



Food and Agriculture  
Organization of the  
United Nations



General Fisheries  
Commission for  
the Mediterranean

# Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters

A multidisciplinary approach

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GENERAL FISHERIES COMMISSION FOR THE MEDITERRANEAN

# Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters

A multidisciplinary approach

Edited by

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Required citation:

Hidalgo, M., Hernández, P. & Vasconcellos, M., eds. 2024. *Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters - A multidisciplinary approach*. Studies and Reviews No. 104 (General Fisheries Commission for the Mediterranean). Rome, FAO. <https://doi.org/10.4060/cd1122en>

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ISBN: 978-92-5-138856-3

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# PREPARATION OF THIS DOCUMENT

This report stems from the “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” (Transboran) research project carried out under the framework of the regional project CopeMed II of the Food and Agriculture Organization of the United Nations (FAO), in close collaboration with the General Fisheries Commission for the Mediterranean (GFCM).

The research project aimed at enhancing the understanding of stock boundaries and connectivity in the Alboran Sea. The work started with a technical workshop organized in April 2017 in Alicante, Spain, where experts from Algeria, Italy, Morocco, Spain and Tunisia presented and discussed current methodologies for the delimitation of stocks, with a view to designing a research project to identify stocks of sardine (*Sardina pilchardus*) and European hake (*Merluccius merluccius*) and their spatial distribution in the Alboran Sea (geographical subareas [GSAs] 1–4) and adjacent waters. A concept note of this research project was then presented to the nineteenth session of the Scientific Advisory Committee (SAC) in May 2017, which suggested that blackspot seabream (*Pagellus bogaraveo*) should be included in the study along with the other two species. Finally, the three-species proposal was presented and approved by partner countries during the tenth meeting of the CopeMed II Coordination Committee in October 2017.

The activities of the Transboran research project started with a meeting held in December 2017 in Fuengirola, Spain, where experts from Algeria, Italy, Morocco, Spain and Tunisia discussed and agreed on procedures for sample collection, processing

and analysis using a range of different techniques. Subsequently, sampling and data analysis were carried out between 2018 and 2021, involving experts from seven institutions of the five countries participating in CopeMed II: the Spanish Institute of Oceanography (IEO, Spain), the National Institute of Fisheries Research (INRH, Morocco), the National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA, Algeria), the National Institute of Marine Sciences and Technologies (INSTM, Tunisia), the University of Málaga (Spain), the University of Bologna (Italy) and Sapienza University of Rome (Italy). This report presents a synthesis of the main findings of the project and integrates its results in order to formulate hypotheses of the stock boundaries of sardine, European hake and blackspot seabream in the Alboran Sea.

This publication was prepared under the overall supervision of Miguel Bernal (Executive Secretary). The editing and publishing of the report was led by Matthew Kleiner (Publication Specialist), under the coordination of Ysé Bendjeddou (Publication Coordinator). This work also benefited from the expertise of Alex Chepstow-Lusty, who performed the technical editing, and Koen Ivens, who handled the overall graphic design and layout. The editorial process was supervised by Dominique Bourdenet (Knowledge Management Officer).

This document was produced with the financial support of the European Union. The views expressed herein can in no way be taken to reflect the official opinion of the European Union.



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# ABSTRACT

This report presents the main findings of the “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” (Transboran) research project, which was carried out under the framework of the FAO regional project Cope-Med II and in close collaboration with the GFCM. The project applied a multidisciplinary approach to precisely describe the spatial structure of the populations of sardine (*Sardina pilchardus*), European hake (*Merluccius merluccius*) and blackspot seabream (*Pagellus bogaraveo*) in the study area in order to provide the best possible management advice for these three species in the western Mediterranean. Samples from 17 locations across the Alboran Sea and in adjacent areas of the Atlantic Ocean and Mediterranean Sea were analysed using genetics, otolith microchemistry, otolith shape, parasite composition, body morphometry and meristic descriptors. These analyses were complemented by numerical hydrodynamic modelling, fishery data analysis, and data obtained through a dedicated ichthyoplankton survey. Results from the different disciplines were compared and integrated using multivariate analyses and a semi-quantitative stock differentiation index.

Considering the results of all disciplines in an integrative manner, the project did not find scientific grounds to conclude that European hake and sardine in the northern and southern Alboran Sea behave as single and homogeneous

populations that could support joint stock assessment and management. The differentiation between stocks in the northern and southern Alboran Sea was stronger for European hake than for sardine. The results for European hake and sardine also revealed that the differentiation across geographical subareas (GSAs) is not always clearly consistent with geographically contiguous stocks. In the southern Alboran Sea, samples from the eastern part of GSA 4 (Algeria) were generally more similar to GSA 12 (Tunisia), while those obtained from the western part of GSA 4 were more similar to GSA 3 (Morocco). For these two species, the results support the available evidence that European hake and sardine stocks in the northern and southern Alboran Sea (GSAs 1 and 3) behave as independent populations. In the case of blackspot seabream, there is no evidence of population structure within the study area. However, hydrodynamic simulations showed regional differences for the recruitment process, which were affected by the geographical location of spawning within the Strait of Gibraltar, the tidal strength and the depth of spawning. On the other hand, the analysis of otolith microchemistry suggests potential migration to spawning areas in the Strait of Gibraltar, which requires further study.

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# ACKNOWLEDGEMENTS

The “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” (Transboran) research project was led by the FAO regional project CopeMed II and the GFCM and was made possible thanks to funding from the Government of Spain and the European Commission Directorate-General for Maritime Affairs and Fisheries (DG MARE). Gratitude is extended to the following institutions for their contributions to the research project: the Spanish Institute of Oceanography (IEO, Spain), the National Institute of Fisheries Research (INRH, Morocco), the National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA, Algeria), the National Institute of Marine Sciences and Technologies (INSTM, Tunisia), the University

of Málaga (Spain), the University of Bologna and Sapienza University of Rome (Italy). The useful comments from Steve Cadrin and one other anonymous peer reviewer are also recognized.

Acknowledgement and appreciation for their specialized contributions to the different techniques used in the analyses are extended to Massimiliano Babbucci, Meriam Feki, Ana Giráldez, Sabri Jaziri, Alfonso Pita and Naiara Rodríguez-Ezpeleta. Special recognition also goes to Pablo Abaunza (IEO) and Moussa Mennad (CNRDPA) for their significant efforts in coordinating the work of the experts of their respective institutions. Fabio Fiorentino and Henri Farrugio contributed to ample discussions in the elaboration of the concept.

# ABBREVIATIONS

<b>ANOVA</b>	analysis of variance
<b>CNRDPA</b>	National Centre for Research and Development of Fisheries and Aquaculture (Algeria)
<b>CopeMed II</b>	Coordination to Support Fisheries Management in the Western and Central Mediterranean – Phase II
<b>CPUE</b>	catch per unit effort
<b>DG MARE</b>	Directorate-General for Maritime Affairs and Fisheries of the European Commission
<b>DV</b>	difference value
<b>EAG</b>	Eastern Alboran Gyre
<b>EEZ</b>	exclusive economic zone
<b>ELS</b>	early life stages
<b>FAO</b>	Food and Agriculture Organization of the United Nations
<b>F<sub>ST</sub></b>	fixation index
<b>GFCM</b>	General Fisheries Commission for the Mediterranean
<b>GSA</b>	geographical subarea
<b>IEO</b>	Spanish Institute of Oceanography (Spain)
<b>INRH</b>	National Institute of Fisheries Research (Morocco)
<b>INSTM</b>	National Institute of Marine Sciences and Technologies (Tunisia)
<b>LFN</b>	Lagrangian flow network
<b>mPCR</b>	multiplex polymerase chain reaction
<b>PCA</b>	principal coordinate analysis
<b>PERMANOVA</b>	permutational multivariate analysis of variance
<b>PLD</b>	pelagic larval duration
<b>SAC</b>	Scientific Advisory Committee on Fisheries
<b>SDI</b>	stock differentiation index
<b>SNP</b>	single nucleotide polymorphism
<b>s.s.</b>	sensu strictu
<b>Transboran</b>	“Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” research project
<b>WAG</b>	Western Alboran Gyre



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# GLOSSARY

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## Allometry

The relationship between the size of an organism and aspects of its physiology, morphology and life history. (Bradshaw and McMahon, 2008)

---

## Amplicon

A piece of DNA or RNA produced by an amplification reaction. (Zaid *et al.*, 2001)

---

## Ancestry coefficient

Intraclass correlation of pairs of alleles within populations, serving as a measure of genetic distance between populations. For a general class of evolutionary models, it determines the distribution of allele frequencies among populations. (Samanta, Li and Weir, 2008)

---

## Annealing temperature

The temperature at which a substance undergoes an internal change as part of a heating process.

---

## Barycentre

The point at the centre of a system.

---

## Bayesian algorithm

Mathematical formula for determining conditional probability, which is the likelihood of an outcome occurring based on a previous outcome in similar circumstances, providing a way to revise existing predictions or expectations given new or additional evidence. (Walker, 2024)

---

## Biological reference points

Thresholds used to define safe levels of harvesting for marine fish populations, most commonly either minimum acceptable biomass levels or maximum fishing mortality rates. (Collie and Gislason, 2011)

---

## Bongo net

Net consisting of two plankton nets – ring nets with a small mesh width and a long funnel shape – mounted next to each other. The bongo net is pulled obliquely through the water column by a research vessel and allows scientists to work with two different mesh widths simultaneously. (Ocean Innovations, 2024)

---

## Centroid

Geometric centre or the arithmetic mean position of all the points on the surface of a plane or solid figure.

---

## Clopper-Pearson interval

Statistical method for calculating binomial confidence intervals. It is often called an “exact” method, as it attains the nominal coverage level in an exact sense. (Clopper and Pearson, 1934)

---

## Clupeid

Soft-finned bony fish of the family Clupeidae, typically having oily flesh. Clupeids are widely distributed and include herrings and sardines, among others. (Collins, 2024)

---

## Discriminant analysis of principal components

Statistical method of grouping information in clusters using a few synthetic variables. These are constructed as linear combinations of the original variables that have the largest between-group variance and the smallest within-group variance. (Jombart and Collins, 2015)

---

## Dynamic factor analysis

Statistical method used for time-series data to estimate a small number of unobserved processes (“trends”) that can describe observed data. (Ward *et al.*, 2022)

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## Early life stages

In fish, refers to the pre-hatch embryonic period, the post-hatch free embryo or yolk-sac fry, and the larval period, during which the organism feeds. Juvenile fish, which are anatomically rather similar to adults, are not considered an early life stage. (Ohio River Valley Water Sanitation Commission, 2019)

---

## Fisher discriminant analysis

Feature selection algorithm used in statistics to find a linear combination of features characterizing or separating two or more classes of objects or events. (Kavlakoglu, 2024)

---

## Fixation index

The average coefficient of inbreeding in a population. (Rédei, 2008)

---

## Fluorophore

Fluorescent chemical compound that can re-emit light upon light excitation. (National Institute of Biomedical Imaging and Bioengineering, 2024)

---

## Fourier descriptors

Mathematical representation of how the curvature function varies with distance along the boundary of an object. (Davies, 2001)

---

## Generalized additive model

Generalized linear model in which the linear response variable depends linearly on unknown smooth functions of some predictor variables. (Wood, 2017)

---

## Genetic drift

Change in the frequency of an existing gene variant in a population due to random chance.

---

## Genetic marker

A DNA polymorphism that can be easily detected by molecular or phenotypic analysis. Because DNA segments lying near each other on a chromosome tend to be inherited together, markers are often used as indirect ways of tracking the inheritance pattern of a gene that has not yet been identified, but whose approximate location is known. (NIH, 2007)

---

## Gill rakers

Any of the bony structures on a gill arch that divert solid substances away from the gills. (Merriam Webster, 2024)

---

## Hardy-Weinberg equilibrium

Principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. When mating is random in a large population with no disruptive circumstances, the law predicts that both genotype and allele frequencies will remain constant because they are in equilibrium. (Nature Education, 2014a)

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## Heterozygosity

Having two different forms of a gene that controls a particular characteristic, one from each parent, and therefore able to pass on either form. (Cambridge University Press, 2024)

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## Jackknife classification resampling

Statistical resampling method that provides estimates of the bias and a standard error of an estimate by recomputing the estimate from subsamples of the available sample. (Sinharay, 2010)

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## Kurtosis

A measure of the tailedness of a distribution providing information on how often outliers occur. (Turney, 2022)

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## Lagrangian flow network

Modelling framework in which, for example, ocean subareas are represented as nodes in a network interconnected by links representing the transport of particles (e.g. eggs and larvae) by currents. (Monroy *et al.*, 2017)

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## Laser ablation

The process of removing material from a solid surface by irradiating it with a laser beam. (A-Laser, 2024)

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## Linear discriminant analysis

Statistical method used to find a linear combination of features that characterizes or separates two or more classes of objects or events. (IBM, 2023)

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## Linkage disequilibrium

A genetic measure of non-random co-occurrence of alleles at different loci in a population. (Arnatkevičiūtė, Fulcher and Fornito, 2023)

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## Meridional transport

Transport of energy from the tropics to the polar latitudes, either by the atmosphere or the ocean. (Masuda, 1988)

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## Meristics

An area of ichthyology relating to the counting of quantitative features of fish, such as the number of fins or scales. (Boyd, 2016)

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## Mesoscale

Of intermediate size or scale.

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## Microsatellite

A tract of repetitive DNA in which certain DNA motifs are repeated, typically 5–50 times. They occur at thousands of locations within an organism's genome and have a higher mutation rate than other areas of DNA leading to high genetic diversity. (Reshma and Das, 2021)

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## Multiplex polymerase chain reaction

Use of a polymerase chain reaction to amplify several DNA sequences simultaneously. This process amplifies DNA in samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. (Nazir *et al.*, 2019)

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## Multivariate analysis of variance

Statistical procedure providing regression analysis and analysis of variance for multiple dependent variables by one or more factor variables or covariates. (IBM, 2021)

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## Neap tide

A period of moderate tides when the sun and moon are at right angles to each other. (NOAA, 2023)

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## Neutral genetic markers

Loci often used to study the evolutionary history of populations and species. Since they do not affect an organism's fitness, they are assumed to evolve at a constant rate and can be used to estimate the time since two populations or species diverged from a common ancestor. (TutorChase, 2024)

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## Oligonucleotide

A polynucleotide whose molecules contain a relatively small number of nucleotides. (Falcone, 2019)

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## Otolith

Ear stones that are part of a fish's inner ear and allow the animal to hear and sense vibrations in the water and provide a sense of balance. Otoliths grow throughout a fish's life by accumulating animal or environmental material on the outer surface around a core. (NOAA, 2020)

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## Otolith core

The foundation on which all new otolith growth occurs. (Virginia Marine Resources Commission, 2023)

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## Otolith edge

The measurement from the last annulus to the margin of an otolith. (Florida Fish and Wildlife Commission, 2024)

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## Outlier genetic markers

Loci that demonstrate significantly higher or lower within-population genetic differentiation than expected under neutrality. (Feng, Jiang and Fan, 2015)

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## Parsimonious

Describes statistical models using relatively few independent variables to obtain a good fit to the data. (Frost, 2024)

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## Permutational multivariate analysis of variance

Statistical model used in ecology and biology to explain communities with environmental variables. Its goal is similar to a multivariate analysis of variance but is instead based on permutations of distance matrices. (XLSTAT, 2024)

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### **Principal components analysis**

Statistical method used to reduce a cases-by-variables data table to its essential features, called principal components. Principal components are a few linear combinations of the original variables that maximally explain the variance of all variables. (Greenacre *et al.*, 2022)

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### **Principal coordinates analysis**

Statistical technique summarizing and attempting to represent inter-object (dis)similarity in a low-dimensional Euclidean space. (Ebner, 2018)

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### **Procrustes analysis**

Rigid shape analysis that uses isomorphic scaling, translation and rotation to find the best fit between two or more landmarked shapes. (Ross, 2004)

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### **Sensitivity analysis**

Study of how the uncertainty in the output of a mathematical model or system (numerical or otherwise) can be divided and allocated to different sources of uncertainty in its inputs. (European Commission, 2024)

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### **Single nucleotide polymorphism**

Variation at a single position in a DNA sequence occurring among individuals. (Nature Education, 2014b)

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### **Spring tide**

Spring tides occur twice each lunar month all year long without regard to the season. The concept derives from the tide “springing forth”. (NOAA, 2023)

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### **Subinertial forcings**

Forcings resulting from small currents that are independent of the movement of large bodies of water.

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### **Tidal forcings**

Forcings resulting from the gravitational pull of the moon and the sun.

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### **Tukey’s test**

Statistical method that compares the means of every treatment to the means of every other treatment by applying simultaneously to the set of all pairwise comparisons and identifying any difference between two means that is greater than the expected standard error. (Plant and Soil Sciences eLibrary, 2024)

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### **Virtual population analysis**

A general method in fisheries to model the progression of a cohort through time. (Lassen and Medley, 2001)

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### **Voracera**

A special type of longline used historically in the south of Spain to fish blackspot seabream (“voraz” in Spanish).

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# EXECUTIVE SUMMARY

The Alboran Sea lies in a transition zone between the Atlantic Ocean and the Mediterranean Sea and is bordered by three countries: Algeria, Morocco and Spain. For fisheries monitoring and management purposes, the Alboran Sea is divided into four geographical subareas (GSAs) – GSAs 1, 2, 3 and 4. Fish stocks in these GSAs have been considered as independent management units until now, due to knowledge gaps concerning the spatial structure and connectivity of fish populations.

The “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” (Transboran) research project ran from 2017 to 2021 with the objective of precisely describing the spatial structure of sardine (*Sardina pilchardus*), European hake (*Merluccius merluccius*) and blackspot seabream (*Pagellus bogaraveo*) populations in order to provide the best possible management advice for these species in the western Mediterranean.

The project adopted a multidisciplinary approach, combining information from different techniques, including fishery data analysis, hydrodynamic dispersion modelling, genetics, otolith microchemistry, otolith shape, parasite composition, body morphometry, meristic description of gill rakers, and vertebral counts. All of these techniques were applied to sardine and European hake, while only genetics, dispersal modelling and otolith microchemistry were used for blackspot seabream. This multidisciplinary approach allowed for the analysis of information at contrasting spatial and temporal scales to better understand the structure of marine populations. Such an approach is useful for taking account of ecological, hydrodynamic and evolutionary processes and thereby may contribute to defining management boundaries for the three species investigated.

The analyses were based on a comprehensive and coordinated biological sampling programme carried out in 17 locations distributed across the Alboran Sea and neighbouring regions of the Atlantic Ocean and the Mediterranean Sea. Numerical model simulations were used to describe the hydrodynamic effects on the dispersal of early life stages and the connectivity of populations between sampling sites. In addition, complementary field data on the distribution and abundance of fish early life

stages were obtained through an ichthyoplankton survey carried out from 20 February to 14 March 2020 on board the R/V Emma Bardan of the Spanish Ministry of Agriculture, Fisheries and Food. Results from the different disciplines were compared and integrated using multivariate analyses and a semi-quantitative stock differentiation index.

Results do not support the hypothesis that European hake and sardine stocks in the northern and southern Alboran Sea (GSAs 1 and 3) behave as unique and homogeneous populations. On the contrary, there is sufficient scientific evidence showing spatial differentiation between the northern and southern Alboran Sea stocks. With the exception of genetic markers, all other techniques show significant differentiation between European hake stocks in GSAs 1 and 3, which is also consistent with the results from hydrodynamic simulations that reveal low levels of connectivity between the two areas and strong self-recruitment in both GSAs 1 and 3. A similar pattern emerges for sardine, although for this species the similarity found in otolith microchemistry indicates that environmental conditions in spawning areas may be alike. The spatial differentiation for European hake was higher than for sardine, suggesting more efficient gene flow, spatial dynamics and likely migration behaviour of small pelagic species such as sardine. Another important feature highlighted by the results is the level of differentiation between adjacent GSAs in the northern (GSA 6) and southern Alboran Sea (GSA 12). In the northern Alboran Sea, the differentiation between European hake in GSAs 1 and 6 was supported by techniques capturing traits at the evolutionary (genetic markers) and life cycle (parasites and otolith microchemistry) timescales. For sardine, similarities between GSAs 1 and 6 were found in genetic markers and meristics, but not in other techniques. On the other hand, in the southern Alboran Sea, sardine and European hake each showed a general differentiation between the results of most techniques within GSA 4. Samples taken in the eastern part of GSA 4 were generally more similar to those from GSA 12, while those from the western part of GSA 4 were more similar to GSA 3. The connectivity between GSA 3 and the western parts of GSA 4 was also supported by the results of hydrodynamic simulations. Finally, the similarities between GSAs 6 and 12, especially for European hake, reveal some level of connectivity



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between these two areas, apparently mediated by the Almería-Oran front. Almost all the techniques employed, as well as the integrative analyses, provide information on two main structuring mechanisms for European hake and sardine: i) long-term and longitudinal genetic drift; and ii) local processes driven by the environment and/or demographic processes spatially structured at a smaller scale.

As for blackspot seabream, there is no evidence of population structure within the study area, with genetics results also revealing an unexpected absence of genetic structure over a very large geographic distance. However,

hydrodynamic simulations showed regional differences in the recruitment process subject to the geographical location of spawning within the Strait of Gibraltar, the tidal strength and the depth of spawning. By contrast, otolith microchemistry revealed current spatial differences in the otolith edge associated with the time of sampling and the last period of life of the individuals, while such a degree of differentiation is not apparent in the core of the otolith. This pattern suggests potential migration to the Strait of Gibraltar for spawning and thus merits further research.

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# INTRODUCTION

## The global challenge

Quantitative fisheries science has experienced technical advances over the last decade, thanks to the development and considerable enhancement of diverse assessment methods to improve diagnoses of stock status and to predict their development. However, there remain challenges to the effective assessment and management of fisheries, some of which date back decades. Among these hurdles is the partial (and often general) lack of knowledge of the spatial structure of stocks (e.g. Punt, 2019; Cadrin, 2020; Kerr *et al.*, 2017). Even the pioneers of fisheries science recognized the need to account for spatial structure in the analyses that form the basis for fisheries management advice. For example, Beverton and Holt (1957) gave theoretical attention to the wide variation in fishing intensity generated by unfishable grounds that lead fishers to keep returning to other areas. It is now generally acknowledged that ignoring spatial structure can lead to misperceptions of stock status and failures in fisheries management (Baudron and Fernandes, 2015; Cadrin *et al.*, 2019). It can result in wide-ranging consequences, including unintentionally allowing overfishing and the severe depletion of spatial components, potentially triggering stock collapse or making it impossible to rebuild stocks. For this reason, the development of stock assessments must incorporate an evaluation of the most appropriate spatial scope and structure (Cadrin, 2020; Berger *et al.*, 2017).

In addition, the impacts of climate change on fish stocks are rapidly accelerating, particularly on fisheries production and stock distribution (Pinsky *et al.*, 2018; Free *et al.*, 2019). Considering the anticipated changes in species distribution and the related increase in transboundary stock migrations due to warming, future international governance may need to pay special attention to such redistributions (Pinsky *et al.*, 2018; Hoagland *et al.*, 2021). By 2030, it is predicted that 23 percent of transboundary stocks will have shifted and 78 percent of the world's exclusive economic zones (EEZs) will have experienced at least one shifting stock. Indeed, by the end of this century, projections show a total of 45 percent of stocks shifting globally and 81 percent of EEZ waters experiencing at least one shifting stock (Palacios-Abrantes *et al.*, 2022). Ignoring spatial structure could thus impact the effectiveness of adaptation tools to face climate change and ultimately lead to maladaptation (Hidalgo *et al.*, 2022a).

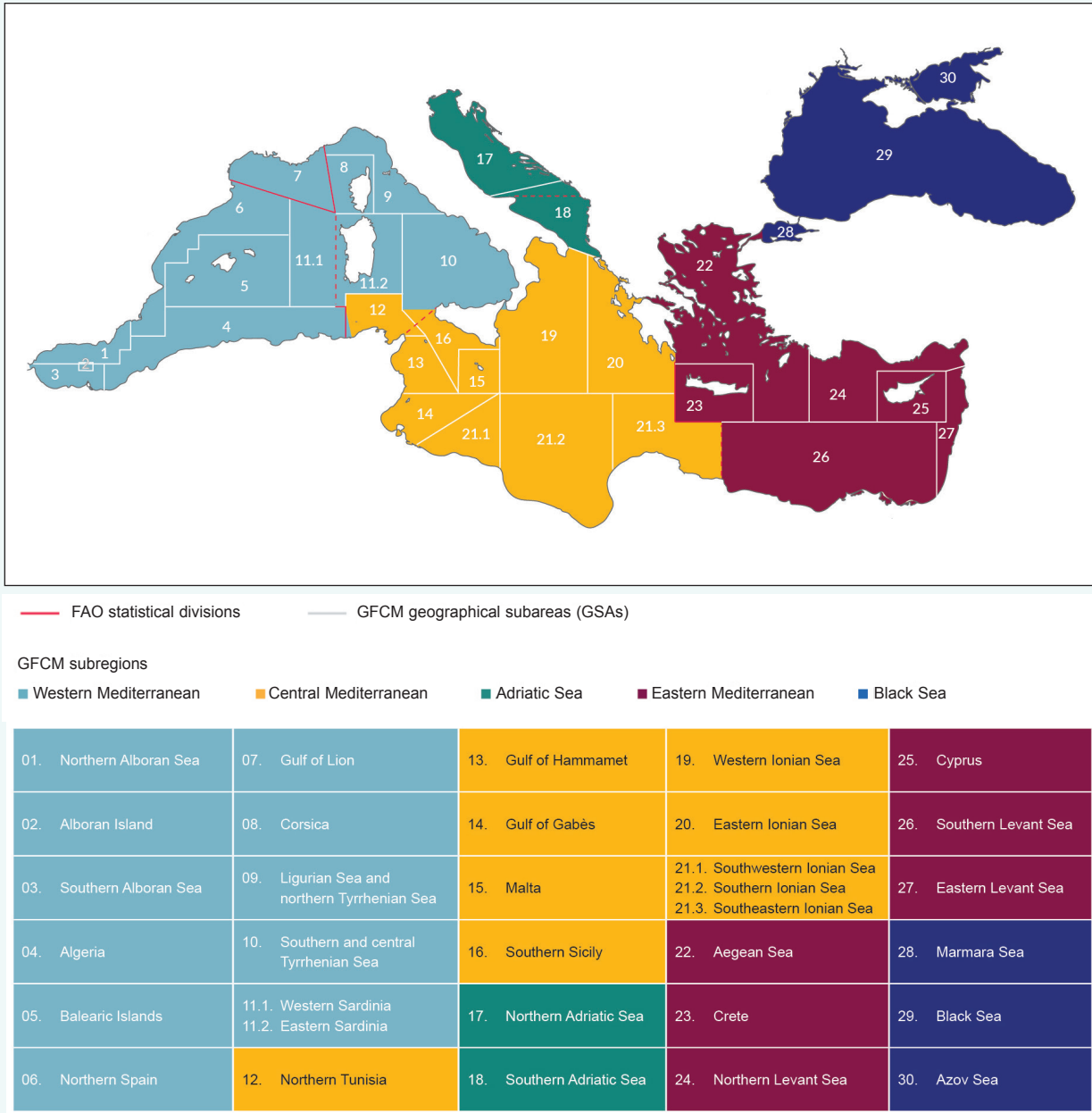
Historically, stock delimitation has followed a top-down approach, by which managers decide the stock boundaries for socioeconomic or political reasons, with fisheries and assessment modellers adapting their work to these boundaries. Today, however, a vast amount of research shows that stock delimitation should follow a bottom-up approach, based on scientific evidence. This paradigm inherently requires multidisciplinary and holistic approaches.

One of the main factors to consider when identifying a stock structure and establishing its boundaries is the strength of interdisciplinary analyses developed across national jurisdictions (ICES, 2021). In recent decades, numerous methods have been developed and improved considerably (e.g. genetics). However, applying different approaches may sometimes lead to competing hypotheses, because alternative methods can reveal differences at contrasting spatial (from local to regional) and temporal (from daily to evolutionary) scales. Nevertheless, there is general agreement on the need to adopt a holistic approach with multiple perspectives to improve information on stock structure for resource management purposes, in order to capture a range of ecological and structuring processes acting at contrasting scales.

