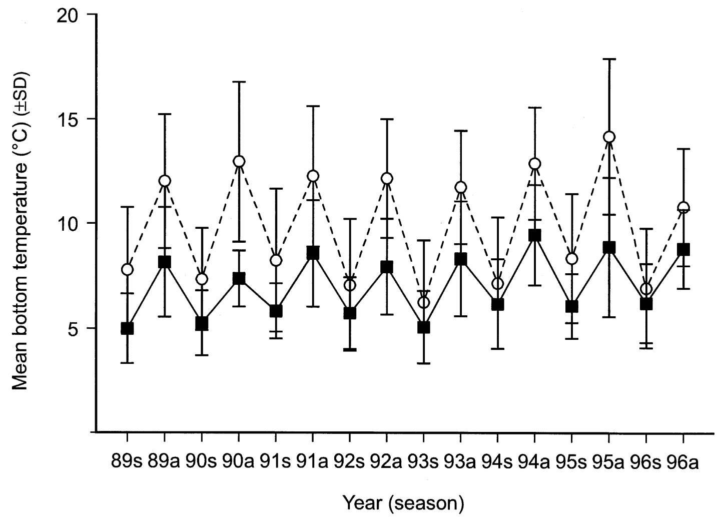

Figure 2

Mean ocean bottom temperatures (°C) (± standard deviation) of northern (squares) and southern (circles) regions collected from the spring (s) and autumn (a) NEFSC bottom-trawl surveys (1989–1996).

Silver hake had significantly different growth rates between sexes, year classes, and the regions from where they were sampled (Fig. 3). Female silver hake grew at a faster rate than males in both the northern and southern regions; and age-length differences between the sexes increased as the fish got older (ANCOVA, P<0.05). Growth rates of each sex, within each region, also differed between sampling years, indicative of the presence of a year class effect (ANCOVA, P<0.005). Likewise, significant differences in growth rates were found between silver hake from the northern and southern regions in each year for both males and females (ANCOVA, P<0.005).

The six otolith morphometric variables (length, width, area, perimeter, circularity, and rectangularity) that were measured for silver hake were $\log_e$-transformed, except for otolith width, to correct for non-normality and heterogeneity of variances. ANCOVAs detected significant (P<0.0001) "region-fish length" interactions for four (otolith area, length, width, and perimeter) of the six variables measured when all samples were examined together, irrespective of age group, year class, or sex. However, the use of individual ANCOVAs for fish of each age group reduced the number of interactions to only one variable for both 1-year-old (rectangularity) and 2-year-old silver hake (width), and two variables (area, length) for samples from 3-year-olds (Table 2). All the remaining variables for the different aged samples were significantly correlated with fish length (P<0.01), and therefore were corrected for variable fish length with their respective common within-group slope (Table 2). Consequently, length-corrected data determined from the individual ANCOVAs for each age group were used for the remaining analyses and indicated the strong effect different ages can have on these types of measurements. Typically, for any given fish length, silver hake from the northern region appeared to have larger otolith dimensions (area, length, width, and perimeter) than those fish from the southern region (Fig. 4).

Multi- and univariate analyses were used to examine confounding sources of variation that may have influenced regional patterns of silver hake (Table 3). In general, otolith morphometrics were not significantly different (P>0.05) between the sexes, indicating that their otoliths develop at a similar rate over the age range examined. In contrast, as for the growth rate analyses, significant differences were found among otolith morphometrics of the same-age silver hake sampled from different year classes for both males and females (Table 3). Likewise, similar year-class differences in otolith morphometrics were found when the sexes were pooled together, confirming congruency in rates of otolith development, and justifying assimilation of sexes for statistical purposes.

