Understanding the Population Structure of the European Anchovy (*Engraulis encrasicolus*) in the Black Sea, Mediterranean Sea and the Northeast Atlantic Ocean by using Otolith Shape Analysis

Gizem Akkus[1], Sharif Jemaa[2,3], Ali Cemal Gucu[1], Paul Marchal[4], Pierluigi Carbonara[5], Mahmoud Bacha[2], Rachid Amara[2], Bruno Ernande[4], Claire Saraux[6], Kélig Mahé[4]

[1]Middle East Technical University, Institute of Marine Sciences, P.O.Box 28, 33731, Erdemli-Mersin, Turkey
[2]Université Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[3]Conseil National des Recherches Scientifiques, Centre National des Sciences Marines, BP 534 Batroun, Lebanon
[4]Ifremer, Centre Manche Mer du Nord, Fisheries laboratory, BP 699, 62321 Boulogne-sur-mer, France
[5]COISPA Tecnologia & Ricerca - Stazione Sperimentale per lo Studio delle Risorse del Mare, Via dei Trulli 18/20 70126 Bari, Italy
[6]MARBEC, Ifremer, Université de Montpellier, CNRS, IRD, 34200 Sète, France


European anchovy


Sagittal Otolith

ABSTRACT

European anchovy, *Engraulis encrasicolus*, is a small pelagic coastal marine fish largely spread from the North Sea to central Africa, including the entire Mediterranean and the Black and Azov Seas. The aim of this study is to identify the potential different populations of European anchovies and their relationship between the Northeast Atlantic, the Western and Eastern Mediterranean and the Black Sea. The outlines of 2535 pairs of sagittal otoliths were collected from 20 regions by combining new samples (English Channel, Atlantic, the Mediterranean and the Black Sea) with existing ones available from a previous study. Elliptical Fourier Analysis (EFA) was used to analyze otolith shape variation among locations. Before examining geographical differentiation by Linear discriminant (LDA) and Hierarchical Clustering Analysis, potentially confounding sources of variation (sex, fish length, otolith side and sampling year) were tested by partial RDA. Sex, sampling year and otolith side had no significant effect on otolith shape. However, after accounting for ontogenic factors, the geographical area had a significant effect on otolith shape. Three different groups of anchovies were identified: Atlantic-Southwestern Mediterranean, Northwestern Mediterranean and Eastern Mediterranean-Black Sea with a classification success of 91%. These results have implications for the stock management of European anchovy populations from the North Sea to the Black Sea.

INTRODUCTION

- The European Anchovy is a small pelagic and coastal marine fish.
- Genetic methods may not be sensitive enough to detect population structure due to high gene flow ➡ otolith shape might be a useful tool to identify population structures as its geographical variation may be related to phenotypic local adaptation.

MATERIALS and METHODS

Area	#	Region	sample size	Total length (cm)
Atlantic Ocean	1	English Channel	119	13.6
	2	Bay of Biscay	55	12.8
	3	Gulf of Cadiz	122	11.7
	4	Casablanca	50	12.4
	5	South Cap Blanc	38	12.9
North West Mediterrannean Sea	6	Gulf of Lion	296	10.8
	7	Catalan Sea	52	12.3
	8	Tyrrhenian Sea	45	11.6
South West Mediterrannean Sea	9	Southern Alboran Sea	73	12.4
	10	Alboran Sea Ghazaouet	198	13.0
	11	Alboran Sea Benisaf	136	12.1
	12	Algero-Provençal Basin	112	11.2
	13	Alboran Sea Bejaia	466	12.7
East Mediterrannean Sea	14	Ionian Sea	54	12.5
	15	Adriatic Sea	80	11.7
	16	Aegean Sea	41	11.5
	17	Marmara Sea	96	11.1
BlackSea	18	Western Black Sea	190	11.0
	19	Middle Black Sea	169	10.8
	20	Eastern Black Sea	143	10.6
		Total Sample Size	***2535***	***11.9***

Table 1: European anchovy otolith samples by regions and average total length of sampled fish.

