Food and Agriculture Organization of the United Nations

COPEMED II
Report of the kick-off meeting for the project on stock identification in Alboran Sea and adjacent waters
19-20 December 2017, Fuengirola, Spain

1. Background and objectives of the meeting

The kick-off meeting was kindly hosted at the laboratory of IEO in Fuengirola and was attended by experts from Algeria, Tunisia, Morocco, Spain and Italy. (List of participants in Annex 1). The objective of this meeting was to decide on basic elements for the development of the two years research Project: *"Transboundary population structure of Sardine, European hake and blackspot seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach (TRANSBORAN)"* following the mandate of the 10th CopeMed II Coordination Committee. More specifically the meeting addressed:

1. Identification of the sampling sites;
2. Frequency of sampling and number of individuals;
3. Protocols for samples manipulation and transportation;
4. Distribution of tasks among the different laboratories;
5. Establishing of a calendar of tasks

Pilar Hernández, fishery expert of CopeMed II Project informed the group on the approval of the Project by the 10th meeting of the Coordination Committee of CopeMed in October 2017 and the modifications added upon request of the 19th Session of SAC. Three species were proposed to be addressed in the project: sardine, hake and black spot seabream. The third one had been added as per SAC request, given that it is a priority species in western Mediterranean. In order to avoid increasing excessively the costs and the workload to the institutions involved, two of the most discriminating techniques (genetics and isotopes) were selected to be applied to blackspot seabream. She also informed that additional funds could be provided by the GFCM Mid Term Strategy to cover the increased costs of including a third species.

Manuel Hidalgo was elected as general Coordinator of the Project and Alberto García was elected Chair of the meeting. The agenda as presented in Annex 2 was approved by all the participants.

The elected Coordinator made a short introduction to several relevant elements to frame the methodological discussions of the meeting and the design of the sampling protocols. This included a review of past, ongoing and future projects dealing with similar topics on the same species and in close by areas.

Other Similar Projects in place:

SARDINHA 2020 (national funded project in Portugal) and SPELMED (EU project coordinated from Spain) are two ongoing projects with similar objectives. Mechanisms of collaboration and information sharing with these projects should be established.

In November 2017, the SARLINK Survey leaded by scientists of the Centre Oceanographic of Vigo (IEO) had collected a number of individuals in the north part of Alborán sea. The objective of the project is to investigate connectivity processes and effective population size of Sardine in south Iberian Atlantic and in the Gulf of Cádiz. The project is basically a genetic approach but will also apply isotopes (muscles and otoliths), and potentially parasites. The availability of 800 individuals currently frozen to be used, if considered useful, for the current Alborán project was discussed. Finally, as the samples had been taken in the Northwest of Alborán sea (Estepona and Málaga in Spain) and samples from the Southern part were missing, and given the impossibility to carry out a similar survey in the coming weeks in the Southern part of Alborán sea (Moroccan and Algerian coasts), the group decided to not use the material of SARLINK for the purposes of the TRANSBORAN project.

Further discussion about the best way to collect samples took into consideration three possible options: the existing surveys, the collection at ports as well as sampling on board professional vessels. The two last ways were preferred options provided that surveys dates are often not coincident among countries. Furthermore, by organizing specific sampling periods simultaneously in the selected areas, would allow for an easiest geographic and temporal control of the sampling.

2. Description of current sampling programs in place: surveys and landings

The four countries introduced their surveys which information is summarized here below:

Sampling programs:

MOROCCO

- **Surveys at sea**
  - *Sardina pilchardus :*

April (recruitment) / September (beginning of spawning)

Larvae are collected during these 2 surveys

  - *Merluccius merluccius :*

January (spawning) / May (beginning of recruitment) / December (spawning)

Larvae are not collected / No information on larvae distributionn *of M. merluccius*

  - *Pagellus bogaraveo :* Not targeted by INRH research vessels

- **Current biological sampling at ports**

INRH Tanger :

- *Merluccius merluccius* (purchase) : 1 box (~ 100 – 200 samples) 1-2 / month
  - ✓ Larache
  - ✓ M'diq in 2018 ?
- *Sardina pilchardus* : 3-4 kg (~ 100 samples) :
  - ✓ M'diq 1 / week
  - ✓ Larache 1 / week

