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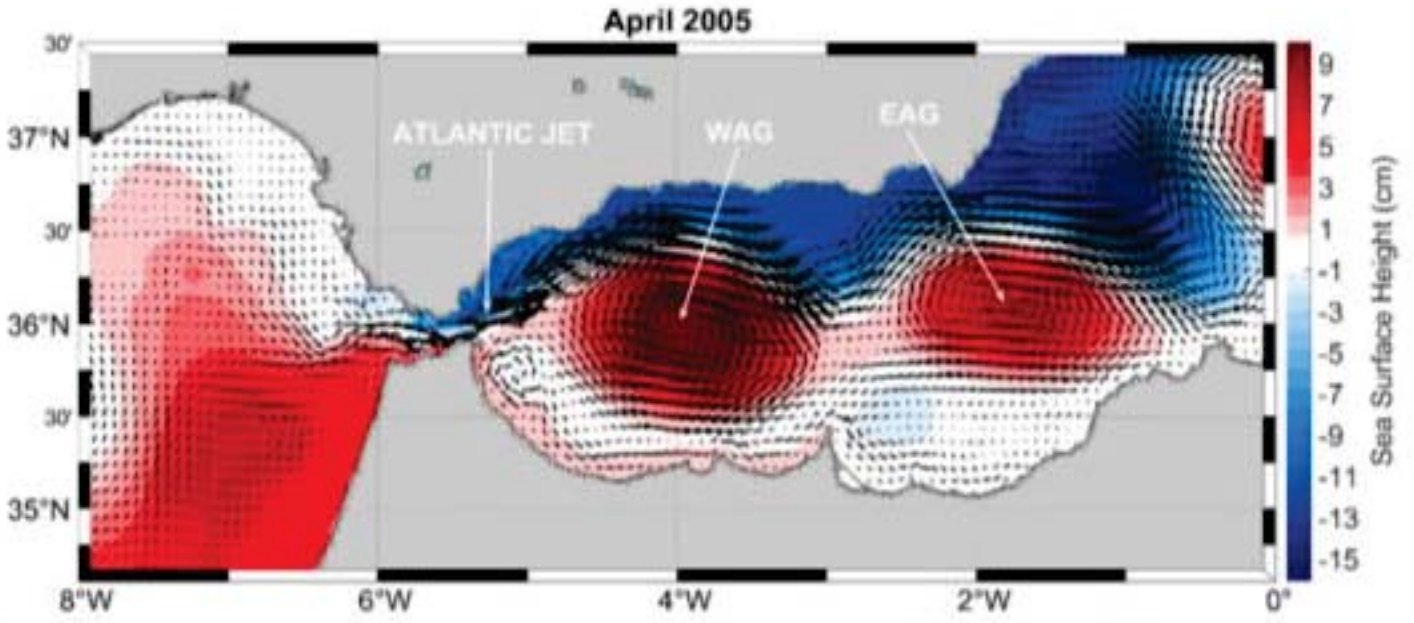

REPORT OF THE MID-TERM WORKSHOP ON TRANSBORAN PROJECT "TRANSBOUNDARY POPULATION STRUCTURE OF SARDINE, EUROPEAN HAKE AND BLACKSPOT SEABREAM IN THE ALBORAN SEA AND ADJACENT WATERS: A MULTIDISCIPLINARY APPROACH"

22-24 July 2019
Malaga, Spain

CopeMed II Technical Documents Nº52
GCP/INT/028/SPA; GCP/INT/270/EC

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MALAGA, SPAIN, 22–24 JULY 2019

July 2019

Preface

The CopeMed II Project on *Co-ordination to Support Fisheries Management in the Western and Central Mediterranean* is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Government of Spain, represented by the Secretaría General de Pesca (Mº de Agricultura, Pesca, Alimentación y MedioAmbiente, MAPAMA), and the European Union, represented by the European Commission (EC).The premises of the project at the Subdelegación del Gobierno in Málaga (Spain) are part of the Spanish contribution included in the agreement with the FAO.

The objective of the project is to maintain the sustainability of the marine fisheries in the central and western Mediterranean Sea and its ecosystem, taking into consideration environmental, biological, economic, social and institutional issues. In addition, the project will continue to reinforce the collaboration among the participating countries of the sub-region by facilitating their participation in the activities of the Scientific Advisory Committee (SAC) and in the General Fisheries Commission for the Mediterranean (GFCM).

Regions covered by CopeMed II are the western and central sub-regions of the Mediterranean. Participating countries are Algeria, France, Italy, Libya, Malta, Morocco, Tunisia and Spain. The main beneficiaries are the fishery policy-makers, managers and fishery administrations in the western and central Mediterranean countries. The project is also contributing to the strengthening of regional collaboration by supporting the participation of the countries in relevant regional scientific organizations, such as the FAO's General Fisheries Commission for the Mediterranean (GFCM). Secondary beneficiaries include the national research institutes, fishers and fishers' associations, and industrial organizations.

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CopeMed II (GCP/INT/028/SPA – GCP/INT/270/EC) Publications

CopeMed II project publications are issued in the CopeMed Technical Documents series and are related to meetings, missions and research organized or conducted within the framework of the CopeMed II Project.

Comments on this document would be welcomed and should be sent to the Project premises:

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Cover picture: Numerical reconstruction of the surface circulation in the Alboran Sea on April 2005.
Author: Physical Oceanography Group of the University of Malaga.

Preparation of this document

This document is the final version of the Report of the CopeMed II Mid-Term Workshop on TRANSBORAN Project: "Transboundary population structure of Sardine, European hake and Blackspot Seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach". Málaga, Spain, 22–24 July 2019.

Acknowledgements

CopeMed II acknowledges the participation and valuable contributions of all experts from Algreria, (CNRDPA), Italy (Universitá la Sapienza and Universitá de Bologna), Morocco (INRH), Spain (IEO and Universidad de Málaga), Tunisia (INSTM), who attendend the workshop.

