Supplementary Material on the Methodology Part XII

Analysis of European hake otolith shape

European hake otolith samples were collected during October–December 2018 from 15 locations distributed over GSAs 1, 3, 4 and 6, as well as the North and South Atlantic. The aim of this work was to investigate the viability of using otolith contour shape analyses as an aid in the assessment of hake stock structure in the Alboran Sea and its adjacent waters (i.e. in the Atlantic and Mediterranean).

For this aim, two comparisons were achieved:

- the first between the different GSAs in order to investigate the stock structure in the Mediterranean; and
- the second between the 15 sampling areas over the Atlantic and Mediterranean.

Material and methods

All the otoliths were first photographed in high contrast; images were produced using reflected light, in which otoliths stood out as bright objects on a black background for shape analysis. Shapes of the otoliths were analyzed by OTOLab software by using the OTOTHRESH tool for image segmentation (Figure 1). The physical characteristic descriptors measured were: area, major axis, minor axis, eccentricity, perimeter, circularity compactness, skewness, kurtosis and Fourier descriptors

Fourier descriptors (FDs) describe the outline of the otolith based on harmonics. Each harmonic is characterized by four coefficients, resulting from the projection of each point of the outline on axes (x) and (y). The higher the number of harmonics, the greater the accuracy of the outline description (Kuhl and Giardina, 1982). The OTOTHRESH tool was used to generate 20 harmonics for each otolith. Each harmonic is composed of four coefficients, resulting in 80 coefficients per otolith. Each otolith was normalized by the program for size and orientation, which caused the degeneration of the first three FDs derived from the first harmonic. Therefore, each individual was represented by 76 coefficients for the shape analysis. For multivariate analysis, it is recommended to reduce the number of harmonics to avoid collinearity between shape descriptors. So, the number of harmonics used to reconstruct each otolith, such that its shape is reconstructed at 99.99%, is determined by the calculation of the cumulated Fourier power. After the calculation of the cumulated Fourier power, only 13 harmonics were kept.

The analytical design was built to detect differences in the contour shape and physical characteristics of European hake otoliths collected from the 15 sampling areas through a forward stepwise linear discriminant analysis. A classification accuracy for each individual was evaluated through the percentage of correctly classified individuals using jackknifed approach

Results

First comparison: between GSAs

The forward stepwise linear discriminant analysis (DA) was performed in order to explore the spatial variation of the hake otolith shape among the different GSAs. The results revealed that the two first discriminant functions of the DA performed with normalized elliptical Fourier descriptors (NEFDs) and physical characteristics descriptors accounted for 95%. There was significant overlap among individual samples from all the GSAs, except for GSA 3 (Figure 1). The overall classification success of hake to their site of capture was poor, at 35.5%. Despite this, there was some level of structuring between samples from GSA 3 and the other GSAs. This is corroborated by the highest jackknifed classification accuracy, at about 74.2%, of individuals from GSA 3 to their area of origin, contrary to the other GSAs, which were characterized by lower classification accuracy due to the misclassification of individuals from GSAs 1, 6, 4 and 12 with respect to each other (Figure 1, Table 1).

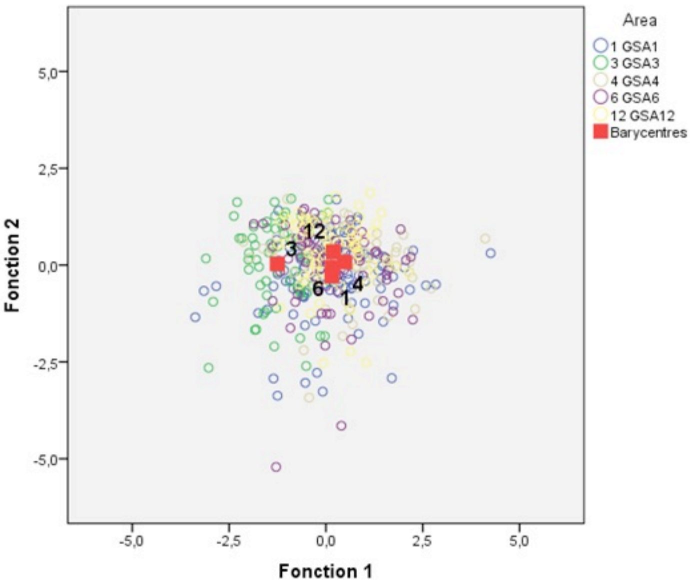

Figure 1. Scatter plot of scores obtained by DA for European hake from GSAs 1, 3, 4, 6 and 12 based on the contour shape of the otolith