- ✓ Chmaala 2 / month (but not sure to keep that site in 2018) (site of artisanal fishing)
- ➢ *Pagellus bogaraveo :* 1-2 / month
  - ✓ Tanger

INRH Nador :

- ➢ *Merluccius merluccius* (purchase) : 1 box (~ 100 – 200 samples) 1 / month
  - ✓ Nador (but origin from Saadia to Al Hoceïma)
- ➢ *Sardina pilchardus* : 3-4 kg (~ 100 samples) :
  - ✓ Al Hoceïma 1 / month
  - ✓ Nador 2-3 / month
  - ✓ Cap de l'Eau (Ras Kebdana) 2 / month
- ➢ *Pagellus bogaraveo :* No sampling at ports

TUNISIA:

- Surveys: Annual hydro-acoustic survey was done until 2010. In 2018 a survey will be done if Hannibal vessel is repaired
- Biological sampling of landings at ports: Sardine and Hake among other priorities species are sampled from landing at the fishing ports of Tabarka and Kelibia. Sampling onboard professional vessels is also possible in Tunisia.

ALGERIA:

- Surveys :
  - Pelagic survey in September
  - Demersal survey in June- July
- Biological sampling of landings at ports :
  - Eastern Part: Annaba
  - Western part (Alboran Sea): Ghazaouet

SPAIN

- Surveys :
  - Pelagic survey in September
  - Demersal survey in June- July
- Biological sampling of landings at ports :
  - DCF National program

3. Sampling needs

All coordinators presented their needs and summarized protocols for samples treatment.

The ensuing discussion concluded with the following sampling strategy: the lack of synchronicity among the different surveys currently (and the lack of some of them) in place prevents the use of surveys as samples providers for the purposes of this project. Therefore, the group decided to do specific and punctual sampling at ports or on board professional vessels all in the same temporal window in the four participating countries with the strategy described below:

HAKE:

40 mature individuals of a size between 25-30 cm. Large individuals have to be avoided, all the specimens have to be approximately of the same age (second year class). The

sample scheme generally attempts to be taken out of the reproductive period, in a range of 8 weeks between October-November in all the countries at the same time. However, the project recognizes that hake is a protracted spawner and the potential sampling of spawning individuals cannot be ensured.

SARDINE:

50 individuals of a size between 14-17 cm (i.e., same age class). They all have to be mature individuals and will be sampled in a period between May-June.

In addition, for isotopes analysis larvae and eggs of sardine will be collected through ichthyoplankton surveys in the countries where they exist. Spain can deploy a series of punctual sampling trips with a small boat in the bays known to be concentration areas of eggs and larvae during the same period than the surveys are carried out in Morocco (and eventually in other countries).

BLACKSPOT SEABREAM:

30 individuals of a size between 30-35 cm between May-June. In addition, for this species, scientist on board research vessel should be aware that if they catch some *P. bogaraveo* (particularly recruits) during the surveys they should sample all individuals for genetics and otoliths.

4. Delimitation of the study area and location of sampling sites

The team of the University of Málaga in charge of hydrodynamic modelling presented the extension of the model and the current knowledge of the dynamics of the water masses in the area (inset Fig. 1). After having analyzed visually a series of landings, the group decided to select a range of 8 years (2004 to 2011) that covers high and low abundances periods for all species to run simulations of particles' dispersion.

According to this information, and the existing knowledge on nursery and spawning grounds, 17 sampling sites have been selected for hake and sardine (Fig. 1). In the case of blackspot seabream, the sampling will be geographically less exhaustive and focused in specific fishery areas where samples are available (Fig. 2).

Trained experts will be in charge of the sampling of the number of individuals agreed for each species and will do the sampling treatments for the different techniques as defined in the below list of nine steps. More detailed documents will be prepared by the Coordinators and distributed before the sampling period starts.