Silver hake sampled from the northern region tended to have larger otolith dimensions than those from the southern region (Fig. 4). Overall, when the appropriate length-corrected morphometric variables were examined together for silver hake of the same age group and year class, irrespective of sex, significant differences were found between samples from the northern and southern regions (MANOVA, P<0.001) (Table 4). Fewer multivariate significant differences were detected for samples from 3-year-olds, although these differences were probably the result of samples for this age group having fewer variables to examine compared with samples from the other age groups. This was supported by univariate tests where significant differences (P<0.05) between silver hake from the northern and southern regions were found for several variables, for all age groups, and for almost all of the age group, sex, and year class combinations (Table 4). Also, the oto-

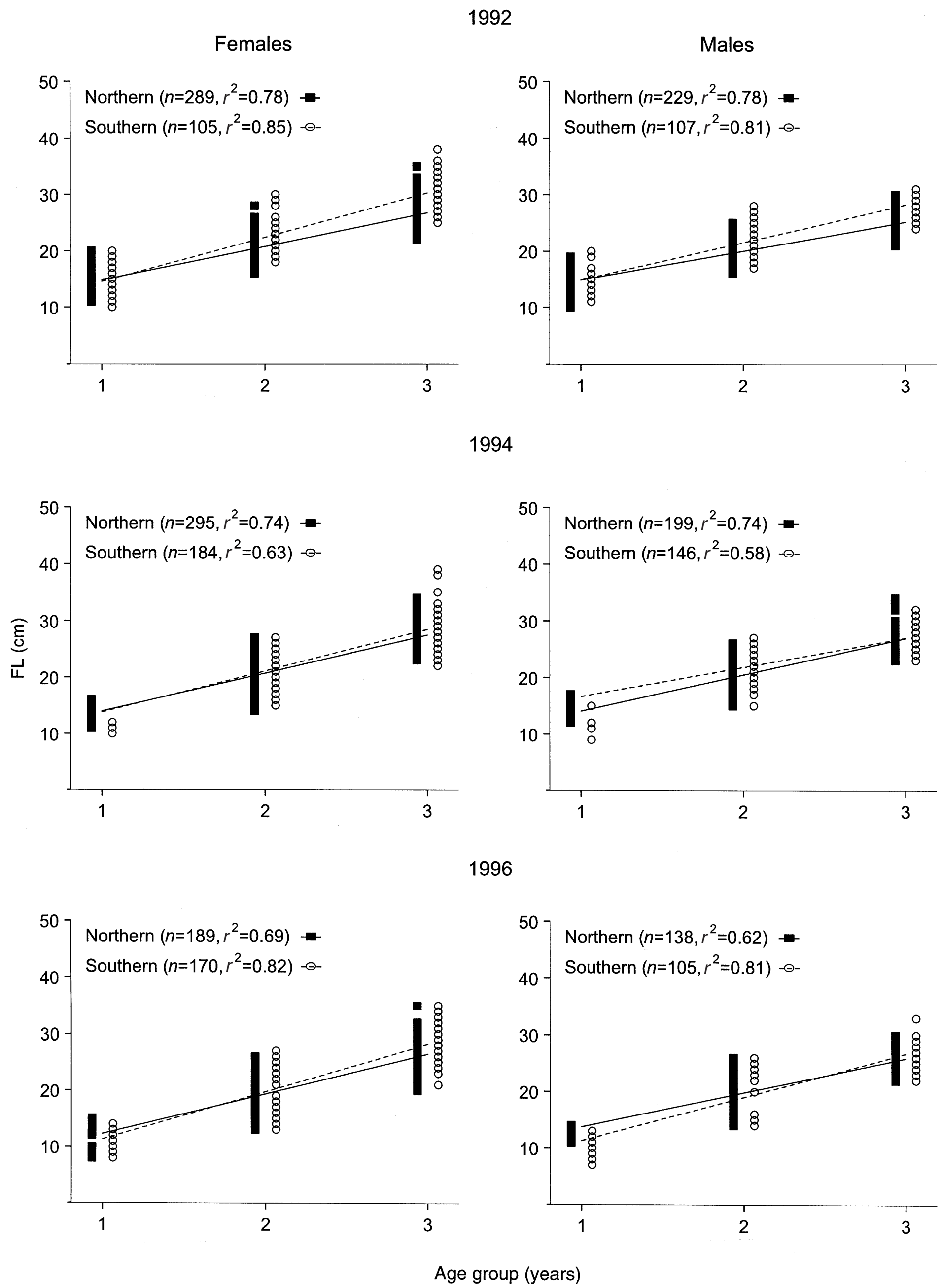

Figure 3

Linear growth relationships of silver hake samples (age groups 1 to 3) from the NEFSC 1992 (northern, F: S=6.41, I=8.15; M: S=4.97, I=9.98; southern, F: S=6.83, I=8.33, M: S=5.73, I=9.58), 1994 (northern, F: S=6.75, I=7.20; M: S=6.07, I=8.33; southern, F: S=7.10, I=6.99, M: S=4.84, I=12.25), and 1996 (northern, F: S=6.91, I=5.57; M: S=5.76, I=8.30; southern, F: S=8.03, I=3.72, M: S=7.15, I=4.65) spring surveys. (F=females; M=males; S=slope; I=intercept; n=sample size; r^2=coefficient of determination).

lith morphometric variables not included in the comparisons of 3-year-olds (those variables based on the ANCOVA results—area and length) were the most significant of the variables examined individually for the other age groups, emphasizing the potential importance of these two variables for stock discrimination.

Discriminant analyses provided further support for the separation between northern and southern stocks among

Table 2

