Food and Agriculture Organization of the United Nations

COPEMED II

# CONCEPT NOTE

## Transboundary population structure of Sardine, European hake and Blackspot Seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach

## (Acronym: **TRANSBORAN**)

# INTRODUCTION

Recent research on fish stock identification methods have revealed inconsistencies between the spatial structure of biological populations and the definition of stock units used in assessment and management. There is common agreement that this mismatch emerges from a lack of acknowledgement of the population spatial structure of harvested species. Indeed, it is now clear that the population structure of marine species falls along a continuum from panmictic to numerous distinct populations, with the majority of species exhibiting complex structure within this range.

Historically, stock delimitation has followed a top-down approach, in which management stewardships decide the stock boundaries attending mainly to political reasons and, consequently, fisheries and assessment modelers adapt their work to these boundaries. However, there are currently vast research evidences showing that stock delimitation should follow a bottom-up approach in which the stock delimitations should be based in scientific evidences. This inherently requires multidisciplinary and holistic approach.

Although in the last years some studies were published overall specific studies on modelling eggs and larval drift from spawning to nursery areas were still lacking In the Mediterranean. These studies are considered essential to investigate connectivity within and among stock units in a modern meta-population paradigm.

Based on all these previous studies, and at the request of Scientific Advisory Committee of the GFCM to deepen in the structure of fish populations, the FAO Regional Project CopeMed II, organized a workshop to propose a specific research program to identify the stock structure and boundaries of Sardine and European hake inhabiting the Alboran sea and the adjacent waters. The workshop was held from 3-6 of April 2017 in Alicante and the group of experts discussed the existing data and methodologies and agreed to undertake a study of two years[1] (extendable according to the availability of funds) using different techniques following a holistic approach.

[1] The proposal of this project, originally designed for two species (sardine and hake) was presented to the Scientific committee of the GFCM in May 2017 which expressed interest and suggested to add a third species to the study: *P. bogaraveo* given that it is a priority species in western Mediterranean. The proposal was accepted by the 10th meeting of the CopeMed II Coordination Committee which gave green light to the project on the 31st of October 2017 to be developed between 2018 and 2019 funded by CopeMed II and by the GFCM Mid Term Strategy.

The proposal of this new project with details of the methodology by the different project components are reported hereunder.

The proposed project aims:

- To investigate the spatial population structure and to identify the most plausible stock units of Sardine, European Hake and blackspot Seabream in the Alboran Sea according a multidisciplinary approach. The project output will reveal whether the current GSA boundaries are the appropriate spatial scale of assessment and management for these three species.;

The methodology will follow these steps, (not necessarily in chronological order):

- to examine existing information on the stock structure of Sardine, European Hake and blackspot Seabream in the Alboran Sea and adjacent areas, including upstream the Atlantic Ocean close to the Gibraltar Strait and downstream the portion of the Western Mediterranean more directly affected by the current entering from the Strait of Gibraltar.
- to propose an *ad hoc* sampling program for a minimum of two years in which the same individuals will serve to different type of analysis (*i.e.:* genetics, parasites, morphometry, isotopes).
- to model the movements of the different life stages of fish coupled with the hydrodynamics of water masses in Alboran area, and compare the outputs with observations of the two species (i.e. surveys, cpue , assessment indicators).
- to analyze the information produced with a multidisciplinary and holistic approach.

One of the main themes in the identification of stock structure and boundaries is the strength of interdisciplinary analyses when they are developed across national boundaries. In the last decades, a lot of methods have been developed and proposed as proper ways to deal with stock identification. However, applying different approaches may lead sometimes to competing hypotheses because different methods are sensitive to show differences at contrasting spatial (from local to regional) and temporal scales (from daily to evolutionary scales). However a general agreement exists on the need to adopt a holistic approach with multiple perspectives to improve information on stock structure for resource management that capture different ecological and structuring processes acting at contrasting scales.

According to Cadrin et al. (2014) the process for multidisciplinary identification of the most likely population structure and recommendations for the most appropriate management units requires a review of available information, synthetic conclusions, and practical considerations for management. Moreover, the whole process must adhere to principles of best scientific information available, which should be relevant, inclusive, objective, transparent, timely, verified, validated and peer-reviewed (Cadrin *et al.,* 2014). However, from a practical perspective, the final management approach used to address misalignments also depends on the complexity of the implementation procedures, which are constrained by available knowledge of the population structure of the species (Kerr et al. 2017, Hidalgo et al. 2017).