CopeMed II. 2019. Report of the CopeMed II Mid-Term Workshop on TRANSBORAN Project: "Transboundary population structure of Sardine, European hake and Blackspot Seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach". Málaga, Spain, 22–24 July 2019.
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ABSTRACT

The Workshop main objectives were to take stock of the work carried out during the first 18 months of functioning of the Transboran project and to provide orientation on the remaining tasks to be done to accomplish all the initial project objectives. The sampling process was assessed and found satisfactory with some minor shortcomings that were solved. The experts shared preliminary results of the different disciplines: genetic markers, morphometry (body and otoliths shape), meristics, microchemical composition, parasites, analysis of fishery data and life history traits and hydrodynamic modeling. The three techniques that have completed the analyses are: i) hake parasites, ii) fisheries information for hake, and iii) hydrodynamic simulations for blackspot seabream. The pace of the different analysis was uneven, depending of the technique with delays basically due to the bureaucratic burden. While some of the preliminary results for hake (parasites and fisheries data) suggest certain demographic segregation between north and south, final interpretation need to be done at the view of all analyses completed, which will provide complementary information of connectivity processes and segregation at different spatial scales.

Report of the CopeMed II Mid-Term Workshop on TRANSBORAN Project "Transboundary population structure of Sardine, European hake and Blackspot Seabream in the Alboran Sea and adjacent waters: a multidisciplinary approach". Málaga, Spain, 22–24 July 2019

1. Opening and objectives of the meeting

The meeting was kindly hosted by the '*Subdelegación del Gobierno de Málaga*' where CopeMed II premises are located. It was attended by 22 participants from 5 countries (Algeria, Italy, Morocco, Spain and Tunisia). The agenda was adopted as presented in Appendix I. The whole list of participants is included as Appendix II.

Copemed II Fishery Expert, Ms Pilar Hernández introduced the objectives and expected outputs of the meeting that can be summarized as follows: i) Take stock of the work done so far, ii) Put forward preliminary results of the different disciplines, iii) Identify methodologies for the Interdisciplinary evaluation and iv) Define the work plan for the next semester. In addition, she presented a summary of the task achieved and the remaining work according to the calendar defined at the beginning of the project in the shape of a table (Table 1).

Table 1. Calendar of tasks achieved (black) and remaining (grey)

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)								
Intermediate Workshop								
Statistical analysis								
Populations simulations								
Final workshop								
Final report								

2. Overview of the status of samples and the sampling process

The scientific coordinator of TransBoran project, Manuel Hidalgo form IEO introduced a summary of the sampling process carried out during 2018.

The collection of samples for the TransBoran project has concluded successfully with enough biologic material to tackle the objectives proposed in the project. A total of 866 samples were collected for sardine in all the 17 ports initially proposed (Figure 1). While some areas, such as Spain or Tunisia, had to wait to obtain sardine at the size objective of the project, fish of this size finally arrived to all ports. In the case of hake, 611 hakes were sampled in 15 out the 17 ports originally proposed; samples were not obtained in Al Hoceïma (Morocco) and Cherchell (Algeria) due to the lack of availability (Figure 2). Nevertheless, the samples obtained are sufficient to accomplish the objectives of the project. By contrast, the sampling of blackspot seabream was more challenging mainly due to the lack of individuals of this species in the Spanish longline fishery around the Gibraltar Strait. Currently, 274 individuals have been sampled while a strong component of them were obtained in areas outside Alboran Sea (e.g. Atlantic Portuguese coast, Cantabric coast, Strait of Sicily and Greece; see more details in figures 3, 4 and in the section below).

To circumvent these limitations in blackspot seabream, particularly the small sampling size in north Alboran, the TransBoran group agreed to provide samples of the Spanish scientific bottom trawl survey already collected this past summer (MEDITS2019). The IEO participants suggested to try to add one-day of sampling from Spanish fishery that could be covered by IEO funds. This proposal remains pending for further discussion with IEO authorities.

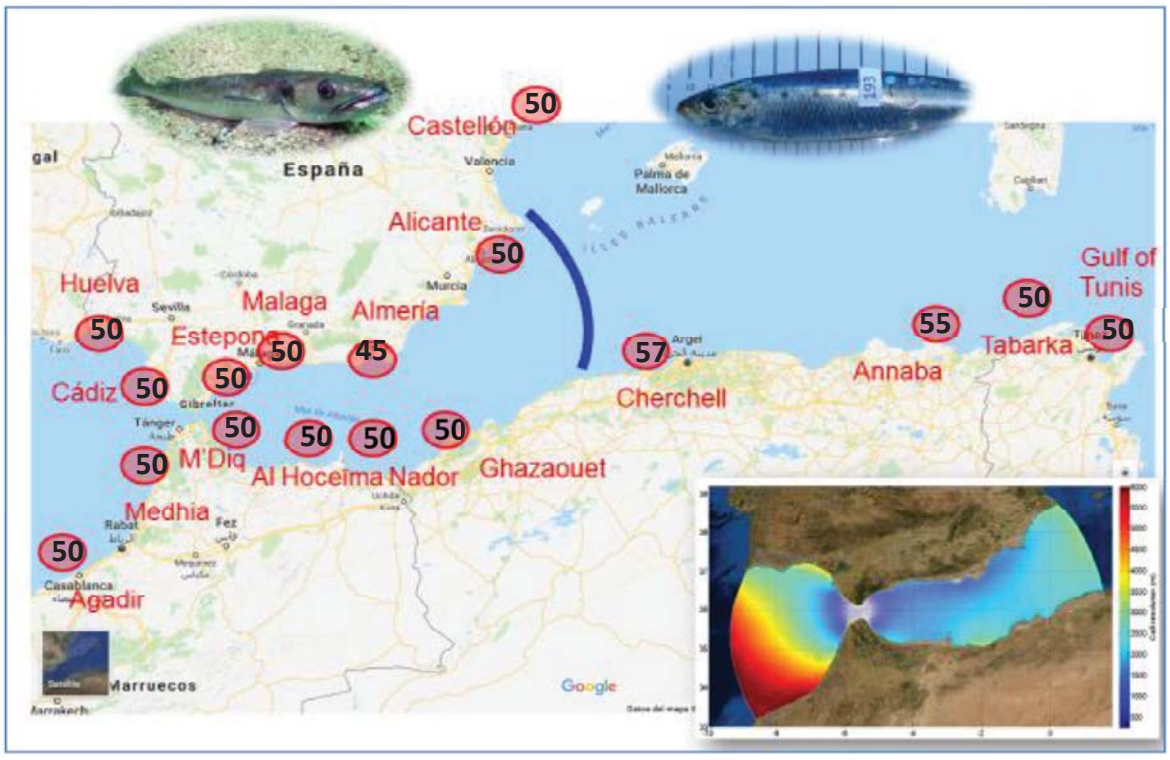

Figure 1. Sardine samples in 17 ports.