Otolith morphometric variables that were significantly correlated with fish length for 1-, 2-, and 3-year-old silver hake samples. N.S. = not significant.

A 1-year-old silver hake

	Fish length × variable (df=2, 83)		Fish length (df=1, 85)		
Variable	*F*	*P*	*F*	*P*	*b*
Otolith length	0.10	N.S.	603.59	0.0001	0.0063
Width	2.65	N.S.	526.37	0.0001	0.0156
Area	1.24	N.S.	683.39	0.0001	0.0118
Perimeter	0.05	N.S.	471.07	0.0001	0.0067
Circularity	1.85	N.S.	15.99	0.0001	0.0016
Rectangularity	6.07	0.0035	—	—	—

B 2-year-old silver hake

	Fish length × variable (df=3, 90)		Fish length (df=1, 93)		
Variable	*F*	*P*	*F*	*P*	*b*
Otolith length	1.46	N.S.	621.48	0.0001	0.0047
Width	3.84	0.0123	—	—	—
Area	2.17	N.S.	715.78	0.0001	0.0084
Perimeter	0.78	N.S.	515.89	0.0001	0.0049
Circularity	0.80	N.S.	30.83	0.0001	0.0014
Rectangularity	1.11	N.S.	6.83	0.0104	–0.0002

C 3-year-old silver hake

	Fish length × variable (df=11, 348)		Fish length (df=1, 359)		
Variable	*F*	*P*	*F*	*P*	*b*
Otolith length	2.56	0.0040	—	—	—
Width	0.68	N.S.	561.32	0.0001	0.0102
Area	2.04	0.0239	—	—	—
Perimeter	1.10	N.S.	1024.62	0.0001	0.0037
Circularity	0.64	N.S.	144.02	0.0001	0.0020
Rectangularity	0.82	N.S.	12.78	0.0004	–0.0001

the silver hake samples, as indicated by the multi- and univariate analyses (Fig. 5). We found significant differences in the discriminant scores (CV I) between those samples from the northern and southern regions for both 1-year-old (t=-6.3075, df=87, P<0.0001) and 2-year-old fish (t=4.7654, df=95, P<0.0001). However, as in the preceding analyses, poor separation was observed between the samples of 3-year-olds (t=1.7827, df=370, not significant). This was again probably due to the reduced number of variables in the analyses, particularly that of otolith area and length. Variation in otolith area, length, and circularity was mainly responsible for the north–south separation in samples of 1- and 2-year-olds, whereas otolith width and circularity accounted for most of the variation in the samples of 3-year-olds (Table 5).

