Supplementary Material on the Methodology Part XIII

Analysis of sardine otolith shape

Sardine otolith samples were collected during the period from June to September from 17 locations distributed over GSAs 1, 6, 12, 4 and 3, as well as the North and South Atlantic. The aim of this work was to investigate the viability of using otolith contour shape analyses to help interpret the stock structure of sardine in the Alboran Sea and its adjacent waters by sampling fish from 13 locations in the Mediterranean and 4 locations in the Atlantic. 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 17 sampling areas over the Atlantic and Mediterranean.

Material and method

All otoliths were first photographed with 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 sardine otoliths collected from the 17 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 a jackknifed approach.

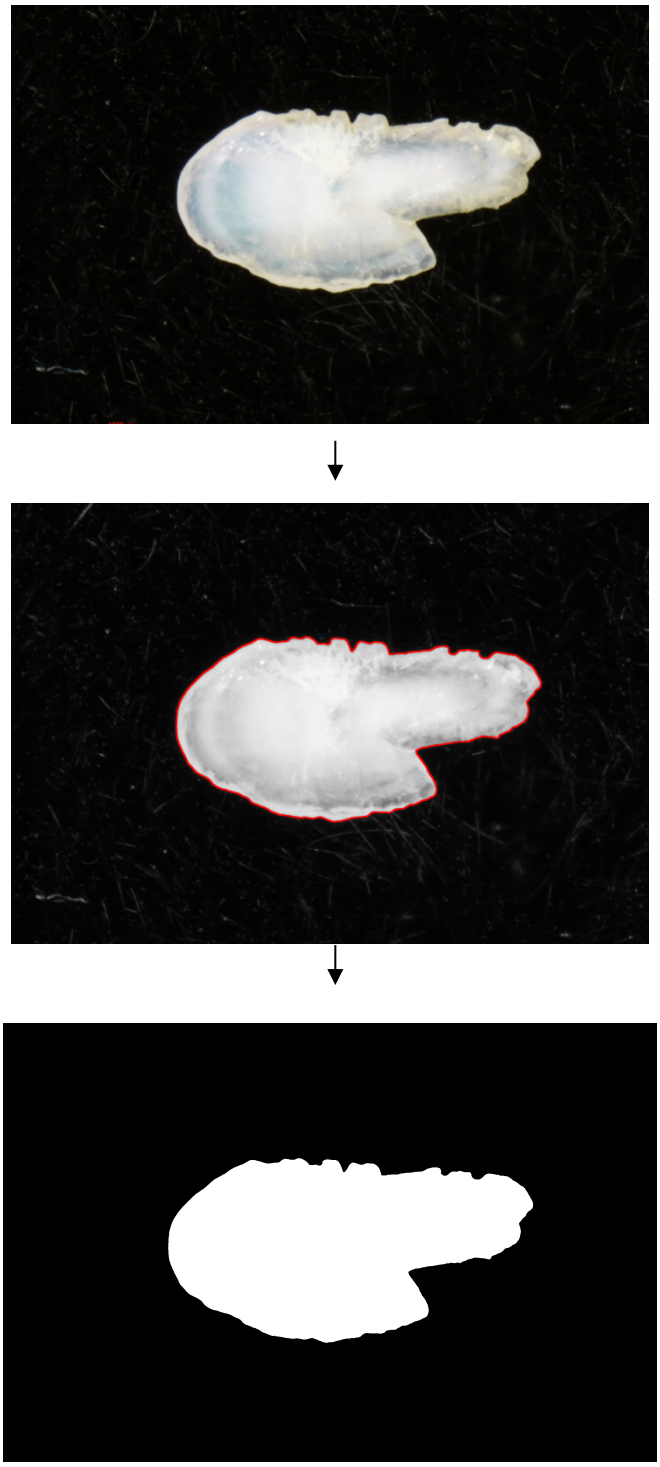

Figure 1. Sardine otolith image processing and analysis with the OTOTHRESH tool, showing the original image above, followed by the contour shape and the resultant binary image used to extract the physical characteristic descriptors and Fourier descriptors

Results

First comparison: between GSAs

In order to elucidate the sardine stock structure in the Mediterranean, the shape of the sardine otolith of all specimens from the different collection locations were pooled by GSA. The forward stepwise linear discriminant analysis (DA) discriminated between the different GSAs with an overall classification accuracy of 42% and a jackknife cross-validation of an average of 40.3%. The first two discriminant functions of the DA performed with normalized elliptical fourrier descriptors (NEFDs) and physical characteristics descriptors accounted for 95% of the variance. Sardine could be discriminated into groups based on the first two functions. The score plots for the first two discriminant functions show a separation between sardines from GSA 1 and those from GSA 6 and versus a group formed by sardines from GSAs 3 and 4. Sardines from GSA 12 overlap with the different GSAs (Figure 2). This is corroborated by the jackknifed classification including all areas, which was moderately accurate in assigning individuals to their respective collection area. Classification accuracy was highest for GSA 1, followed by GSA 6. Classification errors for GSAs 3 and 4 were due to misclassifications of individuals between each other. For Tunisia, the very low classification accuracy was related to misclassifications in all GSAs, especially in GSAs 6 and 4 (Table 1).

