Comparing otolith shape descriptors for population structure inferences in a small pelagic fish, the European sardine *Sardina pilchardus* (Walbaum, 1792)

João NEVES[1,3], Ana VERÍSSIMO[2], António Múrias dos SANTOS[2,3], Susana GARRIDO[1]

[1] Faculty of Sciences, University of Porto, R. Campo Alegre s/n, 4169-007 Porto, PORTUGAL

[2] CIBIO - Research Centre in Biodiversity and Genetic Resources , Campus Agrário de Vairão, 4485-601 Vairão, PORTUGAL

[3] Portuguese Institute for the Sea and Atmosphere (IPMA), Rua Alfredo Magalhães Ramalho, 6, 1449-006 Lisbon, PORTUGAL.

[4] Marine and Environmental Sciences Centre (MARE), Faculty of Sciences, University of Lisbon, Lisbon, PORTUGAL

*Corresponding author e-mail: jfbneves@gmail.com

Introduction / Condensed Abstract

Otolith shape analysis has been one of the most used methods to study population structure in the last decades. Currently, two different shape descriptors are used to perform otolith shape analysis: Elliptic Fourier descriptor, which focuses on the overall otolith shape differences, and Discrete Wavelet descriptor, which is sensitive to local differences along the otolith contour. Here, we conducted a comparative analysis of both descriptors in reconstructing the population structure and connectivity patterns in a small pelagic fish species, the European sardine *Sardina pilchardus* (Walbaum, 1792), to understand the possible population structure differences related to the descriptor, using Discrete Wavelet descriptor for the first time in this species. The two otolith shape descriptors showed similar although limited overall classification success associated with the population dynamic characteristics of the species. Both descriptors point to migration among adjacent areas, such as northern Atlantic locations, eastern Mediterranean, and even beyond well-defined geographical barriers such as the Gibraltar Strait, among Atlantic and western Mediterranean locations. Both descriptors supported the division of the populations of Mediterranean waters into two main groups but differed in the grouping of Atlantic waters.

Objectives

Contribute to the knowledge of population structure of sardines using otolith morphometry

- Use shape analysis to study sardine population structure
- Compare shape analysis results between different descriptors (Fourier vs Wavelet)

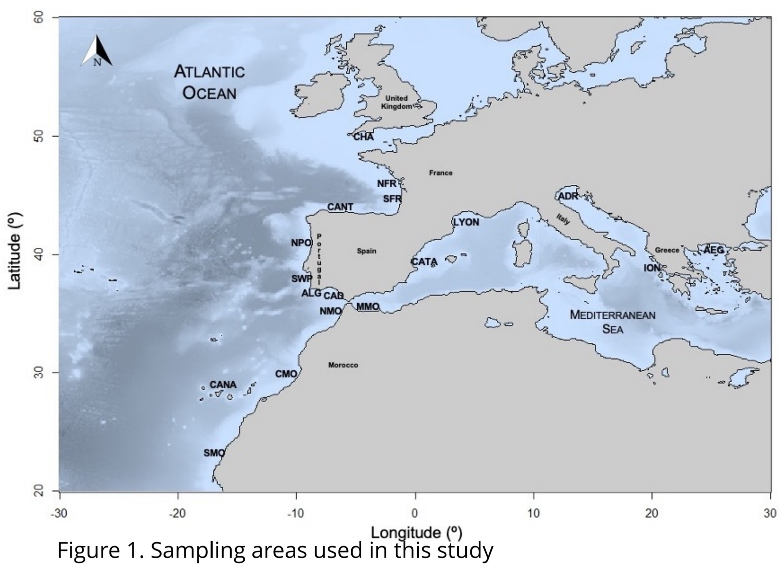


Figure 1. Sampling areas used in this study

Methodology

- Otoliths were collected under the scope of the SARDINOMICS project (Mar2020), in a total of 18 areas, representing almost the whole distribution range (Figure 1).
- Otolith images were analysed using ShapeR package, and shape coefficient descriptors were calculated based on Elliptic Fourier (EFd) and Discrete Wavelet (DWd)
- General shape parameters were extracted, as well as the otolith shape indices (OSi) calculated.
- To evaluate the shape of the otoliths, multivariate statistical analyses of descriptive otolith information were used, such as discriminant and clustering methods.