Classification success, based on each particular discriminant model, decreased with each age group. Overall, individual samples of 1-, 2-, and 3-year-old silver hake were correctly classified 75%, 69%, and 49% of the times, respectively (Table 6). Classification rates increased slightly when samples from each age group were analyzed separately by sex (1-year-old fish, males—78%; 2-year-old fish, females—68%, males—71%; 3-year-old fish, females—54%, males—56%) and year class (3-year-old fish: 1989 year class—54%; 1991 year class—64%; 1993 year class—61%), indicating the confounding affects that these variables can have on these types of analyses when used for stock discrimination.

Discussion

We confirmed that two stocks of silver hake exist in U.S. waters of the northwest Atlantic from differences in whole otolith morphometrics and growth rates. Our method of

Table 3

Examination of confounding sources of variation (sex and year class) on the length-corrected otolith morphometric variables. N.S. = not significant.

	MANOVA				ANOVA		
Comparison	*F*	df	*P*	Significant variable	*F*	df	*P*
Sex							
Females vs. males (South; 1991 year class; age 1)	0.18	4, 39	N.S.	—	—	—	—
Females vs. males (North; 1990 year class; age 2)	2.59	4, 76	0.0434	circularity	8.02	1, 79	0.0059
Females vs. males (South; 1990 year class; age 2)	0.36	4, 12	N.S.	—	—	—	—
Females vs. males (North; 1989 year class; age 3)	1.49	4, 22	N.S.	—	—	—	—
Females vs. males (South; 1989 year class; age 3)	2.54	4, 9	N.S.	—	—	—	—
Females vs. males (North; 1991 year class; age 3)	1.74	4, 71	N.S.	—	—	—	—
Females vs. males (South; 1991 year class; age 3)	7.32	4, 95	0.0001	rectangularity	8.43	1, 98	0.0046
Females vs. males (North; 1993 year class; age 3)	0.53	4, 72	N.S.	—	—	—	—
Females vs. males (South; 1993 year class; age 3)	0.57	4, 73	N.S.	—	—	—	—
Year class							
1989; 1991 vs. 1993 (Females; North; age 3)	4.97	8, 226	0.0001	—	—	—	—
1989; 1991 vs. 1993 (Males; North; age 3)	5.45	8, 114	0.0001	width	4.80	2, 59	0.0118
1989; 1992 vs. 1993 (Females; South; age 3)	2.37	8, 214	0.0183	perimeter	6.67	2, 109	0.0018
				circularity	6.13		0.0030
1989; 1992 vs. 1993 (Males; South; age 3)	2.86	8, 150	0.0055	perimeter	6.18	2, 77	0.0033
1989; 1991 vs. 1993 (Sexes pooled; North; age 3)	8.60	8, 350	0.0001	width	7.81	2, 177	0.0006
1989; 1992 vs. 1993 (Sexes pooled; South; age 3)	3.23	8, 374	0.0014	perimeter	9.65	2, 189	0.0001
				circularity	5.94		0.0031

using otolith morphometrics, coupled with discriminant function analysis, when analyzed with respect to fish age, enabled us to distinguish between a northern stock (from the Gulf of Maine to northern Georges Bank) and a southern stock (from southern Georges Bank to the Middle Atlantic), thus providing a more efficient and accurate method for discriminating between silver hake stocks than had been used previously.

Typically, silver hake of the northern stock grew at a slower rate, and had larger otoliths than fish of the southern stock. Otoliths of silver hake from the northern stock tended to be longer, wider, and greater in area and perimeter than similar-size fish from the southern stock because, for any given fish length, northern stock fish were older and therefore had more time to accumulate otolith material than had southern stock fish. These results were consistent with previous growth studies where slower growing fish generally had larger otoliths than those of similar-size, faster growing fish (Templeman and Squires, 1956; Reznick et al., 1989; Fowler and Short, 1996).