Following Cadrin et al. (2014), the stock identification process can be summarized in 6 steps

1. A clear definition of the current spatial management units.
2. The identification of all *a priori* hypotheses and paradigms about the population structure, and their internal consistency.

3. Comprehensive information of the specific fishery resource, with emphasis on data and results arising from research explicitly intended for stock assessment.
4. An interdisciplinary evaluation, whose goal is an integrated and holistic knowledge of the life history of the stock.
5. A statistical revision, selecting and using all the information useful for rigorous testing, followed by simulations inside the area and in view of its neighboring connectivity (Kerr and Goethel, 2014).
6. All the necessary recommendations for practical units which reflect not only the stock biology but also other aspects of managing and monitoring its fishery. This includes adopting a different spatial scale for assessment and for management; for instance, a whole region conducting a unique assessment while small management areas apply context- dependent management measures.

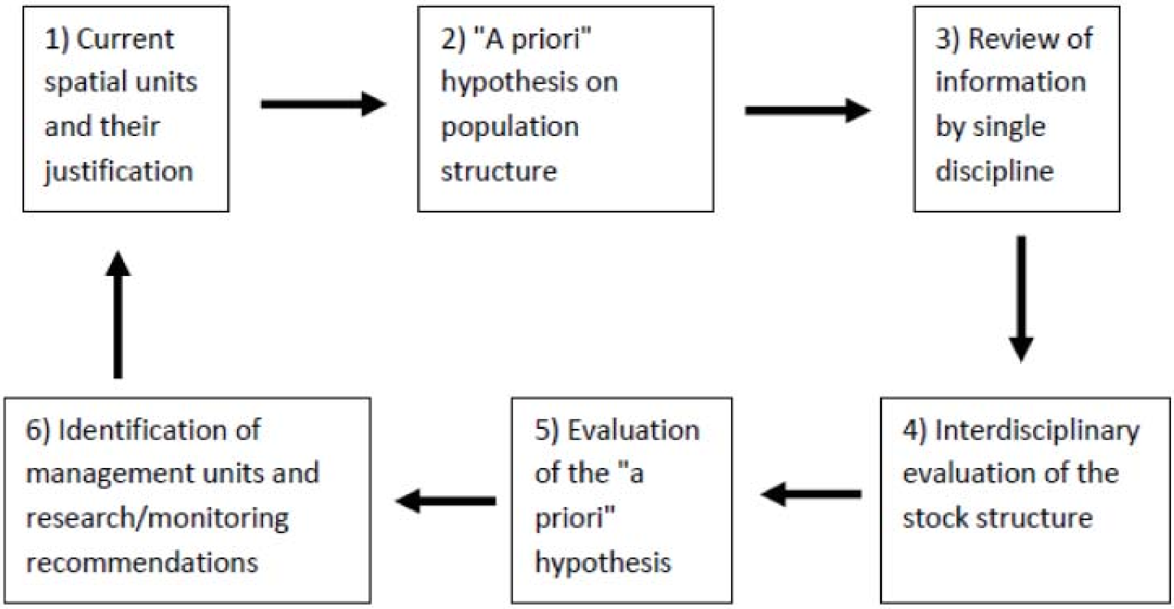


Figure 1 - The logical framework in stock identification followed by SIMWG of ICES. (drawn from Cadrin *et al.,* 2014).

The 3 existing GSAs in the Alboran Sea (1,3 and 4) will represent the "*a priori*" stock hypothesis to be tested. Information coming from single disciplines will be collected and scrutinized with the objective to be analysed by a holistic approach similar to the framework proposed by the STOCKMED project. Differently from the STOCKMED project that was based only on existing data, and similarly to HOMSIR project an *ad hoc* sampling scheme will be designed to collect new data and samples. Sampling will be designed by the experts on an initial phase with the purpose to cover the spatial and temporal scales of the life cycle of the two-species concerned. The same individuals will provide biological material for the different analysis. The disciplines involved are: hydrodynamic connectivity, genetic markers, parasites as markers, otolith shape and elemental composition, body morphometry and meristics, analyses of fishery patterns, demographics indices and life history traits, and isotopes analyses.