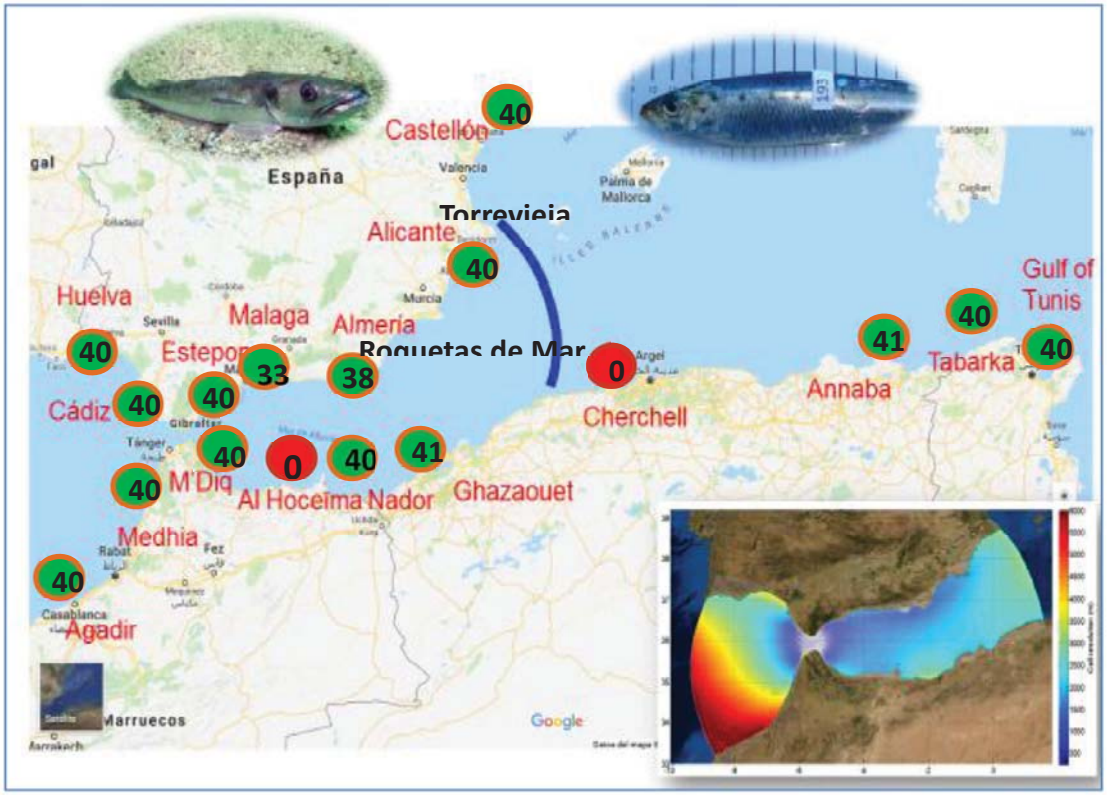

Figure 2. Hake samples in 15 out of 17 ports.

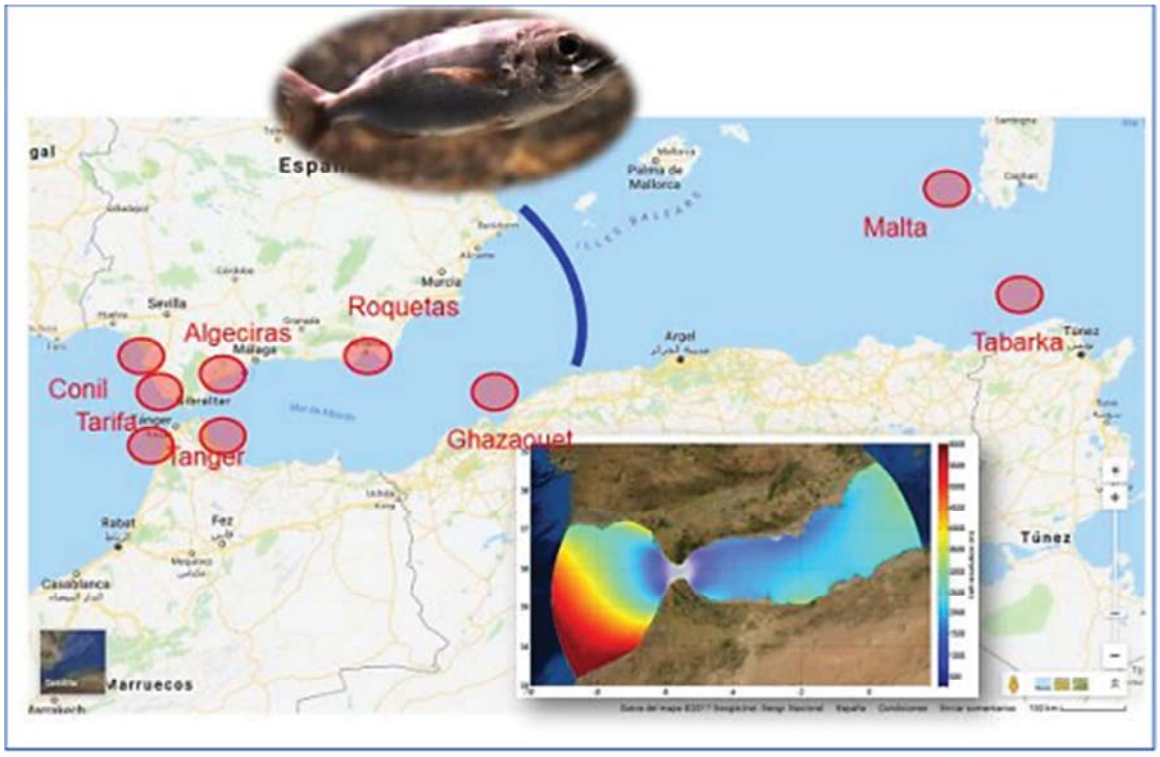

Figure 3. Blackspot seabream sampling ports as initially foreseen.