Likewise, Helser (1996) found that silver hake from the northern stock grew at slower rates than those fish of the southern stock, and like Nichy (1969), Almeida (1978) and Pentilla et al. (1989) found that the northern stock reached larger asymptotic lengths. However, Helser (1996) also found results that did not support the current stock

Table 4

Spatial comparisons of the length-corrected otolith morphometric variables. N.S. = not significant.

Spatial comparison	MANOVA *F*	MANOVA df	MANOVA *P*	Significant variable	ANOVA *F*	ANOVA df	ANOVA *P*
Same sex, age group, and year class							
North vs. South (Males; age 1; 1991)	6.98	4, 62	0.0001	length	13.71	1, 65	0.0004
				width	8.12		0.0059
				area	18.06		0.0001
North vs. South (Females; age 2; 1990)	1.24	4, 51	N.S.	—	—	—	—
North vs. South (Males; age 2; 1990)	6.30	4, 37	0.0006	area	8.68	1, 40	0.0053
				circularity	13.93		0.0006
North vs. South (Females; age 3; 1989)	1.98	4, 19	N.S.	—	—	—	—
North vs. South (Males; age 3; 1989)	2.40	4, 12	N.S.	—	—	—	—
North vs. South (Females; age 3; 1991)	5.80	4, 95	0.0003	—	—	—	—
North vs. South (Males; age 3; 1991)	3.84	4, 71	0.0070	perimeter	10.48	1, 74	0.0018
				circularity	7.35		0.0083
North vs. South (Females; age 3; 1993)	2.16	4, 101	N.S.	—	—	—	—
North vs. South (Males; age 3; 1993)	2.38	4, 44	N.S.	width	7.03	1, 47	0.0109
Same age group, year class; sexes combined							
North vs. South (age 1; 1991)	9.60	4, 84	0.0001	length	17.18	1, 87	0.0001
				width	9.04		0.0035
				area	22.16		0.0001
				circularity	10.61		0.0016
North vs. South (age 2; 1990)	5.05	4, 93	0.0010	length	11.15	1, 96	0.0012
				area	12.78		0.0006
North vs. South (age 3; 1989)	0.78	4, 36	N.S.	—	—	—	—
North vs. South (age 3; 1991)	5.18	4, 171	0.0006	perimeter	10.44	1, 174	0.0015
				circularity	8.47		0.0041
North vs. South (age 3; 1993)	4.05	4, 150	0.0038	width	11.49	1, 153	0.0009

scheme; growth rates differed between silver hake from northern Georges Bank and the Gulf of Maine and between fish from southern Georges Bank and the Middle Atlantic. Such within-stock differences further emphasizes the uncertainty that currently exists over both stock boundaries and the degree of mixing between silver hake stocks in U.S. waters of the northwest Atlantic.

Similarly, the uncertainty in stock boundaries and the degree of stock mixing was evident in most of the past silver hake stock discrimination studies (Conover et al., 1961; Konstantinov and Noskov, 1969; Nichy, 1969; Anderson, 1974), although we believe that our approach has the potential to resolve some of these questions. A major reason for this uncertainty has been due to a lack of intensive genetic studies to investigate the population structure of the species, and therefore a subsequent lack of knowledge concerning the actual reproductive isolation of the species. Schenk (1981) demonstrated two genetically distinct groups but, owing to a limited geographic sample area, was not able to define boundaries between the groups. However, it must be remembered that a "stock" in its working definition for practical fisheries management is a management unit and not a discrete biological population unit. Consequently, stocks of fish with different phenotypic (i.e. morphometric) characteristics or life history (i.e. growth) dynamics should be considered as separate management units and modeled separately regardless of genetic characteristics for stock assessment and management purposes (Cadrin and Friedland, 1999). Although our study did not delineate actual stock boundaries or determine the mixing components between the silver hake stocks, further investigation into the application of this technique may show that otolith morphometrics, when coupled with image processing and discriminant analysis, is capable of achieving these objectives.