The main phases of the project are outlined in the section below. The last section of this document: Project Components includes technical sheets produced by different groups of experts in the different disciplines during the CopeMed II workshop and represent the preliminary description of methods, resources and costs in a more detailed way.

The theoretical spatial concept of the project can be represented in figure 2.

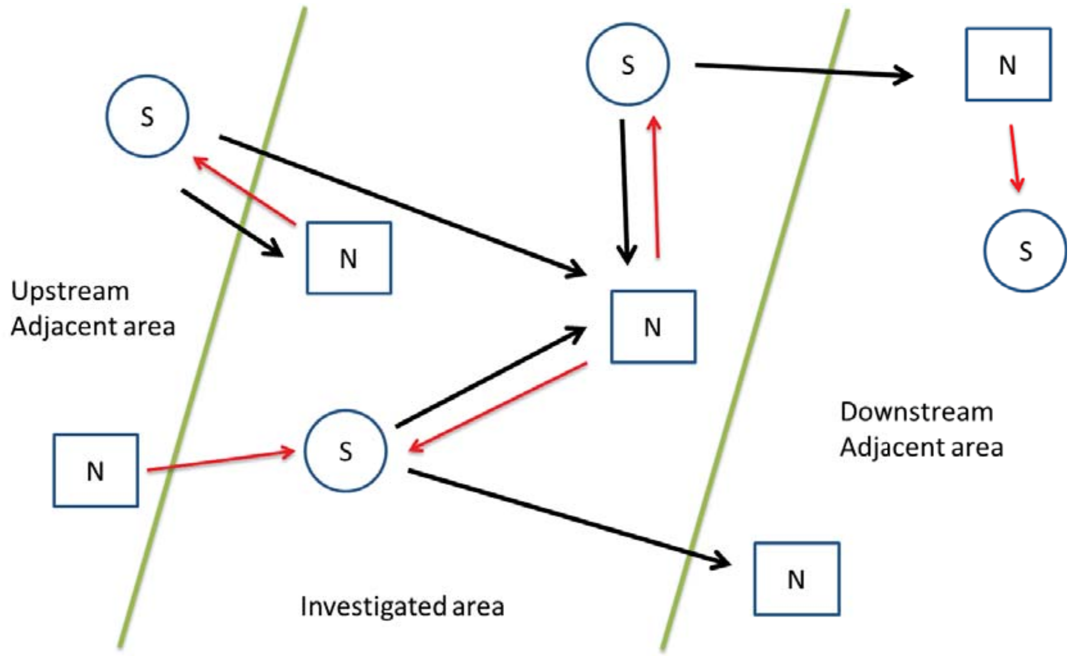


Figure 2: the source-sink dynamics of a species during its life cycle in an area and its adjacent upstream and downstream vicinities. **S**: spawning area; **N**: nursery area; **red arrows**: adults displacements; **black arrows**: larvae displacement towards the nursery areas. (from Fiorentino *et al*., 2017)

**PHASES OF THE PROJECT:**

**Modelling**

A hydrodynamical-biological model describing the movements of simulated particles in the Alboran sea will be used to simulate the dispersal and/or retention of eggs and larvae and examine both potential and effective connectivity between the northern and southern continental shelves of the Alboran basin and the vicinities.

Metrics of connectivity will be calculated for defined areas and periods that reproduce the spawning to the recruitment of each species and the outputs will be compared with observations from surveys and landings. A series of years of data will be selected per specie including contrasting years of high and low abundance as well as contrasting oceanographic conditions to test the model outputs.

**Sampling**

Experts from different disciplines will work jointly according to a determined sampling strategy that will cover the different spatial and temporal scales needed for each analysis.

The necessary number of fish will be collected during the regular sampling programs (surveys and landings) in the four countries: Algeria, Morrocco, Spain and North Tunisia. The biological material will be processed and analysed, or sent to the laboratories responsible for the different analysis: genetic markers, parasites, otoliths shape and elemental composition, stable isotopes, body shape and meristics of hard structures.