## The Mediterranean challenge

In the Mediterranean, the regional fisheries management organization responsible for the sustainable use and management of marine living resources is the General Fisheries Commission for the Mediterranean (GFCM). In 2009, through Resolution GFCM/33/2009/2, the GFCM established 30 geographical subareas (GSAs) covering its entire area of application, from the Strait of Gibraltar to the Black Sea (Figure 1). The extent to which the dimensions and borders of these GSAs reflect the ecological structure of Mediterranean stocks has often been questioned. Furthermore, the high percentage of Mediterranean stocks in a state of unsustainable exploitation has offered an additional argument for attempting to resolve the longstanding challenge of stock delineation in the Mediterranean Sea (FAO, 2023). Indeed, the European Commission has funded two basin-wide projects since the early 2010s with this aim in mind: STOCKMED, from 2011 to 2014 (Fiorentino *et al.*, 2015), and MED\_UNITS, from 2017 to 2021 (European Commission, 2022).

**FIGURE 1.** GFCM area of application, subregions and geographical subareas



In certain critical areas of the Mediterranean, the proximity of opposing coasts makes the challenge of stock differentiation even more important, including in the eastern Mediterranean, the Strait of Sicily, the Adriatic Sea and the Alboran Sea. In the case of the Alboran Sea, the FAO regional project CopeMed II, which was run in collaboration with the GFCM, launched the research project at the heart of this report: “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach” (Transboran), a four-year project co-sponsored by the Directorate-General for Maritime Affairs and

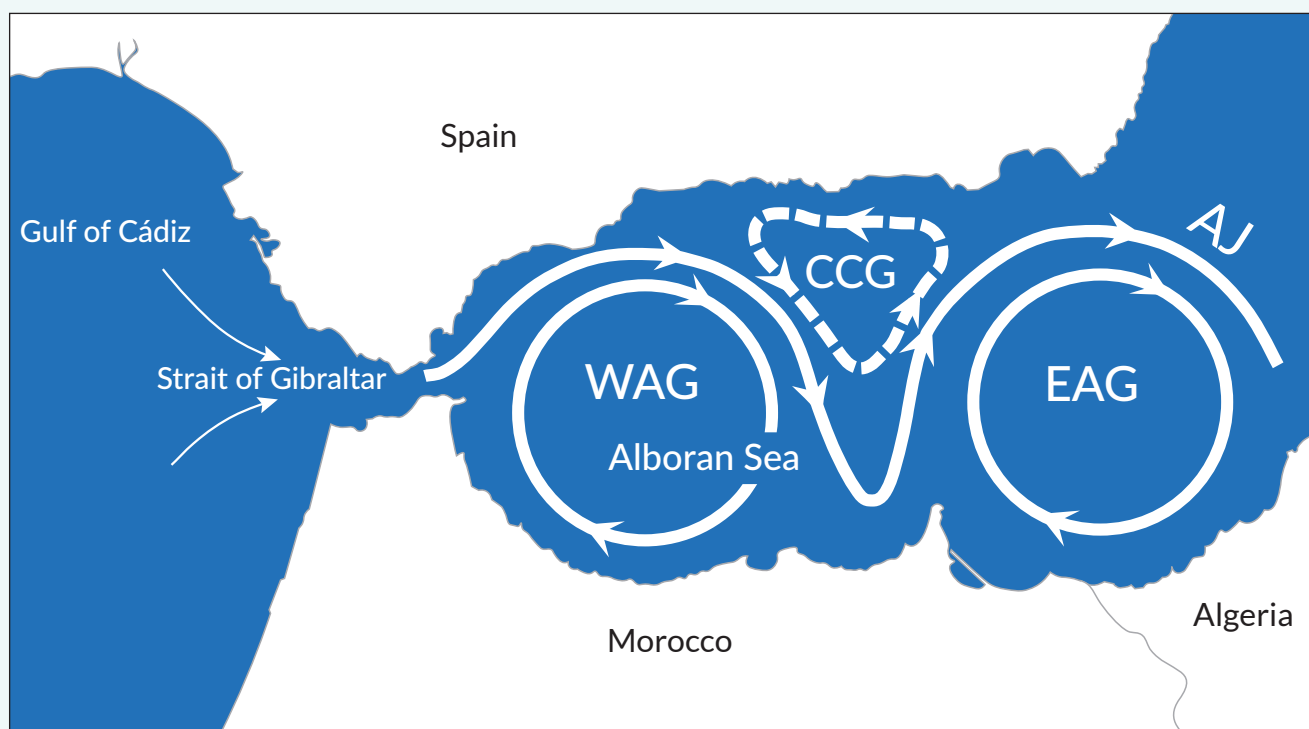
Fisheries (DG MARE) of the European Commission and the Spanish Ministry of Agriculture, Fisheries and Food. A consortium of seven institutions from five countries – Algeria, Italy, Morocco, Spain and Tunisia – contributed to the research: the Spanish Institute of Oceanography (IEO, Spain), the National Institute of Fisheries Research (INRH, Morocco), the National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA, Algeria), the National Institute of Marine Sciences and Technologies (INSTM, Tunisia), the University of Málaga (Spain), the University of Bologna and Sapienza University of Rome (Italy).

## The Alboran Sea case study

The oceanographic, ecological, fisheries and management characteristics of the Alboran Sea make it a perfect – and timely – case study to develop for this project. The study area comprises the Strait of Gibraltar and its adjacent Atlantic waters to the west (including the ports of Huelva and Cádiz on the Spanish coast and Agadir on the Moroccan side) and extends to the Gulf of Tunis in the east, covering GSAs 1, 2, 3, 4, 6 and 12. Given the short distances across the Alboran Sea, it might be expected that populations and ecosystems would be naturally connected by the strong hydrodynamics in the area. However, connectivity pathways are not straightforward. From an oceanographic perspective, and considering the overall dynamic of general circulation in the Mediterranean, the Alboran Sea is a

zonal-oriented basin whose main role is to steer the inflow of Atlantic water through the Strait of Gibraltar towards the interior of the Mediterranean. From this broad point of view, its mean surface circulation consists of a west-to-east flow that isolates the ecosystems of its northern and southern shores from each other, or that at least acts as a considerable obstacle to their connectivity (Figure 2; Sánchez-Garrido and Nadal, 2022; Garcia-Lafuente *et al.*, 2021). While this circulation pattern guarantees east-west connectivity, north-south oceanographic connections are less evident despite the relative proximity of the Alboran Sea's northern and southern coasts.

**FIGURE 2.** Surface circulation of the Alboran Sea and Gulf of Cádiz



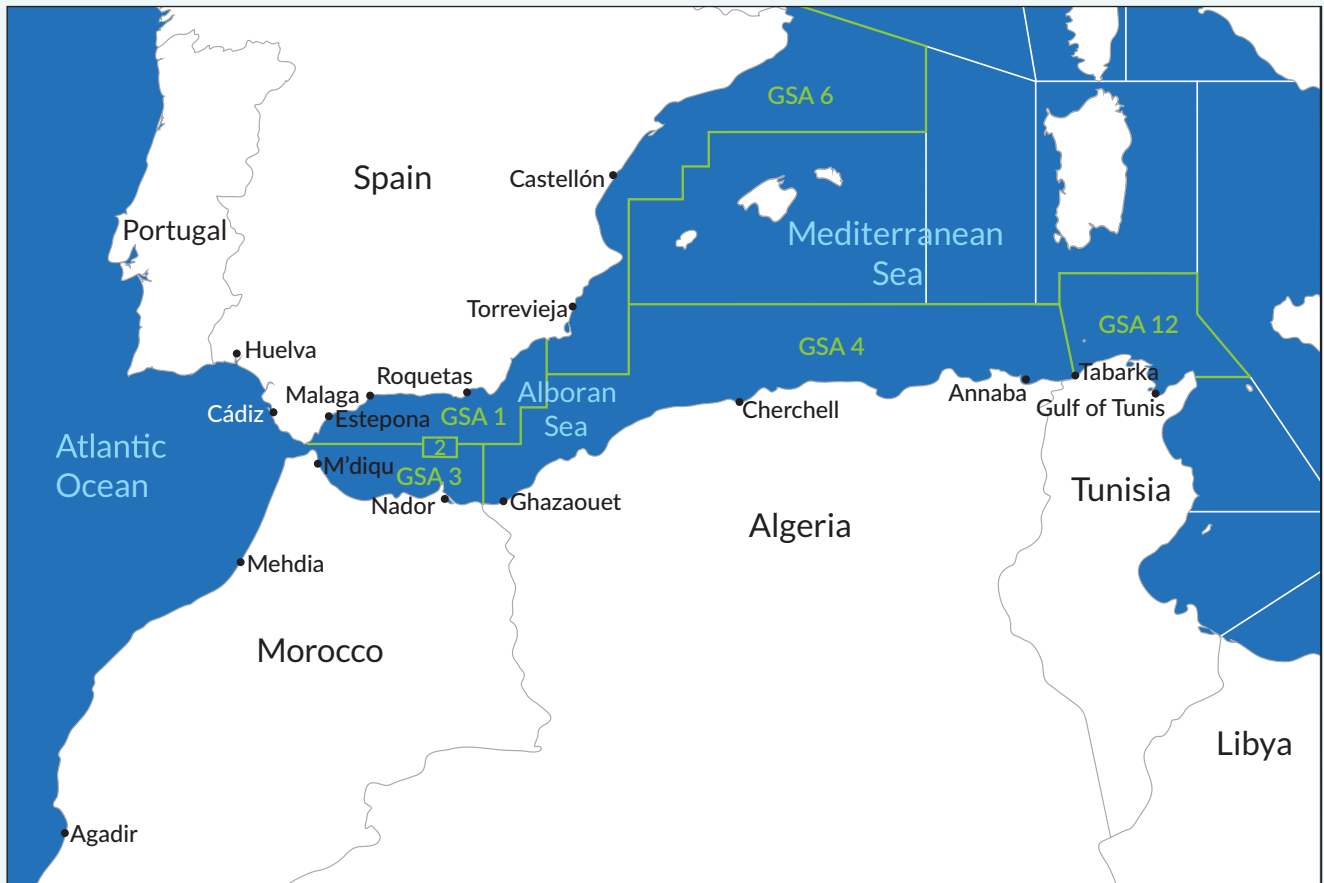
Sources: Base map redrawn from Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401; graphic overlay adapted from Garcia-Lafuente, J., Sánchez-Garrido, J.C., Garcia, A., Hidalgo, M., Sammartino, S. & Laiz, R. 2021. Biophysical Processes Determining the Connectivity of the Alboran Sea Fish Populations. In: J.C. Báez, J-T. Vázquez, J.A. Camiñas & M.M. Idrissi, eds. *Alboran Sea – Ecosystems and Marine Resources*. Springer, Cham, Switzerland.

Notes: Solid thick lines outline the two anticyclonic gyres, the Western Alboran Gyre (WAG) and Eastern Alboran Gyre (EAG), respectively, and the Atlantic jet (AJ); the dashed line represents the Central Cyclonic Gyre (CCG).

The Alboran Sea cuts across four different GSAs (GSAs 1, 2, 3 and 4; Figure 3) that are considered as independent fisheries management units. These units, though they do not correspond to actual species distribution boundaries, dictate the spatial scope at which stock assessment

and management are addressed, i.e. the stocks are considered as four independent units, while they are fished by the fleets of the three surrounding countries: Algeria, Morocco and Spain.

**FIGURE 3.** The Alboran Sea (geographical subareas 1 to 4), adjacent Atlantic waters, neighbouring geographical subareas 6 and 12 and sampling ports for European hake and sardine



Source: Base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Different population groups of the same species may be identified according to their biological features (e.g. growth rate, spawning period) and may react differently to fishing pressure, therefore presenting different resilience levels. As a consequence, the effects of fishing pressure or environmental stressors on a species in one area can differ

from the effects on the same species in a neighbouring area, and these differences should be taken into account when establishing management measures – particularly when they concern fleets from different countries.

### Species studied

The fish species studied in this report are the three with the highest landings and value in the Alboran Sea and its adjacent waters and those selected by the GFCM as priority species in the western Mediterranean: European hake (*Merluccius merluccius*), sardine (*Sardina pilchardus*)

and blackspot seabream (*Pagellus bogaraveo*). The aim of the Transboran project was to investigate the cross-scale connectivity processes and the population structure of each species using contrasting case studies. Alboran Sea sardine is currently assessed by Spain, Morocco and

Algeria as different country-specific stocks; on the other hand, from 2010 to 2021 the GFCM assessed European hake as a shared stock across GSAs 1 and 3, and blackspot seabream continues to be assessed jointly. Following assessments of the three species over the last 10 years, the GFCM has warned that hake is overexploited and blackspot seabream is depleted (FAO, 2023).

Sardine is a pelagic species that stays closer to the coast than European hake and blackspot seabream. It shows a relatively shallow dispersal, meaning that the stock's potential north-south connectivity could be directly affected by the mesoscale shallow circulation in the Alboran Sea. European hake and blackspot seabream display a deeper dispersal and are less affected by surface circulation, while the sluggishness of the flow at depth could decrease the likelihood of effective north-south stock connectivity (Garcia-Lafuente *et al.*, 2021).

Blackspot seabream presented a special case study, as little is known about its life cycle. Most adults and spawners are fished in a very small area of the Strait of Gibraltar – where most of the spawning is assumed to occur – while larvae are thought to be mainly dispersed in the Alboran Sea. Therefore, the Alboran Sea might be considered as a species hatchery and nursery area, in which juveniles are mostly caught close to the shore by recreational angling (Gil, Silva and Sobrino, 2001). Adults, which are harvested by Spanish and Moroccan “*voracera*” longline fishing fleets, spawn during the first quarter of the year in the Strait of Gibraltar (Gil-Herrera, 2010). This interconnected cycle of recruitment takes place on both sides of the Strait of Gibraltar and nearby coastal areas and is followed by juveniles returning to the grounds where the fishery is active (Gil-Herrera *et al.*, 2021).





# CHAPTER 1

## SUMMARY OF THE METHODOLOGY



The Transboran project adopted a multidisciplinary approach, combining information from eight techniques. Together with studying fishery data, the following techniques were applied: i) hydrodynamic dispersion modelling; ii) genetics; iii) otolith microchemistry; iv) otolith shape; v) parasite composition; vi) body morphometry; vii) meristic description of gill rakers; and viii) vertebral counts. All of these techniques were applied to sardine and European hake, while for blackspot seabream, only genetics, dispersion modelling and otolith microchemistry were used. This multidisciplinary approach allowed for the retrieval of information at contrasting spatial and temporal scales (Table 1), which, when combined, are useful for considering the ecological, hydrodynamic and evolutionary processes contributing to the population structure of the three

species. The approach includes, for instance, microchemistry and oceanographic modelling, which are assumed to operate at short scales, together with meristic methods, assumed to respond to evolutionary and multidecadal processes. Meanwhile, the rapid development of genetic tools is enabling information to be obtained over broader spatial and temporal windows, depending on the genetic marker used. As sampling of blackspot seabream was not geographically structured around the Alboran Sea, only three contrasting (in terms of the spatial and temporal scale of response) techniques were selected – hydrodynamics, otolith microchemistry and genetics – and were supported by external samples obtained from other regions far from the Alboran Sea (i.e. the North Atlantic and eastern Mediterranean).

**TABLE 1.** Techniques used to analyse the stock structure of European hake, sardine and blackspot seabream in the Alboran Sea, along with the timescale of the information captured by each technique and its relation to the species’ life cycles

Technique	Timescale	Relation to life cycle
<b>Genetic markers</b>	Multigenerational and evolutionary (from decades to thousands of years)	Indicates the degree of isolation between populations at large and evolutionary scales. Depending on the markers, local adaptations to habitat conditions at smaller scales can also be revealed.
<b>Meristics</b>	Multigenerational and evolutionary (from decades to thousands of years)	Indicates differences in meristic counts. Differences are determined by the genetic basis and to a lesser degree by environmental circumstances.
<b>Body morphometrics</b>	Individuals’ lifetimes to multigenerational (from one year to decades)	Indicates differences in body measurements, which are affected by a fish’s condition and its reproductive stage, as well as environmental conditions during life and local adaptations.
<b>Otolith shape</b>	Individuals’ lifetimes to multigenerational (from one year to decades)	Reflects differences in environmental conditions during the course of the life cycle, such as temperature, prey density and photoperiod, including multigenerational local adaptations to habitat conditions.
<b>Parasites</b>	Individuals’ lifetimes (from one year to a decade)	Indicates if a fish has inhabited areas that are endemic for certain types of parasites within its life cycle, starting from its arrival in the nursery.
<b>Otolith microchemistry (edge)</b>	Individuals’ recent past (days to months)	Indicates similarities and differences in habitat conditions in the recent period prior to capture.
<b>Otolith microchemistry (core)</b>	Individuals’ early life stage (days to months)	Indicates similarities and differences in habitat conditions in early life stages prior to nursery or bottom settlement. Useful for distinction of spawning areas.
<b>Hydrodynamics</b>	Individuals’ early life stage (days to months)	Simulations help scientists understand how the transport of fish in their early life stages on currents could connect distinct geographical areas.

## 1.1 Sampling

An exhaustive biological sampling exercise was conducted over the whole study area, as well as in neighbouring regions of the Atlantic Ocean and the Mediterranean. Sardine and European hake were sampled at the 17 locations previously indicated in Figure 3 (Introduction), which were geographically segregated considering that fishing trips in the study area only last one day. Fifty sardines between 14 and 17 cm in length (i.e. of the same age class and all

mature) were collected at each location between May and June, while 40 mature European hake individuals, 25–30 cm long (second year class), were collected over eight weeks from October to November. In the case of blackspot seabream, 30 individuals between 30–35 cm in length were collected at the locations indicated in Figure 4 between May and June.

**FIGURE 4.** Sampling ports for blackspot seabream complementing the sampling performed for European hake and sardine



Source: Base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

The sampling protocol applied to each fish is explained in detail in Supplementary material on the methodology Part I (FAO, 2024). A sampling training workshop was hosted at the IEO from 23 to 25 April 2018, in order to ensure standardized sampling across all locations. Each sample passed

through the analyses mentioned above (genetic markers, meristics, body morphometrics, otolith shape, parasites, and otolith edge and core microchemistry).

## 1.2 Hydrodynamic modelling

A different approach was used for the simulation exercises of the three species due to existence of two different spawning locations for both European hake and sardine in the Alboran Sea and the Gulf of Cádiz, respectively, while blackspot seabream’s spawning grounds are concentrated in the Strait of Gibraltar.

For European hake and sardine, the methodology employed combined numerical model simulations and a Lagrangian flow network (LFN) analysis procedure applied to the numerical results (Dubois *et al.*, 2016; Monroy *et al.*, 2017). The LFN analysis relies on calculations of early life stage (ELS) drift trajectories in order to evaluate the con-

nectivity between selected habitat patches. Due to the unavailability of fine spatial distributions of spawning grounds to be potentially used as starting points for particles (eggs and larvae) released by each species, these habitat patches correspond to putative spawning and nursery grounds of the target species and are referred to as “boxes” in LFN terminology. In line with the available information on the spawning areas of the species studied, a total of ten boxes were considered for sardine and European hake, distributed across the Alboran Sea (eight boxes) and the Gulf of Cádiz (two boxes) (Figure 5).

**FIGURE 5.** Selected boxes for the Lagrangian flow network analysis



Source: Base map redrawn from Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

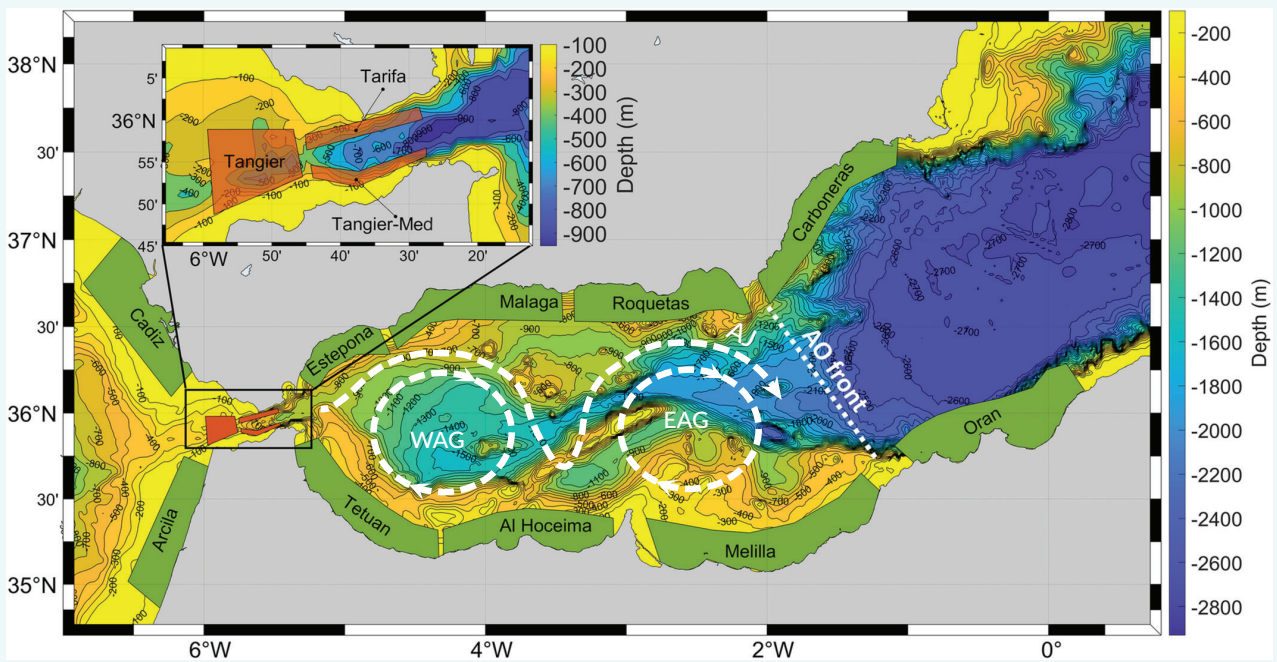


The ocean model used corresponded to a regional application of the Massachusetts Institute of Technology general circulation model (Marshall *et al.*, 1997). The model configuration and its physical parameterizations were the same as those described in Sánchez-Garrido *et al.* (2013), who developed an operational application for the Strait of Gibraltar and the Alboran Sea. Specific details of the parameters used for the dispersion experiments for each species – in terms of the numbers of particles dispersed, timing, pelagic larval duration (PLD) assumed, and depth of dispersion – are available in Supplementary material on the methodology Parts II and III (FAO, 2024).

Blackspot seabream ELS dispersal was simulated by applying a Lagrangian particle tracking algorithm to the outputs

of a hydrodynamic model for the Strait of Gibraltar/Alboran Sea region, run from December 2004 to April 2005. Three release areas and ten landing areas were used to assess the dynamic connectivity of this species from its spawning areas to its potential recruiting zones (Figure 6). Five different release depths were considered (1 m, 12 m, 25 m, 52 m and 81 m), and a total of 480 runs were executed, with the aim of evaluating the temporal and spatial variability of the connectivity patterns, under tidal and subinertial forcings. Further details on the procedure applied to the modelling of blackspot seabream ELS dispersion are available in Nadal *et al.* (2022).

**FIGURE 6.** Release (spawning) areas and landing (recruitment) areas used for hydrodynamic modelling



Source: Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

Note: Spawning areas are shown in orange, and recruitment areas are shown in green; the dashed-line circles represent the Western Alboran Gyre (WAG) and the Eastern Alboran Gyre (EAG); the curved dashed line with an arrow represents the Atlantic Jet (AJ); the straight dashed line represents the Almeria-Oran (AO) front.

### 1.3 Genetics

For European hake, all individuals were genotyped at six microsatellite loci. Amplified fragments were analysed on an ABI 3500 Genetic Analyzer with GeneScan 600 LIZ internal size standard (Applied Biosystems). In addition, a custom genotyping by sequencing assay was developed by Thermo Fisher Scientific from a set of 917 hake single nucleotide polymorphisms (SNPs) selected by Milano *et al.* (2014) and Leone *et al.* (2019). From those, a total of 574 SNPs were designed, contained within 554 amplicons/target areas. Next, targeted sequencing was performed using the custom-developed genotyping by sequencing hake panel. After SNP genotyping (see Supplementary material on the methodology Part V; FAO, 2024), linkage disequilibrium, Hardy-Weinberg equilibrium and genetic drivers were tested with tests implemented in GENEPOP 4.0 (Rousset, 2008) and GENETIX 4.0.5 (Belkhir *et al.*, 2004). Outlier loci, with the potential to be selected, were identified using the independent approaches in Bayescan 2.1 (Foll and Gaggiotti, 2008) and R package pcadapt (Luu, Bazin and Blum, 2017) with the whole dataset (more details are available in Supplementary material on the methodology Part V; FAO, 2024). The method implemented in the R package pcadapt was used to detect local adaptation. This method relies on principal components analysis (PCA) to detect population structure, and it allows for the identification of genetic markers putatively involved in biological adaptation by considering their relationship with the population structure (more details are available in Supplementary material on the methodology Part V; FAO, 2024). The population genetic structure analyses were performed on microsatellite datasets and, on the whole, neutral and outlier datasets for SNPs. Gene–environment association analysis was also performed. For each macroarea, the centroid and its geographical coordinates (latitude and longitude) were identified and were used for individual coordinates.

For sardine, 20 microsatellite loci were selected from the literature (see Supplementary material on the methodology Part VI; FAO, 2024). Microsatellite loci were grouped in multiplex polymerase chain reactions (mPCRs) using four fluorophore dyes. Microsatellite markers with nonspecific amplification or without amplification were excluded. The

final mPCR design allowed for the characterization of the genotype of 859 sardines collected across 17 sampling ports (in seven GSAs) at 9 microsatellite loci – for further details, see Supplementary material on the methodology Part VII (FAO, 2024) and Pérez-Sánchez, 2021. Microsatellite genetic data for 805 sardines at 9 loci were examined with GenAlEx software 6.5 (Peakall and Smouse, 2012) to obtain allele frequency and estimate genetic diversity through observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity. The inbreeding coefficient (Weir and Cockerham, 1984) and compliance with the Hardy-Weinberg equilibrium were analysed using GENEPOP 4.7 (Rousset, 2008; Raymond and Rousset, 1995). Partitioning of genetic diversity at microsatellite loci within subpopulations relative to the total population was analysed using Wright's fixation index statistic ( $F_{ST}$ ) obtained with GenAlEx 6.5 software (Peakall and Smouse, 2012), which was also used to assess the number of migrants. Clustering of genetic diversity was investigated using three different approaches: principal coordinate analysis, discriminant analysis of principal components, and Bayesian algorithms to proportionally assign individuals to inferred population clusters (see Supplementary material on the methodology Part VII; FAO, 2024). Principal coordinate analysis was performed with GenAlEx software 6.5 (Peakall and Smouse, 2012), whereas the R package adegenet was used for discriminant analysis of principal components (Jombart, 2008).

For blackspot seabream, 29 microsatellite markers were used from a total of 320 individuals in order to estimate both genetic variability and differentiation. In total, six different multilocus reactions were designed based on loci annealing temperature and expected amplicon size, and the mPCR tested. Polymerase chain reactions were performed with the Platinum Multiplex PCR Master Mix from Thermo Fisher Scientific, and oligonucleotide sequences were provided by reference bibliography and labelling according to the set-up of multiplex amplification. The same statistical analyses as those described for sardine were performed – for further details, see Supplementary material on the methodology Part VIII (FAO, 2024) and Spiga, 2020.

### 1.4 Otolith microchemistry

For the three species, laser ablation and inductively coupled plasma mass spectrometry were used to analyse the elemental composition of two parts of the otolith: the core

(representing the early life stages) and the edge (representing the recent living period prior to capture). The core region was also examined to assess the plausible differ-



ences between individuals' spawning/nursery areas. Furthermore, a clear differentiation of adult fish otolith core elements between regions could suggest that mixing of fish from different juvenile source areas was minimal, supporting the existence of stock structure. The edges were analysed to assess whether the elemental chemistry of recently deposited otolith material varied between regions.

For the edge, the data of three edge spots from each individual otolith were averaged to provide the ratios between edge elements and calcium used for all statistical analyses. For the core and edge otolith regions, the same series of analyses were conducted. First, a univariate analysis of variance (ANOVA) was carried out to assess whether in-

## 1.5 Otolith shape analyses

For European hake and sardine, all the otoliths were first photographed in high contrast. Images were produced using reflected light, making the otoliths appear as bright objects on a black background for shape analysis. The shapes of the otoliths were analysed by OTOLab software using the OTOTHRESH tool for image segmentation (Nava *et al.*, 2018). The physical characteristic descriptors measured were area, major axis, minor axis, eccentricity, perimeter, circularity, compactness, skewness, kurtosis, and Fourier descriptors.