Subsequently, to understand better the fishery dynamics of silver hake stocks and to manage them accordingly, we must question what caused these differences—whether they are differences in fish growth, or internal or external morphometric characters, both between and within stocks. Otolith morphometric differences between silver hake stocks are probably the result of both environmental and genetic influences. For example, the hydrodynamics of northwest Atlantic waters are considered to be heterogeneous; environmental conditions differ between the Gulf of Maine and the Middle Atlantic (Brooks, 1996). Certainly, we observed significant and consistent differences in the bottom temperatures between the two stock regions inhabited by silver hake, where those fish in the northern region experience lower temperatures than those in the southern region. Because fish growth and external body morphometrics are known to be influenced by such environmental factors (Ihssen et al., 1981; Pawson and Jennings, 1996), one would also expect that internal morphometrics, such as otolith length and width, would also differ in response to these variables because fish growth and otolith growth tend to be related. In accord with these assumptions, otolith shape has been found to vary in response to environmental conditions and is also highly correlated with fish growth (Campana and Casselman, 1993). Similarly, the results of our study showed significant correlations between fish and otolith growth for all the otolith morphometric characteristics measured. Therefore, it is likely that the differences found between silver hake stocks in growth rates (Helser, 1996; our study), external morphometrics (Conover et al., 1961; Almeida, 1987), and internal morphometrics (our study) are to some extent reflective of localized environmental conditions.

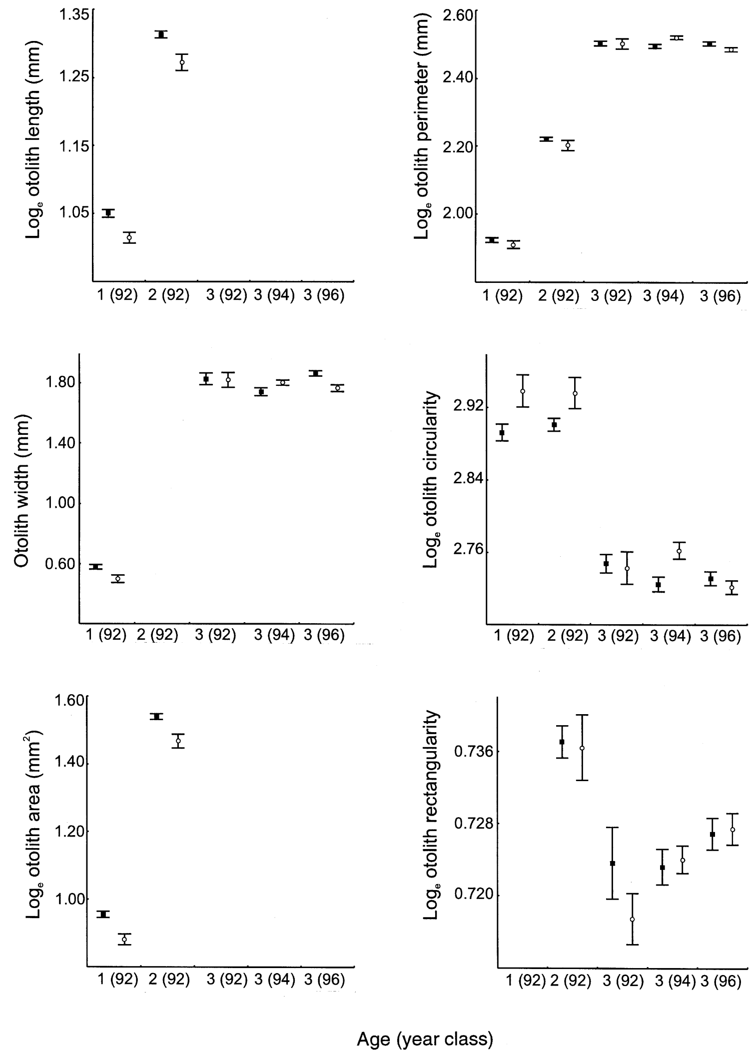

Figure 4

Otolith morphometrics (mean ±standard error) for 1-, 2-, and 3-year-old silver hake samples pooled across sexes for individual year classes showing differences between those fish from the northern (squares) and southern (circles) regions.