Figure 4: Actual sampling locations of blackspot seabream

3. Current advances by the different methods used

3.1 Genetic markers: Three presentations were introduced by the experts on this subject. The summary and further discussions are summarized below:

- ***Blackspot Seabream.*** *(Lead: A. Cariani, University of Bologna, Unibo, Italy; Others: Martina Spiga ; Alice Ferrari ; Federica Piattoni)*

The original sampling design as defined by the sampling protocol failed in some Spanish ports due to the absence of blackspot seabream during the fishing season of 2018. Samples from Nador (Morocco), Algeciras and Roquetas (Spain) were missing. Part of this lack was covered by other samples provided by collaborative projects from Portugal, Greece, Santander (Spain), Annaba (Algeria) and from Medits survey (Spain), altogether helping to reveal a large scale genetic structure of the species. Finally, 14 locations were sampled (figure 4),with a total of 274 individuals of blackspot seabream. Table 2 shows locations and individuals collected. The following locations present few individuals: Ghazaouet (9), Malta (4), Tarifa (3) and Medits survey (10), which are not robust enough for molecular analysis given the low number of collected individuals. To overcome this situation, more samples have been requested from the Spanish coast if possible. While samples from 2019 survey have been provided, samples from the Spanish fishery will be unlikely obtained.

Blackspot seabream population genetic structure will be studied using microsatellite loci (already developed by Stockley et al. 2000 ; Pinera et al. 2006 for *Pagellus bogaraveo* and Ramsak et al. 2003, for *Pagellus erythrinus*). Genetics laboratory work is performed by BMR Genomics company (DNA extraction, PCR amplification and genotyping) and allele scoring will be performed at Unibo.

Eighteen microsatellite loci from *P. bogaraveo* out of the twenty-two loci assessed have been focused. Three loci are under focus and one locus has been excluded due to insufficient polymorphism. The seven microsatellite loci isolated from *P. erythrinus* have been put on hold for the moment. The 274 sampled individuals are ready to be processed by BMR Genomics.The future planned work and deadlinesare as follows:

-DNA extraction, PCR amplification and genotyping of the 274 existing samples at BMR Genomics (as soon as FAO procedure to get the purchase order is performed and then 2 months service at BMR).

-Allele scoring and data analysis (3 months)

New samples from Spanish coast (Gibraltar Strait) are requested to improve the statistical coverage of the molecular analysis.

The group was also informed that a Master thesis is ongoing by Martina Spiga who will conduct a short stay at the IEO funded by the University of Bologna to perform the analysis on the results by the end of 2019- beginning 2020.

Table 2. Location of the samples for blackspot seabream and number of samples per location. Note that samples initially reflected to Roquetas refer to the Medits samples collected in summer 2018.

Country	Location Name	Location code	Number of Individuals Expected	Number of Individuals Received	Sampling Period
Morocco	Tanger	TNG	30	18	June 2018
Morocco	Ksar Sghir	TNG		15	June 2018
Morocco	Eddalya	TNG		35	October 2018
Morocco	Nador	NDR	30	0	
Algeria	Ghazaouet	GHZ	30	9	June 2018
Algeria		ANB		30	July 2018
Tunisia	Tabarka	TBK	30	30	December 2018
Malta	Malta	MLT	30	4	September 2018
Malta	Malta	MLT		13	March 2019
Spain	Conil	COL	30	15	July 2018
Spain	Tarifa	TRF	30	3	July 2018
Spain	MEDITS	MEDITS-18 SBR		10	April-May 2018
Spain	Santander	STD		26	November 2018
Spain	Algeciras	ALG	30	0	
Spain	Roquetas	RQT	30	0	
Greece		ION		30	August 2018
Portugal		PT		36	January-February 2019
			Total number of individuals:	**274**	

- ***European hake*** *(Lead: K. Mokhtar-Jamaï, Institut National de Recherche Halieutique, INRH Morocco; Others: Hamza Bensaber (PhD Student), Mohamed Malouli Idrissi)*

The sampling for genetics of hake has been completed. Almost all the expected sampling locations from the original sampling protocol have been collected (except Al Hoceima in Morocco and Cherchell in Algeria). In total 15 locations were sampled (~40 hake individuals per location) with a total of 593 individuals of hake. The detail of sampling is provided in the map (Fig. 2). DNA extraction is in progress using PureLink Genomic DNA Mini Kit (Invitrogen). Hake population genetic structure will be studied using two different genetic markers: microsatellite loci (already developed by Moran et al. 1999, Rico et al. 1997, D'Amato et al. 1999 and Magoulas et al. (Unpublished but sequences are available in Genbank and SNP (Single Nucleotide Polymorphism) (Milano et al. 2011, 2013).

Five microsatellite loci out of twelve existing loci are focused at INRH genetics laboratory. The other seven microsatellite loci are under evaluation. By late September, the final locus of the loci and the loci choice will be performed (depending on the

focus). PCR amplification and genotyping for microsatellite loci will be finished by late December 2019.

Regarding SNP loci, the work is being done in collaboration with Alessia Cariani from Unibo. Sixty SNP loci out of 395 polymorphic loci have been selected using the following criteria: outlier loci selected, no linkage disequilibrium between loci, no minor allele frequency. The technique to analyze SNP has been identified as well as the supplier (Open Array 12K system at Thermo Scientific laboratory in Paris, France). The supplier initiated the design of the Open Array. The team will be available to perform the SNP analysis at Thermo Fisher Scientific facility from 11 to 15th of November 2019, and the purchase order from FAO should be delivered at that time.

The future planned work and deadlines are as following:

-DNA extraction of the 593 samples (in progress).

-PCR amplification of the microsatellite loci (final number will depend on the focus in progress).

-Genotyping of the microsatellite loci and scoring (December 2019).

-Microsatellite loci Statistical analyses (beginning of 2020).

-SNP analysis (November 2019 depending on FAO procedure to get the purchase order).

-SNP loci Statistical analyses (mid 2020).