Fourier descriptors describe the outline of the otolith on the basis of harmonics. Each harmonic is characterized by four coefficients, resulting from the projection of each point of the outline on x- and y-axes. A higher number of harmonics gives greater accuracy of the outline description (Kuhl and Giardina, 1982). The OTOTHRESH tool was used to generate 20 harmonics for each otolith, resulting in 80 coefficients per otolith. Each otolith was normalized by

## 1.6 Body morphometrics

All photos received for European hake (611) and sardine (866) were digitalized using 14 landmarks for sardine and 19 for hake, along with three treatments using tpsDig v. 2.57 (Rohlf, 2015). A single operator developed the analyses in order to remove any errors stemming from manipulation by multiple operators. The digitalized dataset was iteratively checked once more for obviously misplaced or swapped points, with the aid of the procedure for the identification of outliers. Three digitalized datasets were averaged per specimen and subjected to a generalized Procrustes analysis with sliding of semi-landmarks, using ten iterations and minimizing Procrustes distances as criteria (Bookstein, 1997).

dividual element to calcium ratios differed significantly between regions. Post-hoc Fisher's least significant difference tests were used to determine the nature of significant differences between areas. A forward stepwise linear discriminant analysis was built to classify each individual to one of the sites from which they were collected. Classification accuracies for each species and environment were evaluated through the percentage of correctly classified individuals using jackknife classification. As jackknife classifications are particularly sensitive to low sample size, GSAs as groups (>30 individuals per group) were considered for all the analyses (see Supplementary material on the methodology Parts IX, X and XI; FAO, 2024).

the programme for size and orientation, which caused the degeneration of the first three Fourier descriptors derived from the first harmonic. This meant that each individual was represented by 76 coefficients for the shape analysis. The number of harmonics used to reconstruct each otolith, such that its shape is reconstructed at 99.99 percent, is determined by the calculation of the cumulated Fourier power. After the calculation, only 10 harmonics were kept for European hake and 13 for sardine.

The analytical design was built to detect differences in the contour shape and physical characteristics of sardine and European hake otoliths collected from the 15 sampling areas for hake and 17 areas for sardine, through a forward stepwise linear discriminant analysis. A classification accuracy for each individual was evaluated through the percentage of correctly classified individuals using a jackknife approach (see Supplementary material on the methodology Parts XII and XIII; FAO, 2024).

Dorsoventral arching was removed by orthogonal projection (Burnaby, 1966), which was also used to check the lack of sexual dimorphism influence. To account for allometric variation in shape, a multivariate regression was performed, and residuals were used in subsequent analyses. Permutational multivariate analysis of variables (MANOVA) was applied to assess the variation between areas and sites, along with factorial discriminant analyses (see Supplementary material on the methodology Part XIV; FAO, 2024).

## 1.7 Meristics

Two meristic characteristics – namely the number of vertebrae and the number of gill rakers – were used. A total of 736 individual sardines and 551 individual European hakes were processed in order for their vertebrae to be counted, and a total of 862 individual sardines and 592 individual hakes were processed for their gill rakers to be counted. A workshop for readers was organized in order to standardize the reading of vertebrae and gills before processing the samples. To minimize the error rate, three readings per sample were carried out.

## 1.8 Parasites

Parasites were only detected in European hake, while no infection was detected in sardine. All hakes were gutted, and the viscera of each individual separated. Muscle flesh was cut into two to four portions, separating the hypaxial (ventral) and epaxial (dorsal) regions, following the horizontal septum. It was then observed by the naked eye, with the use of a transilluminator camera. With an optical microscope, the parasite larvae were counted and identified to the genus level, as *Anisakis* spp. types I and II, according to the morphological keys available. Larvae were washed in saline solution and stored in Eppendorf tubes at  $-30^{\circ}\text{C}$ , or stored directly in alcohol, until they could be genetically identified to the species level.

The genetic identification was applied to a total of 304 *Anisakis* spp. larvae recovered in the fish specimens from the selected fishing grounds, which were identified to the species level by a multimarker genotyping approach. The diagnostic allozyme loci (Adk-2, Pep C-1 and Pep C-2) were analysed following the previously established procedures (Mattiucci, Paoletti and Webb, 2009; Mattiucci *et al.*, 2014) on 61 *Anisakis* spp. larvae. In addition, the larvae identified by allozymes were sequenced at the mitochondrial (mtDNA *cox2*, 629 bp) and the nuclear (elongation factor EF1  $\alpha$ -1 of nDNA, 409 bp) gene loci (Mattiucci *et al.*, 2014, 2016). Meanwhile the *Anisakis* spp. larvae previously stored in alcohol (N = 243) were sequenced at the

## 1.9 Fisheries

Analyses based on fisheries data were performed on European hake only through the research for two MSc theses, in 2019 and 2021 (El Yaagoubi, 2019; Galindo-Ponce, 2021). For sardine, analyses first presented in Jghab *et al.* (2019), combining catch per unit effort (CPUE) in the Alboran Sea

A global analysis of variance was used to test differences among the different GSAs, with a pairwise comparison of Tukey's test applied to cross-check. A PCA was also carried out over the mean values grouped at the port level to test potential differentiation between ports within each GSA (see Supplementary material on the methodology Part XV; FAO, 2024).

mtDNA *cox2*, and at the nuclear elongation factor EF1  $\alpha$ -1 of nDNA (see Supplementary material on the methodology Part XII; FAO, 2024). With the information obtained, a Bayesian inference (BI) tree was elaborated, based on mtDNA *cox2* gene sequences of *A. pegreffii* larvae from hake sampled in the fishing grounds, with respect to the other previously sequenced *Anisakis* spp.

To assess the epidemiological data, the levels of infection by *Anisakis* spp. larvae were calculated for the hake in each sampling area. The epidemiological parameters considered were prevalence (P, expressed as a percentage) with confidence limits (Clopper-Pearson), abundance, mean intensity, and range of larvae present (min–max). The statistical significance of the differences observed in the prevalence and abundance values for infection by *Anisakis* spp. larvae was assessed by the Fisher's exact test and bootstrap t-test.

Finally, a haplotype network based on mtDNA *cox2* fragment for *A. pegreffii* was performed using the statistical parsimony procedure (95 percent parsimony connection limit), implemented in TCS version 3.5.1.2 (Clement, Posada and Crandall, 2000). The number of mutations were displayed in circles, and the network was plotted with tcsBU (dos Santos *et al.*, 2016). Pairwise genetic differentiation of *A. pegreffii* was estimated using  $F_{ST}$  (Mattiucci, 2019).

with environmental information, are also succinctly presented in this report.

Fisheries data on European hake were provided by IEO (Spain) for GSA 1 and by INRH (Morocco) for GSA 3. The spatio-temporal variation of fishery patterns, demographic

indices and length distributions of this species were analysed by applying several methodologies. Generalized additive models and dynamic factor analysis were applied to monthly time series of CPUE for 13 ports in the study area to describe the seasonal and interannual variability. In addition, length distribution of this species was compared between GSAs and years. Finally, spatial distribution metrics (i.e. centres of gravity) in longitude, latitude and depth were calculated for both GSAs to compare the spatial distribution of European hake and then examine its temporal variability and potential density-dependent influences (El Yaagoubi, 2019).

A calibrated virtual population analysis stock assessment model, namely an extended survivors analysis, was used to

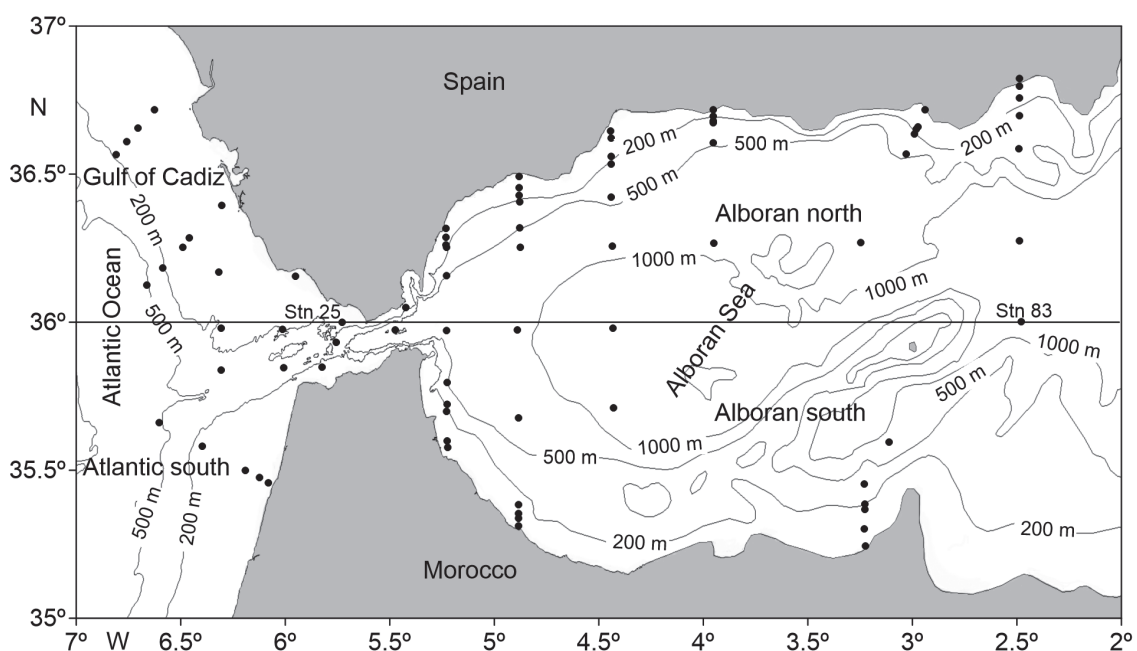
evaluate and compare the consequences of two alternative stock structure scenarios for the assessment of the resource status: i) joint shared stock, by combining information from GSAs 1 and 3; and ii) two independent stocks, by running GSAs 1 and 3 separately. Finally, the project also assessed the sensitivity of the extended survivors analysis model to a set of recruitment strength scenarios that may arise from the impacts of connectivity or from self-recruitment processes (Galindo-Ponce, 2021). The model assumptions (e.g. natural mortality rate, selectivity curves) were identical across scenarios and areas, so no confounding effects could affect this sensitivity analysis.

## 1.10 Ichthyoplankton survey

The Transboran 2020 survey was carried out from 20 February to 14 March 2020, on board the R/V *Emma Bardan*, provided by Spain's Ministry of Agriculture, Fisheries and Food. The objective was to contribute to the delimitation of the spatial population structure of the three species assessed, as well as to the detection of potential connection paths between population units through the location of their early life stages. While the survey was designed to mainly cover the sardine spawning season, opportunistic

sampling for European hake and blackspot seabream was conducted associated with the whole ichthyoplankton community. A total of 81 stations were sampled, arranged in 14 transects perpendicular to the coastline (Figure 7). Each transect included five stations located at depths of ~30 m, ~70 m, ~100 m, ~200 m and ~500 m (further details are available in Additional results Part I; FAO, 2024).

**FIGURE 7.** Bathymetry map of the study area, with sampling stations



Ichthyoplankton samples were collected using a bongo net with a mouth diameter of 60 cm and a mesh size of 500 µm. Each mouth of the bongo net was equipped with a Hydro-Bios flow meter to measure the volume of water filtered. One of the bongo samples was preserved in 97 percent ethanol for further larval growth and genetic studies. The other sample, used for the ichthyoplankton community studies, was sorted on board to separate sardine and European anchovy (*Engraulis encrasicolus*) larvae. In the laboratory, fish eggs and larvae were sorted from the formalin-preserved samples. All fish larvae were identified

## 1.11 Integrative analyses

Two different approaches were used to interactively combine and quantitatively analyse the results of all the techniques.

The first, stock differentiation index (SDI), is a semi-quantitative method (e.g. Welch *et al.*, 2009, 2015; Izzo *et al.*, 2017). It relies on the construction of a matrix of differentiation showing differences among the qualitative pairwise comparisons of all ports based on the results of each technique used. Thus, for each technique and each possible pair of ports, it was qualitatively determined whether the reported findings suggested differences between ports. When a difference between two ports was identified, that pair was assigned a difference value (DV) of 1. Conversely, when two ports were similar, a DV of 0 was assigned. Statistical tests (or a connectivity matrix in the case of oceanographic modelling) were used to assign a DV for each pair and each technique. The final SDI was then calculated combining the matrices developed for all the techniques used for each species following the equation  $SDI = \sum DV / count\ DV$ , where the sum of the DVs for one pair of areas is divided by the total number of data sources (i.e. techniques) assessed for that pair (count DV). Once the final mean matrix was calculated, an average per GSA and for each of the North and South Atlantic was calculated. The final SDI value then provided a measure of the relative difference between areas (i.e. SDI = 1 shows maximum dif-

ference, and SDI = 0 shows no difference). If no difference between areas was detected, the null hypothesis of a single stock would be retained, even without direct evidence. The same criteria were adopted as in Izzo *et al.* (2017), with an SDI > 0.66 considered to provide strong evidence for separation of subgroups into different stocks,  $0.33 \leq SDI \leq 0.66$  showing moderate evidence for separation, and SDI < 0.33 showing weak evidence.

Second, a PCA was also developed, integrating information from all the techniques, in which appropriate discriminant analyses were applied depending on the characteristics of the data in each case. For each technique, first and second axes of the discriminant technique were used, ensuring that they explained more than 70 percent of the variance of each dataset. In the case of otolith microchemistry, the core and the edge of the otolith were considered as independent techniques, as they provided information about different fish life stages and therefore different drivers affecting the species population structure. The same applies to the “neutral” and “outlier” signal of the SNP genetic analyses performed for European hake. The contribution and quality of the representation of each technique over the discriminant axis were assessed, as well as the significance and relevance of the separation between GSAs.







# CHAPTER

## 2

## MAIN RESULTS BY SPECIES AND TECHNIQUE





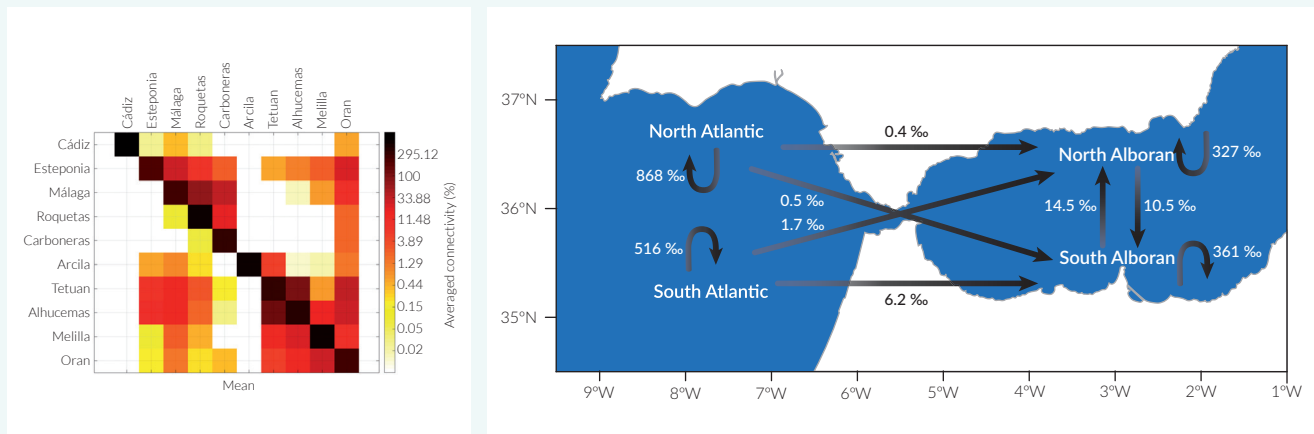
## 2.1 European hake

### 2.1.1 Hydrodynamic modelling

The LFN analysis carried out to identify the most likely connectivity links between selected habitat patches of European hake in the Alboran Sea provides robust patterns for this species, mainly associated with strong self-recruitment and generally reduced connectivity between the northern and southern Alboran Sea. The numerical simulations indicate that self-recruitment is the most likely link of the LFN, with average values of 327 permille and 361 permille for the northern and southern Alboran Sea, respectively (Figure 8). It was also found that the probability of ELS hake crossing the Alboran Sea from north to south and vice versa is only 10–15 permille in the time period representative

of its PLD, which is about 40 days. In terms of spawning biomass – and assuming from a potential single population that 65 percent would correspond to the northern Alboran Sea (GSA 1) and 35 percent to the southern Alboran Sea (GSA 3) – it was estimated that meridional transport is minimal, with around 0.6 percent and 0.51 percent of the total spawning biomass crossing the Alboran Sea, from north to south and south to north, respectively (Figure 8). This meridional transport of hake spawning material appears to be mediated by the two anticyclonic gyres typically present in the Alboran Sea.

**Figure 8. Connectivity matrix (left) and general patterns of European hake transport, including self-recruitment (right)**



Source: Right panel base map redrawn from Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

The connectivity probability between the Atlantic boxes of the LFN (off Cádiz and Arcila) and the Alboran Sea boxes was estimated at 4.4 permille overall. This link was mainly accounted for by the box off Arcila (7.9 permille) rather than by the box off Cádiz (0.9 permille) (Figure 7). The analyses also identified "forbidden" network pathways: the Atlantic boxes are isolated from each other, and ELS never reach the Atlantic from the Alboran Sea. These two constraints are explained by the permanent presence of the swift Atlantic current through the Strait of Gibraltar (the so-called Atlantic Jet). Sensitivity runs performed on the results indicate that either greater drifting depths or shorter PLD increase the chances of ELS crossing the Alboran Sea, whereas these same conditions make the in-

put of ELS from the Atlantic less likely. Greater depths also substantially increase the probability of self-recruitment. More detailed information is available in Supplementary material on the methodology Part III (FAO, 2024).

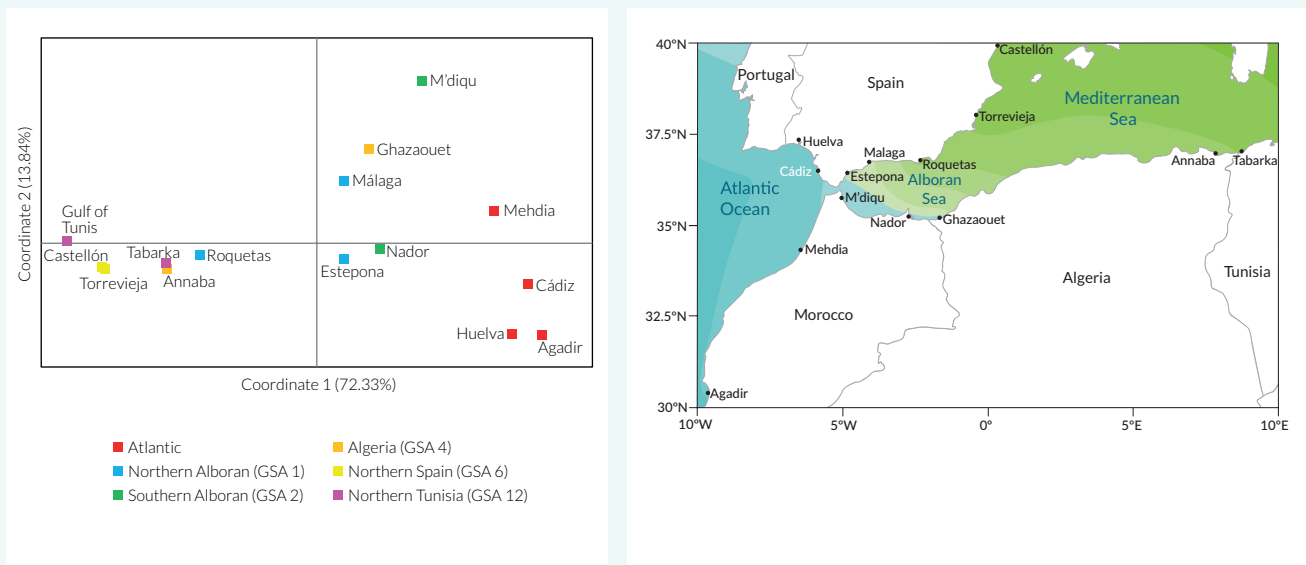
While the analysis indicates that the northern and southern habitat locations of European hake in the Alboran Sea are not completely hydrodynamically isolated from each other, it is fair to assume that the flux of spawning material between the two sides (north and south) of the basin is not likely to be sufficient to establish an actual biological link and a meaningful demographic impact between hake subpopulations.

## 2.1.2 Genetics

Genetics analyses, based on SNP markers, provide two types of information: one is based on the neutral markers that give information on long-term genetic drift, and the other is based on outliers that provide information on potential local adaptation and/or the influence of environmental drivers. Neutral signals identify three main regions:

i) the Atlantic component; ii) a transition area in the Alboran Sea excluding Roquetas; and iii) a final component of exclusively Mediterranean character (including Roquetas) (Figure 9). A general west–east genetic drift was identified, with minimal and heterogeneous differentiation between the northern and southern Alboran Sea (Figure 9).

**Figure 9.** Principal coordinate analysis for the dataset of European hake neutral genetic markers (left) and interpolation of the values of the ancestry coefficient over the study area (right)



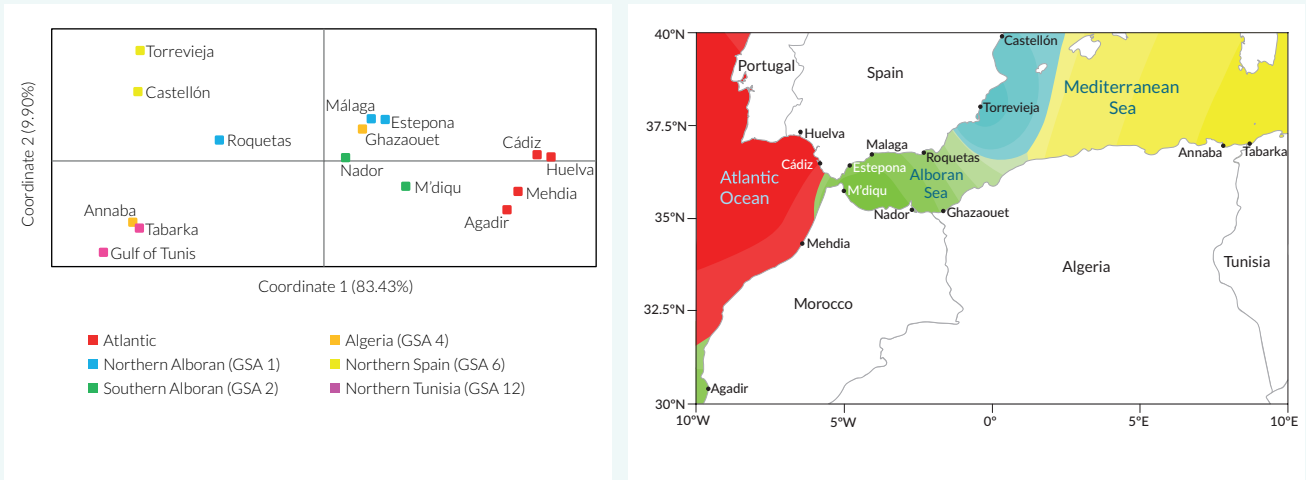
Source: Right panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Note: In the left panel, the ports of each area are identified with the colour assigned to the six management areas of the study, as shown in the legend; the percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

The outliers signal revealed a clearer subregional structure, while no differentiation was observed in the Alboran Sea. It was able to differentiate between the Atlantic, the

Alboran region, northern Spain, and the eastern Algeria and Tunisia region. However, Roquetas, as well as M'diqu, also reveal a mixed signal between regions (Figure 10).

**Figure 10.** Principal coordinate analysis for the dataset of European hake outlier genetic markers (left) and interpolation of the values of the ancestry coefficient over the study area (right)



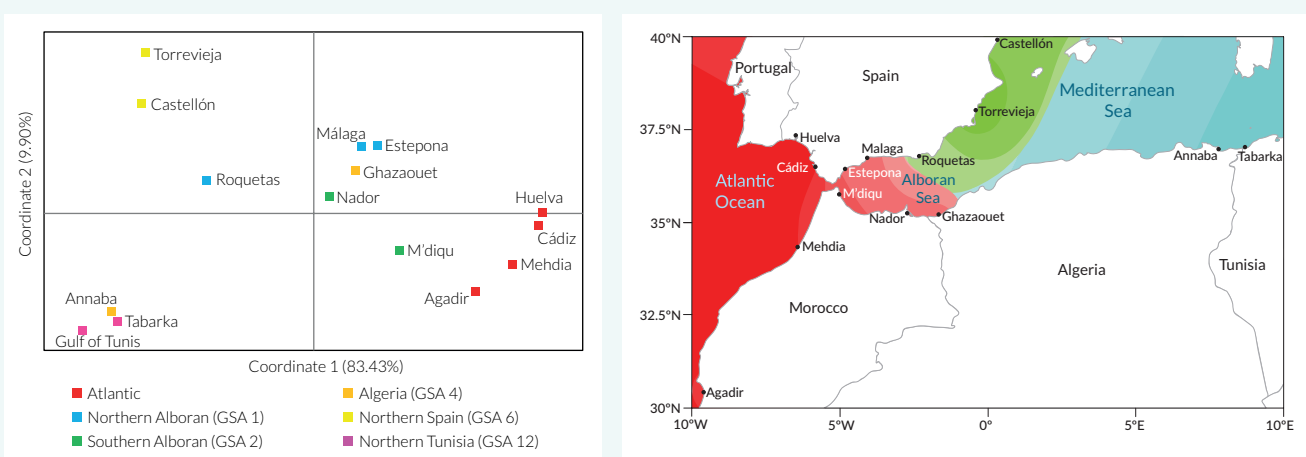
Source: Right panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Note: In the left panel, the ports of each area are identified with the colour assigned to the six management areas of the study, as shown in the legend; the percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

The whole SNP signal (i.e. the combination of neutral and outlier markers) reveals spatial segregation mainly driven by the outliers pattern. Although the results suggest a west–east genetic drift pattern, there is no differentiation in the Alboran Sea, while subregional differentiation between the northern Spain, Alboran and eastern locations (i.e. eastern Algeria and Tunisia) is detected (Figure 11).

The results also identify Roquetas as a transitional location between northern Spain and the Alboran Sea and M’diq as a transitional connection between the Atlantic and the Alboran Sea (i.e. Mediterranean Sea). Microsatellites markers were also used. Their results, along with more detailed information, can be found in Supplementary material on the methodology Part V (FAO, 2024).

**Figure 11.** Principal coordinate analysis for the whole European hake genetic dataset (left) and interpolation of the values of the ancestry coefficient over the study area (right)



Source: Right panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Note: In the left panel, the ports of each area are identified with the colour assigned to the six management areas of the study, as shown in the legend; the percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

### 2.1.3 Otolith microchemistry

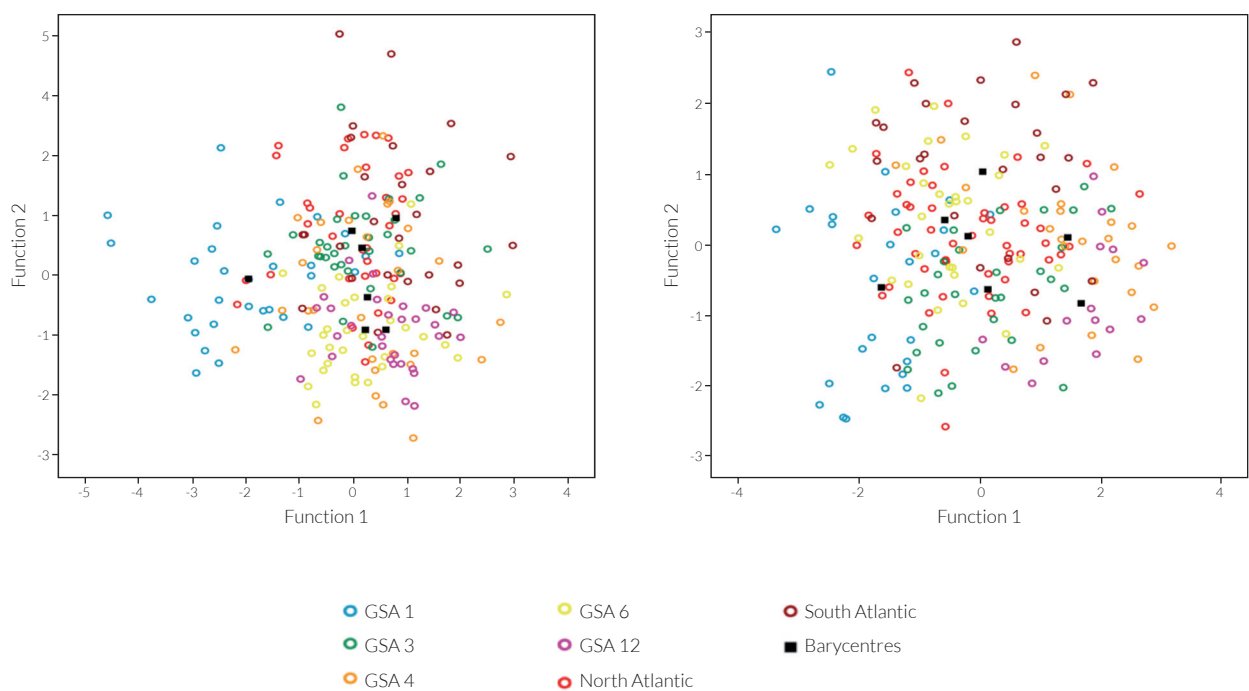
Otolith microchemical analyses provide information at two ontogenetic scales: i) early life stages from the core of the otolith; and ii) the most recent period of life from the edge of the otolith. Among other analyses (see Supplementary material on the methodology Part X; FAO, 2024), a forward stepwise linear discriminant model analysis was built for otolith core and edge data to classify European hake from the Atlantic and the Mediterranean. The two sampled otolith regions – the northern Alboran Sea (GSA 1) and southern Alboran Sea (GSA 3) – show differences, while clustering across neighbouring areas slightly differs for edge and core analyses.