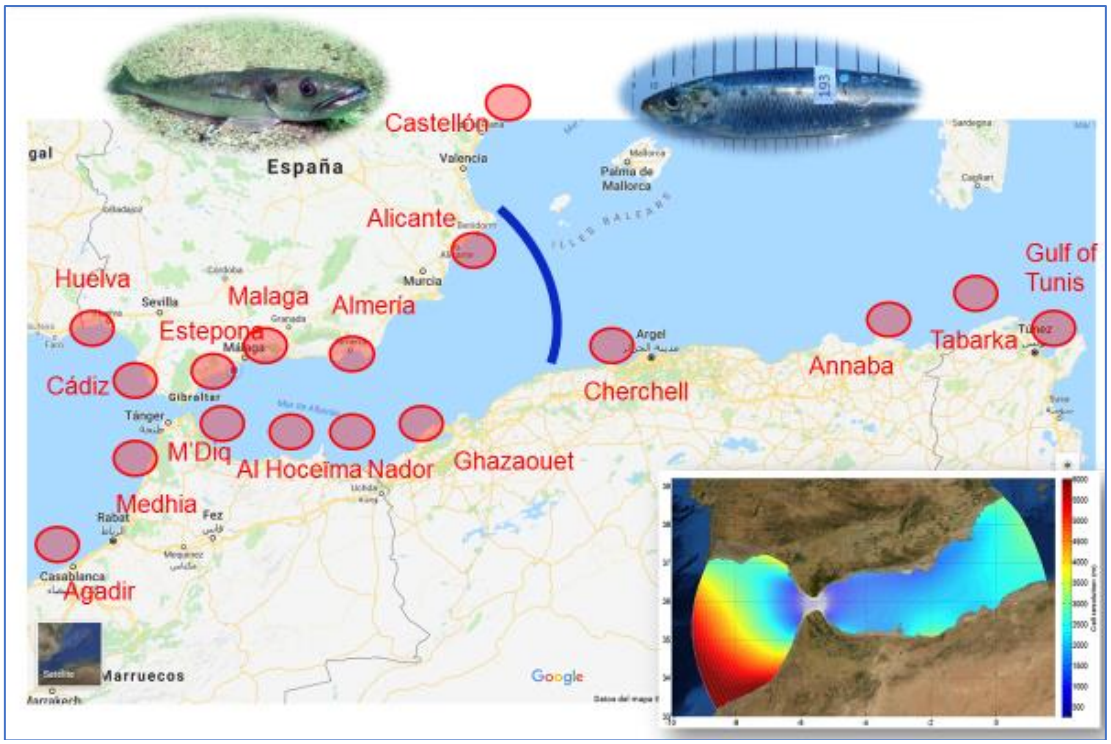


Figure 1: Sampling ports for hake and sardine (blue line is the limit of the hydrodynamic model as illustrated in the inset map).