Discriminant and Clustering analysis

Linear Discriminant Analysis

- backward stepwise selection adopted
- 38 EFd , 30 DWd and 4 OSi variables
- Some **areas seems to agregate**, related with **proximity/geographic location** (Figure 2)

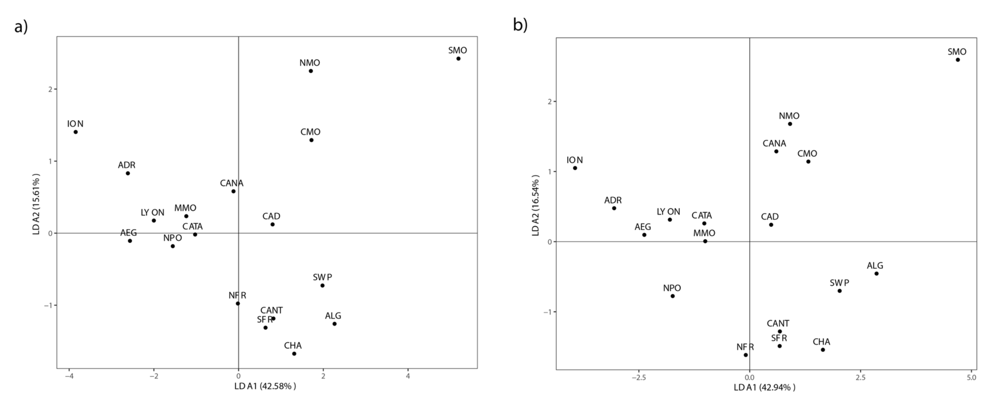


Figure 2. LDA scores for the classification of European sardine otoliths by sampling areas based on a) Elliptic Fourier and b) Discrete Wavelet descriptors

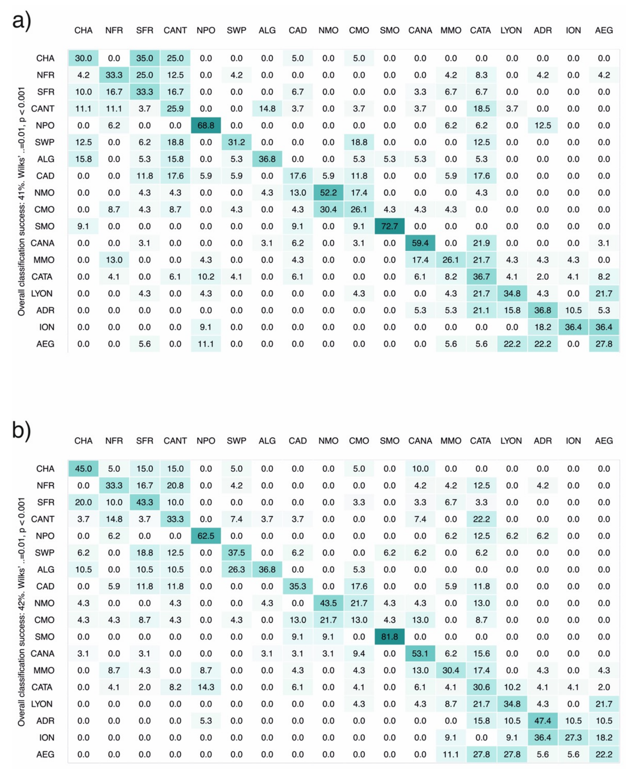


Figure 3. Jackknifed classification matrix of the LDA among sampling areas based on a) Elliptic Fourier and b) Discrete Wavelet descriptors set

Elliptic Fourier (Fig. 3a)

- 41% overall classification success
- individuals correctly classified by origin varied from ~18% (Gulf of Cadiz - CAD) to ~73% (South Morocco - SMO)

Discrete Wavelet (Fig. 3b)

- 42% overall classification success
- individuals correctly classified by origin varied from 13% (Central Morocco - CMO) to 82% (South Morocco - SMO).

Highest percentage of misclassified individuals

- Mediterranean locations (up to ~36%),
- northern distribution areas (up to ~35%)
- between North and Central Morocco (up to ~30%).

Statistically significant differences between sample locations (PERMANOVA, 4999 permutations)

- Elliptic Fourier - pseudo-f: 10.84, p = 0.0002
- Discrete Wavelet - pseudo-f: 5.19, p = 0.0002

Hierarchical Cluster Analysis

- discriminant analysis pattern **sustained by the cluster analysis**
  - suggesting **4 main clusters** for both sets (Figure 4)
- **Each group has a recruitment hotspot (except F3)**