**Information Processing**

- Spatial analysis

The information produced in the steps above together with all data compiled referred to the fisheries indicators, biological parameters and demographic and spatial metrics will be collated and processed with integrative statistical tools.

- Multivariate statistics:

Multivariate statistical techniques as the Multi Criteria Decision Analysis (MCDA) will be used at a later extent. These techniques include: selection of best descriptors, assignation of weights by Non-Structural Fuzzy Decision Support System - NSFDSS, perform integration of multiple thematic descriptors by the Cohen's Kappa coefficient of agreement, perform sensitivity analysis by the Stochastic Multi-criteria Acceptability Analysis -SMAA-. This exercise of synthesis will help in the elaboration of alternative hypothesis of stock boundaries.

**Proposal of stock units**

The final proposal of new possible configurations of stock units will be integrated with maps of distribution of fishing effort (by fleet segment). The areas where new stock units have been identified will be compared with the current configuration of GFCM GSAs.

The final aim is to describe the spatial distribution of the resources in the most precise way to be applied in the regular assessments and to inform the management advice for Sardine, European Hake and blackspot Seabream in Western Mediterranean.

# The Project Components

The study area will cover the Alboran sea and adjacent waters including the Atlantic Ocean and southern Mediterranean coast up to northern Tunisia trying to cover the whole distribution area of the two species selected (Sardine, European Hake and blackspot Seabream) with the aim to better understand the movements of fish during all their life cycle. Samples will be taken from a set of locations well distributed along the whole study area in periods that cover the different life stages. The same specimens will serve to take different measures, otoliths and tissue samples for different analysis: genetic, elemental composition, isotopes and parasites. In addition, time series from fishery and surveys data will be incorporated.

Scientific teams have been created to oversee each one of the project components (or research lines) established. The outlines of these research lines and resources existing and needed are summarised below:

## 1. HYDRODYNAMIC CONNECTIVITY

This task will focus on the description of the hydrodynamics features that shape the connectivity between spawning and nursery areas in Alborán and its source and sink outer areas. The hypothesis generated by this model will be validated by other methods.

The study will be based on a hydrodynamical-biological coupled model (ocean physics plus lower trophic ecosystem component) that will cover both Gulf of Cadiz and Alboran Sea (until the Gulf of Vera in the north and 1º East in the south) (Figure 1). At temporal scale, the model will solve from tidal to inter-annual scales. This work is already well advanced by the Physical Oceanography group of the University of Málaga can simulate the drifting of fish larvae in both space and time through Lagrangian drifter experiments. The experiments will be conducted during the spawning season of each species. Phytoplankton, zooplankton and sea water temperatures, which are too variables provided by the model, will be used to further assess the probability of larvae survival along their drift (e.g. starvation mortality). The model has a mean spatial resolution of 1.5 km in the Alboran Sea, which enables for analysis of sets of cells up to the 30’x30’ statistical grid.

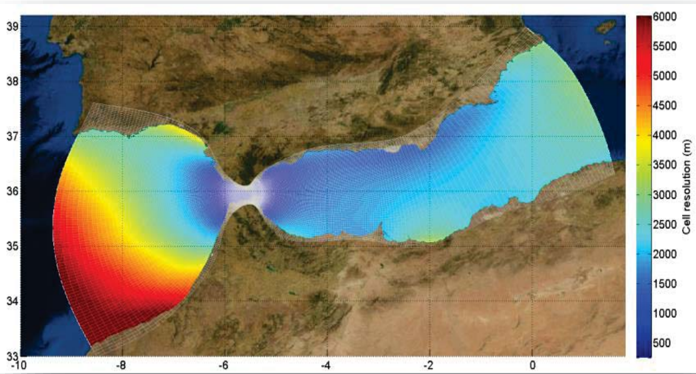


Figure 1. Geographic domain of the hydrodynamical-biological coupled model.

Demographic and size information from acoustic (sardine) and trawling (hake and blackspot Seabream) surveys and landings will be collected for as many years as available in the most recent period. Connectivity and self-recruitment estimates derived from the hydrodynamical-biological coupled model will be obtained based on tools proposed by Dubois et al (2016) and Hidalgo et al (in review), and statistically compared with demographic information obtained from scientific surveys and landings.