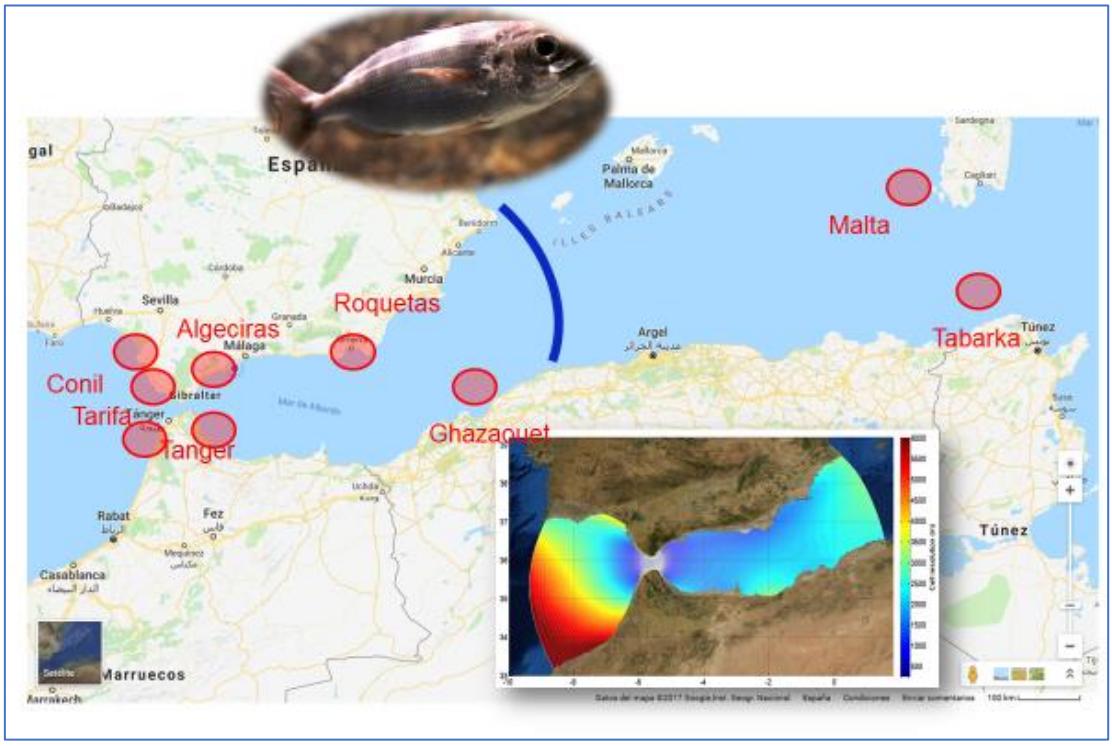
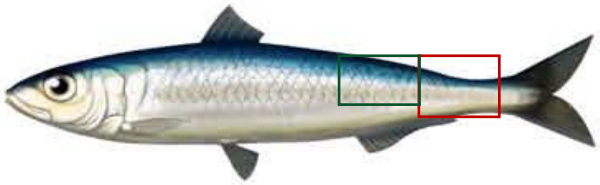


Figure 2: Sampling ports for blackspot seabream (blue line is the limit of the hydrodynamic model as illustrated in the inset map).

PROTOCOLS FOR SAMPLES TREATMENT

These are the basic steps to be done on each specimen sampled (more detailed protocol for each technique will be distributed before the sampling starts)

1. Take a picture of the whole individual on an ichthyometer.
2. Take a piece of muscle for Isotopes (look at the details in the enclosed figure).
3. Take a piece of white skeletal muscle (0.5 cm^3) on the sides of the fish. Store frozen in 2-5 ml tubes with screwcap, labelled.
4. Extract both otoliths and store clean and dry.
5. For Shape analysis: Take a picture of the right otolith with the proximal face looking up on a dark background with reflected light.
6. For microchemistry (20 specimens as minimum) of the left otoliths, the extraction must be done with ceramic or plastic forceps (avoid metallic to prevent from contamination) and the otoliths must be stored dry after drying in a laminar flow hood in an eppendorf decontaminated with nitric acid 1%
7. Cut both gill arches and store in formaldehyde.
8. Parasite extraction
9. Picture of fish-bone with all its vertebrae once flesh has been removed



5. Codification of samples

The codes of each individual sampled will consist of:

- Three alpha code for the name of the species
- Three alpha code for the name of the port
- Date in the format day/mo/year
- Number of the specimen
- Sex
- Size (in mm)

Table 1: example of two individuals sampled at the port of Agadir

Species	Port/survey	Haul nb. from surveys)	date	nb of individual	sex	size
HKE	AGA		21/11/2018	1	M	25,3
HKE	AGA		22/11/2018	2	M	28

Table 2: list of codes

CODES		
Species	*M. Merluccius*	**HKE**
	S. pilchardus	**PIL**
	P. bogaraveo	**SBR**
Ports	Agadir	**AGA**

	Medhia	**MDH**
	Huelva	**HUE**
	Cádiz	**CDZ**
	Tanger	**TNG**
	Conil	**COL**
	Tarifa	**TRF**
	Algeciras	**ALG**
	Estepona	**ETP**
	Málaga	**MLG**
	Almeria	**AMR**
	Roquetas	**RQT**
	M´diqu	**MDQ**
	AlHoceima	**ALH**
	Nador	**NDR**
	Ghazaouet	**GHZ**
	Cherchell	**CHE**
	Annaba	**ANB**
	Tabarka	**TBK**
	G. of Tunis	**GTU**
	Malta	**MLT**
Survey	Name	**nb of haul**
Date	Day/Mo/YR	
Size	**Total length in mm**	