Table 1. Results of the jackknife classification of individuals based on the contour shape and physical characteristics of the otolith

	Area classified to (% sample)				
Jacknife classification	GSA1	GSA 3	GSA4	GSA6	GSA12
GSA1	**29.4**	22.5	22.5	2	23.5
GSA 3	14.5	**74.2**	0	1.6	9.6
GSA 4	16.7	12.5	**37.5**	5.6	27.8

GSA6	18.2	18.3	28.6	**5.2**	29.9
GSA12	16.4	13.7	24.7	6.8	**38.4**

Second comparison: between GSAs and the North and South Atlantic

To investigate the stock structure of the European hake through the Atlantic and Mediterranean, a forward stepwise CDA was performed using the NEFDs physical characteristics descriptors as the explanatory variable.

The canonical variates plot indicated some overlap, especially among samples from GSAs 1, 12, 4 and 6. The samples from GSA 3 were separated from the other GSAs, but overlapped with the North and South Atlantic (Figure 2).

The jackknifed classification procedure involving all areas was poorly accurate in assigning individuals to their areas of origin, leading to the high errors of classification, especially misclassification of samples from GSA 3 to AtlS and AtlN, as well as the misclassification of samples from GSAs 1, 4, 6, and 12 with respect to each other (Table 2).

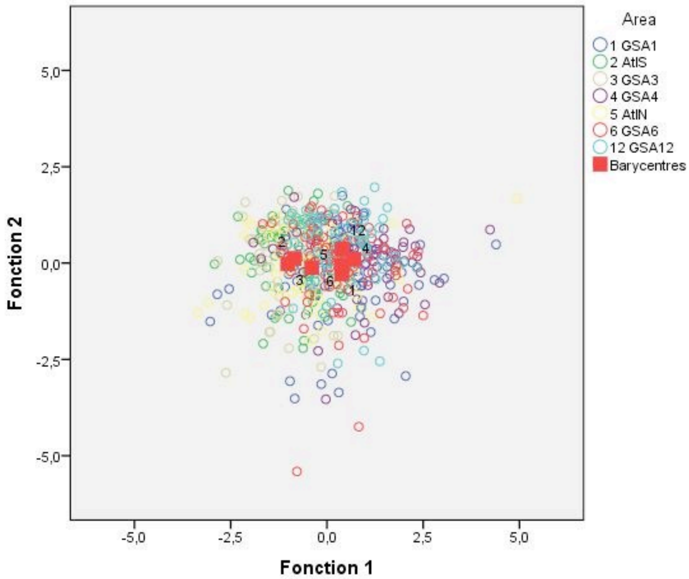

Figure 2. Scatter plot of scores obtained by DA for European hake from GSAs 1, 3, 4, 6 and 12 and AtlN, AtlS based on the contour shape of the otolith

Table 2. Results of the jackknife classification of individual based on the contour shape and physical characteristics of the otolith

	Area classified to (% sample)						
Jacknife classification	GSA1	AtlS	GSA 3	GSA4	AtlN	GSA6	GSA12
GSA1	**24.5**	3.9	8.8	22.5	15.7	2	22.5

AtlS	8.7	**26.1**	33.3	1.4	18.8	0	11.6
GSA 3	8.1	30.6	**38.7**	0	16.1	0	6.5
GSA 4	16.7	5.6	5.6	**37.5**	6.9	2.8	25
AtlN	19.4	10.4	26.9	6	**17.9**	4.5	14.9
GSA6	16.9	13	3.9	28.6	7.8	**3.9**	26
GSA12	12.3	12.3	0	24.7	12.3	4.1	**34.2**

Conclusion

The otolith shape analysis for European hake was not sufficient to draw firm conclusions regarding hake stock structure in the Alboran Sea and its adjacent waters. However, the samples from GSA 3 seemed to be relatively separated from the samples of the other GSAs, clustering closer to the North and South Atlantic samples. Hake samples from GSAs 1, 6, 4 and 12 could be composed of one mixing population, probably related to hydrographic conditions.

Reference

Kuhl, F.P. & Giradina, C.R. 1982. Elliptic Fourier Features of a closed contour. *Computer Graphics and Image Processing*, 18: 236–258.