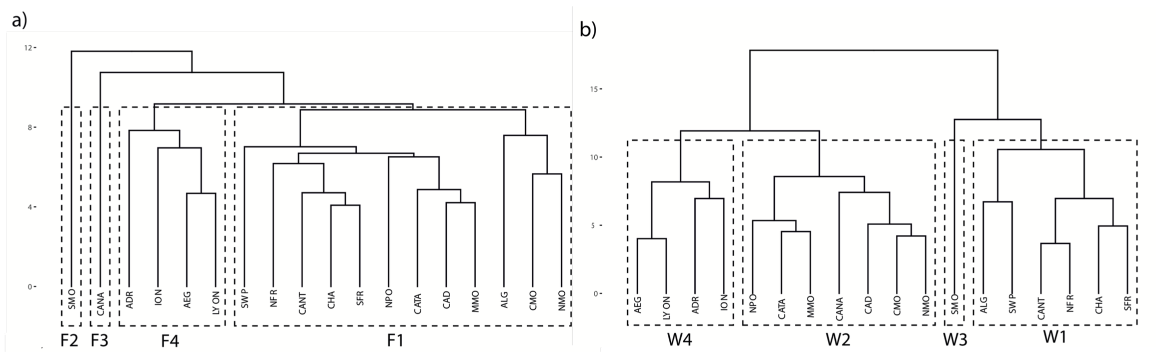


Figure 4. Cluster analysis defining the linkage dendrogram based on Euclidean distances using mean a) Elliptic Fourier and b) Discrete Wavelet descriptors data

Differences among defined groups

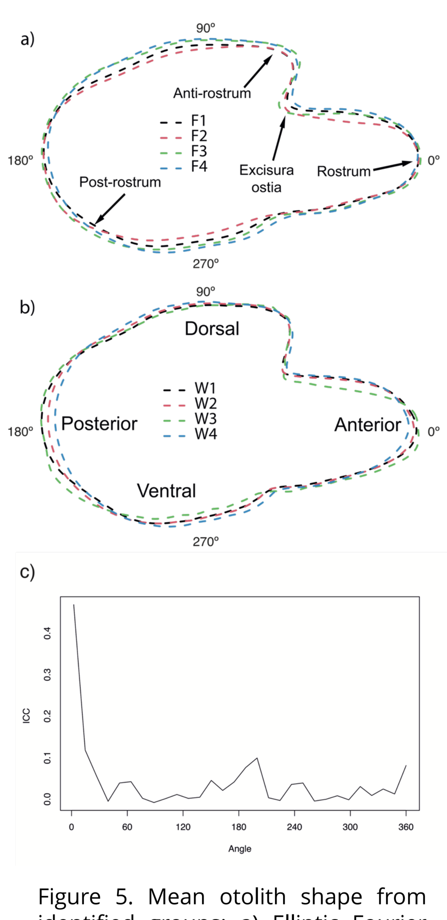


Figure 5. Mean otolith shape from identified groups: a) Elliptic Fourier and b) Discrete Wavelet; and c) intraclass correlation (ICC) among groups based on Discrete Wavelet

Elliptic Fourier (Fig. 5a)

- dorsal and ventral extensions
- rostrum and excisura ostia
- Atlantic and West Mediterranean waters (F1+F2)
  - slightly narrower and concave dorsal zone

Discrete Wavelet (Fig. 5b)

- rostrum and posterior parts
  - supported by inter-class correlation (Fig. 5c)
    - 345º-20º **(rostrum)**
    - 160º-210º **(posterior part)**
- ventral extension
- Eastern Mediterranean cluster (W4)
  - rounder and wider otoliths

South Morocco (F2 and W3) - **most divergent**

- longer and narrower shape overall
- narrower and thinner rostrum
- more prominent ventral post-rostrum

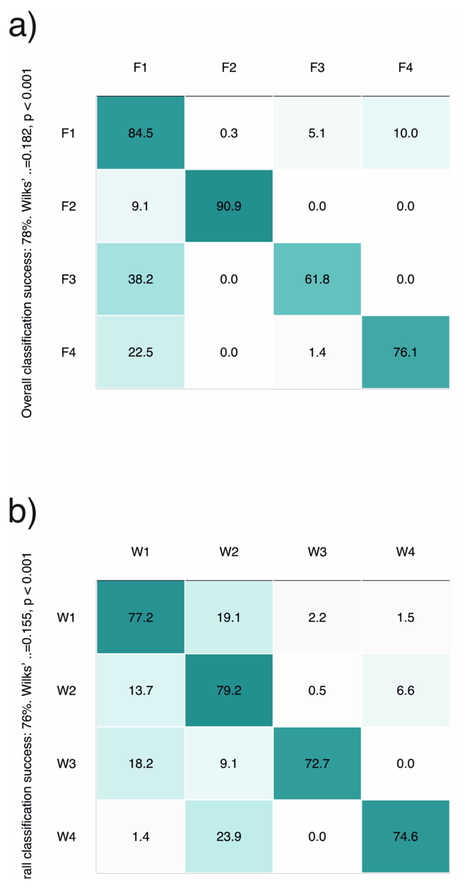


Figure 6. Jackknifed classification matrix of the LDA among groups based on a) Elliptic Fourier and b) Discrete Wavelet descriptors set

Elliptic Fourier (Fig. 3a)

- 78 % overall classification success

Discrete Wavelet (Fig. 3b)

- 76% overall classification success

Highest misclassifications:

- F3 vs F1 (~38%)
- W4 vs W2 (~24%)
- suggesting a **connection between groups** (possible identical morphologies), even after the grouping/clustering