Samples of larvae and adults (for sardine) and recruits (for hake and blackspot seabream) will be collected for two consecutive years (2018 and 2019) and analysed with stable isotopes techniques to validate and adjust the hydrodynamical-biological coupled model.

## Existing resources

The University of Malaga (UMA) will use its supercomputing resources for the model simulations, other instruments for physical measurements could be used if required.
IEO will collect samples from the different sampling sites during its current surveys and will perform isotope analyses of organic tissues and otoliths.

## Methods and needs.

The bio-physical modeling will have two steps:

1. Selection of four years per species with enough contrast of high and low abundance, but also contrasting in terms of the general oceanographic scenario.
2. Estimation of connectivity metrics for defined areas and periods that simulates the spawning to the recruitment tracks of each species. The outputs will be compared with observations from surveys and landings.

In addition, analyses of two stable isotopes: bulk-SIA and CSIA will be combined to estimate the ecological segregation in diet and spatial distribution. *S. pilcardus* early life stages (post-larvae) will be collected in the nursery areas from the Gulf of Cadiz and North and South of Alboran Sea. Isotopic values of sardine post-larvae will be related to their respective baseline isoscape signatures (two zooplankton size fractions as putative food sources). Juveniles and/or adults sardines coming from the whole study area will be sampled for otolith SIA and CSIA analyses for retrospective studies of diet and movement across isoscapes. For *M. merluccius*, bulk-SIA and CSIA from tissue and otoliths will be combined in juveniles and adults coming from the whole study area. These outputs together with demographic information from surveys and assessment outputs will contribute to validate the bio-physical model outputs.

## Participants Institutions and coordinator(s)

IEO Málaga, Baleares and University of Málaga (UMA) from Spain; INRH (Morocco); CNRDPA (Algeria), INSTM (Tunisa) and collaboration of scientists from CNR-IAMC (Italy)
Coordinator of Modeling of larvae dispersal and survival: Manuel Hidalgo IEO Málaga
Coordinator of Isotope analysis: Raul Laiz IEO Málaga
Oceanographic modeller and laboratory assistant are needed.

## 2. GENETIC MARKERS

Fish will be collected at specific sites from the Alboran Sea, as well as from neighbouring Atlantic Ocean (Spain and Morocco) and neighbouring Mediterranean Sea up to the Northern coast of Tunisia. Specimens must be both, spawners from adult high concentration areas and early life specimens to investigate overlap with boundaries defined by adults, and connectivity with spawning and nursery grounds (source-sink concept from spawning to nursery areas).

Specimens collected by the different teams (minimum of 40 individuals per sampling site) should be stored in non-denatured ethanol (96%) and transmitted to the laboratories in charge of the genetic analysis.
Approximately 33 samples have been taken into account to estimate the cost of the activities; however the sampling strategy should be designed by experts according to currently undergoing surveys and data collection programs.

### Existing resources

INRH: Molecular biology laboratory
University of Bologna (UniBo): Molecular biology laboratory.
IEO: Molecular biology laboratory at Aquaculture facilities in Vigo and in Málaga

The microsatellite laboratory experiments on *M. merluccius* are already underway at INRH (including samples from the Alboran and the Atlantic Ocean). All the information related to *M. merluccius* SNPs are legacy of FishPopTrace Project and can be transferred to any laboratory. *M. merluccius* microsatellite genotyping is ongoing at IEO, Vigo, for aquaculture applications.
*S. pilchardus* microsatellite genotyping was carried out in UniBo in the past years.

### Method and needs.

*M. merluccius:*
Neutral microsatellite loci (from bibliography and EST-derived possibly non-neutral) (15 loci)
Non-neutral (outlier) SNPs (48 loci)

*S. pilchardus*
Neutral microsatellite loci (15 loci)
SNPs development for *Sardina pilchardus* is ongoing

### Participants Institutions and coordinator(s)

Participants Institutions: IEO Málaga, Vigo (Spain); INRH Nador, Casablanca (Morocco); CNRDPA (Algeria); INSTM (Tunisa); University of Bologna (Italy) and University of Vigo (Spain).

Coordinators of analysis: IEO: C. Johnstone, INRH: K. Mokhtar-Jamaï and UniBo: A. Cariani
Laboratory assistants are needed.