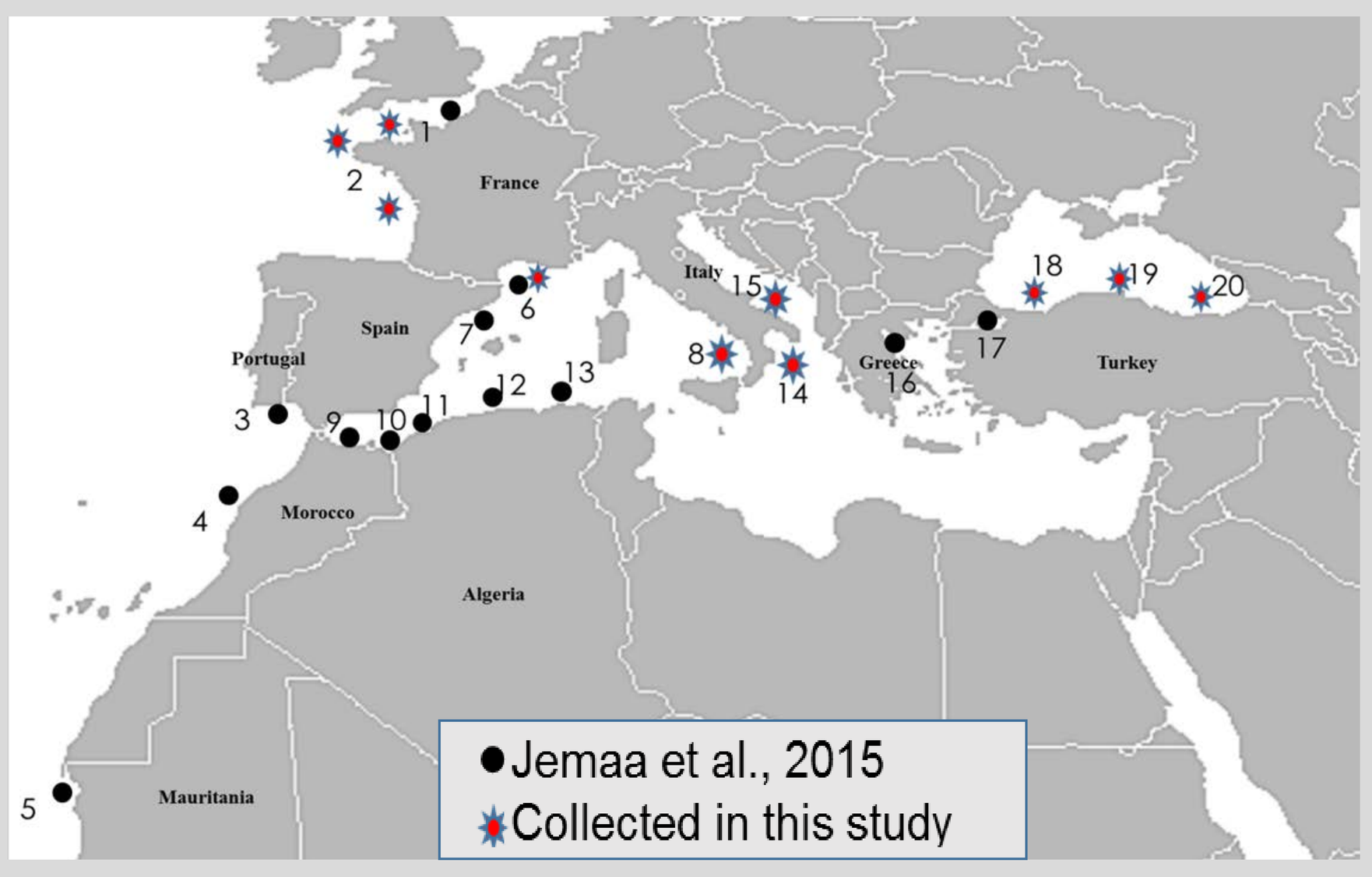


Figure 1: Sampling regions.

- Only mature anchovies were included in this study to avoid otolith shape variation related to sexual maturity.
- 2535 pairs of sagittal otoliths were collected from 20 different regions in 2014-2016.