For the core, classification accuracy was highest for GSA 1 (71.3 percent), followed by GSA 3 (60.6 percent), and was

relatively low for the other locations. The plot indicates some overlap, especially among samples from GSAs 4, 6 and 12 with the North Atlantic and South Atlantic, and among samples from GSA 3 with the North Atlantic and South Atlantic. The samples from GSA 1 were clearly separated from those from the other locations (Figure 12).

For the edge, on the other hand, classification accuracy was highest for GSA 12 samples, at 80 percent, followed by GSA 3, at 63 percent. The canonical variate plot indicated some overlap among individuals sampled from the North Atlantic, South Atlantic and GSAs 1 and 6, as well as an overlap between individuals from GSA 4 and those from GSA 12 (Figure 12).

**Figure 12.** Scatter plot representations of the discriminant analyses of the multi-element chemistry of otolith cores (left) and otolith edges (right) collected from European hake, grouped by management area



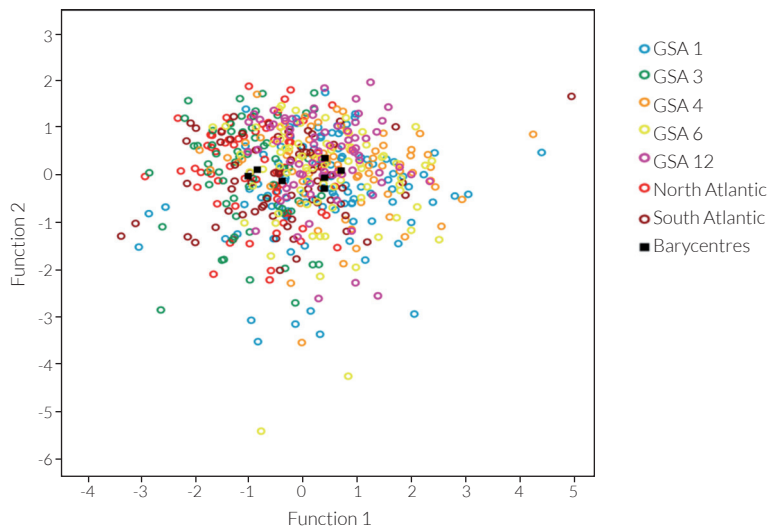
### 2.1.4 Otolith shape

A forward stepwise canonical discriminant analysis was performed using the normalized elliptical Fourier descriptors and physical characteristics descriptors as explanatory variables. For the Alboran Sea, the results show north-south differences, but these differences are not shown in any other subareas. The canonical variates plot indicated some overlap, especially among samples from GSAs 1, 4, 6 and 12. The samples from GSA 3 were separated from the other GSAs but showed overlap with the North and South Atlantic (Figure 13). The applied jackknife classification procedure, involving all areas, performed poorly in assigning individuals to their areas of origin, leading to significant errors in classification, especially the misclassification of

samples from GSA 3 to the North and South Atlantic. Furthermore, there was misclassification among samples from GSAs 1, 4, 6 and 12 (see Supplementary material on the methodology Part XII; FAO, 2024).

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

**Figure 13.** Scatter plot representation of the discriminant analyses of the contour shape of European hake otoliths, grouped by management area

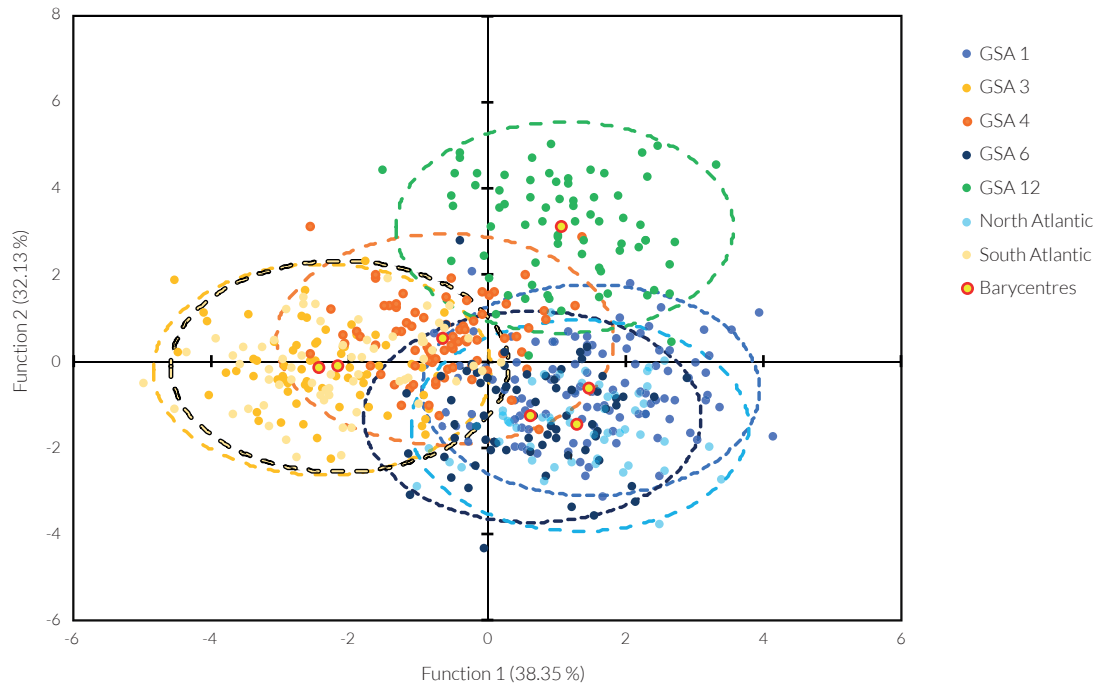


### 2.1.5 Body morphometrics

Fisher discriminant analysis was used to analyse body information after removing the effect of body size. The analysis evidenced differences between the northern and southern Alboran Sea. The northern Alboran Sea (GSA 1) shows strong similarities with northern Spain (GSA 6) and

the North Atlantic (Figure 14). The same occurs with the southern Alboran Sea (GSA 3) and Algeria (GSA 4), while the Tunisian coast (GSA 12) appears well differentiated (see Supplementary material on the methodology Part XIV; FAO, 2024).

**Figure 14.** Scatter plot representation of the Fisher discriminant analysis performed with body information from European hake, grouped by management area



Notes: The Fisher discriminant analysis used 19 landmarks, with each landmark position (x, y coordinates) calculated as the average of three readings for each of 602 individuals to remove bias due to digitalization error.

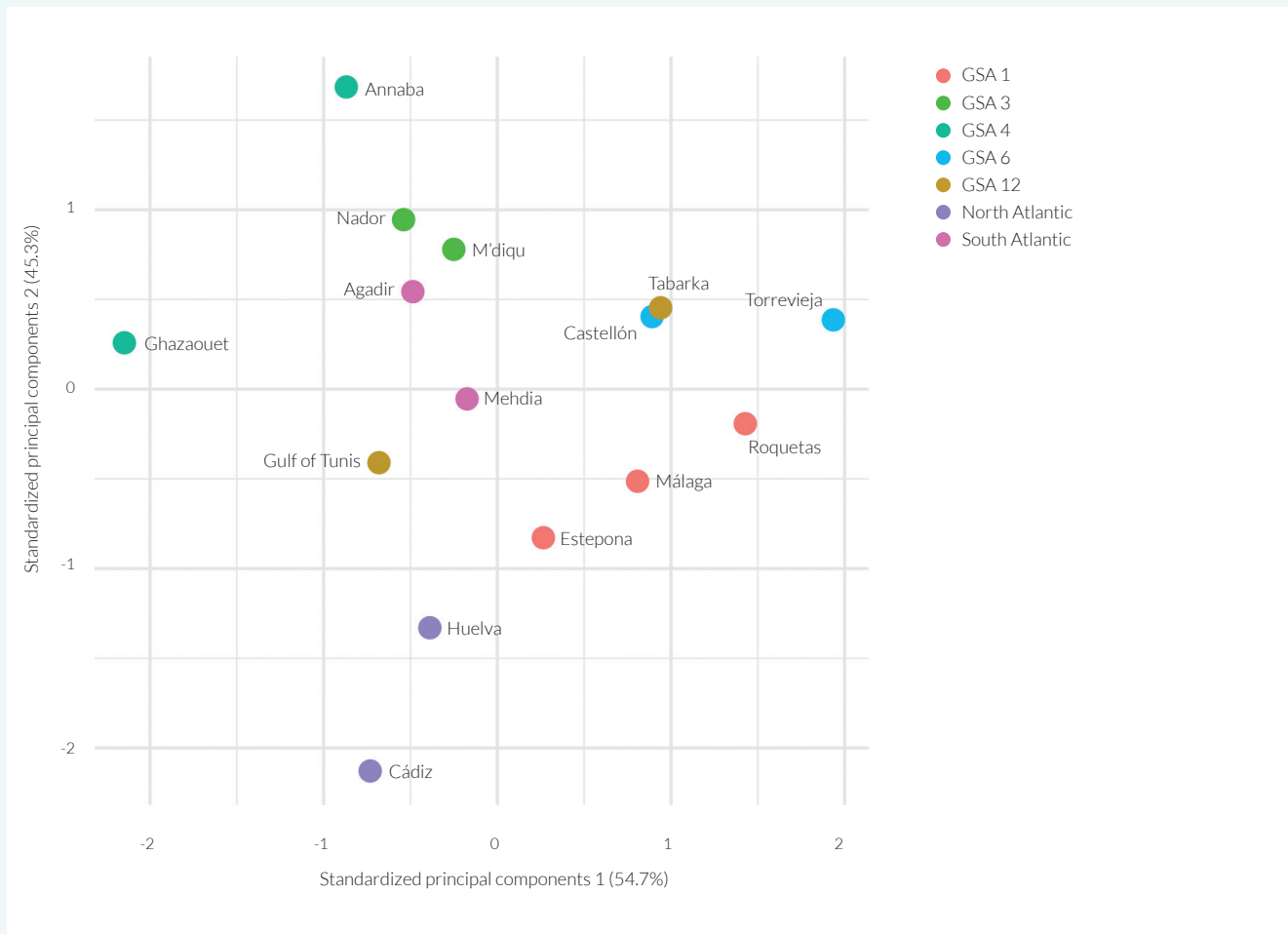
The percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

### 2.1.6 Meristics

Two meristic characters were used: the number of vertebrae and the number of gill rakers. A total of 551 vertebrae and 592 gills from 593 European hake individuals were analysed. While the number of vertebrae did not show clear differences across areas and ports, the number of gill rakers did (for further details of the analyses performed on each metric, see Supplementary material on the methodology Part XV; FAO, 2024). For a better integration of the

information provided by the two metrics, a PCA was performed. According to the results of the PCA (Figure 15), the northern Alboran Sea (GSA 1) and the southern Alboran Sea (GSA 3) show differences, while GSAs 1, 6 and 12 display some similarities. The North Atlantic area appears separated from the rest, while the South Atlantic looks similar to the southern Alboran Sea.

**Figure 15.** Scatter plot representation of the principal components analysis performed with European hake vertebrae and gill rakers, grouped by management area



Note: Percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

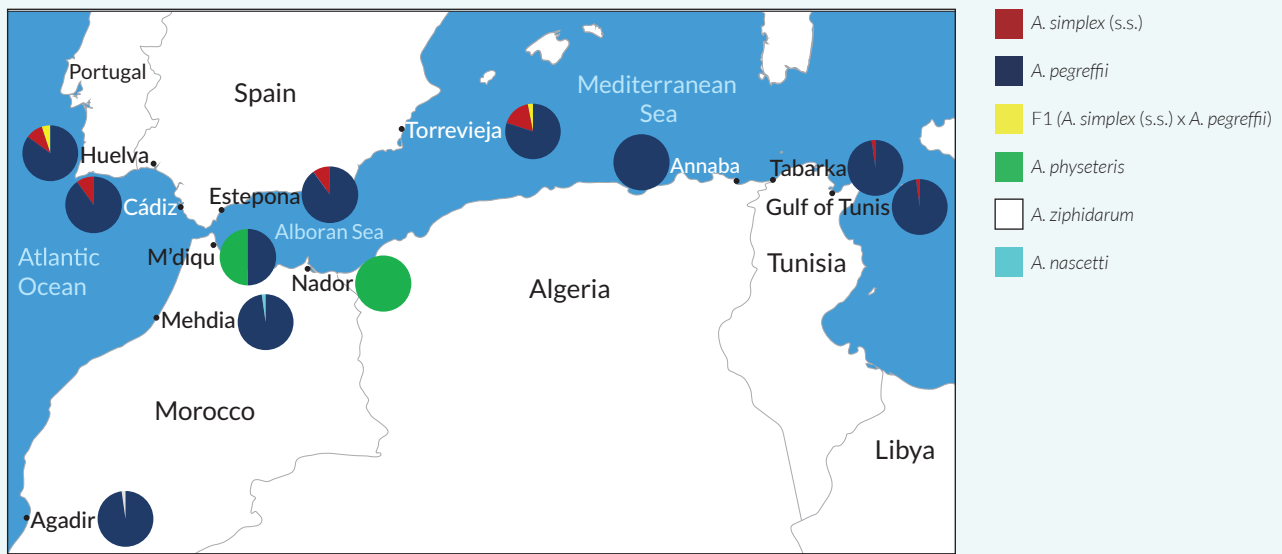
### 2.1.7 Parasites

Analysis of the parasite communities shows clear spatial segregation (Figure 16). Of the five species of *Anisakis* identified, the two sibling species, *A. pegreffii* and *A. simplex* (s.s.), were found to be the dominant species in the fish samples, while lower levels of *A. physeteris*, *A. ziphidarum* and *A. nascettii* were identified. *Anisakis pegreffii* and *A. simplex* (s.s.) showed statistically significant differences ( $p = 0.009$ ) in their relative proportions in fish sampled along the Spanish Atlantic coast with respect to the Atlantic and Mediterranean coasts of Morocco. In the latter sampling locality,

a higher percentage of *A. physeteris* was identified. Furthermore, *A. ziphidarum* and *A. nascettii* were identified in only European hake sampled along the Atlantic Moroccan coast. No high statistical significance was found between the relative proportions of the two sibling species infecting European hake sampled along the Spanish Mediterranean coast included in the project (i.e. off Estepona and Torrevieja) compared to those observed in fish sampled along the Spanish Atlantic coast (i.e. off Huelva and Cádiz).



**Figure 16.** Relative proportions of different species of *Anisakis* spp. genetically identified from European hake captured around ports along the Atlantic coast and Mediterranean coast



Source: Base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

According to the genetic analyses using mitochondrial markers (mtDNA *cox2* sequences analysis) and the haplotype network created, clear differences are found when comparing the following four areas: i) the North Atlantic; ii) the South Atlantic; iii) the northern Alboran Sea and north-

ern Spain (GSAs 1 and 6); and iv) the southern Alboran Sea and eastern regions (GSAs 3, 4 and 12) (Figure 17). These results along with more detailed information are presented in Mattiucci (2019).

**Figure 17.** Main clusters found based on genetic information gathered from the European hake parasite analyses (right) and fixation index comparison values between each pair of these groups (left)



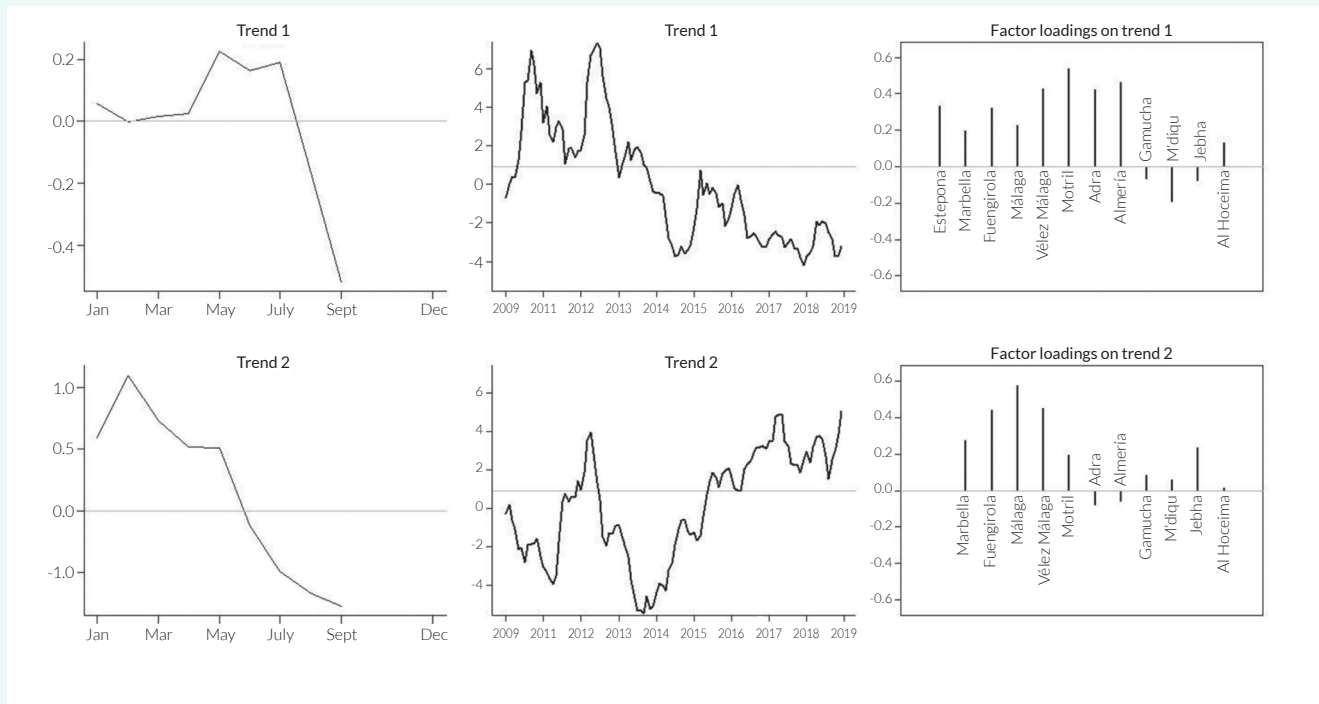
Source: Right panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

### 2.1.8 Fisheries

The application of dynamic factor analyses over monthly CPUE time series of the Spanish (GSA 1) and Moroccan (GSA 3) ports revealed differences between the northern and southern Alboran Sea (Figure 18). The first trend – depicting a long-term decrease and maximum values during the summer – was associated with northern ports,

while the second trend – with a more recent increase and higher values during the late winter and early spring – was associated with eastern ports in both the northern and southern Alboran Sea. Additional analyses and details can be found in El Yaagoubi (2019).

**Figure 18.** Results of the dynamic factor analysis of European hake landings per unit effort time series, with seasonal long-term trends and factor loadings



Source: El Yaagoubi, H. 2019. Spatiotemporal variation of fishery patterns, demographic indices and spatial distribution of European hake, *Merluccius merluccius*, in the GSA 01 and GSA 03. Alicante, Spain, University of Alicante. MSc Thesis.

Note: All y-axes are unitless.

Additional analyses were performed to compare the outcomes of stock assessments of European hake in GSAs 1 and 3, as both independent and combined stocks. This simulation exercise revealed clear north–south differences in the Alboran Sea. Although the three assessments reveal an exploitation status of high overfishing, indicators of the stock status appear buffered and provide a more optimis-

tic outlook for GSA 3 when the two subpopulations are assessed jointly as one single stock (Table 2). Additional analyses, simulations and details can be found in Galindo-Ponce (2021).

**Table 2.** European hake technical reference point values and overexploitation status by management area, based on 2003–2018 data

	GSA 1	GSAs 1 and 3	GSA 3
$F_{0.1}$	0.186	0.143	0.133
$F_{SQ}$	1.144	1.62	1.77
$F_{SQ}/F_{0.1}$	6.148	11.3	13.32
Exploitation status	High overfishing	High overfishing	High overfishing

Source: Galindo-Ponce, M. 2021. Comparative analysis of fisheries assessment of transboundary stocks and the impact of recruitment on population dynamics of *M. merluccius* in the Alboran Sea (GSAs 1 and 3). Alicante, Spain, University of Alicante. MSc Thesis.

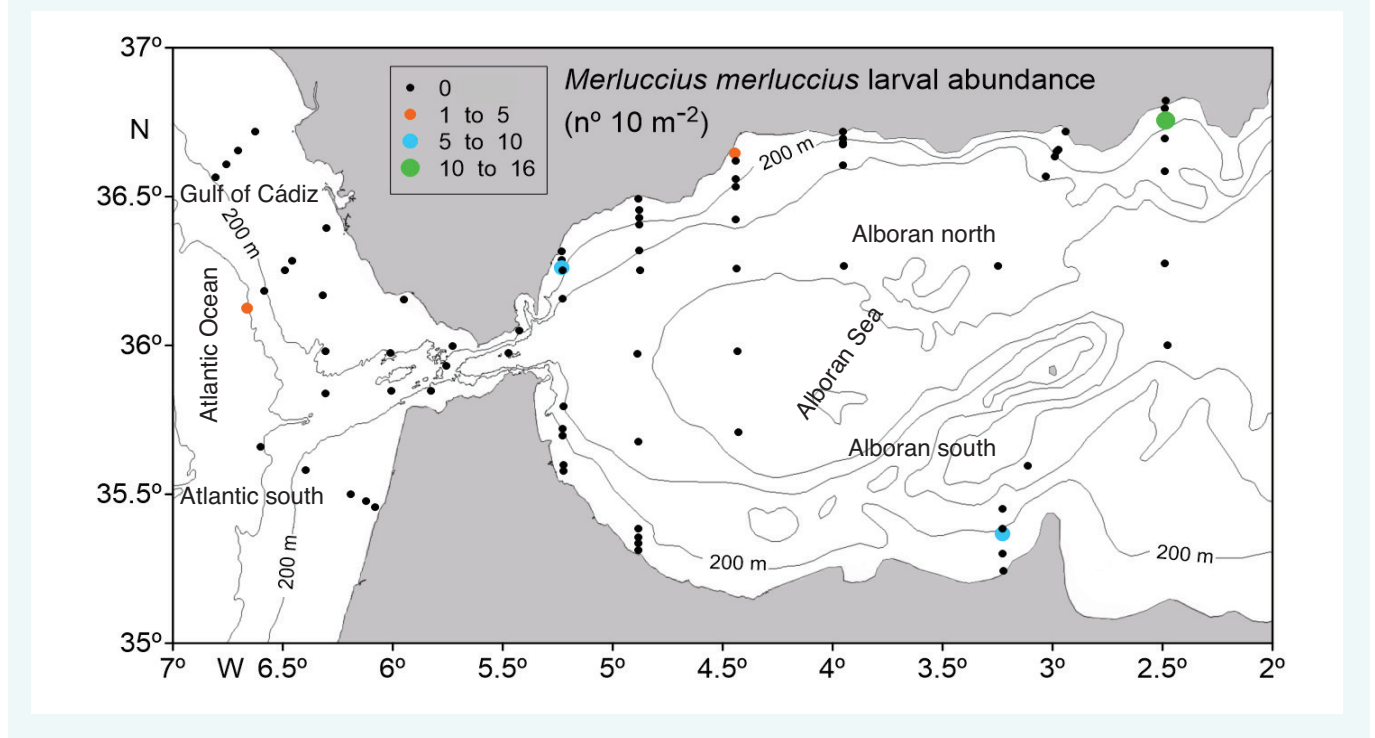
Notes:  $F_{0.1}$  = fishing mortality rate corresponding to 10 percent of the slope of the yield per recruit curve as a function of F when F = 0;  $F_{SQ}$  = current fishing mortality rate.

### 2.1.9 Ichthyoplankton survey

While the sampling scheme of the survey was not optimized to sample ELS of European hake, sporadic catches of larvae were obtained in separate spawning areas. No larvae were found offshore, with all captured within the 200 m isobath

in the Alboran Sea, suggesting strong self-recruitment and/or low survival far from the coast in this region (Figure 19). The presence of larvae may indicate areas that are favourable for retention.

**Figure 19.** Horizontal distribution of European hake larval abundance



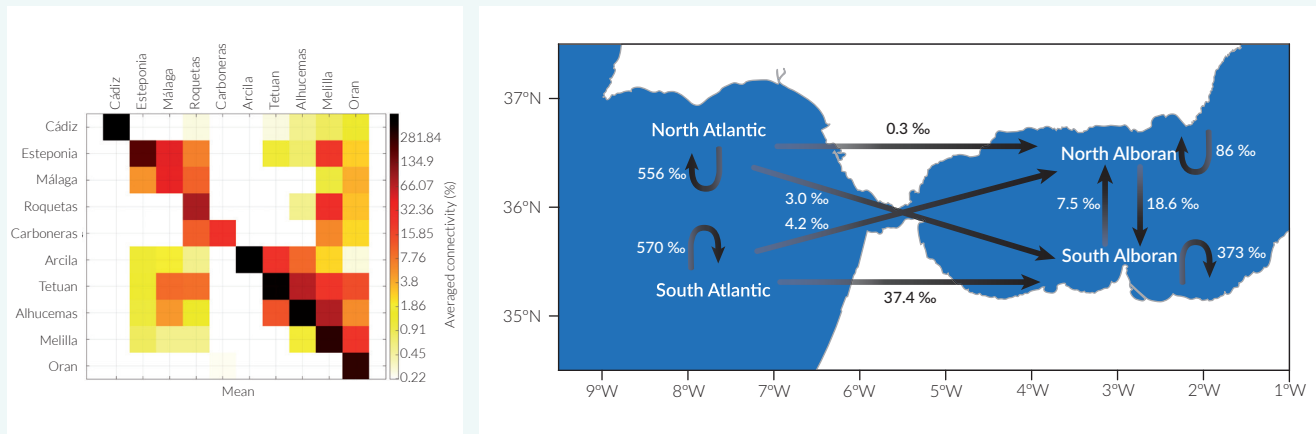
## 2.2 Sardine

### 2.2.1 Hydrodynamic modelling

The results of numerical simulations for sardine indicate that, as with European hake, self-recruitment is the most likely connectivity link of the LFN, with an average value of 86.3 permille in boxes of the northern Alboran Sea and 373.2 permille in boxes of the southern Alboran Sea (Figure 20). The probability for north–south connectivity in the Alboran Sea was of the order of 10–20 permille. The transport of ELS from north to south is more likely than from south to north. Assuming that out of a potential single population, 25 percent corresponds to the northern Albo-

ran Sea (GSA 1) and 75 percent to the southern Alboran Sea (GSA 3), it was estimated that only a minor proportion (about 0.5 percent) of the total spawning biomass of sardine in the Alboran Sea can be transported in either direction. It was also found that ELS from the Atlantic (boxes off Cádiz and Arcila) can be driven into boxes of the Alboran Sea, especially from the southern Atlantic margin (off Arcila) and very rarely from Cádiz. The two Atlantic regions of the network were not connected with each other in any of the simulations (Figure 20).