- ***Sardine*** *(Lead : C. Johnstone, M. Pérez Spanish Institute of Oceanography (IEO, Spain) others: Manuel Nande; Nair Vilas.*

The sampling for *Sardina pilchardus* has been completed. In total 17 locations with approximately 50 individuals per port (Fig. 1) were sampled constituting a collection of 859 tissue samples for genetics stored at the IEO. Several DNA extraction protocols for purification of DNA and microsatellite amplification have been tested and the cost effective FENOSALT method was chosen. DNA extraction from all the sardine tissue samples has been completed.

Scientific literature revision provided a list of 20 microsatellite loci to consider for the Transboran project. Amplification of the 20 microsatellites has been performed and 12 loci selected for configuration of multiplex PCR designed « in silico ». Non specific amplification was found for three loci that were excluded in the final multiplex PCR design. Fluorescently labelled oligonucleotides for two PCR multiplex reactions to characterize nine sardine microsatellite loci have been recently purchased. Genotyping and allele scoring will be initiated in IEO after approval of FAO purchase order for external fragment analysis and laboratory consumables.

Future work and deadline:

- Multiplex PCR amplification at nine microsatellite loci at IEO and fragment analysis with external services (6 months after FAO procedure for purchase orders are completed).

-Allele scoring and data analysis (12 months after FAO procedure for purchase orders are completed)

A new consultancy may be necessary depending on IEO staff availability and time schedule of FAO purchase order approval.

After the three presentations were delivered, the group discussed about the progress on this discipline. The group praised the work of samplers in the four countries for the good provision in the scheduled time and to the three groups for the good progress on the initial steps on identification of Loci and methods. One of the main limitations to follow the initial schedule is the long procedures for the purchases and payments to be done by FAO. This issue was recurrent in all the disciplines. The CopeMed fishery expert informed of the recent changes in the FAO procedures for procurement that are more and more complex and asked for patience while committing on following up this procedures very closely.

The group also agreed to make the necessary efforts to increase the number of individuals sampled of *P. Bogaraveo.* On this regard, the IEO representatives suggested to contact a fisherman for one day contract in Tarifa during the best period of catches to fish additional specimens. This contract could be covered by the IEO as a contribution to the project. After the summer break, all the administrative arrangements will be initiated for this fishing day. At the date of finalizing this report (end of October 2019), IEO funding is not available.

3.2 Morphometry and meristics.

- ***Current advances on body morphometry of sardine and hake sampled in TransBoran Project*** *(Lead: Tahar Filali, Centre National pour la recherche et le development de la Pêche et l'Aquaculture, CNRDPA)*

All pictures of sardine and hake stored in the SharePoint of TransBoran have been revised and screened for quality. 14 landmarks have been selected as shown in figure 5.

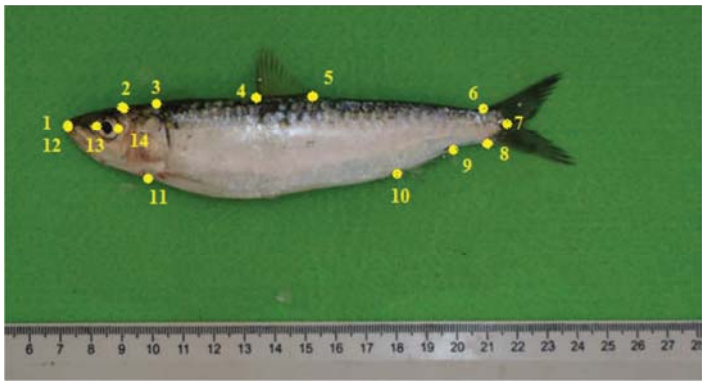

Figure 5. Landmarks identified for sardine.

Standardization of landmarks and Principal Component Analysis is in progress. Preliminary results comparing four extreme ports (Agadir, Estepona, Anaba and Ghazaouet) show good discrimination power of this technique as can be shown in the preliminary outcomes of Principal Component Analysis from four ports which is showed in figure 6 as an example of the firthcoming outputs. However, no conclusions can be extracted from this preliminary plot due to the low number of samples per port and the low number of ports.