TNPC Software
www.tnpc.fr

- Images of the whole sagittal otoliths were scanned automatically by the image analysis system TNPC.
- The first 99 elliptical Fourier harmonics (Hi) were extracted to describe individual otoliths' shape and normalized with respect to the first harmonic.
- The number of Hi, n_k, required to bring the individual cumulated Fourier Power (PF) to 99% for each otolith was kept for further analyses

$$PF(n_k) = \sum_{HI=1}^{n_k} \frac{A_{HI}^2 + B_{HI}^2 + C_{HI}^2 + D_{HI}^2}{2}$$

Left vs Right Side Otoliths

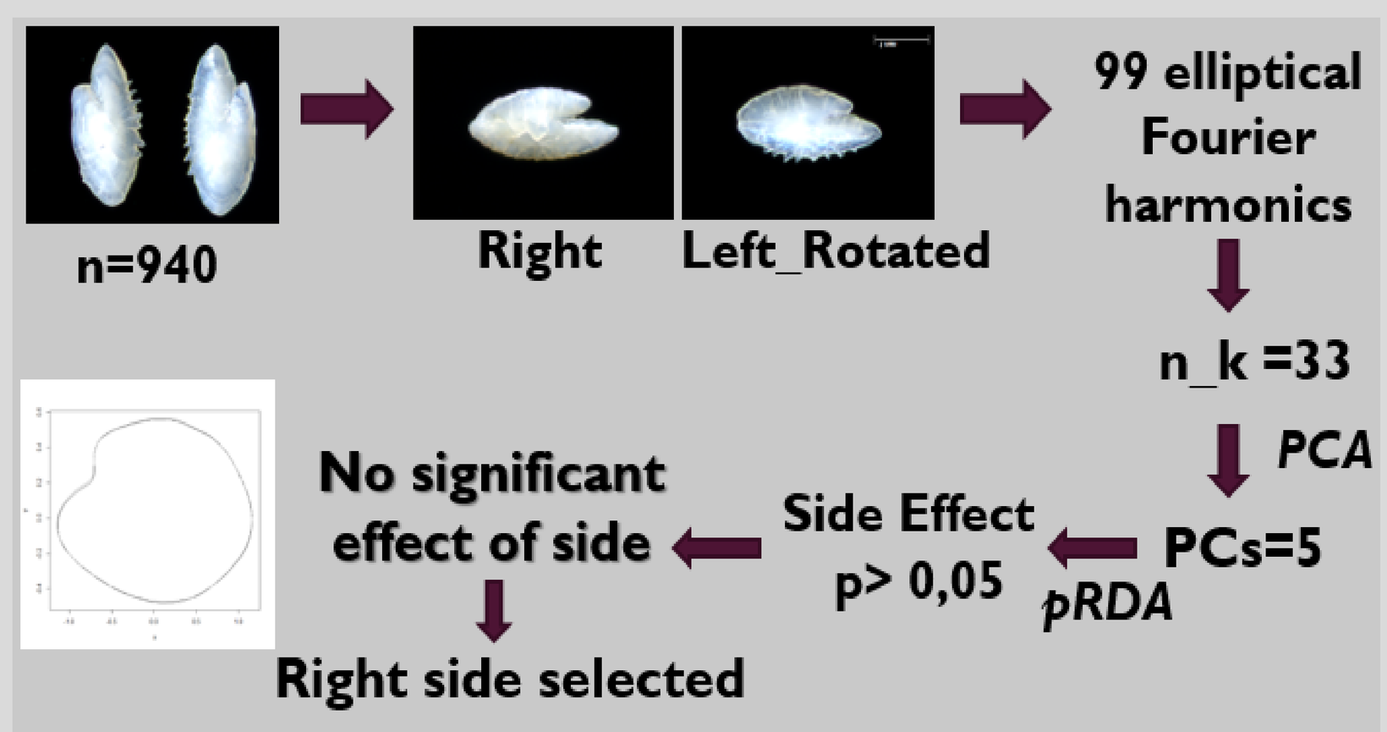


Figure 2: Left *vs* Right side differences.

✓A mirror image of the left side otolith was used in order to compare left and right otoliths.

✓Redundancy analysis (RDA) was applied and no significant differences was found between the right and left otolith's shape.