**Figure 20.** Connectivity matrix (left) and general patterns of sardine transport, including self-recruitment (right)



Source: Right panel base map redrawn from Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

Sensitivity analyses to depth of dispersion and PLD were performed, showing that connectivity results are sensitive to the depth at which sardine ELS are transported by the currents. While the overall pattern of connectivity did not change dramatically with drifting depth, connectivity values increased substantially overall as sardine ELS were located at greater depths (at least 25 m) (see Supplementary material on the methodology Part II; FAO, 2024). Connectivity values were also sensitive to the time at which ELS were released in each box of the LFN, suggesting that the mesoscale variability of the Alboran Sea circulation (and perhaps also the variability of the currents at short-

er timescales) can substantially modulate the exchange of sardine ELS between the selected spawning patches.

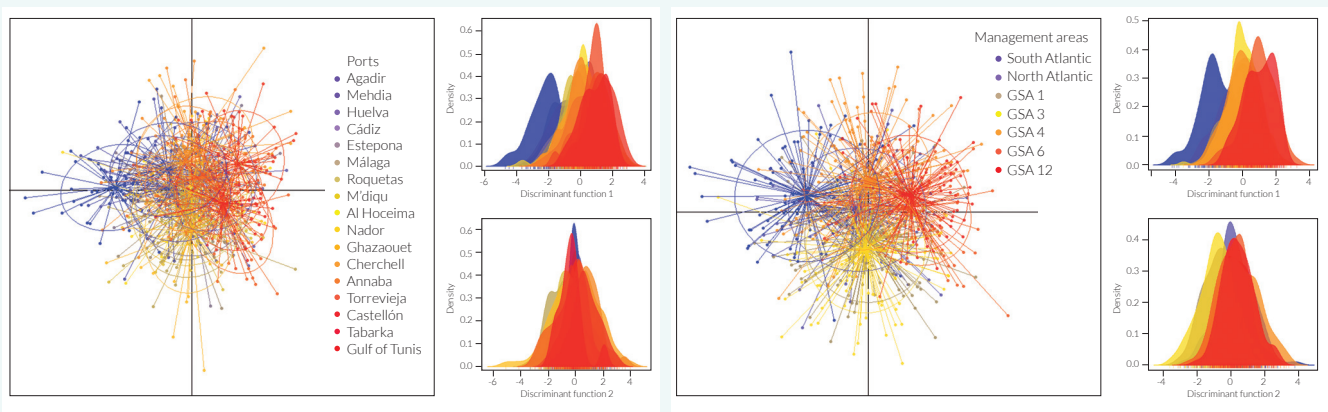
Although the analysis indicates that northern and southern sardine habitat locations in the Alboran Sea are not hydrodynamically isolated from each other, and that self-recruitment is not as strong as for European hake, it remains to be determined whether the flux of spawning material from one location to another is large enough, and likewise whether the feeding conditions and survival rates of the drifting ELS are sufficient, to establish an actual biological link between sardine subpopulations.

## 2.2.2 Genetics

The analyses performed using microsatellite markers support existing gene flow between the sampling locations under study, with high connectivity within the Alboran Sea and adjacent waters. Structuring of sardine genetic diversity at microsatellite loci assessed through  $F_{ST}$  indicates a possible barrier to gene flow in the South Atlantic and between the ports of Agadir and Medhia, or a pattern of

isolation-by-distance. Discriminant analysis of principal components (Figure 21) captures the weak structuring of genetic diversity between Agadir – or the South Atlantic geographical subregion – and the rest of the sampled areas, as obtained for  $F_{ST}$  pairwise comparisons (see Supplementary material on the methodology Part VII; FAO, 2024).

**Figure 21.** Discriminant analysis of principal components performed with genetic data from 805 sardines genotyped at 7 microsatellite loci and grouped according to provenance from 17 sampling ports (left) and, more broadly, 7 management areas (right)



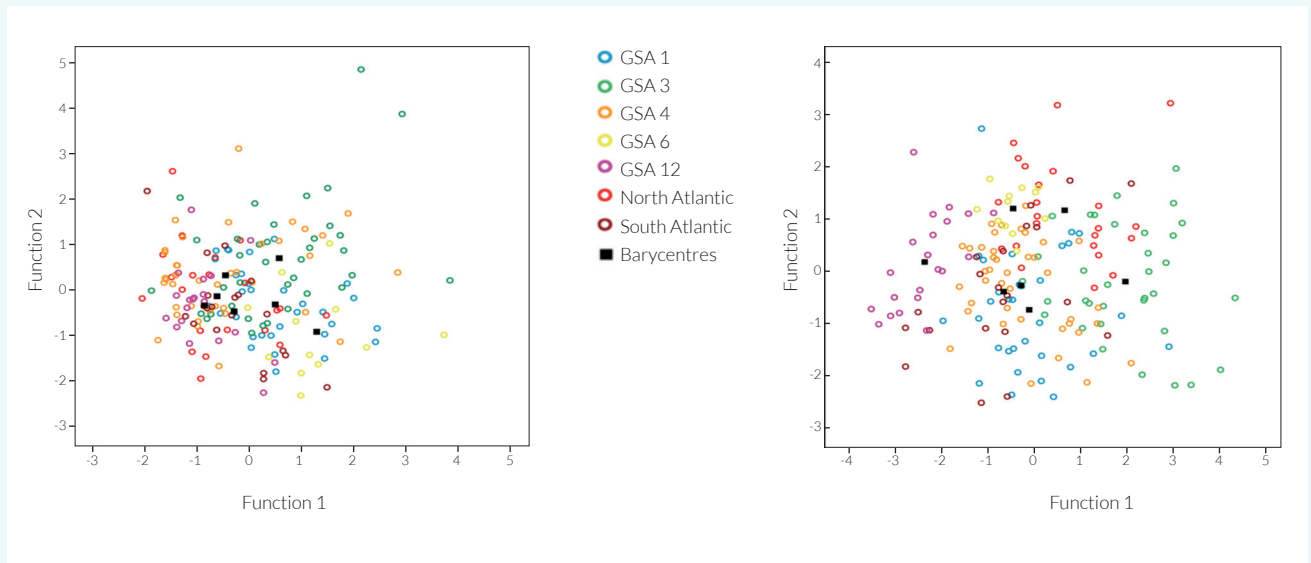
## 2.2.3 Otolith microchemistry

Otolith microchemical analyses provide information at two ontogenetic scales: i) early life stages from the core of the otolith; and ii) the most recent period of life from the edge of the otolith. Among other analyses (see Supplementary material on the methodology Part IX; FAO, 2024), a forward stepwise linear discriminant model analysis was built for core and edge otolith data to classify sardine from the Atlantic and the Mediterranean. The two sampled otolith areas provide a contrasting pattern for the Alboran Sea region. While the edge of the otolith showed north–south differences in the Alboran Sea, these differences were not observed in the core analyses (Figure 22).

At the regional scale, additional patterns emerged. The discriminant analysis plot indicates some overlap, especially among samples from GSAs 4 and 12 and between GSAs 1 and 3. The samples from GSAs 1 and 4 were more variable

than those from the other GSAs. However, the samples from GSA 6 were separated from the other GSAs along canonical variate 1 (Figure 22). This separation was due to the tendency for higher Ba:Ca and lower Mg:Ca ratios in the core of sardine otoliths from GSA 6, which show the highest discrimination accuracy (75 percent), while GSAs 1 and 3 had the lowest discrimination accuracy, at 34.4 percent and 43.2 percent, respectively (see Supplementary material on the methodology Part IX; FAO, 2024). For the edge, classification accuracy was moderately accurate for GSAs 3, 6 and 12 (91.7 percent, 82.4 percent and 81 percent, respectively). However, the canonical variate plot indicated some overlap among individuals sampled from the North Atlantic and GSAs 1 and 4. In addition, individuals from the South Atlantic overlapped with those from GSAs 3 and 6 (Figure 22).

**Figure 22.** Scatter plot representations of the discriminant analyses of the multi-element chemistry of otolith cores (left) and edges (right) collected from sardines, grouped by management area



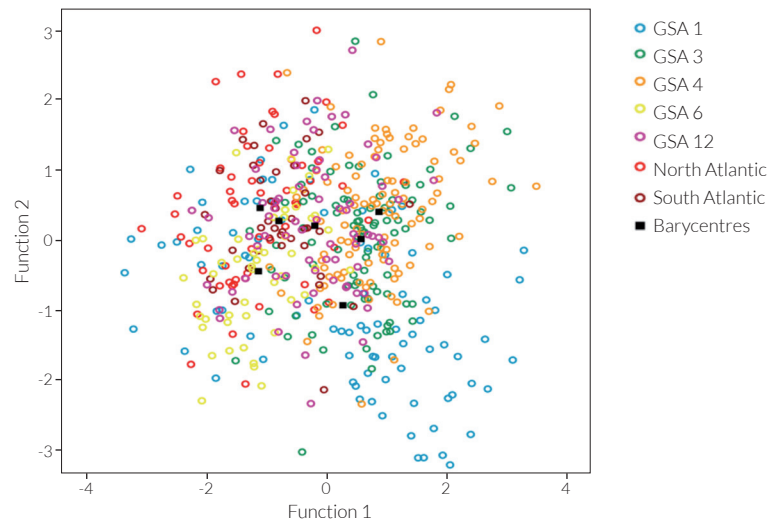
### 2.2.4 Otolith shape

The results of the canonical discriminant analyses revealed that the first two discriminant functions performed with normalized elliptical Fourier descriptors and physical characteristics descriptors accounted for 84 percent of the variance. A forward stepwise linear discriminant analysis was built to classify each individual to one of the sites from which they were collected. Classification accuracies for each species and environment were evaluated through the percentage of correctly classified individuals using a jackknife classification. An overall classification of sardines to their site of capture was 42.5 percent, with a jackknife cross-validation of an average of 39.7 percent (see Supplementary material on the methodology Part XIII; FAO, 2024). In the Alboran Sea, the results show north-south differences between GSAs 1 and 3, but these differences are not shown in the rest of the areas. As for sample size, while analyses of ten Fourier harmonics were appropriate for the sample sizes used for detecting large shape differences, a greater number of samples would be needed to

support more harmonics and detect potential subtle shape differences.

Despite this weak classification, the results indicated some level of structuring between the different areas. For instance, a group of samples from the Atlantic (North and South), a group of samples from GSAs 3 and 4, and the groups of sardines from GSAs 1 and 6 were relatively individualized. Additionally, the GSA 12 sardines overlapped with all areas characterized by the highest level of misclassification, especially with GSAs 4 and 6 (Figure 23). While shape analysis of the otoliths did not show consistent differentiation between fish from the different sampling areas, there was a weak structuring between the Atlantic (North and South) and the other areas of the Mediterranean. The shapes of the sardine otoliths from GSA 12 were relatively closer to those from GSAs 4 and 6 – indeed, 25 percent and 22.2 percent of samples from GSA 12 were misclassified as being from GSA 6 and GSA 4, respectively.

**Figure 23.** Scatter plot representation of the discriminant analyses of the contour shape of sardine otoliths, grouped by management area

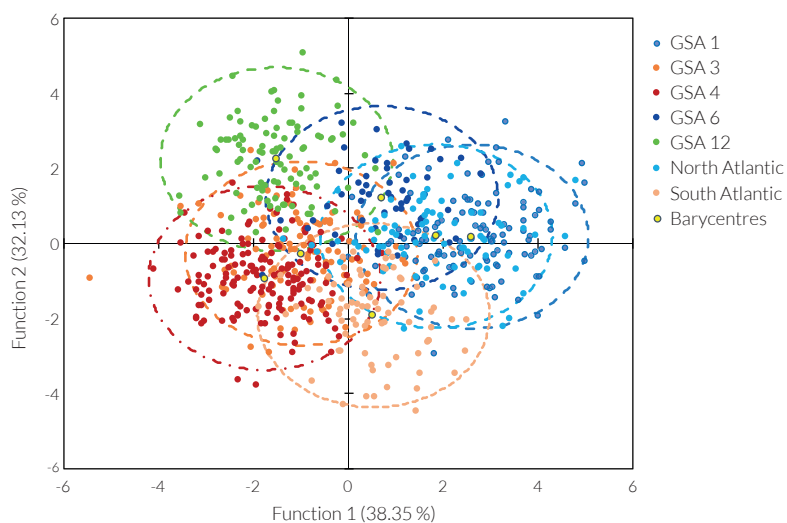


## 2.2.5 Body morphometrics

Fisher discriminant analysis was used to analyse body information after removing the effect of body size. The analysis evidenced differences between the northern and southern Alboran Sea. Indeed, the northern Alboran Sea (GSA 1) showed strong similarities with the North Atlan-

tic (Figure 24). The same was true for the southern Alboran Sea (GSA 3) and Algeria (GSA 4). The Tunisian coast (GSA 12) appeared to have similarities with northern Spain (GSA 6), but not to a significant degree (see Supplementary material on the methodology Part XIV; FAO, 2024).

**Figure 24.** Scatter plot representation of the Fisher discriminant analysis performed with sardine body information, grouped by management area



*Notes:* The Fisher discriminant analysis used 14 landmarks, with each landmark position (x, y coordinates) calculated as the average of three readings for each of 841 individuals to remove bias due to digitalization error.

The percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

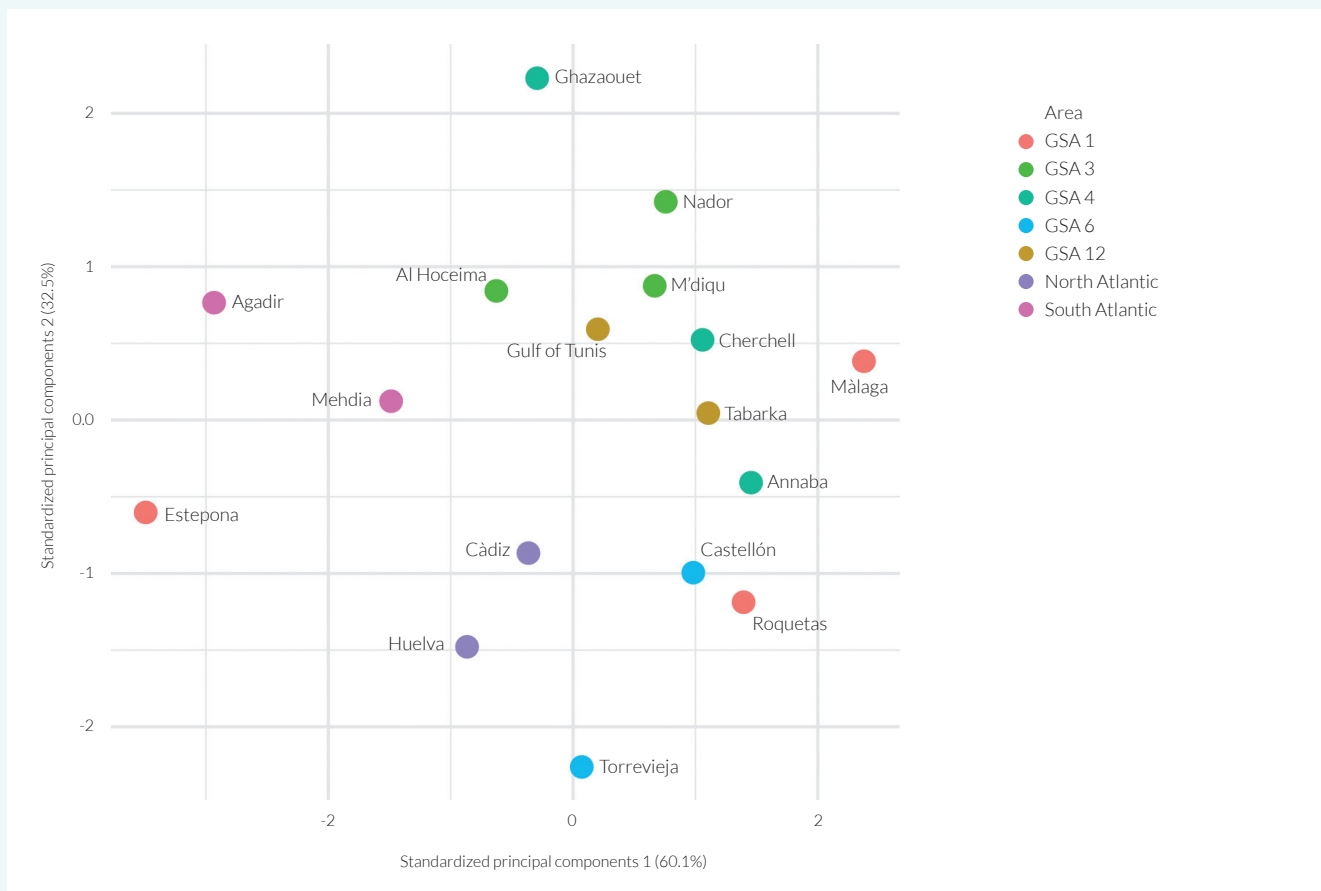


### 2.2.6 Meristics

Two meristic characters were used: the number of vertebrae and the number of gill rakers. In total, only 736 vertebrae and 862 gills from 863 sardine individuals were analysed. With some minimal differences, the two approaches detected mostly the same similarities and differences between areas (for further information on the analyses performed on each metric, see Supplementary material on the

methodology Part XV; FAO, 2024). To better integrate the information of the two metrics, a PCA was performed. According to the results of the PCA (Figure 25), the northern Alboran Sea (GSA 1) and southern Alboran Sea (GSA 3) showed differences. Geographical subareas 1 and 6 and the Atlantic regions displayed some similarities, and the same was true for GSAs 3, 4 and 12.

**Figure 25.** Scatter plot representation of the principal components analysis performed with sardine vertebrae and gill raker information, grouped by management area

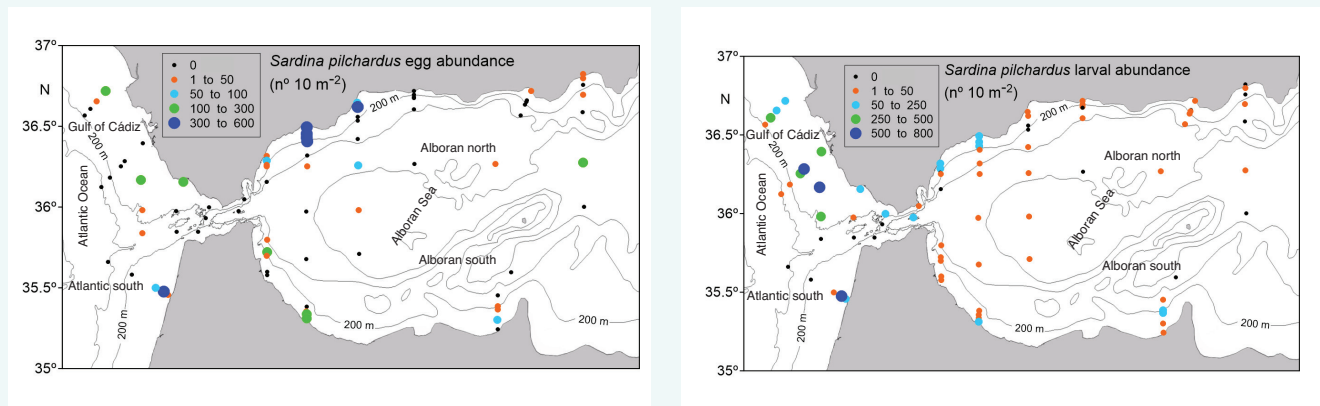


Note: The percentages in parentheses at the end of each axis label represent the percentage of variance explained by each axis.

### 2.2.7 Ichthyoplankton survey

A higher concentration of sardine larvae and eggs was observed in the shelf areas of the study area, while they were also found in the middle of the Alboran Sea, which may imply potential spawning far from the coast, though the phenomenon is most likely due to dispersion by surface currents (Figure 26). A higher concentration of eggs

was found in the Alboran Sea, while larval concentration was higher in the Atlantic region, suggesting that spawning may potentially occur later in the Alboran Sea than in the Atlantic. It would also be plausible to assume that the Gulf of Cádiz has better retention that increases larval survival.

**Figure 26.** Horizontal distribution of sardine eggs (left) and larval abundances (right)

## 2.3 Blackspot seabream

### 2.3.1 Hydrodynamic modelling

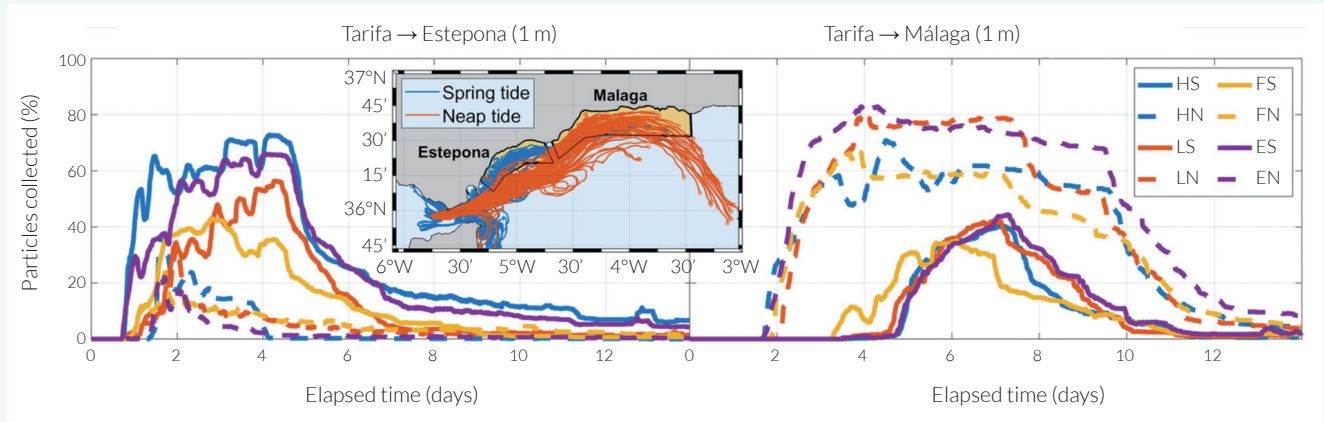
Blackspot seabream ELS dispersal was simulated applying a Lagrangian particle tracking algorithm to the outputs of a hydrodynamic model over the Strait of Gibraltar and Alboran Sea region – for further details, see Supplementary material on the methodology Part IV (FAO, 2024) and Nadal *et al.*, 2022. The design of the particle tracking experiments was not the same as for European hake and sardine: three release areas and ten landing areas were used to assess the dynamic connectivity of this species from its potential spawning areas to its potential recruiting zones. All runs executed over different depths also considered tidal and subinertial forcings.

The results show that the spawning areas of the northern coast of the Strait of Gibraltar are strongly connected with the nurseries in the northern margin of the Alboran Sea, due to the straight connection of the Atlantic Jet and the northern rim of the Western Alboran Gyre (WAG), which flows swiftly along the northern margin of the basin. However, tides, which dominate the dynamics of the spawning area at the Strait of Gibraltar, strongly modulate this pattern. The spread of ELS in the Estepona coastal area, for instance, is especially enhanced by spring tides, which promote the broadening of the cyclonic eddies generated along the northern rim of the WAG (Figure 27). The rest of the northern coast is mostly reached during neap tides, which spread particles to a lesser extent, allowing them to reach the greatest distances. During spring tides, the higher spread of the Atlantic Jet at its exit also promotes

the formation of a southern coastal gyre, which feeds the southern coast directly and connects the northern part of the Strait of Gibraltar to the African coast much more quickly and efficiently. In this scenario, ELS that reach the easternmost portion of the southern coast proceed from the west. Conversely, under neap tides, the connection along the southern coast is mostly westwards: ELS moving there proceed mostly from the east. In both cases, it takes longer for ELS to spread along the southern coast, especially for the second mechanism, which occurs under the action of weaker currents (Figure 27).

The spawning areas on the southern coast of the Strait of Gibraltar are mostly connected to the nurseries on the southern African coast. The main mechanism is the mostly permanent coastal gyre that forms at the very southern exit of the Strait of Gibraltar. Almost regardless of the modulation of tidal intensity, ELS are swiftly advected southwards and flow along the whole African coast with eastward connectivity. Spring tides give rise to the only case of northward connectivity in the basin. When the coastal gyre is especially wide and strong (during spring tides), there is quite a high probability that its external portion will detach and start flowing northeastwards. Early life stages, initially destined for the African coast, may end up being spread along the northern coast. When this occurs, ELS are able to follow the WAG pathway and spread both eastwards and southwards, feeding the African coast with a less efficient westward connectivity.

**Figure 27.** Time series of the percentage of particles released at the surface in the Tarifa releasing box (north of the Strait of Gibraltar) and collected in the Estepona (left) and Málaga (right) landing boxes across all tidal combinations



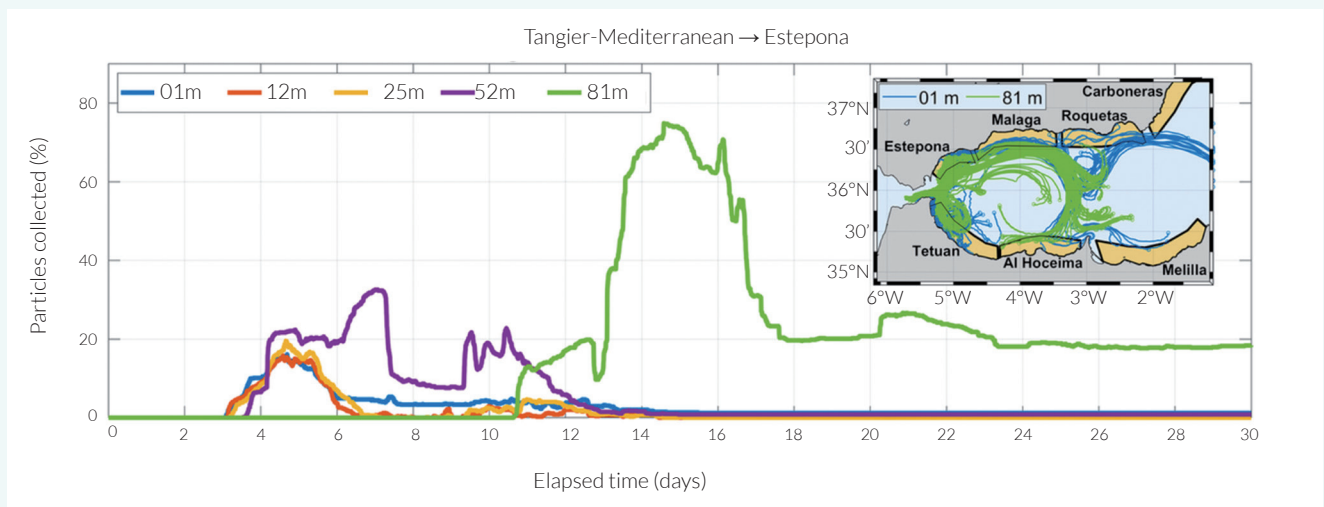
Source: Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

Notes: The suffixes H, L, F and E indicate high, low, flood and ebb tides, respectively, while S and N indicate spring and neap tides, respectively. Inset map shows trajectories for the whole combination of phases in spring (blue lines) and neap (red lines) tides during the first 6 days after the release.

Although the mean patterns described so far can be reasonably assumed to be valid for most situations, it is clear that depth and subinertial variability, especially of a meteorological nature, can easily reverse the pattern. Generally speaking, depth fosters the greatest connectivity within the spawning and nursery areas of the Alboran Sea

(Figure 28). The deeper the depth, the weaker and more stable the current. This dynamic helps to limit the scattering of ELS along longer pathways and enhances the connection between more distant sites, a phenomenon that can be observed for all three release areas.

**Figure 28.** Time series of the percentage of particles released in the Tanger-Mediterranean releasing box (south of the Strait of Gibraltar) under high neap tidal conditions and collected in the Estepona landing box at five different depths



Source: Nadal, I., Sammartino, S., García-Lafuente, J., Sánchez-Garrido, J.C., Gil-Herrera, J., Hidalgo, M. & Hernández, P. 2022. Hydrodynamic connectivity and dispersal patterns of a transboundary species (*Pagellus bogaraveo*) in the Strait of Gibraltar and adjacent basins. *Fisheries Oceanography*, 31(4): 384–401.