Assuming that environmental variables can effect otolith growth, we analyzed bottom ocean temperature between the two stock regions across the life history range of our samples to investigate whether the differences in otolith morphometrics found in this study were due to a weather phenomenon during any given sampling year (Fig. 2). Temperature was found to differ consistently on a seasonal basis during all years, as would be assumed, and there was no marked change in temperature between any given years, within any given season. As a result, the possibility of a severe temperature shift that could influence otolith morphometrics and make this method unsuitable for stock discrimination was not apparent in our study. However, because temperature was shown to differ between the proposed stock regions and because silver hake distribute themselves in response to temperature (Almeida, 1987; Helser, 1996), it can be assumed that this variable could effect otolith growth.

Alternatively, growth and otolith morphometric differences found between silver hake stocks could also be apparent, regardless of environmental variation, and reflect a genetic influence. This could be a valid assumption given

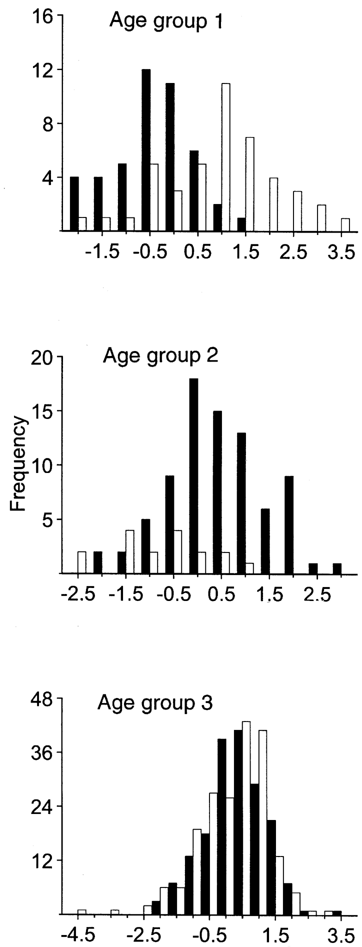

Figure 5

Discrimination of silver hake samples from the northern (solid bars) and southern regions (open bars) based on frequency of canonical variate scores (CV I) for 1-, 2-, and 3-year-old fish.

that a certain degree of reproductive isolation has been confirmed in spawning and postspawning silver hake (Almeida, 1987). Such isolation could restrict gene flow to a level that effectively isolates population units (Iles and Sinclair, 1982). However, some degree of intermixture probably occurs owing to the wide migratory patterns of silver hake (Almeida, 1987). Therefore, further investigations are required to interpret the degree to which morphological differences between silver hake stocks are caused by environmental or genetic influences, but as long as these differences persist between stocks, the use of otolith morphometrics remains a viable tool for stock discrimination.

Otolith morphometrics in combination with an image analysis system has been found to be a relatively inexpensive, objective, and efficient tool by which to distinguish fish stocks (Messieh et al., 1989; Jearld, 1995). Rapid and precise measurements can be obtained in less time with this technique, compared with alternative manual-based procedures, because all the measurements are automatically determined from an outline of the otolith perimeter traced with an edge-detection algorithm (OPTIMAS, 1996). In addition otolith morphometric data can easily be obtained because

Table 5

Length-corrected otolith morphometric variables responsible for the discrimination of 1-, 2-, and 3-year old silver hake samples determined by the standardized within-group variances of the canonical variates (CV I).