Possible Effects of Total Fish Length, Sex, Sampling year, Geographical Area

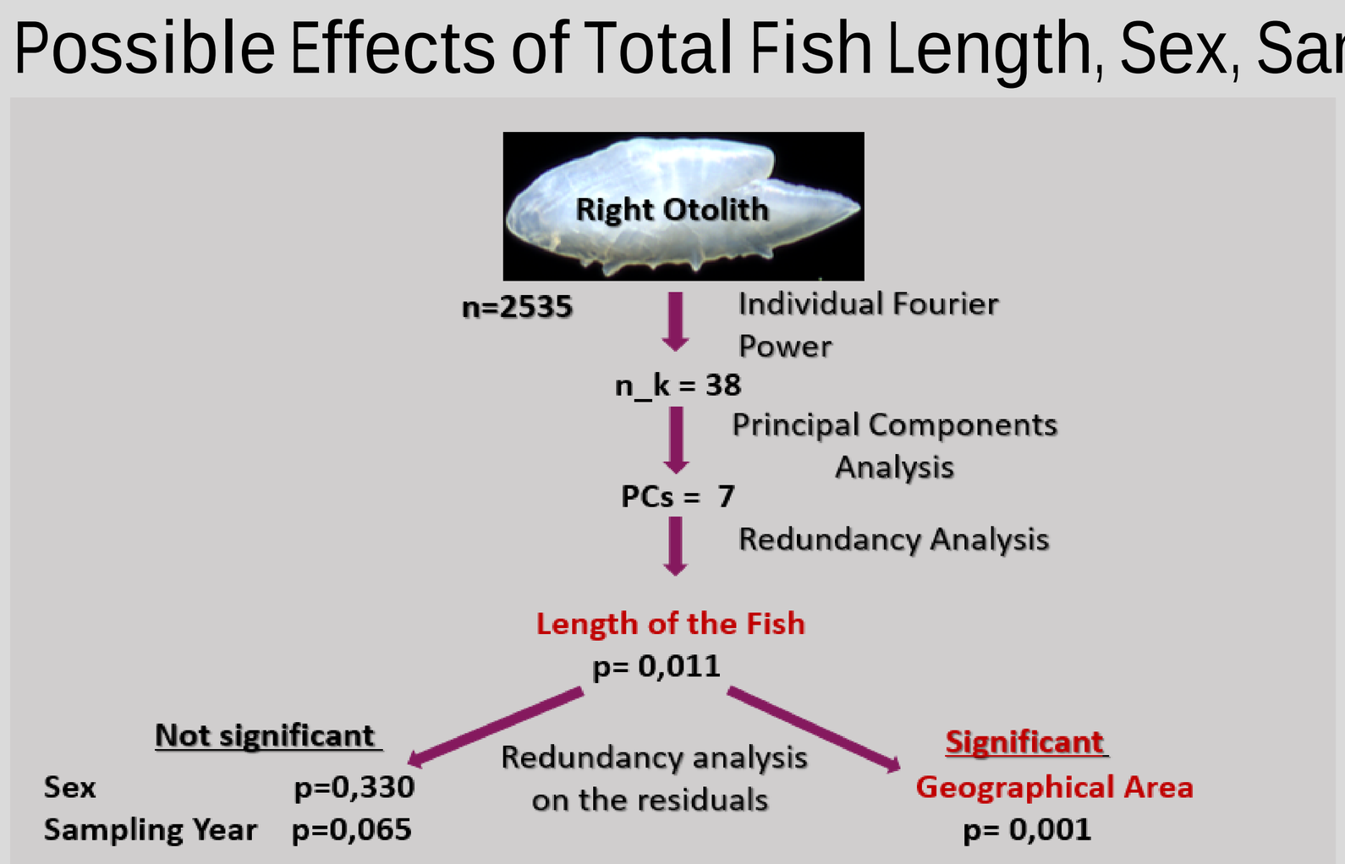


Figure 3: RDA for possible effects on shape analysis

- ✓ RDA with permutation test was applied for testing total length (TL) effect; then sex, sampling year and geographical area effects were tested.
- ✓ Geographical area has a significant effect on otolith shape