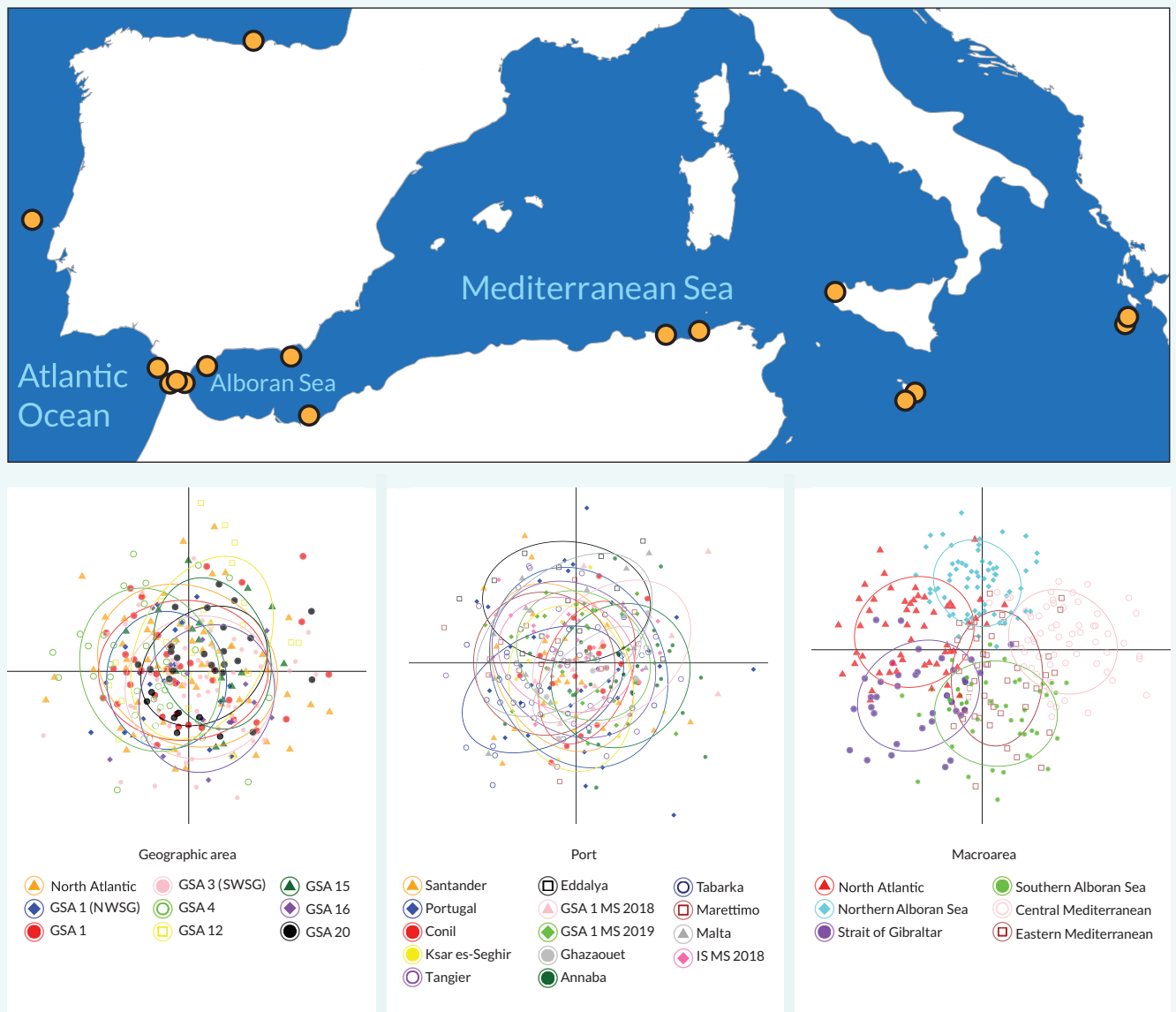
Notes: The five depths considered were 1 m, 12 m, 25 m, 52 m and 81 m. Inset map shows trajectories for surface and 81 m depth spawning levels after 30 days of simulation.

### 2.3.2 Genetics

Although the sampling performed for blackspot seabream was geographically extensive, no genetic structure was observed for this species (Figure 29). Distant samples such as those in northern Spain in the Atlantic and those in the

central and eastern Mediterranean reveal a strong similarity. Indeed, the highest variation (i.e. high genetic diversity) was observed at the individual level (Ferrari *et al.*, 2023)

**Figure 29.** Locations of collected blackspot seabream samples (top) and scatter plot representations of the discriminant analyses of principal components performed with genetic data at 7 microsatellite loci and grouped according to provenance from 9 geographical areas (bottom left), 14 locations (bottom middle) and 6 macroareas (bottom right)



Sources: Top panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Notes: The following abbreviations appear in the legends beneath the bottom panels: NWWSG = northwestern Strait of Gibraltar; SWWSG = southwestern Strait of Gibraltar; MS = MEDITS survey; IS = Ionian Sea.

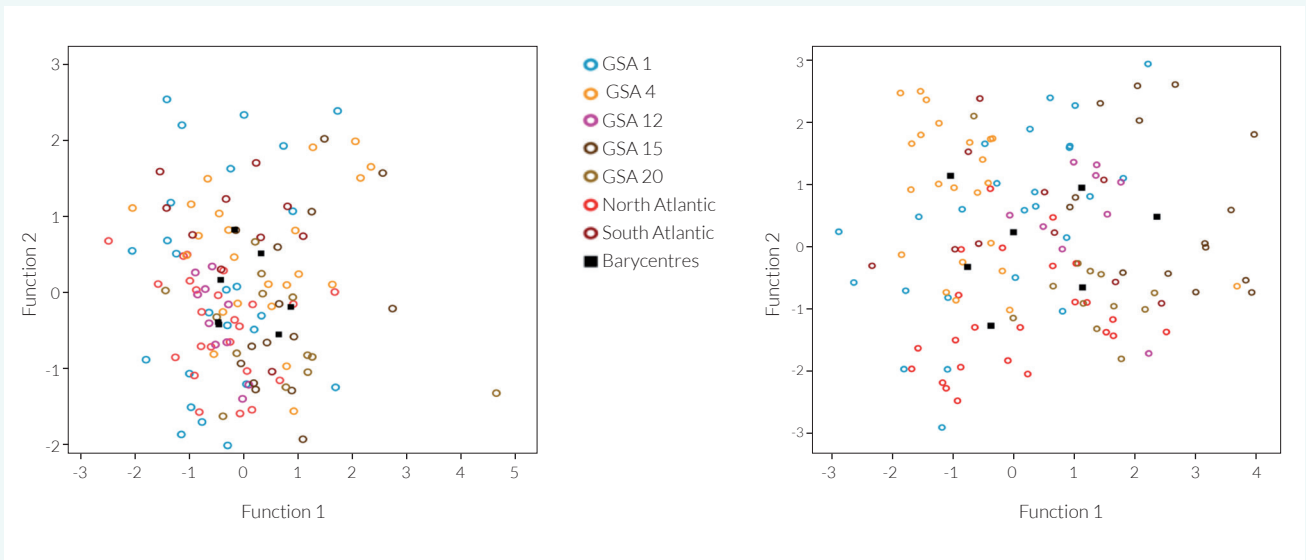
### 2.3.3 Otolith microchemistry

Otolith microchemical analyses provide information at two ontogenetic scales: i) early life stages from the core of the otolith; and ii) the most recent period of life from the edge of the otolith. Among other analyses (see Supplementary material on the methodology Part XI; FAO, 2024), the discriminant analysis performed on the core information indicated a significant overlap, especially among blackspot seabream from the northern Alboran Sea (GSA 1), northern Tunisia (GSA 12) and the North Atlantic, and to a lesser extent from the southern Alboran Sea (GSA 4) and the South Atlantic (Figure 30). In addition, the samples from Malta (GSA 15) and Greece (GSA 20) were separated from the other areas likely due to the higher Ba:Ca and Sr:Ca ratios in the otolith core samples from these two GSAs (Figure 30) (see Supplementary material on the methodology Part XI; FAO, 2024). The jackknife classification involving all areas was very poor at assigning individuals to their areas of origin. The highest relative classification accuracy

was observed for GSA 20, at 30.8 percent, followed by GSA 15 at 26.7 percent (see Supplementary material on the methodology Part XI; FAO, 2024).

Discriminant analysis of otolith edge information indicated weak clusters of samples associated with the Strait of Gibraltar (GSAs 1 and 4 and the North and South Atlantic) compared to those further east (GSAs 12, 15 and 20) (Figure 30). The jackknife classification procedure, with all areas included, was moderately accurate at assigning individuals. Classification accuracy was highest for the northern Tunisian samples (GSA 12), followed by samples from the eastern areas (GSAs 15 and 20). The lowest classification accuracy was observed in the Strait of Gibraltar-associated areas, with minimal values for the South Atlantic and northern Alboran Sea (GSA 1) (see Supplementary material on the methodology Part XI; FAO, 2024).

**Figure 30.** Scatter plot representations of the discriminant analyses of the multi-element chemistry of blackspot seabream otolith cores (left) and edges (right), grouped by management area

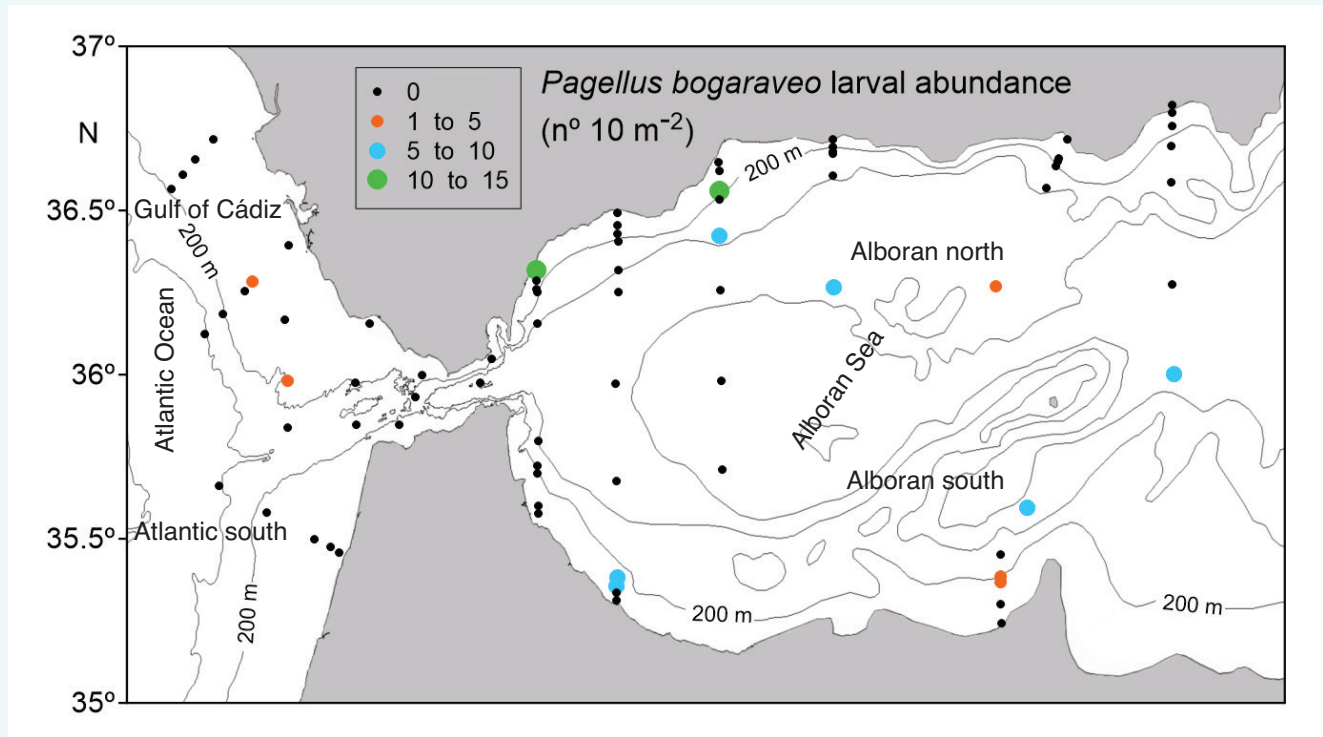


### 2.3.4 Ichthyoplankton survey

While the sampling scheme of the survey was not optimized to sample blackspot seabream ELS, there was moderate occurrence of larvae of this species, including high numbers at some locations (Figure 31). While larvae were observed both in coastal areas and offshore, higher num-

bers were observed in the northern Alboran Sea and relatively close to the Strait of Gibraltar, such as near Estepona and Málaga. This distributional pattern is consistent with particle tracking experiments (Section 2.3.1 on "Hydrodynamic modelling").

**Figure 31.** Horizontal distribution of blackspot seabream larval abundances







# CHAPTER

# 3

## INTEGRATIVE ANALYSES



### 3.1 European hake

#### 3.1.1 Stock differentiation index

The quantification of matrices and the calculation of SDIs for European hake are available in Additional results Part II (FAO, 2024). Table 3 displays SDI calculations at the port level, with Table 4 showing average values at the GSA level. In general terms, there was a high differentiation level for European hake, with a mean SDI value across the whole study of 0.71 and, in general, no combination of GSAs with a low SDI value (i.e. SDI < 0.33). The exception to this pattern, beyond the pairwise port comparisons within GSAs, was the low differentiation between GSA 3 ports and those of neighbouring GSAs: between Annaba and GSA 12 ports, and between Ghazaouet and GSA 3 ports. Notably, Tabarka in GSA 12 shows some level of similarity with GSA 6 ports, suggesting the potential role of the Almería-Oran Front as a hydrographic curtain facilitating an eventual connection between these two areas.

At the GSA level, the northern Alboran Sea (GSA 1) and the southern Alboran Sea (GSA 3) show high-level differentiation, with an SDI of 0.89 (Table 4). Neighbouring management areas display moderate SDI values, such as 0.54 for GSAs 1 and 6, 0.44 for GSAs 3 and 4, and 0.53 for the North and South Atlantic in the Gulf of Cádiz, or low values in the case of GSAs 4 and 12. The connectivity between the Atlantic and the Mediterranean can be better seen in the south, with a moderate SDI value between the South Atlantic and GSA 3 (0.53), while the North Atlantic and GSA 1 seem highly differentiated (0.81). Geographical subareas 6 and 12 are distant areas but show moderate to low differentiation (SDI = 0.36).

**Table 3.** Matrix of port-level stock differentiation index comparisons considering the ten techniques used for European hake

	GSA	1	1	1	12	12	3	3	4	4	6	6	NA	NA	SA	SA
GSA	Port	Estepona	Málaga	Roquetas	Gulf of Tunis	Tabarka	M'diqu	Nador	Annaba	Ghazaouet	Castellón	Torreveija	Cádiz	Huelva	Agadir	Mehdia
1	Estepona															
1	Málaga	0.00														
1	Roquetas	0.22	0.22													
12	Gulf of Tunis	0.67	0.78	0.67												
12	Tabarka	0.67	0.67	0.67	0.14											
3	M'diqu	0.89	0.89	1.00	0.86	0.71										
3	Nador	0.89	0.78	0.89	0.71	0.71	0.00									
4	Annaba	0.89	0.89	0.78	0.14	0.14	0.56	0.56								
4	Ghazaouet	0.78	0.78	0.89	0.43	0.43	0.33	0.33	0.14							
6	Castellón	0.56	0.56	0.44	0.43	0.29	1.00	0.89	0.57	0.57						
6	Torreveija	0.56	0.56	0.56	0.43	0.29	1.00	0.78	0.71	0.57	0.00					
NA	Cádiz	0.89	0.89	0.89	1.00	1.00	0.78	0.78	1.00	1.00	0.86	0.86				
NA	Huelva	0.67	0.78	0.78	0.86	0.86	0.78	0.78	1.00	1.00	0.71	0.71	0.13			
SA	Agadir	0.78	0.89	0.67	0.86	0.71	0.56	0.56	0.71	0.71	0.86	0.71	0.63	0.63		
SA	Mehdia	0.78	0.78	0.78	0.86	0.86	0.56	0.44	0.71	0.86	0.86	0.71	0.50	0.38	0.00	



Note: NA = North Atlantic; SA = South Atlantic.

**Table 4.** Matrix of average stock differentiation index values at the geographical subarea level considering the ten techniques used for European hake

GSA	1	12	3	4	6	NA	SA
1	0.15						
12	0.69	0.14					
3	0.89	0.75	0.00				
4	0.83	0.29	0.44	0.14			
6	0.54	0.36	0.92	0.61	0.00		
NA	0.81	0.93	0.78	1.00	0.79	0.13	
SA	0.78	0.82	0.53	0.75	0.79	0.53	0.00



Notes: NA = North Atlantic; SA = South Atlantic.

Yellow values in boxes along the diagonal indicate stock differentiation index (SDI) values within each GSA.

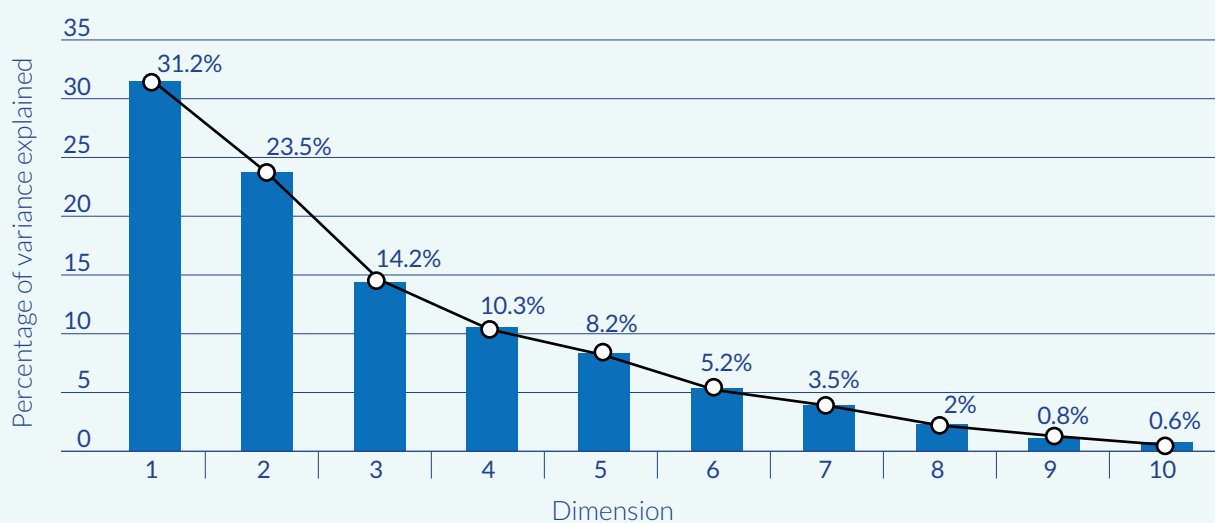
### 3.1.2 Multitechnique discriminant analyses

The multitechnique discriminant analyses performed included the outcomes (with the three first axes explaining almost 70 percent of the variance) of seven techniques that used discriminant analyses. These techniques were neutral components of genetics (SNPs), outlier components of genetics (SNPs), otolith shape, body morphometry, meristics (combining information from vertebrae and gill rakers), and

otolith core and edge microchemistry as independent outcomes.

The PCAs performed indicate that the two first dimensions account for 55 percent of the explained variance. If the third dimension is included, the total amounts to 69 percent (Figure 32).

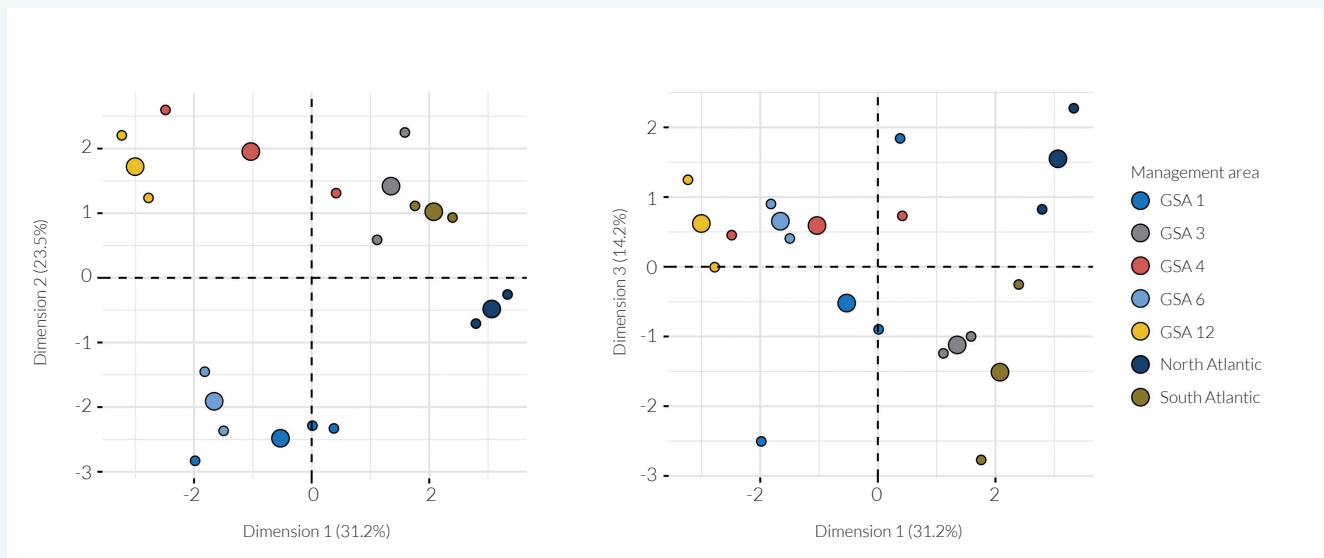
**Figure 32.** Percentage of the variance explained by the different dimensions of the principal components analysis applied to the outcomes of the seven techniques used for European hake



Results of the PCA show that the northern Alboran Sea (GSA 1) and northern Spain (GSA 6) appear well differentiated from the other GSAs when the two first dimensions are considered (Figure 33). In addition, along the southern Mediterranean, the results of the two first dimensions demonstrate an east (from Tunisia, GSA 12) to west (South and North Atlantic) gradient (Figure 33). The third dimension does not provide any obvious pattern for

European hake (Figure 33). Thus, from here onwards, the focus will be on the interpretation of dimensions 1 and 2. A PERMANOVA test shows significant differences at the GSA level ( $F = 5.99, p < 0.001$ ), while the low number of observations retained under the integrative analyses limits the performance of a pairwise PCA among all GSA combinations.

**Figure 33.** Biplot representations of dimensions 1 and 2 (left) and 1 and 3 (right) of the principal components analysis applied to the outcomes of the seven techniques used for European hake



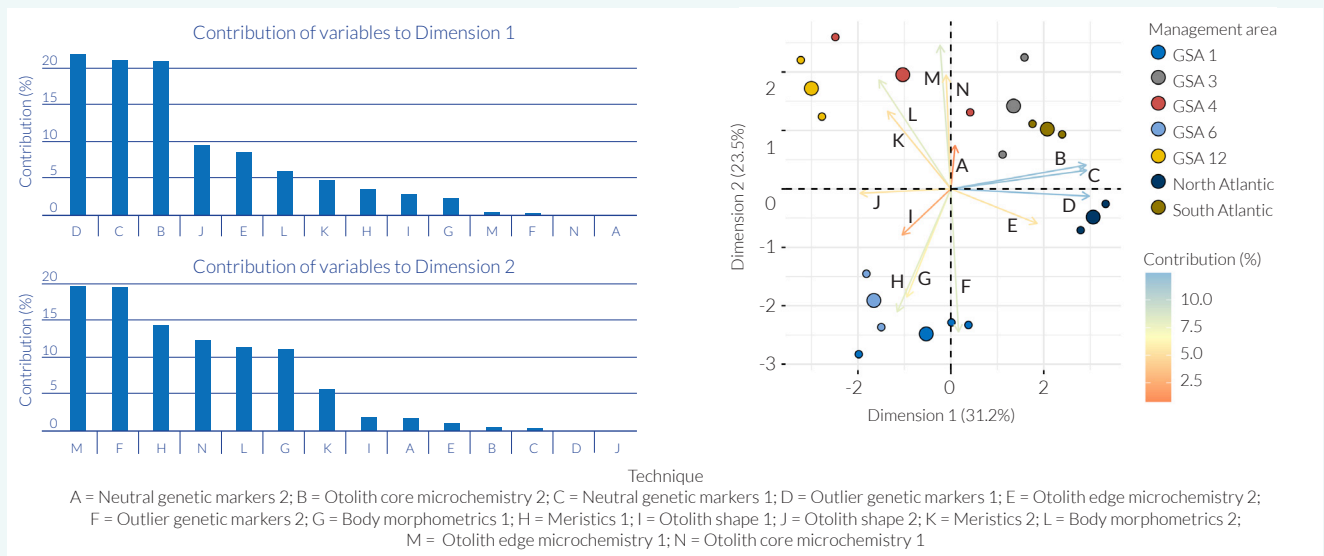
*Notes:* The values in the parentheses at the end of each axis label indicate the variance explained for each dimension, while the larger circles represent the barycentre for each management area and the smaller circles represent the sample sites (ports) of each management area. Colours are coded for each management area.

The first dimension is mainly related to a clear longitudinal gradient, starting strong in GSA 12 (northern Tunisia), followed by intermediate values in Algeria (GSA 4) and northern Spain (GSA 6), then separated from the southern Alboran Sea (Morocco, GSA 3) and the northern Alboran Sea (Spain, GSA 1), and ending with the North Atlantic and finally (a little more separated) the South Atlantic (Figure 34). The gradient revealed by this first dimension is likely related to genetic drift, as it is associated with the first dimension of neutral and outlier genetic information and the second dimension of otolith core microchemistry (Figure 34). The second dimension clearly separates the northern Alboran Sea (GSA 1) and northern Spain (GSA 6) from the rest of the GSAs, while there is a likely transition with the North Atlantic (Figure 34). This second

dimension is mainly associated with more local adaptation and small-scale environmental drivers, as it mostly depends on the first dimension of otolith microchemistry (both edge and core) and the second dimension of genetic outliers, followed by the first dimensions of meristics and otolith core microchemistry and by both dimensions of body morphometry (Figure 34).

Finally, as groups (i.e. GSAs) were not big enough to test potential clustering, the Atlantic and the northern and southern Mediterranean were tested as potential large areas. The biplot for the first two dimensions shows that the northern and southern Mediterranean clustered separately, with the Atlantic crossing both and partially overlapping the southern Mediterranean (Figure 35).

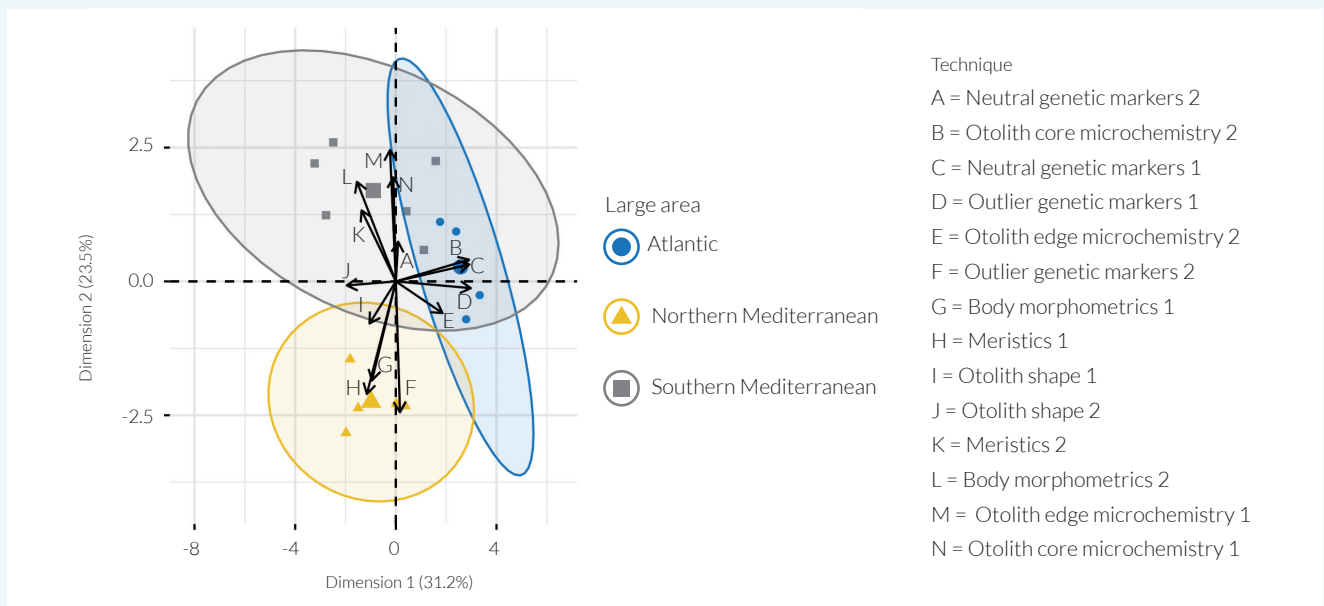
**Figure 34.** Relative contributions of each technique to the total variance of the two dimensions considered in the integrative analysis for European hake (left) and the degree to which each technique contributes, as represented in the biplot space (right)



**Notes:** The values in the parentheses at the end of each axis label in the right panel indicate the variance explained by each dimension, while the larger circles represent the barycentre for each management area and the smaller circles represent the sample sites (ports) of each management area. Colours are coded for each management area.