Statistically significant differences between defined groups (PERMANOVA, 4999 permutations)

- Elliptic Fourier - pseudo-f: 19.44, p < 0.0002
- Discrete Wavelet - pseudo-f: 14.31, p < 0.0002

Significant differences between all pairwise comparisons

Population Structure Comparison

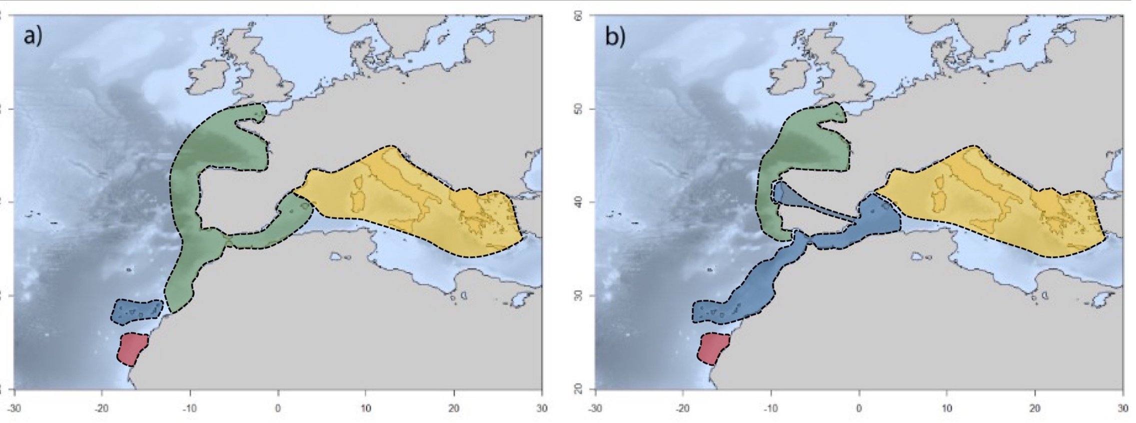


Figure 7. Map showing the identified groups based on a) Elliptic Fourier and b) Discrete Wavelet otolith shape analysis

Both descriptors presents a **similar latitudinal distribution**

➔ Mediterranean separated in two groups (West and East)

➔ South of Morocco isolated group

➔ No separation among Atlantic and Mediterranean waters

Major differences:

➔ Canary islands isolated group (Fourier)

➔ Atlantic + West Mediterranean unique group (Fourier)

➔ European and African (+ WestMed) Atlantic waters separated groups

Conclusions

➔ Otolith shape **variation** among populations

➔ 18 populations → **4 major groups** for each descriptor set

Significant differences among groups

➔ **Different descriptors → Slightly different population structure**

Seems to be related to the descriptors characteristics:

- Elliptic Fourier **targeting the overall otolith shape**

- Discrete Wavelet **sensitive to local differences along the otolith contour**

Future work

- Interpret results in relation to **environmental parameters** (e.g SST, Chla)
- Add **intrinsic parameters** (e.g. age, year-class, annual growth)
- Comparing additional classification **machine learning approaches** (e.g. QDA, Random Forest, Support Vector Machines)

References

Campana, S. et al . (1993) "Stock discrimination using otolith shape analysis." Canadian Journal of Fisheries and Aquatic Sciences 50.5: 1062-1083.

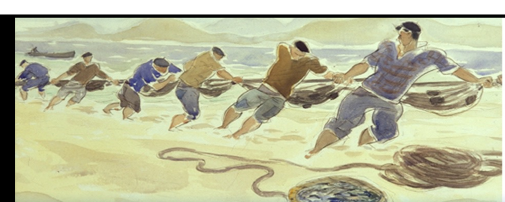
Libungan, L. A. and Pálsson, S. (2015) 'ShapeR: An R package to study otolith shape variation among fish populations', *PLoS ONE*, 10(3).

Libungan, L. A., and Pálsson, S. (2015). ShapeR: Collection and Analysis of Otolith Shape Data, version 0.1-5. R package. 2015.

Neves, J., Silva, A. A., Moreno, A., Veríssimo, A., Santos, A. M., & Garrido, S. (2021). Population structure of the European sardine *Sardina pilchardus* from Atlantic and Mediterranean waters based on otolith shape analysis. *Fisheries Research*, *243*, 106050.

Acknowledgements

This work was performed under the framework of the project SARDINOMICS funded by the program MAR2020. JN was funded by Portuguese funds through the FCT - Portuguese Foundation for Science and Technology SFRH/BD/04621/2021. AV was funded by Portuguese funds through the Portuguese Foundation for Science and Technology (DL57/2016).



Small Pelagic Fish: New Frontiers in Science and Sustainable Management
November 7 - 11, 2022
Lisbon, Portugal