6. Compilation and analysis of data about fisheries (for sardine and hake)

For the collection of this type of information, all the existing resources in the different institutes will be made available to the project. Historical and current available data will be analysed in a specific workshop at the end of the second year of project. These are the type of data needed:

- **Distributions:** Collecting density and biomass per survey (acoustics and trawling) station, year and age-class.
- **Landings and CPUE:** Monthly time series of landings and CPUEs of sardine will be collected for each port of sampling (Fig. 1).
- **Size frequencies**: Monthly size frequencies of landings per port and the whole country.
- **Life history traits**: Size at maturity, spawning period, sex ratio, GSI (including annual dynamics), VBTF parameters, LW parameters, monthly individual weights and length to calculate condition indices.

7. Distribution of tasks among the different laboratories

Hydrodynamics and connectivity: University of Málaga.

Isotope in otoliths and in muscle: IEO Málaga

Genetic markers: IEO (Málaga and Vigo), INRH Casablanca, University of Bologna

The three species and different markers will be distributed as shown in table 3

Table 3. Distribution of genetic analysis

		Year 1	**Year 2**
M. Merluccius	**12-16 microsatellite loci 48 SNPs**	**INRH** (microsatellites) **INRH - UniBo (**SNPs**)**	
S. Pilchardus	**12-16 microsatellite loci**	**IEO** (microsatellites)	**(SNPs / mt DNA sequencing?)**
P. bogaraveo	**12-16 microsatellite loci**	**UniBo** (microsatellites)	**mt DNA sequencing?**

Fish parasites: University of Rome La Sapienza

Otolith shape and elemental composition: INSTM la Goulette and IEO Málaga

Body morphometry and meristics: CNRDPA Bou Ismail.

Analyses fishery patterns, demographics indices and life history traits: IEO Málaga, INRH, CNRDPA, INSTM. A workshop will be organized to analyze these data that will be provided by the four institutes to the Project Coordinator

Population simulations and synthesis exercises: IEO Málaga, INRH, CNRDPA, INSTM

8. Conclusions and follow up recommendations.

The group agreed on the following procedure for the samples collection and treatment:

An expert will be identified in each of the four participating countries. The respective institutes will be responsible for the selection and training (if needed) of the selected person who will be in charge of the whole treatment as described in steps 1-9 above.

Fully extensive and well detailed protocols for the treatment of samples will be elaborated by the Coordinators of each technique and will be distributed among the participating institutions well in advance the first sampling period (*i.e.*: May 2018)

To avoid shipping excessive costs and issues with customs, the transportation of samples will be done personally. An expert from the laboratory in charge of each technique will travel to gather all the material that will be centralized in the most easy-accessible city of each country and will transport the samples with the corresponding certificates and letters needed for the customs.

The laboratories in charge of the different analysis are ready to receive trainees for on-the-job training stays. CopeMed will coordinate with the scientist to devise a calendar of training stays compatible with the periods of sampling and analysis.

Proposed calendar (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								

Estimated budget for two years is: Eur 355.000
From this total, the part corresponding to black spot Seabream is: Eur 66.500

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Annex 2

Agenda

Tuesday 19th December 9:00-18:00

1. Background and objectives of the meeting.

2. Description of current sampling programs in place: surveys and landings

3. Sampling needs by the coordinators of each of the disciplines

    - Stable isotopes: bulk-SIA and CSIA in tissue and otoliths
    - Otoliths elemental composition
    - Genetic markers (mitochondrial and nuclear) *(Carolina Johnstone)*
    - Parasites
    - Otolith shape
    - Body morphometry
    - Meristics

4. Delimitation of the study area and location of sampling sites

5. Codification of samples

Wednesday 20th December 9:00 to 17:00

6. Compilation and analysis of data about fisheries (history of fisheries, spatial distribution of fishing-grounds, historical trends of CPUE and stock status indicators) and life history traits (growth, reproduction, length-at-age, body condition)

7. Distribution of tasks among the different laboratories

8. Conclusions and follow up recommendations