Linear Discriminant Analysis (LDA)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Class.Succ. (%
Atl	1_English Channel	65	0	2	0	0	4	1	0	2	1	11	0	30	0	0	0	0	2	1	0	54
	2_Bay of Biscay	6	0	2	0	0	15	1	0	0	0	11	0	19	0	0	0	0	0	1	0	0
	3_Gulf of Cadiz	9	0	27	0	0	18	0	0	0	0	5	0	48	0	1	0	0	10	4	0	22
	4_Casablanca	2	0	2	0	0	18	0	0	0	0	5	0	22	0	0	0	0	0	1	0	0
	5_South Cap Blanc	0	0	2	0	0	2	0	0	1	0	1	0	32	0	0	0	0	0	0	0	0
NW-Med	6_Gulf of Lion	3	0	3	0	0	185	5	0	0	0	0	0	59	0	0	3	0	23	15	0	63
	7_Catalan Sea	0	0	0	0	0	30	8	0	1	0	0	0	7	0	0	1	0	1	4	0	15
	8_Tyrrhenian Sea	0	0	0	0	0	24	0	0	0	0	0	0	18	0	0	0	0	2	1	0	0
SW-Med	9_Southern Alboran Sea	0	0	0	0	0	5	0	0	5	4	12	0	47	0	0	0	0	0	0	0	6
	10_Alboran Sea_Ghazaou	8	0	6	0	4	6	1	0	1	82	20	2	65	0	3	0	0	0	0	0	41
	11_Alboran Sea_ Benisaf	28	0	7	0	0	4	0	0	3	28	24	0	42	0	0	0	0	0	0	0	17
	12_Algero-Provençal Bas	3	0	5	0	0	20	0	0	1	4	1	0	70	0	0	0	0	5	3	0	0
	13_Alboran Sea_ Bejaia	13	0	8	0	1	45	0	0	2	41	5	0	337	0	0	0	0	10	3	0	72
E-Med	14_Ionian Sea	1	0	0	0	0	28	1	0	0	0	0	0	21	0	0	0	0	2	1	0	0
	15_Adriatic Sea	8	0	5	0	0	32	0	0	0	0	1	0	27	0	0	0	0	4	3	0	0
	16_Aegean Sea	0	0	0	0	0	27	3	0	0	0	0	0	8	0	0	3	0	0	0	0	7
	17_Marmara Sea	0	0	5	0	0	57	1	0	0	0	0	0	18	0	0	1	0	10	4	0	0
BlackSea	18_Western Black Sea	16	0	7	0	0	67	0	0	0	0	2	0	43	0	0	0	0	30	25	0	15
	19_Middle Black Sea	10	0	3	0	0	59	0	0	0	0	0	0	41	0	0	0	0	20	36	0	21
	20_Eastern Black Sea	2	0	7	0	0	34	0	0	0	1	0	0	20	0	0	0	0	13	18	0	0

Table 2: Table of LDA results, Jackknife Classification matrix of anchovy otolith shapes from 20 different sampling regions (N=2535).

- Jackknife Classification Success is 32% for 20 different regions.

TESTED SCENARIOS	JACKKNIFED CLASSIFICATION SUCCESS (%)
for 20 sampling location	32%
Atl, NW-Med, SW-Med, E-Med. BS	54%
ATL , NW-E Med , SW Med, and BS	57%
ATL- SW Med , NW Med, E Med , and BS	67%
ATL- SW Med , NW-E Med , and BS	68%
ATL- SW Med , NW Med , and E Med-BS	91%

Table 3. Jackknife Classification Success Percentage for different grouping scenarios.

From these scenerios

LDA and Hierarchical Clustering Analysis for Atlantic-South Western Mediterranean, North Western Mediterranean and Eastern Mediterranean-Black Sea