Otolith morphometric variable	Canonical variate I		
	Age group 1	Age group 2	Age group 3
Length	–1.01	0.57	—
Width	0.04	—	0.62
Area	0.10	0.38	—
Perimeter	—	—	–0.32
Circularity	0.82	–0.66	–0.59
Rectangularity	—	–0.16	0.09

Table 6

Jack-knifed classification matrix of the frequency of assigned cases in each region (pooled sexes and year classes) used to differentiate samples.

	Classification of individual silver hake								
	Age group 1			Age group 2			Age group 3		
	Correct %	North	South	Correct %	North	South	Correct %	North	South
North	80	36	9	70	57	24	54	98	82
South	70	13	31	65	6	11	43	109	83
Total	75	49	40	69	63	35	49	207	165

otoliths are routinely collected by most fisheries agencies to determine the age of their respective principal fish species for assessment and management purposes. However, one drawback in using otolith morphometrics for stock discrimination is that otoliths frequently are broken or lost during routine collection and processing, effectively limiting the number of samples available for analysis.

In these types of studies, it is also essential to consider any confounding variation that may be present owing to differences between samples in age group, year class, or sex, so as to not mistake stock differences for sample differences. Failure to account for such extraneous influences may result in falsely attributing morphological differences between separate stocks to a "stock effect," whereas the differences may in fact simply be reflecting variation between samples in age structure, sex ratio, or sampling year (Castonguay et al., 1991). Significant variation in otolith morphometrics of silver hake observed in our study between different age groups, year classes, and to a lesser extent sex, emphasized the need to examine these factors before evaluating the stock status of the species with this technique. Likewise, significant differences in these effects have been detected in other morphometric-based stock identification studies for marine fish species, such as Atlantic salmon (L'Abée-Lund, 1988), Atlantic mackerel (Castonguay et al., 1991), and Atlantic cod, *Gadus morhua* (Campana and Casselman, 1993). Thus, although it is clearly necessary to correct for these confounding variables, which can influence any stock identification procedure, they do not limit the use of otolith morphometrics for stock discrimination provided they are examined and accounted for before making any conclusions about the stock structure of a species. Indeed, calculating linear discriminant functions of otolith morphometric characteristics for 1-year-old silver hake on a yearly basis, differentiated by sex, should provide a level of discrimination not yet seen for silver hake stocks in U.S. waters of the northwest Atlantic and may assist in estimating levels of stock mixing (an objective yet to be realized for silver hake or for most of our commercially exploited fish stocks).

There was clearly a need for a more advanced and efficient method in distinguishing between silver hake stocks or in determining mixing levels between stocks. Assessment and management of silver hake stocks are currently based on stock structure results from the late 1970s and early 1980s, on discriminant analysis of external morphometric characters, coupled with a qualitative analysis of research survey and commercial catch data (Almeida, 1987). Since that time, the status of silver hake has certainly changed—spawning stock biomass and recruitment levels have declined (Helser and Brodziak, 1998)—and stock discrimination technologies through image processing have certainly improved (Cadrin and Friedland, 1999). We suggest that the use of whole otolith morphometrics in the analysis of silver hake stocks will give fishery scientists and managers greater logistical benefits (in terms of time and expense), not to mention greater discrimination success, than the stock discrimination methods used previously.

Our study indicated that successful identification of silver hake stocks can be achieved by using whole otolith morphometrics in combination with image processing and discriminant function analysis. We are confident that this is a relatively inexpensive, objective method which can facilitate routine discrimination of silver hake stocks, as well as other marine fish species, by efficiently obtaining accurate measurements to produce valid, repeatable results. This method may prove to be particularly useful for rapid (in-season) assessments for determining levels of stock mixing that are often a requirement of contemporary management plans. Discrimination between stocks is important for fisheries stock-rebuilding strategies, such as those for silver hake, because genetic and stock biodiversity of the species needs to be maintained to ensure all divisions of a stock contribute to the replenishment of the resource.

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