The techniques included in the integrative analysis were neutral genetic markers, outlier genetic markers, body morphometry, otolith edge and core microchemistry, meristics and otolith shape. The number (1 or 2) accompanying each technique represents the dimension (1 or 2) for the discriminant analyses.

**Figure 35.** Biplot representation of dimensions 1 and 2 of the principal components analysis applied to the outcomes of the seven techniques used for European hake, with clustering of three potential large areas



**Notes:** The values in parentheses at the end of each axis label indicate the variance explained by each dimension, while the larger shapes represent the barycentre for each large area and the smaller shapes represent the sample sites (ports) of each large area.

The techniques included in the integrative analysis were neutral genetic markers, outlier genetic markers, body morphometry, otolith edge and core microchemistry, meristics and otolith shape. The number that accompanies each technique represents the technique-specific dimension 1 or 2.



## 3.2 Sardine

### 3.2.1 Stock differentiation index

The quantification matrices and the calculation of SDIs for sardine are available in Additional results Part III (FAO, 2024). Table 5 displays the SDI calculations at the port level, with average values at the GSA level shown in Table 6. In general terms, there was a moderate differentiation level for sardine, particularly when compared to European hake, with a mean SDI value across the whole study of 0.56. Low to moderate differentiation is observed for some neighbouring ports, such as between Mehdia and GSA 3 ports and between some North Atlantic and GSA 1 ports, and is also more generalized across GSAs, such as in the cases of low SDIs among GSAs 3, 4 and 12 ports. Indeed, in terms of GSA differentiation, only three combinations show GSA pairs with a low SDI value (i.e.  $SDI < 0.33$ ): GSA 4 with GSAs 3 and 12, and GSA 3 with the South Atlantic. This low differentiation of GSA 3 with its neighbouring areas in the south could likely represent high dynamic connectivity,

including demographic connectivity. The northern Alboran Sea (GSA 1) and the southern Alboran Sea (GSA 3) show moderate evidence of stock differentiation ( $SDI = 0.52$ ). Beyond the neighbouring interactions mentioned in the southern part of the study area, the North Atlantic–South Atlantic transition in the Gulf of Cádiz displays a moderate SDI value, at 0.54, while the transition between the North Atlantic and the northern Alboran Sea shows low to moderate differentiation ( $SDI = 0.38$ ). By contrast, other pairs of neighbouring areas, such as GSAs 1 and 6 display high values of differentiation ( $SDI = 0.67$ ). The effect of the Almería-Oran Front as a hydrographic curtain and frontier is also suggested by the results, with moderate SDI values between GSA 1 and GSA 4 ( $SDI = 0.56$ ), and between GSA 6 and GSA 12 ( $SDI = 0.50$ ).

**Table 5.** Matrix of port-level stock differentiation index comparisons considering the ten techniques used for sardine

	GSA	1	1	1	12	12	3	3	3	4	4	4	6	6	NA	NA	SA	SA
GSA	Port	Estepona	Málaga	Roquetas	Gulf of Tunis	Tabarka	M'diqu	Alhoceima	Nador	Annaba	Cherchell	Ghazaouet	Castellón	Torre Vieja	Cádiz	Huelva	Agadir	Mehdia
1	Estepona																	
1	Málaga	0.57																
1	Roquetas	0.29	0.00															
12	Gulf of Tunis	0.83	0.83	0.83														
12	Tabarka	0.83	0.83	0.67	0.00													
3	M'diqu	0.57	0.43	0.43	0.33	0.40												
3	Alhoceima	0.57	0.57	0.57	0.33	0.50	0.00											
3	Nador	0.57	0.43	0.57	0.67	0.67	0.29	0.17										
4	Annaba	0.67	0.67	0.50	0.00	0.17	0.17	0.17	0.20									
4	Cherchell	0.67	0.50	0.50	0.50	0.17	0.17	0.00	0.50	0.00								
4	Ghazaouet	0.50	0.50	0.50	0.50	0.33	0.17	0.17	0.17	0.33	0.40							
6	Castellón	0.83	0.50	0.50	0.50	0.67	0.67	0.67	0.67	0.50	0.83	1.00						
6	Torre Vieja	0.83	0.67	0.67	0.33	0.50	0.67	0.67	0.67	0.50	0.67	0.67	0.20					
NA	Cádiz	0.57	0.29	0.29	0.67	0.83	0.57	0.57	0.57	0.33	0.50	0.67	0.50	0.40				
NA	Huelva	0.57	0.29	0.29	0.67	0.67	0.71	0.86	0.57	0.50	0.50	0.67	0.67	0.83	0.33			
SA	Agadir	0.14	0.71	0.57	0.83	0.83	0.43	0.43	0.43	0.83	0.67	0.83	0.83	0.83	0.57	0.57		
SA	Mehdia	0.57	0.71	0.71	0.50	0.50	0.29	0.29	0.14	0.50	0.67	0.67	0.50	0.50	0.43	0.57	0.29	



Note: NA = North Atlantic; SA = South Atlantic.

**Table 6.** Matrix of average stock differentiation index values at the geographical subarea level considering the ten techniques used for sardine

GSA	1	12	3	4	6	NA	SA
1	0.29						
12	0.81	0.00					
3	0.52	0.48	0.15				
4	0.56	0.28	0.19	0.24			
6	0.67	0.50	0.67	0.69	0.20		
NA	0.38	0.71	0.64	0.53	0.60	0.33	
SA	0.57	0.67	0.33	0.69	0.67	0.54	0.29



Notes: NA = North Atlantic; SA = South Atlantic.

Yellow values in boxes along the diagonal indicate stock differentiation index (SDI) values within each GSA.

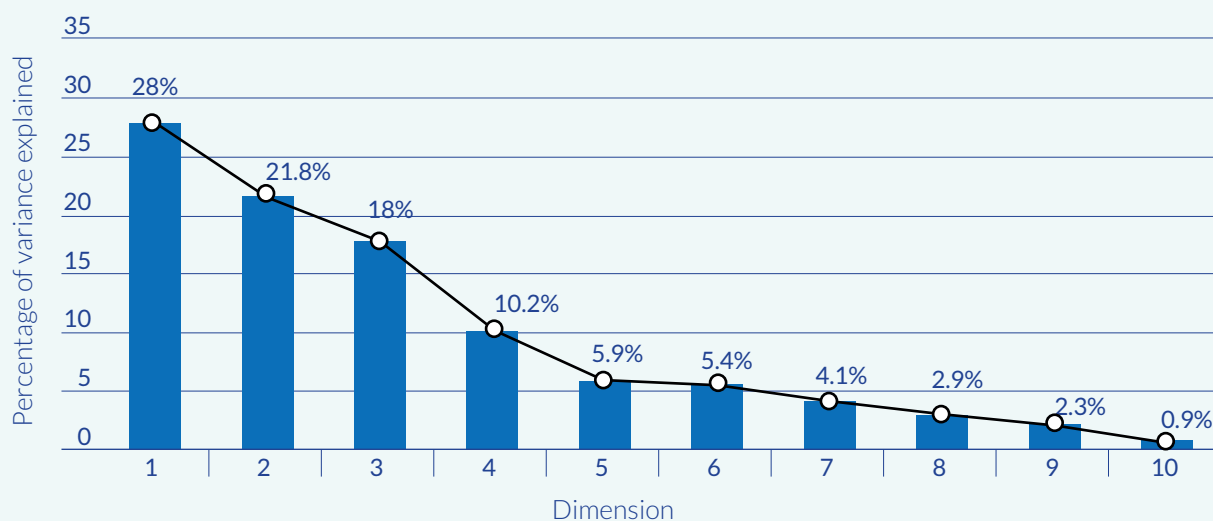
### 3.2.2 Multitechnique discriminant analyses

The multitechnique discriminant analyses performed included the outcomes of six techniques that used discriminant analyses, with the two first axes explaining almost 70 percent of the variance for the analyses in each technique (Figure 36). These six techniques were genetics (microsatellites), otolith shape, body morphometry, meristics (combining information from vertebrae and gill rakers), and

otolith core and edge microchemistry as independent outcomes.

The principal components analyses performed indicate that the first two axes account for 50 percent of the variance, with the explained total variance reaching 68 percent if the third axis is also included (Figure 36).

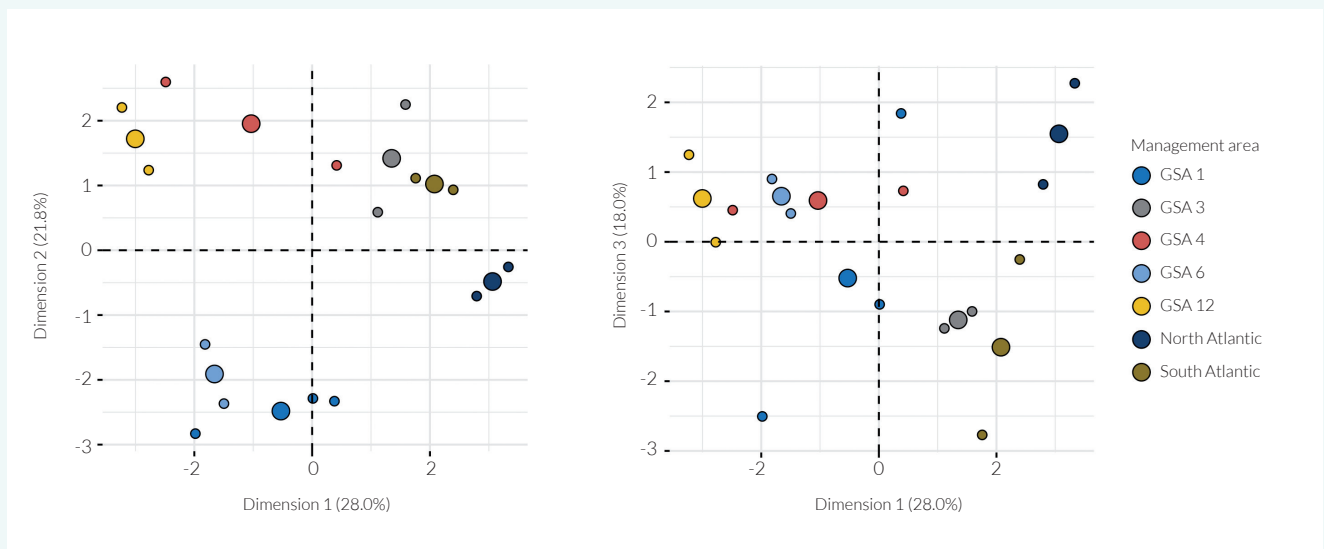
**Figure 36.** Percentage of the variance explained by the different dimensions of the principal components analysis applied to the outcomes of the six techniques used for sardine



The PCA results show that the Moroccan and the Algerian coast (GSAs 3 and 4) are well differentiated from the other areas (Figure 37). On dimensions 1 and 3, the South Atlantic is also associated with GSAs 3 and 4, while on dimension 2, the South Atlantic appears more closely linked to the North Atlantic. From the eastern side of the study system, GSA 12 (Tunisia) seems to be equally connected to GSAs 1, 4 and 6, suggesting the role of the Almería-Oran

Front as a hydrographic curtain and frontier (Figure 37). A multivariate PERMANOVA test shows significant differences at the GSA level ( $F = 3.48, p = 0.002$ ), while the low number of observations retained under the integrative analyses limits the performance of a pairwise permutation contrast analysis among all GSA combinations.

**Figure 37.** Biplot representations of dimensions 1 and 2 (left) and 1 and 3 (right) of the principal components analysis applied to the outcomes of the six techniques used for sardine

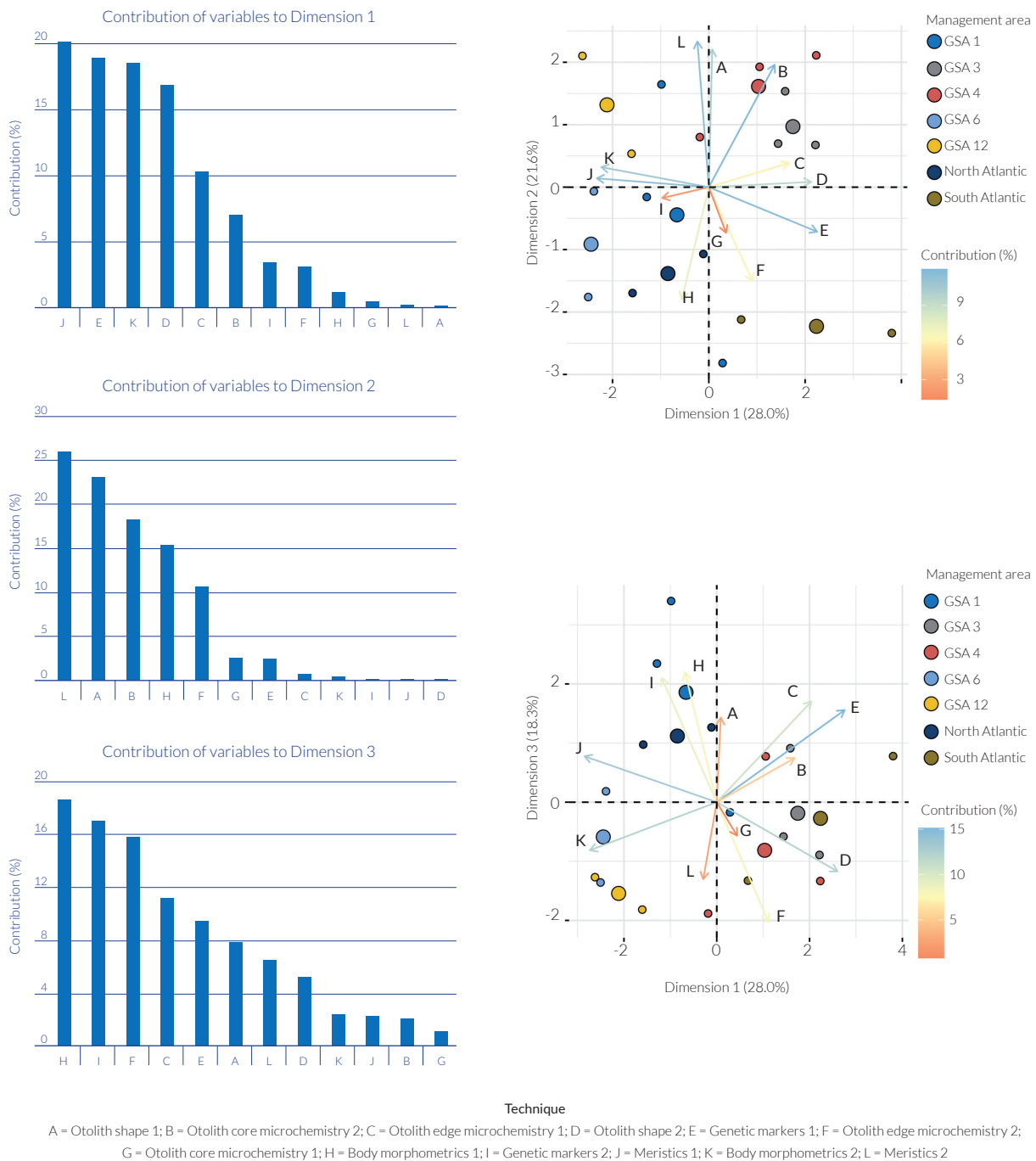


Notes: The values in the parentheses at the end of each axis label indicate the variance explained for each dimension, while the larger circles represent the barycentre for each management area and the smaller circles represent the sample sites (ports) of each management area. Colours are coded for each management area.

The first dimension, which mainly segregates GSAs 3 and 4 and the South Atlantic, was associated with the first dimension of meristics and genetics and with the second dimension of body morphometry and otolith shape, suggesting relatively dynamic drift from the Atlantic to the eastern Mediterranean through the southern Alboran Sea (Figure 38). The variables associated with dimension 2 may potentially indicate local adaptation and plastic responses to the local environments of the South Atlantic and south-

ern Alboran Sea, including connectivity to Tunisia: these variables are the first dimensions of otolith shape and body morphometry and the second dimensions of meristics and otolith core microchemistry. The third dimension was mainly represented by the first dimension of body morphometry, the second dimension of genetics, and the two axes of otolith edge microchemistry, which can also indicate local adaptation in the North Atlantic and the northern Alboran Sea (Figure 38).

**Figure 38.** Relative contributions of each technique to the total variance of each of the three dimensions considered in the integrative analysis for sardine (left), and the degree to which each technique contributes, as represented in the biplot space (right)



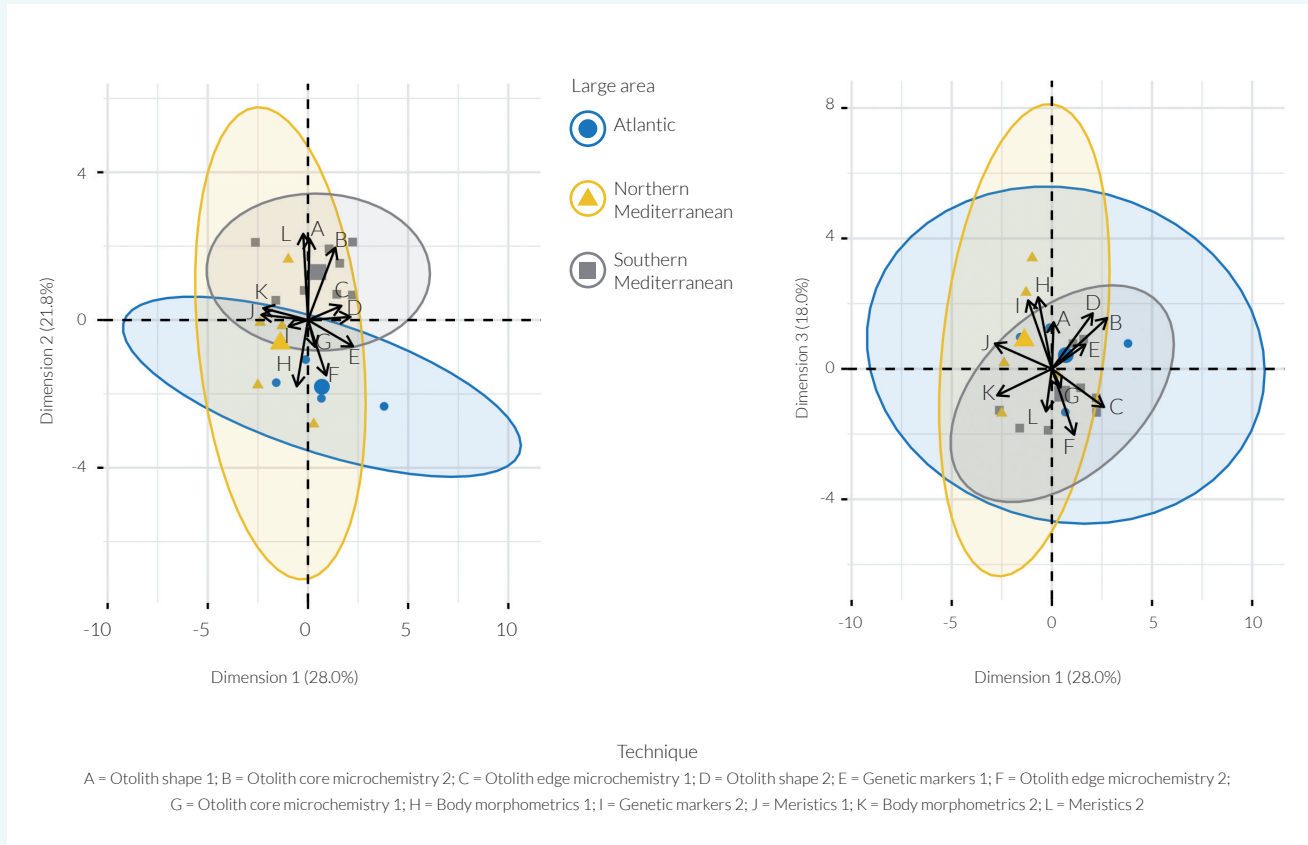
Notes: The values in parentheses at the end of each axis label in the right panels indicate the variance explained by each dimension, while the larger circles represent the barycentre for each management area and the smaller circles represent the sample sites (ports) of each management area. Colours are coded for each management area.

The techniques included in the integrative analysis were genetics, body morphometry, otolith edge and core microchemistry, meristics and otolith shape. The number (1 or 2) accompanying each technique represents the dimension (1 or 2) for the discriminant analyses.

Finally, as groups (i.e. GSAs) were not big enough to test potential clustering, the Atlantic and the northern and southern Mediterranean were tested as potential large areas. The biplot for the first two dimensions shows that the Atlantic and southern Mediterranean clustered separately,

with the northern Mediterranean overlapping and crossing both (Figure 39). In the biplot for the first and third dimension, no large areas segregate and the three of them overlap (Figure 39).

**Figure 39.** Biplot representations of dimensions 1 and 2 (left) and 1 and 3 (right) of the principal components analysis applied to the outcomes of the six techniques used for sardine, with clustering of three potential large areas



Notes: The values in parentheses at the end of each axis label indicate the variance explained by each dimension, while the larger shapes represent the barycentre for each large area and the smaller shapes represent the sample sites (ports) of each large area. The techniques included in the integrative analysis were genetics, body morphometry, otolith edge and core microchemistry, meristics and otolith shape. The number that accompanies each technique represents the technique-specific dimension 1 or 2.



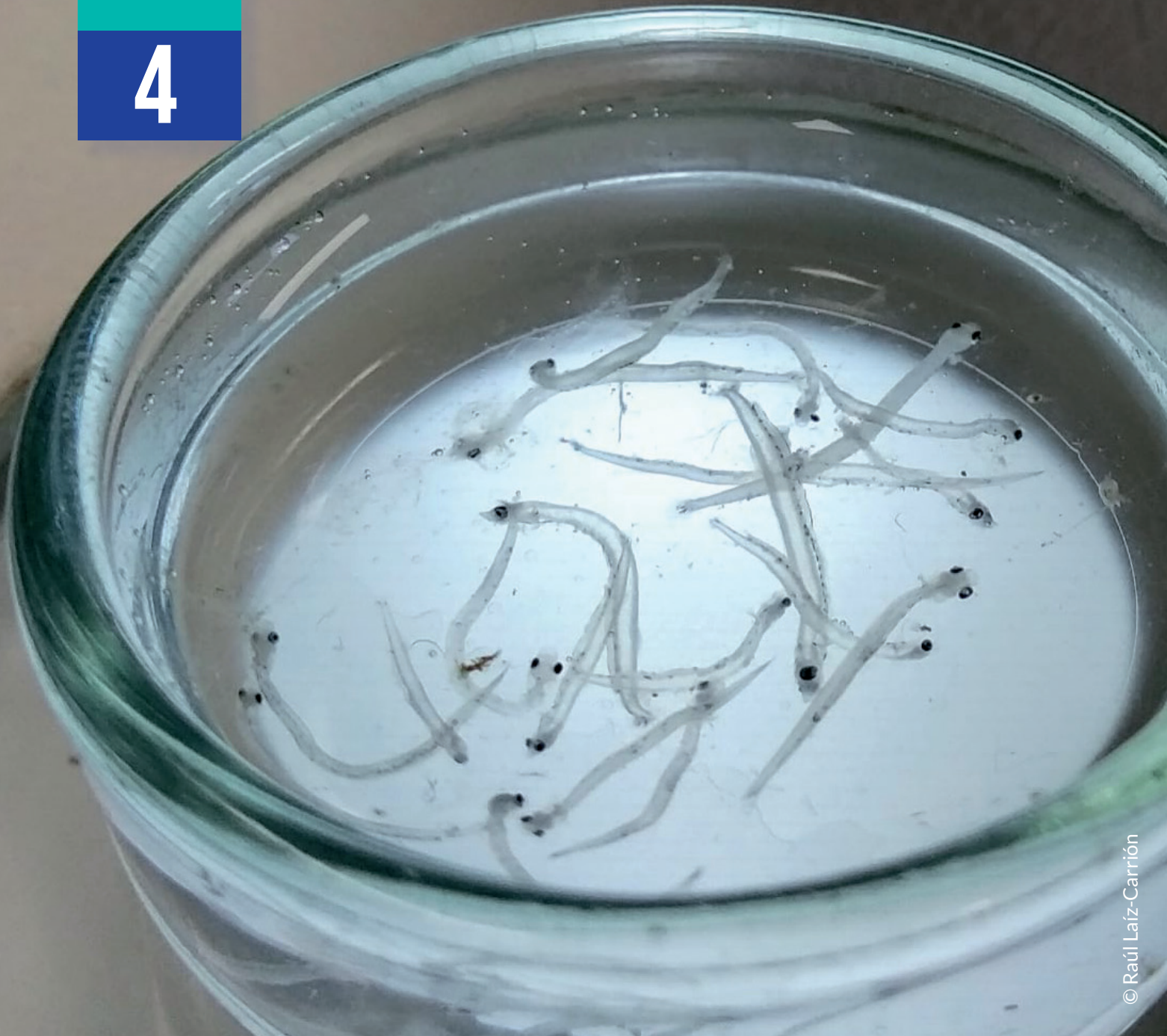




# CHAPTER

# 4

## OUTCOMES OF THE ANALYSES



Stock identification is an iterative process, with each stage having different sampling designs and analytical approaches, and successive stages integrating information from multiple disciplines (Cadrin, 2020). The exploratory stage of stock identification should develop a population structure hypothesis that is consistent with the available information. This first stage was performed for the Transboran project during the project preparation phase by clearly developing these hypotheses and selecting a series of techniques and disciplines to be used. Confirmatory analysis of geographic variation then involves estimating the significance of differences among areas and recognizing, if needed, the relative size effects of each approach (Abaunza, Murta and Stransky, 2014) to identify the most informative variables among the multiple disciplines in view of a final stage of stock differentiation, delineation and composition analyses.

The results of the Transboran project indicate that developing a holistic view of the population structure of harvested species is needed to properly inform and guide scientifically well-founded decisions on the most plausible stock structure for assessment and management purposes (ICES, 2021). The project found that all techniques complemented each other in providing scientific support for the population structure of these species (see the synthesis in Table 7), which was reinforced by the results of the integrative analyses revealing the relevant ecological and evolutionary processes. The Transboran project offered the opportunity to simultaneously analyse species with contrasting life history characteristics and fishery dynamics, broadening the scope of its implications and impact. Nevertheless, beyond general conclusions, an in-depth knowledge of the stock structure is always required at the species level and for a given region. The same species may have contrasting dynamics in neighbouring ecosystems, while two species within the same ecosystem can also display different spatial structures and dynamics.

One of the main differential characteristics relevant to this project is the transitional nature of the study system: the Alboran Sea and its adjacent waters. As discussed below

for each species, results show consistencies with other studies, but also discrepancies, which may be related to the high dynamism of the Alboran Sea compared to other study areas, as well as to its role as an ecological transitional system and biogeographical bottleneck. Strong hydrodynamism, coupled with the ecosystem complexity of the Alboran Sea, affect population dynamics by forcing the development of local adaptations (often needed over a short timescale) to maximize survival and long-term population persistence in a highly stochastic and unpredictable environment (e.g. Hidalgo *et al.*, 2014; Carim *et al.*, 2017). In this context, the implication is that the balance and trade-off between the variation in neutral (adaptive) genetic markers (i.e. those not subject to selection and indicating reproductive isolation) and the variation in functional genetic markers (i.e. those subject to selection and indicating both isolation and adaptation to local environments) must be higher compared to other systems (Mariani and Bekkevold, 2014).

Indeed, differences in neutral genetic characters indicate that populations have been reproductively isolated for many generations, which is far longer than the ecological timescales relevant to stock assessment or fishery management (Waples and Gaggiotti, 2006). Therefore, populations that have been recently isolated or have adapted to local conditions on an evolutionary timescale may not exhibit genetic differences but may rather show demographic independence at ecological timescales (Cadrin, 2020), which is likely in transitional systems such as the Alboran Sea. In addition, a balance between plastic and genetic (i.e. adaptive) components of life history traits might be expected around the Alboran Sea (Felmy *et al.*, 2021), which receives a runoff of species from the Atlantic side (Real *et al.*, 2021), often triggering a high turnover, species replacement and high interspecies life history diversity (Hidalgo *et al.*, 2022b). Hence, it is also fair to assume that, in this biogeographical context, plastic and genetic (i.e. adaptive) components of life history may balance their importance, which is supported by the results of the Transboran project.