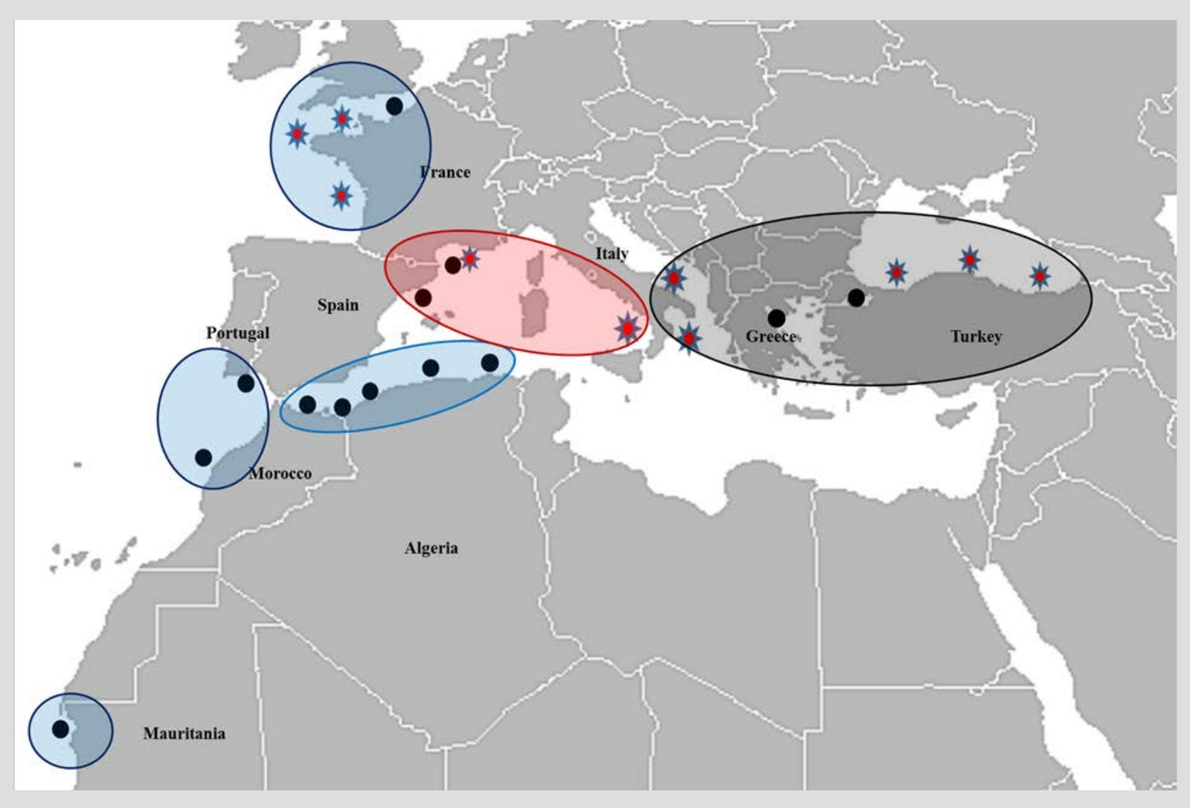
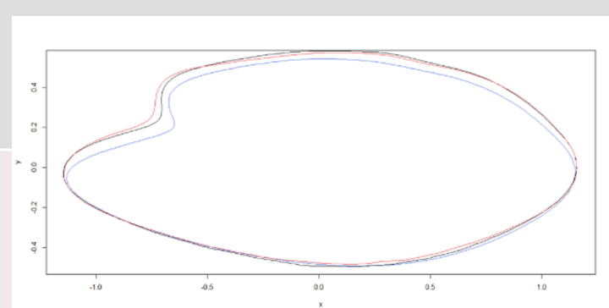


Figure 4: Atl-SW Med, NW Med and E Med-BS.



Areas	Number	Average Total Length (cm)
Atl-SWM	1369	12.5
E Med-BlackSea	773	11.3
NW Med	393	11.6

Table 3: Sample size and average TL of Samples.

Classified

Observed: LDA	Atl-SW	E Med- BS	NW Med	Class.Succ. %
Atl-SW Med	1346	0	22	98
E Med- BS	69	645	11	88
NW Med	97	0	296	75

Table 4: Table of LDA result, Jackknife Classification matrix of anchovy otolith shapes for 3 different groups.

✓ Jackknifed Classification Success **91% for 3 regions**

Hierarchical Clustering	1	2	3	Total
Atl-SW Med	610	632	127	***1369***
E Med- BS	40	384	349	***773***
NW Med	6	168	219	***393***
Total	***656***	***1184***	***695***	***2535***

Table 5: Hierarchical Clustering Analysis results for 3 different clusters.