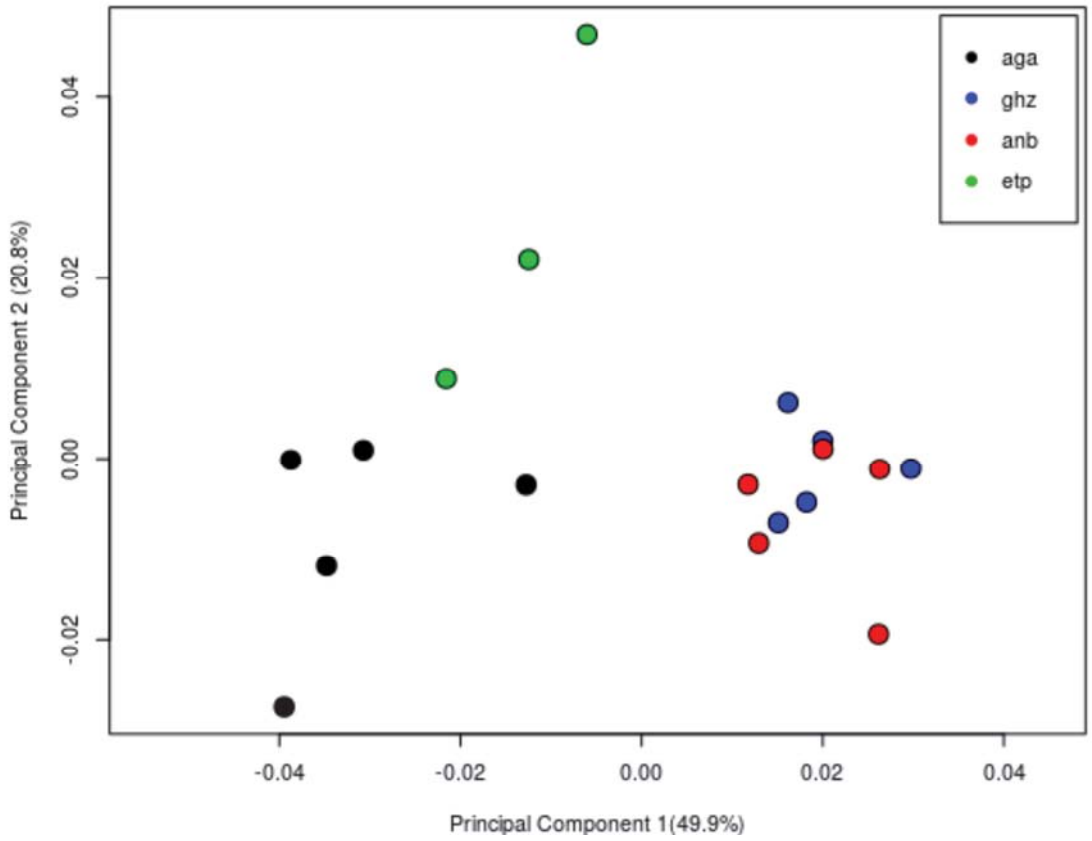

Figure 6. Preliminary outcomes of Principal Component Analysis for the body morphometrics of sardine from four ports Agadir, Ghazaouet Anaba and Estepona.

Some deficiencies in the pictures have prevented identification of two landmarks in some specimens. The group advised to first check how many of the samples show these deficiencies and then if the number is significant, and if these two landmarks are not the most discriminant ones eliminate them from the set of fourteen.

- ***Vertebra and gill-rakers.*** *(Lead: F. Wahbi, Institut National de Recherche Halieutique, INRH Morocco; Others: Meryem Benziane, Moulay Hachem Idrissi, Mohamed Malouli)*

The sampling for the meristic approach provided a total of 862 vertebrae and gills for *Sardina pilchardus* and 593 vertebrae and gills for *Merluccius merluccius*. A workshop was organized in Casablanca between readers in order to standardize the reading of vertebrae and gills before processing the samples. For minimizing the error rate, 3 readings per sample will be performed. The vertebrae photos displaying problems of readability will be removed from the final reading. For now, some of the vertebrae photos and gills have already been processed and the other ones are in progress (see detail in Table 3). The readings and statistical analysis will be finalized in December 2019-january 2020.

Table 3. State of reading for the meristic approach.

Country	Sardina pilchardus (% of reading)		Merluccius merluccius (% ofreading)	
	Vertebrae	Gills	Vertebrae	Gills
Morocco	**100%**	80%	**100%**	90%
Algeria	70%	50%	70%	40%
Tunisia	**100%**	40%	80%	50%
Spain	50%	40%	50%	0%

3.3 Otoliths Elemental composition and shape analysis.

- ***Preliminary results of the shape analysis and microchemistry of the otolith of Sardine Collected in Tunisia, Algeria, Morocco and Spain*** *(Lead*: *Sana Khemiri, Adel Gaamour from Institut National des sciences et technologies de la Mer (INSTM), Tunisia. Others: Sabri Jaziri, Miriam Domínguez, Javier Rey)*

The otoliths of sardine were extracted from the fish collected from the 17 sampling locations in the Alboran Sea and its adjacent waters. All the otoliths were, first photographed with high-contrast. Images were produced using reflected light, where otoliths were bright objects on a black background for shape analysis. Shapes of the otoliths were analyzed by OTOLab software by using the OTOTHRESH tool for image segmentation. The shape parameters measured were: Area, Major axis, Minor axis, Eccentricity, Perimeter, Circularity Compactness and Fourier Descriptors (sShenF1, sShenF2 sShenF3 sShenF13 and FF).

The otoliths were then analyzed for elemental quantification of Strontium, Barium, Manganese and Magnesium in the core and in the edge by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) following standardized methodologies.

Preliminary results revealed a certain heterogeneity in the shape, more evident in the microchemical composition of the otoliths between the samples collected from 4 locations situated in Tunisia, Algeria, Morocco and Spain, performed as a preliminary analysis. Figure 7 shows the output of canonical variate plots for the core of otholiths in the four ports from different countries selected as an example. Note, however, that no conclusion at all attending to the objectives of the project can be extracted from this plot as it is exploratory and preliminary.

For the hake and the blackspot seabream, the otoliths were already extracted and photographed but not yet analyzed by OTOLab software nor by laser ablation inductively coupled plasma mass spectrometry.

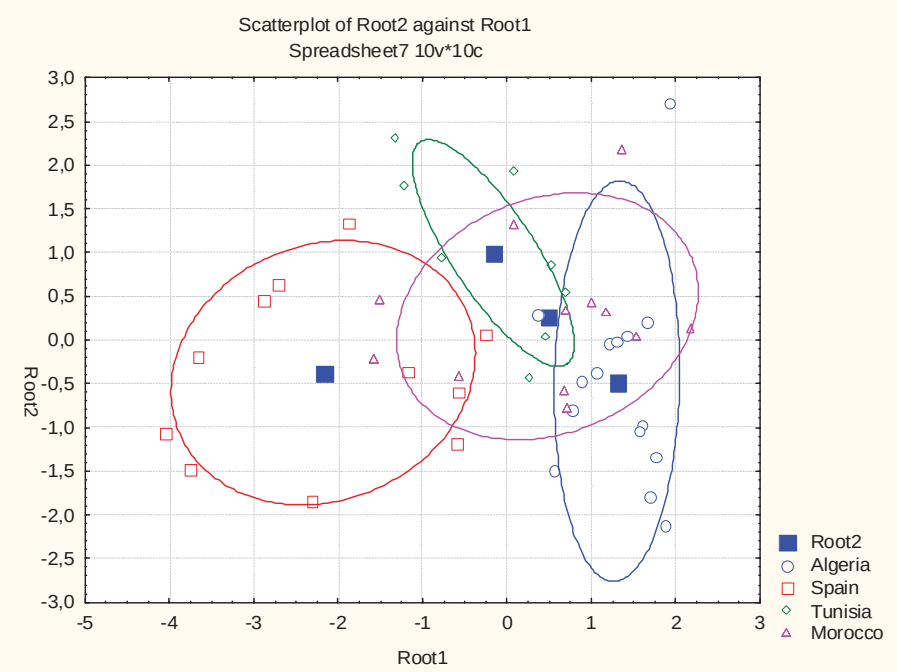

Figure 7. Example of preliminary analysis showing the output of canonical variate plots for the microchemical composition of core of otholiths in the four selected ports.