## 3. PARASITES AS MARKERS

The parasite based methodology (including parasite community structure of the fish species along its geographical distribution, parasitic infection levels and genetic/molecular characterization of parasite species) represents an important approach in defining a fish stock. Indeed a parasite can be used as a suitable biological tag for fish stock identification when its geographical distribution and life cycle are known, and when the parasite's residence time in the host is long enough, compared with the lifespan of the fish host.

Sampling sites (a minimum of 8) will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. Samples must be obtained during the peak of spawning season which for sardine is in winter and for hake is in Spring.

Samples must contain adults. Preferred larger sizes of both species. Estimated numbers of individuals are 50 specimens per sampling site and year of sardine and 35 specimens of hake and blackspot seabream per sampling site and year.

### Existing resources

La Sapienza University can provide infrastructure for genetics of parasites. The samples will be provided by the other institutions following a protocol well established for the preservation of the material. Previous experience in hake and sardine will be included as a comparative analysis. There are research surveys through the study area that can be used to obtain the samples.

### Method and needs.

Traditional parasitological analysis including UV-press system; traditional morphological analysis of some parasite species; genetic / molecular identification of anisakids and other nematodes; population genetic analysis of some parasitic species. Statistical analysis of the genetic data and epidemiological parameters including simple and multivariate methods. Comparison with the results from other methods.

### Participants Institutions and coordinator(s)

IEO (Spain); INRH (Morocco); CNRDPA (Algeria), INSTM (Tunisa), La Sapienza-University of Rome, Italy and Faculty of Sciences Sfax University of Tunisia.

Coordinator of parasites identification: Simonetta Mattiucci from La Sapienza University (Italy)

Coordinator of sampling and analysis of data: Pablo Abaunza from IEO , Madrid (Spain)

Laboratory assistants are needed

Experts from the Faculty of Science (Tunisia) will be in close contact, including short-term visits for the molecular identification, with the University La Sapienza (Italy).

## 4. OTOLITH SHAPE AND ELEMENTAL COMPOSITION

Otolith shape and microchemistry analyses are useful techniques for discrimination of populations. The chemical composition of whole otoliths is analyzed using solution-based inductively coupled plasma mass spectrometry (ICPMS). In addition, the otoliths shape is analyzed using Fourier analysis.
A minimum of 8 sampling sites will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. For each species 120 specimens are needed (length range: 11 to 16 cm for sardine; 25 to 35 cm for hake): 100 for shape analysis and 20 for microchemistry.

### Existing resources:

All the equipment for image acquisition of otoliths is available at the IEO, INRH and CNRDPA furthermore, the software needed for the otoliths shape analysis is available at the INSTM.

### Method and needs.

Otolith Shape: High-contrast images of complete otoliths will be acquired using reflected light on a black background. A camera linked to a computer will be used to acquire digital images. The shape of each otolith will be assessed with the elliptic Fourier analysis.

Otolith microchemistry: Upon extraction, sagittal otoliths are cleaned in a laminar flow hood. Whole otolith analysis is done by solution-based inductively coupled plasma mass spectrometry (ICP-MS) to permit simultaneous measurement of the concentrations of many elements that are useful for stock identification. Once the otoliths are cleaned and preserved, will be sent to an external laboratory for the ICP-MS.

### Participants Institutions and coordinator(s)

IEO (Spain), INRH (Morocco), CNRDPA (Algeria) and INSTM (Tunisia)
Coordinator for otoliths shape and analysis: S. Khemiri from INSTM

## 5. BODY MORPHOMETRY AND MERISTICS

Morphometric and meristic characters will be used to investigate fish populations.
A minimum of 8 sampling sites will be selected in agreement with the other groups to make sure that the totality of the study area will be covered. Samples will be taken from Alboran Sea and adjacent areas: Spain, Morocco, Tunisia and Algeria, from survey and landings, with a frequency of twice a year and out of the reproduction period (between April and September for all countries). Sample size will be up to 50 specimens per station.

### Existing resources:

Basic equipment and expertise exist in the four centres. Complementary basic equipment and technical assistance for the analysis of data can be needed.

### Method and needs.

Morphometry of body including measurements of some body dimensions will be done with a calliper and through image analysis from pictures. Meristic characters such as the number of vertebrae and branchispines in dry material will be used to investigate fish populations variability.