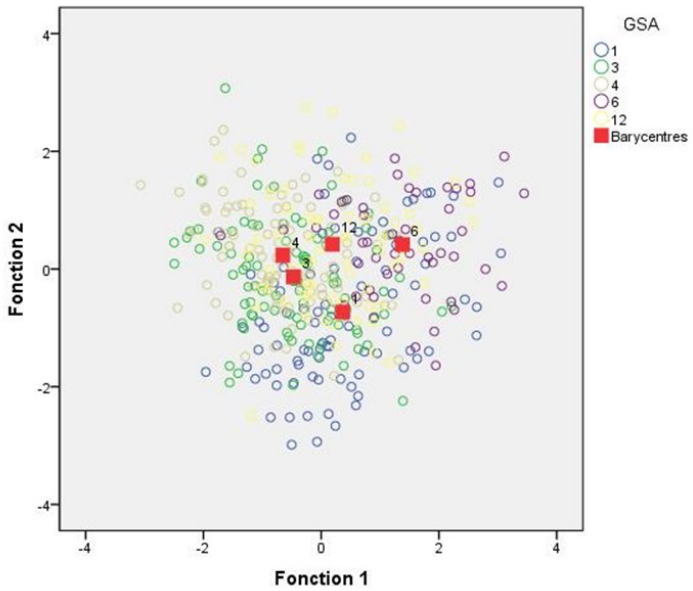

Figure 2. Scatter plot of scores obtained by DA for sardine from GSAs 1, 3, 4, 6 and 12 based on the contour shape and physical characteristics of the otolith

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

	Area classified to (% sample)				
Jacknife classification	GSA1	GSA 3	GSA4	GSA6	GSA12
GSA1	**50.6**	6.9	6.9	23	12.6

GSA 3	18.5	**34.8**	31.5	7.6	7.6
GSA 4	12.1	31.8	**43**	3.7	9.3
GSA6	16.3	2	2	**59.2**	20.4
GSA12	18.1	16.7	22.2	25	**18.1**

Second comparison: between GSAs and the North and South Atlantic

The DAs were performed to investigate the stock structure of the sardine through the Atlantic and Mediterranean. The results revealed that the first two discriminant functions of the CDA performed with NEFDs) and physical characteristics descriptors accounted for 84%. An overall classification success of sardine to their site of capture was 42.5%, with a jackknife cross-validation of an average of 39.7%. Despite this weak classification, the results indicated some level of structuring between the different areas: a group of samples from the Atlantic (AtlS and AtlN), a group of samples from GSAs 3 and 4, and the groups of sardine from GSAs 1 and 6 were relatively individualized, while sardines from GSA 12 overlap with all the areas, making it the GSA characterized by the highest level of misclassification, especially with GSAs 6 and 4. (Figure 3, Table 2)

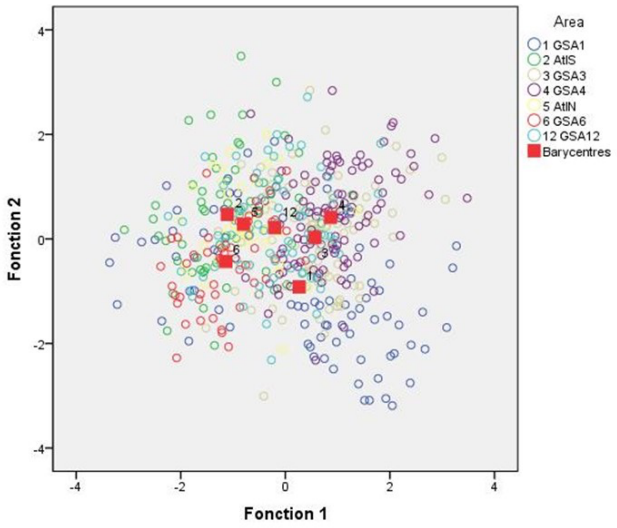

Figure 3. Scatter plot of scores obtained by DA for sardine from GSAs 1, 3, 4, 6 and 12 and AtlN, AtlS based on the contour shape and physical characteristics of the otolith

Table 2. 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	AtlS	GSA 3	GSA4	AtlN	GSA6	GSA12

GSA1	**46**	10.3	10.3	6.9	9.2	11.5	5.7
AtlS	5.2	**37.9**	1.7	3.4	24.1	19	8.6
GSA 3	23.9	6.5	**28.3**	25	7.6	2.2	6.5
GSA 4	13.1	3.7	18.7	**47.7**	2.8	1.9	12.1
AtlN	9.8	17.1	2.4	0	**43.9**	17.1	9.8
GSA6	6.1	12.2	2	0	14.3	**46.9**	18.4
GSA12	15.3	9.7	6.9	18.1	8.3	12.5	**29.2**

Conclusion

Shape analysis of the sardine otolith did not show consistent discrimination between fish from the different sampling areas, however there is a weak structuring between Atlantic (North and South) and the other areas of Mediterranean Sea. Also, the shape of the otolith sardine from GSA 12 was relatively closer to those from GSAs 4 and 6; indeed 25 % and 22.2% of samples from GSA 12 were misclassified, respectively, to GSA 6 and GSA 4.

Reference

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