3.4 Parasites as natural markers.

- ***Parasites of the genus* Anisakis *as "biological tags" in fish stock characterization of*** *European hake* ***Merluccius merluccius*** from *the Alboran Sea and adjacent waters. (Lead: Simonetta Mattiucci from University La Sapienza, Italy amd mariem Feki INSTM, Tunisia. Others: Maria Letizia Palomba, Chiara Abattista)*

The genus Anisakis includes species of heteroxenous parasites of marine organisms, with crustaceans as first intermediate hosts, fishes and squids as intermediate and/or paratenic hosts, and, finally, cetaceans as definitive ones. The larval stages of Anisakis spp. commonly infect the viscera and musculature of many teleost species, also of commercial-economic importance. Their third larval stage, recovered from fish hosts and invertebrates, exhibit morphotypes indicated as Anisakis Type I or Type II (*sensu* Berland, 1961), but they cannot be identified, at the species level, based on conventional morphological analysis. Among the genetic methodologies used for the species identification of Anisakis larvae, nuclear (allozymes, DNA microsatellite loci, EF1 α−1 gene locus) and mitochondrial (mtDNA cox2) markers represent the most valuable tools for distinguishing Anisakis species.

During the first year of the TRANSBORAN Project, a total of 283 Anisakis spp. larvae were identified by a multi-markers genotyping approach collected in European hake from the selected localities of the project. These results allowed to: i) provide data on differential distribution of distinct species of Anisakis in hakes caught in different fishing grounds; ii) provide data on the infection levels with Anisakis spp. in different sub-populations of hakes; iii) use the differential distribution of Anisakis spp. genetically identified, and infection levels, as biological tags of hake sub-populations in Alboran Sea and adjacent waters; and iv) infer phylogeographical analysis of sub-populations of a target parasite species, i.e. *Anisakis pegreffii*.

In particular, five species of Anisakis were genetically identified: *A. pegreffii, A. simplex (s. s.), A. physeteris, A. ziphidarum and A. nascettii*. Among those, *A. pegreffii*

and *A. simplex (s. s.)* were the dominant species in all the fish samples; whereas, *A. physeteris, A. ziphidarum* and *A. nascettii* were identified at lower percentage. *A. pegreffii* and *A. simplex (s. s.)* showed statistically significant differences in their relative proportions from hakes sampled along the Spanish Atlantic coast, with respect those from those of the Atlantic and Mediterranean coast of Morocco. In the latter sampling locality (Mediterranean Morocco), also a higher percentage of *A. physeteris* was identified. The species *A. ziphidarum* and *A. nascettii* were identified only in hakes sampled along the Atlantic Moroccan coast. No high statistical significance was found between the relative proportions of *A. pegreffii* and *A. simplex (s. s.)* infecting hakes sampled from the Mediterranean coast of Spain (i.e. Estepona and Torrevieja) versus those observed in fish sampled along the Spanish Atlantic coast (i.e. Huelva and Cádiz). Further, in these last localities, which is included in the Atlantic contact zone between the two species, few hybrids (likely F1) between *A. pegreffii* and *A. simplex (s. s.),* were genetically detected. The similar relative proportion of *A. pegreffii* and *A. simplex* (s. s.) was observed in hakes from Spanish Atlantic coast (i.e Huelva and Cádiz) with respect to that from Mediterranean waters (i.e. Estepona and Torrevieja), indicating that those populations of hakes are more similar to each other, in terms of their parasites composition, rather than to other fish sub-populations captured in other Atlantic and Mediterranean sea waters. Cartographic display of these preliminary results are showed in figure 8. Also, the occurrence of F1 hybrid individuals in fish samples from Spanish Atlantic and Mediterranean Sea waters seems to further suggest that those hakes would belong to the same Atlantic population. The rare occurrence of few larvae of *A. simplex* (s. s.) up to the Tunisia coast may result from the occurrence of first intermediate hosts - on which the hakes preyed upon in that area - likely infected by larval stage of the parasite species, perhaps deriving from the oceanographic front of Alboran. Finally, results suggest that a southern population of hake along the Atlantic coast of Morocco would exist, just according to the species of Anisakis identified from the area of Medhia, also due to the absence of *A. simplex (s. s.)* detected in both Mehdia and Agadir samples of hake.

In addition, preliminary data on the population structure of the parasites species *A. pegreffii*, inferred from mtDNA cox2 sequences, were also obtained. Genetic diversity data so far acquired seem to indicate that although *A. pegreffii* is existing as a large panmictic unit, a slight genetic differentiation was found among the meta-population subunits attending to the hakes collected from different fishing grounds. These data on the genetic structure of a parasite species could be used in future comparative host-parasite co-phylogeographical analysis.

Figure 8: Geographical distribution of proportion of *Anisakis spp.* in hakes sampled in different localities

3.5 Analysis of fishery data and life history traits

- ***Spatiotemporal variation of fishery patterns, demographic indices and life history traits of European hake, Merluccius merluccius, in the Alboran sea*** *.(Lead: H. Eyaagoubi, University of Alicante, Spain; others: Manuel Hidalgo, Pilar Hernández)*

Three types of fisheries-dependent and -independent data were used to investigate potential similar of dissimilar variation on information gathered from north and south Alboran (Mediterranean Spanish and Moroccan waters) for the European hake: *i)* the spatiotemporal variation of fishery patterns using monthly CPUE per port, *ii)* demographic and spatial distribution indices from scientific bottom trawl surveys (MEDITS) and *iii)* length distribution of the Mediterranean Spanish and Moroccan waters.

Three different techniques were used for each type of data: i) Dynamic Factor Analyses (DFA) were used to analyze potential synchronic variation as well as to describe the main common modes of seasonal and inter-annual variation on CPUE series; ii) centers of gravity of standardized density obtained from the scientific bottom trawl surveys to assess temporal variation of hake distributions, iii) size distribution tests to analyze temporal and spatial variation of monthly size frequencies of landings per port and reveal potential spatial segregation in the mean size and in the seasonal evolution of the demography (the later only available for GSA 01).