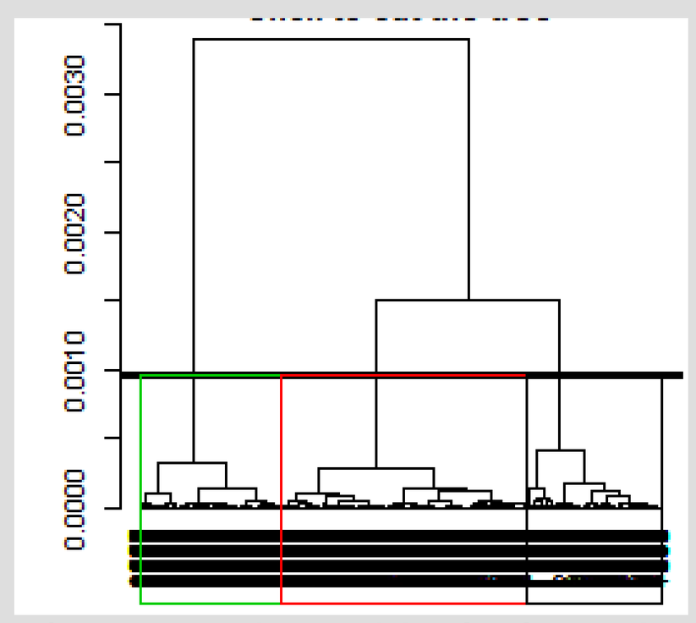
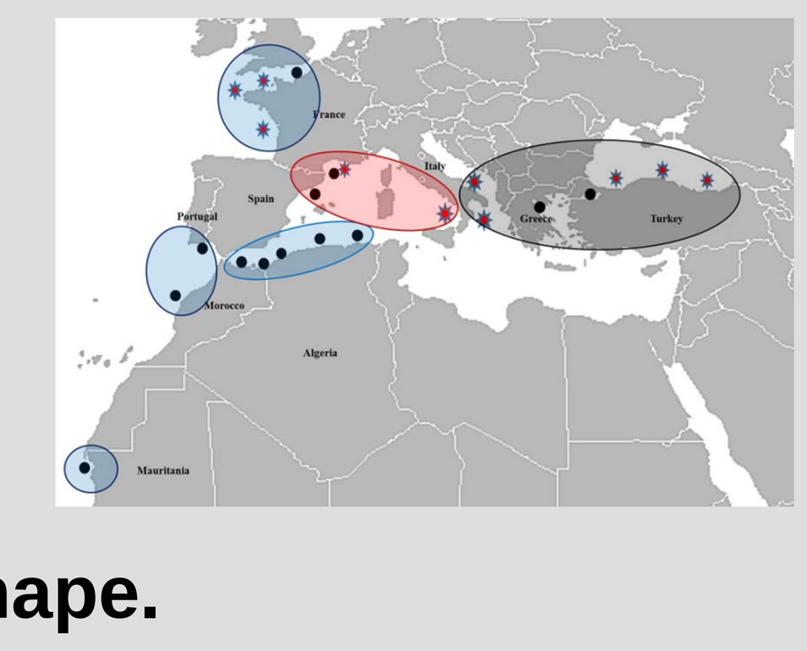


Figure 5: Hierarchical Clustering Analysis .

-> A scenario with three major groups of anchovies was favored.

CONCLUSIONS



- **Three different groups of European anchovies were identified :**
  - **Atlantic-Southwestern Mediterranean**
  - **Northwestern Mediterranean**
  - **Eastern Mediterranean- Black Sea**
- **These results have implications for stock management from the North Sea to the Black Sea.**
- **Further study will focus on the Black Sea to investigate growth patterns and environmental effects on otolith shape.**

CONTACT

Gizem Akkuş
Middle East Technical University, Institute of Marine Sciences,
Email: gizemakkuss@gmail.com

Kélig Mahé
Ifremer, Centre Manche Mer du Nord, Laboratoire Ressources Halieutiques,
Email: kelig.mahe@ifremer.fr

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Reference
Jemaa, S., Bacha, M., Khalaf, G., & Amara, R. (2015). Evidence for population complexity of the European anchovy (*Engraulis encrasicolus*) along its distributional range. *Fisheries research*, *168*, 109-116.