### Participants Institutions and coordinator(s)

A coordinator will be nominated in each country for the morphometry analysis IEO (Spain), INRH (Morocco), CNRDPA (Algeria) and INSTM (Tunisia)
Coordinator for meristics: T. Filali, CNRDPA (Algeria)
Assistance of expert in data analysis is needed.

## 6. ANALYSES FISHERY PATTERNS, DEMOGRAPHICS INDICES AND LIFE HISTORY TRAITS.

Existing data and published Information on the biology and fisheries of the two species in the region will be compiled and analysed. The progress on this type of analysis currently done at the Working Groups of stock assessment of the CopeMed II Project will be taken as a basis. Update will be ensured by the partners' laboratories, which will provide the annual data from surveys and commercial fisheries.

Existing resources: (equipment, software, human resources, current surveys, sampling programs, suitable to include program activities in the different institutions)

Information and expertise exist in the four centres. Complementary technical assistance for some specific analysis can be needed.

### Method and needs.

Pattern of biomass in space and time, Identification of Essential Fish Habitats of the two target species, Analysis of life traits variability in time and space; Pattern of fishing effort in space and time, Statistical framework for identification of stock units and their boundaries by a multi-dimensional approach.

Scientists from the various national institutions will be in close contact to exchange data sets including one or two working groups to process the data.

### Participants Institutions and coordinator(s)

IEO (Spain); INRH (Morocco); CNRDPA (Algeria), INSTM (Tunisa) and collaboration of scientists from CNR-IAMC (Italy)
Coordinators for the compilation of information: A. Giráldez (IEO), MH. Idrissi (INRH)

## 7. POPULATION DYNAMICS SIMULATIONS

Under the different scenarios of stock discrimination obtained and before formulating any type of recommendation, population dynamics simulations will be carried out to evaluate the consequences of the different assumptions about stock boundaries to the assessment of the resource status and the outcomes of management strategies. This component will be further developed based on the outcomes of the previous components. It is envisaged the use of methodologies for management strategy evaluation.

Coordinator: M. Hidalgo (IEO), Spain

## Proposed Calendar of activities (two years 2018-2019)

| Year | 2018 | | | | 2019 | | | |
|---|---|---|---|---|---|---|---|---|
| Quarter | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th |
| **Tasks** | | | | | | | | |
| Detailed protocols | | | | | | | | |
| Selection of personnel | | | | | | | | |
| Training | | | | | | | | |
| Sampling | | | | | | | | |
| Hydrodynamic modelling | | | | | | | | |
| Fisheries data compilation | | | | | | | | |
| Analysis (genetics, isotopes, parasites, etc.) | | | | | | | | |
| Intermediate Workshop | | | | | | | | |
| Statistical analysis | | | | | | | | |
| Populations simulations | | | | | | | | |
| Final workshop | | | | | | | | |
| Final report | | | | | | | | |

## References


Dubois M, et al (2016). Linking basin-scale connectivity, oceanography and population dynamics for the conservation and management of marine ecosystems. *Global Ecol Biogeogr* 25:503–515.

Cadrin, Steven X., Lisa A. Kerr, and Stefano Mariani, eds. Stock identification methods: applications in fishery science. *Academic Press*, 2014.

Fiorentino F., E. Massutí, F. Tinti, S. Somarakis, G. Garofalo, T. Russo, M.T. Facchini, P.Carbonara, K. Kapiris, P. Tugores, R. Cannas, C. Tsigenopoulos, B. Patti, F. Colloca, M. Sbrana, R. Mifsud, V. Valavanis, and M.T. Spedicato, 2015. Stock units: Identification of distinct biological units (stock units) for different fish and shellfish species and among different GFCM-GSA. STOCKMED Deliverable 03: FINAL REPORT. January 2015, 215 p.

Hidalgo et al (2017) Ocean connectivity: a key challenge for fisheries management, spatial planning and ecosystems conservation. (In press.)

Hidalgo et al (in review) Reconciling ocean connectivity and hydroclimate with the management of transboundary metapopulations.

Kerr L, et al (2017) Lessons learned from practical approaches to reconcile mismatches between biological population structure and stock units of marine fish. *ICES J Mar Sci* doi:10.1093/icesjms/ fsw188.