## 4.1 Overview by species

### 4.1.1 European hake and sardine

Addressing the main question of the Transboran project – i.e. which is the most appropriate spatial configuration for the assessment and management of the three species

under investigation – scientific evidence is lacking to conclude that European hake and sardine stocks in the northern Alboran Sea (GSA 1) and southern Alboran Sea (GSA 3)

behave as single, homogeneous populations that could support joint stock assessment and management. Instead, sufficient scientific evidence of spatial differentiation between northern and southern stocks in the Alboran Sea was found (Table 7). In the case of European hake, with the exception of genetic markers, all other techniques showed significant differentiation between stocks in GSAs 1 and 3, which is also consistent with the results of hydrodynamic simulations indicating low levels of connectivity between the two areas. A similar pattern emerged for sardine, although for this species the similarity likewise found in otolith core microchemistry indicates that the stocks may share spawning areas with similar environmental conditions but grow up in areas with different characteristics. The spatial differentiation was higher for European hake than for sardine, suggesting that more efficient gene flow and spatial dynamics, shallower early life stage dispersion, and likely migration behaviour occurs in small pelagic species such as sardine.

Another important feature highlighted by the results is the level of differentiation between neighbouring GSAs and

the northern and southern Alboran Sea. With regard to the northern Alboran Sea, the differentiation in European hake between GSAs 1 and 6 was shown by techniques capturing traits at the evolutionary (genetic markers) and life-cycle (parasites and otolith microchemistry) timescales. For sardine, similarities between GSAs 1 and 6 were found in genetic markers and meristics, though not with other techniques. Conversely, in the southern Alboran Sea, the most relevant result from the project was the general differentiation found within GSA 4 for most of the techniques used to investigate these two species (Table 7). Within this GSA, the eastern part of GSA 4 was generally more similar to GSA 12, while the western part was more similar to GSA 3 (see the more detailed species-specific discussion in Sections 4.2.1 and 4.2.2). The connectivity between GSA 3 and the western parts of GSA 4 is also supported by the results of hydrodynamic simulations (see Sections 2.1.1 and 2.2.1 on "Hydrodynamic modelling"). Finally, the similarities between GSAs 6 and 12 for both species, outside the boundaries of the Alboran Sea, reveal some level of connectivity between these two areas apparently mediated by the Almería-Oran Front, as discussed in Chapter 3.

**Table 7. Pairwise geographical subarea comparisons of all techniques used for European hake and sardine**

	Technique							
	Genetics	Parasites	Morphometrics	Otolith shape	Meristics	Otolith (core)	Hydrodynamics	Otolith (edge)
Timescale of trait	Evolutionary	Lifetime	Lifetime	Lifetime	Early life stages	Early life stages	Early life stages	Recent past
<b>European hake</b>								
GSA 1 and GSA 3	✓	✗	✗	✗	✗	✗	✗	✗
GSA 1 and GSA 6	✗	✗	✓	✓	✓	✗		✓
GSA 3 and western GSA 4	✓	✓	✓	✗	✓	✓	✓	✗
Eastern GSA 4 and GSA 12	✓	✓	✓	✓	✗	✓		✓
GSA 6 and GSA 12	✓	✗	✓	✓	✓	✓		✗
<b>Sardine</b>								
GSA 1 and GSA 3	✓		✗	✗	✗	✓	✗	✗
GSA 1 and GSA 6	✓		✗	✗	✓	✗		✗
GSA 3 and western GSA 4	✓		✓	✓	✓	✓	✓	✗
Eastern GSA 4 and GSA 12	✓		✓	✓	✓	✓		✗
GSA 6 and GSA 12	✓		✓	✓	✗	✗		✗

Notes: Check marks indicate that two areas are similar (i.e. no significant differentiation) according to the results of the technique, while X marks indicate that a significant differentiation was found between geographical subareas (GSAs).

Beyond pairwise comparisons between the northern and southern Alboran Sea (GSAs 1 and 3), other meaningful GSA cross-comparisons are presented. Following the results obtained, GSA 4 was divided into an eastern part and a western part. Techniques are ordered from longer timescale scales (left) to shorter timescales (right).

The integrative results also reveal that each independent technique always provides information at two temporal scales: short and long term. In terms of geography, some techniques primarily differentiate between locations at the local scale, with larger scales on a secondary order (i.e. dimensions, e.g. otolith edge microchemistry or body morphometry), while other techniques structure the data the other way around (e.g. genetics). Nevertheless, once the integrative analyses combined information from all techniques, a similar differentiation pattern was indeed found for both European hake and sardine. The first dimension of the integrative analysis was generally associated with long distances and geographic gradients, and thus long-term evolutionary differentiation. By contrast, the second dimension was mainly associated with small-scale differentiation in terms of local adaptation and/or local environmental influences. Furthermore, while the long-term pattern is the same for all techniques and species, the short-term patterns identified for each species can vary for different techniques. This may explain why local adaptations are always associated with the second dimension in the integrative analyses of the two species. This second pattern is, in contrast, prioritized by most techniques and has strong implications for the population dynamics at the inter-annual level that sustains fish assessment (Abaunza, Murta and Stransky, 2014; Cadrin, 2020).

The similarities observed between neighbouring GSAs reveal that differentiation across geographically contiguous

stocks (i.e. GSAs) is not always clear. This is the case for the lack of differentiation often observed for sardine between GSAs 3 and 4, and between GSAs 4 and 12. Indeed, these similarities are often driven by the ports closer to the neighbouring GSAs. In the case of GSA 4, Annaba often shows similarities with GSA 12, while Ghazaouet shows similar signals to GSA 3. The same occurs to some extent with Roquetas in the northern Alboran Sea (GSA 1), which displayed similar signals to GSA 6 for some of the techniques. The example of the Algerian ports suggests that the Almería-Oran Front, which occurs slightly east of Ghazaouet, impedes longitudinal connectivity (i.e. west-east) along the Algerian coast. By contrast, beyond its frontier the Almería-Oran Front can be a connectivity facilitator between the northern (GSA 6) and southern Mediterranean (GSA 12), as has been reported for European hake. Many techniques also show certain Atlantic influences on the Mediterranean, with longitudinal similarities often found between the North Atlantic and the northern Alboran Sea, and between the South Atlantic and the southern Alboran Sea. However, little evidence was found to suggest any demographic (i.e. stock) connection between Atlantic and Mediterranean populations that could have implications for population or stock dynamics. The transitional role of the Gulf of Cádiz–Strait of Gibraltar–Alboran Sea transition area is well known (Real *et al.*, 2021), and results reveal how the Atlantic–Mediterranean transitions operate differently for each species investigated in this project.

### 4.1.2 Blackspot seabream

In the case of blackspot seabream, no clear signal of population structure was found, with the genetics results revealing an unexpected absence of genetic structure over large geographic distances. However, hydrodynamic simulations revealed regional differences for the recruitment process subject to the geographic location of spawning within the Strait of Gibraltar, the tidal strength and the depth of spawning. By contrast, otolith microchemistry was able to

reveal current spatial differences through the analysis of the otolith edge, associated with the last period of life of individuals, while such differentiation was not found in the otolith core, suggesting potential migration to the Strait of Gibraltar for spawning, a question that requires further research.

## 4.2 Detailed results by species

### 4.2.1 European hake

Of all the techniques used to analyse European hake, only genetics suggest an absence of differentiation between the northern and southern Alboran Sea, which reveals a latitudinal gradient and long-term genetic drift as the primary pattern, a result expected for all species transitioning

between the Atlantic and the Mediterranean through the Strait of Gibraltar. This is consistent with the gradient retrieved in the first dimension of the integrative analysis, largely associated with the southern Mediterranean as the main pathway for long-term connectivity between the



Atlantic and the Mediterranean (Figure 40). By contrast, the north–south differentiation, as consistently revealed by the other techniques, emerges as the secondary pattern in the integrative analysis, with a similar amount of variance explained by the first (31 percent) and second (23 percent) dimensions of the integrative analyses (Figure 33, Chapter 3). It can be argued that this order is caused by each technique being sensitive to different local differences (not only the northern–southern Alboran Sea), while the long-term pattern is the same across all techniques.

Thus, the grouping of GSAs in response to local adaptation depend on each technique. For instance, similarities between GSAs 4, 6 and 12 in otolith core microchemistry may be associated with the connectivity facilitated by the Almería-Oran Front. In contrast, similarities between GSAs 1 and 6 and the North Atlantic may be associated with regional environmental variability and certain demographic connections, particularly between Roquetas, in GSA 1, and GSA 6.

**Figure 40.** Schematic biplot (left) and geographic (right) representations of the grouping, differentiation and connectivity patterns for European hake according to the integrative analyses



Sources: Right panel base map redrawn from UN, 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Notes: The left-hand biplot shows the differentiation and connectivity patterns on the biplot of the integrative analyses.

In the right-hand map, the green line shows a long-term differentiation gradient mainly associated with the southern Mediterranean, while the continuous red line shows a regional segregation of the northern Mediterranean geographical subareas (GSAs) 1 and 6. Potential secondary connectivity between GSA 1 and the North Atlantic and between GSAs 4, 6 and 12 are shown by the dashed red lines.

Comparisons of Transboran project results with those of other studies analysing European hake stock structure and connectivity patterns provide consistent but also contrasting patterns. For instance, hydrodynamic simulations indicate the important role of self-recruitment beyond north–south connectivity, which is consistent with recent studies on European hake in GSAs 6 and 7, demonstrating that self-recruitment off the Catalan coast is critical for recruitment success in the whole of GSA 6 (Hidalgo *et al.*, 2019). Otolith microchemistry studies on European hake are often able to detect large-scale spatial differences (e.g. Tanner *et al.*, 2012, 2014), while often failing to detect regional differences (i.e. within the Mediterranean basin scale; Morales-Nin *et al.*, 2014), though they are sometimes observed through stable isotopes (Hidalgo *et al.*, 2008).

Transboran otolith information, as well as parasites and meristics, suggest spatial segregation upon arrival to the nursery areas consistent with other complex stocks of other gadoid species such as cod (*Gadus morhua*) (e.g. Wright *et al.*, 2006, 2021). Multiple studies on European hake genetics also report large-scale differences (North Atlantic–South Atlantic, Atlantic–Mediterranean, eastern Mediterranean–western Mediterranean; e.g. Cimmaruta, Bondanelli and Nascetti, 2005; Tanner *et al.*, 2014; Leone *et al.*, 2019), while all of them suggest regional movement of individuals (e.g. between northern and southern hake stocks in the Atlantic). This is consistent with Transboran genetics results, as they also detect regional differences over shorter distances (i.e. between the Alboran Sea and the eastern Mediterranean) compared to other studies, but not between the northern and southern Alboran stocks.



Regional-scale movements have been reported in many other European hake stocks, both in the Atlantic and the Mediterranean (e.g. Tanner *et al.*, 2014; Leone *et al.*, 2019; Morales-Nin *et al.*, 2014; Hidalgo *et al.*, 2019). While Galindo-Ponce (2021) suggests that movements of adults between GSAs 1 and 3 are unlikely when comparing stock assessments, these movements cannot be disregarded when considering the whole picture of the Transboran results. However, it is not always clear to what extent these movements have implications for the population dynamics of neighbouring (sub)populations. For instance, integrative analyses and some other techniques suggest similarities between GSAs 1 and 6. Nevertheless, it is well known that the main spawning season, and thus the recruitment window that mostly contributes to the annual recruitment, are seasonally opposed between GSAs 1 and 6 (Hidalgo *et al.*, 2009; El Yaagoubi, 2019; Galindo-Ponce, 2021). This situation makes it highly unlikely that these contiguous stocks demographically interact on an interannual basis. In fact, the differences in genetic markers between European hake sampled in GSAs 1 and 6 corroborate the hypothesis of separated stocks in this area of the Alboran Sea (see Section 2.1.2 on "Genetics"). Indeed, if they are merged as a single stock, there is a risk of creating a misperception of the stock dynamics, which would have even more negative consequences given the state of overexploitation of all European hake stocks in the Mediterranean. It is known that geographic variation in size or maturity-at-age can delineate groups with limited mixing, regardless of the degree of heritability in those traits (e.g. Begg, 2004; Cadrin, 2010). Temporally stable patterns of life history variation are particularly relevant to determining spatial structure for stock

assessment, because population dynamics models need to accurately represent these vital rates: this is the case in the spawning phenology of European hake, indicating a population boundary between GSAs 1 and 6.

The recruitment–spawning stock relationship is the demographic cornerstone by which ecological processes affect stock assessment. Thus, sustaining the meaningful functioning of this relationship is also a major element to consider before making the important decision to merge (or not) population components as a single stock. As a part of the Transboran project, different assessment scenarios (i.e. merging and not merging northern and southern stocks in GSAs 1 and 3) were performed (Galindo-Ponce, 2021). Results pointing in the same direction as the indicators of the stock status are buffered and provide a more optimistic situation for GSA 3 when the two subpopulations are assessed jointly as a single stock. However, as the movement of individuals (mainly premature and mature individuals) cannot be disregarded for this species, the occurrence of other more complex spatial structures, and their implications for stock assessment, should be further explored in the future. As potential future lines of research, spatial population dynamics simulations have proven to be a useful tool for assessing the implications of alternative population structures on assessment outcomes and biological reference points, including movements across management subareas (e.g. Cadrin, 2020; Goethel and Berger, 2017; Goethel *et al.*, 2021). Indeed, spatial stock assessment is a field under continuous development (Punt, 2019), and it could be further explored for several Mediterranean stocks of European hake in the future (Hidalgo *et al.*, 2019).

#### 4.2.2 Sardine

The general pattern of differentiation for sardine observed through both the integrative analyses and the independent techniques is similar to that observed for European hake. However, the degree of differentiation is comparatively lower in sardine due to the more dynamic behaviour of this species and its likely (though still unknown) regional migrations. The first dimension of the integrative analysis is also related to the large distance and long-term gene flow (explaining 28 percent of variance), while the second dimen-

sion is related to regional and north–south differentiation. In particular, the southern Alboran GSAs 3 and 4 are more clearly separated from the rest of the areas that display a longitudinal gradient over the northern Mediterranean, from the Atlantic to the eastern Mediterranean (Figure 37, Chapter 3). However, several techniques show certain similarities between the South Atlantic and the southern Alboran Sea (GSAs 3 and 4) and between GSAs 4 and 12 (Figure 41).

**Figure 41.** Schematic biplot (left) and geographic (right) representations of the grouping, differentiation and connectivity patterns of sardine according to the integrative analyses



Source: Right panel base map redrawn from UN. 2024. United Nations Geospatial Clear Map. In: *United Nations*. New York, USA. [Cited 6 May 2024]. <https://geoservices.un.org/Html5Viewer/index.html?viewer=clearmap>

Notes: The left-hand biplot shows the differentiation and connectivity patterns on the biplot of the integrative analyses.

In the right-hand map, the continuous red line shows a long-term differentiation gradient mainly associated with the northern Mediterranean, while the green line shows a regional segregation of the southern Mediterranean geographical subareas (GSAs) 3 and 4. The dashed red lines represent the most likely connections in light of the results of the independent techniques and those of the integrative analyses.

Of all the techniques used, genetics and otolith core micro-chemistry did not find any differential structure between the northern and the southern Alboran Sea, with otolith shape revealing only a weak structuring. The other techniques found differences between the northern and southern Alboran Sea. However, the final differentiation for the whole system is moderate, as also indicated by the SDI. Indeed, the SDI between GSA 1 and GSAs 3 and 4 or between GSA 1 and the Atlantic areas is also moderate, while the differentiation between GSAs 3 and 4 and the South Atlantic is low, consistent with the integrative multivariate analyses (Figure 37, Chapter 3, and Figure 41). This strong similarity between GSAs 3 and 4, with a moderate connectivity pattern towards GSA 12, is the main signal of regional differentiation and adaptation for sardine compared to the other areas. Thus, a potential complex population structure along this southern margin of the Alboran Sea seems most plausible compared to the north–south connections, though the latter cannot be disregarded.

The lack of differentiation observed in the genetics analyses over the whole area (with the exception of the South Atlantic) is generally consistent with most studies, and it reflects the results obtained from the multiple techniques used in the Transboran project. This is likely due to the narrow geographical area for investigating spatial

differences between the Atlantic and the Mediterranean, the transitional nature of the Alboran Sea, and the lower capacity of some of the genetic markers used to differentiate between adaptive and neutral genetic variation (the latter being highly important in sardine and this dynamic system and for understanding the results within a multi-scale and multidisciplinary framework). Recently, Antoniou *et al.* (2021) analysed genomic data of sardine populations from the northwest Atlantic and the northern Mediterranean, detecting Atlantic–Mediterranean differentiation yet also demonstrating the existence of a transitional area in the Alboran Sea. The results of the Transboran project are consistent with this picture, though they add a different role for the southern Mediterranean areas that had been omitted in previous large-scale studies, showing how these southern areas connect with northern areas and the Atlantic regions (this differs for different species, e.g. between sardine and European hake).

The role of the Almería–Oran Front for structuring sardine populations appears consistent with previous literature on sardine and other species (e.g. Bargelloni *et al.*, 2003; Abaunza *et al.*, 2008; Antoniou *et al.*, 2021), suggesting that the Almería–Oran Front is the main connectivity barrier between the Atlantic Ocean–Alboran Sea transition area and the rest of the Mediterranean. The similarities

reported by Transboran results between GSAs 1 and 4, but not with GSA 6, support this interpretation, and this dynamic contrasts with the facilitation role of the Almería-Oran Front indicated for European hake.

The results for sardine show a likely opposing influence between migration, selection and local adaptation. Regional migrations along coastal areas have been reported in many clupeid species, including Atlantic (sub)populations of sardine (Jardim *et al.*, 2018; Silva *et al.*, 2019). The weak differentiation in otolith core microchemistry, in addition to the Almería-Oran Front effect detected in otolith edge microchemistry between GSAs 1 and 4, suggests potential regional migrations, which was also observed through genetics and the analysis of numbers of migrants. Furthermore, similarities detected between the Atlantic and Mediterranean areas, but separating the northern and southern regions (e.g. body morphometry), indicate potential migration across the Strait of Gibraltar. Besides that, the results of the integrative analysis and the independent techniques suggest that a metapopulation structure, understood as a group of spatially separated populations of the same species that interact at some level throughout their life cycle, could be in place in the Alboran Sea, as has been reported in Atlantic regions (Jardim *et al.*, 2018; Silva

*et al.*, 2019). Assuming that this could be the case, further studies are needed to understand the demographic role of different subpopulations (e.g. source or sink), as this can affect which management measures should be proposed (Silva *et al.*, 2019). As recommended for European hake, simulation studies could help to assess the implications of alternative population structures on assessment outcomes and biological reference points (Cadriñ, 2020).

Finally, there are elements that have not been considered in the project but are nevertheless important in deciding how to delineate sardine stocks. For instance, data collection should be prepared and harmonized across all locations contributing to the stock. If data collection is not harmonized, it may bias the assessment. Therefore, adopting spatial sampling designs and stratified estimates according to the inputs required for the assessment models must be encouraged (Cadriñ, 2020). Likewise, management schemes must be prepared and aligned to the assessment outcomes and the spatial structure of the stock in question. From the oceanographic simulations perspective, the influence of the interannual variability of the Alboran Sea circulation should be further investigated as it could affect estimated connectivity rates (Quattrocchi *et al.*, 2019).

### 4.2.3 Blackspot seabream

The three disciplines applied to blackspot seabream cover the three spatio-temporal scales that generally inform stock identification (Table 1, Chapter 1): large scale (genetics), middle scale (otolith microchemistry) and local (hydrodynamic dispersal simulations). The outcomes of these techniques are highly complementary but are difficult to integrate into a single analysis. At the large scale, genetic studies show unexpected genetic homogeneity (i.e. lack of genetic structure) over a very large geographic distance, from northern Spain in the Atlantic Ocean to the Ionian Sea in the eastern Mediterranean, with the highest genetic diversity found at the individual level. This result could suggest an exposure to a diversity of habitats over the blackspot seabream life cycle and high dispersal and/or migration rates over larger distances. Recent studies report similar conclusions, combining samples from northern France in the Atlantic to the Alboran Sea, with the only difference being between the Azores and other samples (Robalo *et al.*, 2021). The marked genetic structure between the Azores archipelago and Atlantic continental locations is not new and corroborates the results obtained by Stockley *et al.* (2005). The differentiation of an Azorean population

has been found in many other species, such as black scabbardfish (*Aphanopus carbo*) (Stefanni and Knutsen, 2007).

On the other hand, while no evidence of large geographical migrations over the continental shelf have been reported for blackspot seabream, other species such as Atlantic pomfret (*Brama brama*) display migrations of this kind in the same region between spawning and feeding areas (Quinzán *et al.*, 2016). Otolith microchemistry analyses suggest that these potential migrations may also take place at intermediate scales. While large spatial segregation of the sampling is observed in otolith edge microchemistry, the lack of segregation shown by otolith core microchemistry might point to a spawning location with a common environmental signal, consistent with the hypothesis that the Strait of Gibraltar is the site of a common spawning aggregation.

At short scales, hydrodynamic simulations do not clarify the stock structure of blackspot seabream. However, they reveal important and novel insights (Nadal *et al.*, 2022). The eastward transport by the Atlantic Jet exiting the Strait of Gibraltar is the process with the greatest influence on

dispersion from the spawning locations in the strait. This dispersion always occurs over the Alboran Sea, but not towards the Gulf of Cádiz. While the Atlantic Jet has been assessed as an oceanographic feature that hinders meridional transport between the northern and southern Alboran Sea (Garcia-Lafuente *et al.*, 2021), it is certainly the main driver of ELS dispersion throughout the Alboran Sea. Gil, Silva and Sobrino (2001) suggest that the most plausible scenario is that there are interconnected cycles of recruitment of individuals settling in nursery areas on both sides of the Alboran Sea, followed by juveniles returning to the grounds where the fishery is active. Dispersion experiments and larvae found during the Transboran survey support this hypothesis. The selection of the highly dispersive environment of the Strait of Gibraltar as a spawning ground makes the hypothesis that blackspot seabream attempts to maximize self-recruitment unlikely – opposed to what can be observed in the other two species investigated in the Transboran project, particularly European hake.

A second important element is the key role that tidal cycles play in the dispersion of blackspot seabream. Fortnightly modulation is the prevailing factor determining the horizontal paths, with spring tides causing the greatest scattering. This suggests that the species might be adapted to

make the most of the tidal cycle in order to maximize larval dispersal over the Alboran Sea, as the specific instant of spawning in a given semidiurnal cycle (tidal phase) affects the initial ELS dispersion trajectories at the very beginning of the spawning (Nadal *et al.*, 2022). Another major element is the depth of spawning, although its overall influence on connectivity is less marked than that of tides and it does not exhibit a regular pattern. Nevertheless, for a given tidal condition, the patterns associated with releasing eggs at the surface would differ from those at depth in a similar way to how patterns produced during spring tides differ from neap tides, thus affecting the relative proportions of ELS dispersed towards the northern and southern Alboran Sea, respectively (Nadal *et al.*, 2022). Finally, the study also reveals that the geographic location of the spawning, i.e. to the north (towards Tarifa) or south (towards Tangier) of the Strait of Gibraltar, may also be relevant for the final location of dispersed ELS. Therefore, while no stock segregation has been attributed to blackspot seabream, the dispersion experiments reveal that the final seascape of dispersed particles (without considering survival rates and larval behaviour) results from the significant interactions between the geographic location of the spawning, fortnightly tidal variability, and depth of spawning.



# CONCLUSIONS

The multidisciplinary approach of the Transboran project enabled the identification of the mechanisms, at different spatio-temporal scales, structuring the populations of the three studied species: European hake, sardine and blackspot seabream. Almost all techniques, along with integrative analyses, provided information on two main structuring mechanisms for the three species: i) longitudinal genetic drift over the long term; and ii) local environmental influences and spatially structured demographic processes at shorter temporal scales. The transitional nature of the Alboran Sea, as well as its high ecological and oceanographic dynamism, contribute to balancing the roles of long-term differentiation (not subject to selection) versus adaptation to the local environment in the spatial structuring of the three species.

Differences have been found in European hake and sardine coming from the northern Alboran Sea (GSA 1) and southern Alboran Sea (GSA 3). Differentiation in European hake between the north and south was stronger than for sardine, although it was still remarkable for the latter species when compared to the high similarity observed for sardine across the southern Alboran Sea (from the North Atlantic through GSAs 3 and 4 to GSA 12).

In terms of the dispersal of eggs and larvae, European hake is strongly influenced by self-recruitment during ELS and exhibits spatially segregated recruitment dynamics in different nursery and feeding grounds. This makes the north-south differentiation of higher importance than longitudinal differentiation between neighbouring subregions. The results for sardine, which is a pelagic species, suggest complementary influences of migration, genetic drift and local adaptation to environmental conditions.

For both European hake and sardine, the similarity observed between neighbouring GSAs revealed that differentiation across longitudinally contiguous stocks (i.e. GSAs) is not always clear. This is the case, for example, with the lack of differentiation often observed for sardine between the eastern part of GSA 3 (Morocco) and the western part of GSA 4 (Algeria), and between the eastern part of GSA 4 and

the western part of the GSA 12 (Tunisia), which are mainly associated with the ports closer to the neighbouring GSAs. Meanwhile, the Almería-Oran Front acts as a connectivity barrier for sardine, while it appears to operate as a connectivity facilitator for European hake, particularly connecting northern areas (GSA 6) with the southeast (GSA 12), likely through potential active movements.

The genetic studies on blackspot seabream showed unexpected genetic homogeneity (i.e. a lack of genetic structure) over a very large geographic distance from northern Spain in the Atlantic Ocean to the Ionian Sea in the eastern Mediterranean, with the highest genetic diversity found at the individual level. The eastward transport by the Atlantic Jet exiting the Strait of Gibraltar is the process with the greatest influence on the dispersion of ELS blackspot seabream from spawning locations in the strait to areas across the whole Alboran Sea, with the final seascape of dispersed particles determined by strong interactions between the geographical location of spawning, i.e. to the north (towards Tarifa) or south (towards Tangier) of the Strait of Gibraltar, fortnightly tidal variability (higher during spring tides), and the depth of spawning. Contrasting spatial segregations in blackspot seabream otolith microchemistry suggest potential spawning migrations to the Strait of Gibraltar and high connectivity of this species between the Atlantic and the Mediterranean compared to European hake and sardine.

In summary, considering all disciplines in an integrative manner, the Transboran project did not find scientific grounds to conclude that European hake and sardine in the northern and southern Alboran Sea behave as single and homogeneous populations, with GSAs 1 and 3 proving to be among the most differentiated pairs of management areas for both species. Therefore, the suggestion is to maintain, for assessment and management purposes, the current configuration of European hake and sardine stocks in the northern (GSA 1) and southern (GSA 3) Alboran Sea as separate units.





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The boundaries of commercially exploited stocks in the Alboran Sea in the western Mediterranean have long been the object of scientific debate, due to gaps in knowledge of the spatial structure and connectivity of fish populations in the area. In an effort to resolve some of these questions, the “Transboundary population structure of sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters” (Transboran) research project, a collaborative project led jointly by FAO-Copemed II and the GFCM, ran for three years between 2017 and 2021. It aimed to describe the spatial structure of sardine (*Sardina pilchardus*), European hake (*Merluccius merluccius*) and blackspot seabream (*Pagellus bogaraveo*) populations in the western Mediterranean to help inform the best possible management advice for these species.

Seven research institutions from five partner countries (Algeria, Italy, Morocco, Spain and Tunisia) joined forces on a comprehensive and multidisciplinary approach that covered different techniques for stock identification and was complemented by numerical modelling of hydrodynamic dispersal, spatial information on the fleets targeting the studied species, and field data from an ichthyoplankton survey. All of these disciplines provided information at various contrasting scales to take into account life-cycle, demographic and evolutionary processes.

This report presents the main findings of the Transboran project, which show differentiation of sardine and European hake stocks between the northern and southern Alboran Sea and reveal a homogenous population structure for blackspot seabream across the study area. The results therefore support maintaining the current configuration of stocks within their respective geographical subareas for assessment and management purposes.

ISBN 978-92-5-138856-3 ISSN 1020-9549



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CD1122EN/1/06.24