The results suggest:

i) A general differentiation was observed associated to local dynamics at the north and south Alboran, with a secondary segregation between eastern and western ports over the Alboran Sea. While the north-south pattern is consistent with preliminary results obtained by other techniques (i.e. hake parasites results above described), the east-west differences are likely associated to the connection of north and south

populations subunits during the dispersive phase that results in the recruitment scenario observed (see below).

ii) Certain asynchronic, but connected, behavior in the spatial distribution of hake recruits at nursery areas between north and south Alboran Sea. In short, results of spatial metrics showed marked density-dependent and inter-annual fluctuations in hake distributions, which were asynchronically related between GSAs (Morocco and Spain), suggesting potential but intermittent linkages between GSAs driven by early life stages connectivity as the most plausible mechanism. That is, years with lower connectivity of larvae from north to south resulted in higher than average recruits abundance in the north (Spain) with a consequent competition for space and resources that triggered a change in the mean distribution of hake recruits. By contrast, in years with higher connectivity, there was higher than average recruitment in the south (Morocco) with space and food competition reflected in deeper distributions in the south.

iii) Different size distributions where observed between the two GSAs with more temporal stability in Spain than in Morocco and a different temporal dynamics in the increasing pattern of the mean size. However, the establishment of minimum landing size (at 20 cm) in both countries in different years, may hamper the correct interpretation of these results. Further comparison of size distribution from surveys may help to confirm initial guessing.

Future planned work will focus in including comparisons of trawl surveys size distributions and Algerian data, that were not initially included and subject to availability.

This work has been conducted by Hanane El Yaagoubi as a part of her Master Thesis project defended in past September in the Alicante University and supervised by Manuel Hidalgo and Pilar Hernandez.

- ***DFA (dynamic factor analysis) based on CPUEs for sardine in the Morocco and Spain*** *(Lead: M. Hidalgo, Spanish Institute of Oceanography, IEO, Spain; Others: Ana Giraldez, Hanane Elyaagoubi, Pilar Hernández).*

Focusing on similar and long-term available information for the north (Spain, GSA 1) and south (Morocco, GSA 3) of the Alboran Sea, the main objective was to analyze potential synchronic variation of sardine, *Sardina pilchardus*, as well as to describe the main common modes of seasonal and inter-annual variation on CPUE series applying the same technique (Dynamic Factor Analyses, DFA), used in CPUE of hake.

The results show that seasonal variability of CPUE is similar in both GSAs and it follows a single trend with maximum catches in summer in the whole Alboran system. This is a contrary pattern to that observed in hake that show north-south and east-west spatial segregation in the Alboran sea. It might suggest a more common dynamics as a whole in the Alboran Sea for sardine. It also suggests that environmental drivers affecting Morocco CPUEs already descried in Jhab et al. (2018) are the same in the Spanish CPUE and acting at a regional (i.e. Alboran) scale.

3.6 Hydrodynamic modelling of the Strait of Gibraltar (Physical Oceanography Group, University of Málaga)

- ***The model.*** *(Lead: Jesús García Lafuente, Jose Carlos Sánchez Garrido, Simone Sammartino and Irene Nadal from Physycal Oceanography Group of University of Málaga)*

The core of the study addresses the connectivity patterns of the Early Life Stages (ELS) of the three studied species by a numerical hydrodynamic model of the region of interest. It is based on the code of the Massachusetts Institute of Technology General Circulation Model (MITgcm), specifically adapted to the physics of the Strait of Gibraltar. The model grid is curvilinear, with uneven resolution, maximal at the own Strait, and spans from the Gulf of Cadiz to the eastern margin of the Alboran Sea. The model solves the momentum equations for fluids in a fully 3D domain, with uneven vertical resolution, maximal at surface (Figure 9).

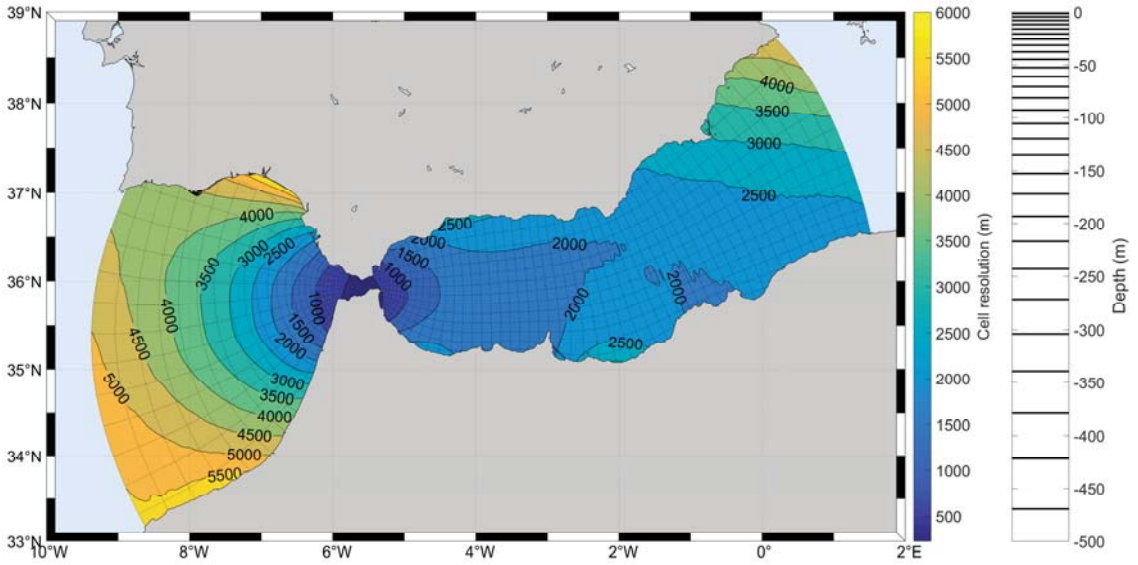

Figure 9. Horizontal and vertical grid of the hydrodynamic model employed in this study.

In order to simulate the Blackspot seabream ELS dispersal, the model has been run from December 2004 to April 2005, with a spin-up time (initial time needed to attain stable solutions) of one month. Horizontal velocities for 5 different depths (surface, 12, 25, 52 and 81 meters) have been outputted every half an hour, as inputs for the Lagrangian algorithm used to reconstruct ELS trajectories.

By observing the temporal evolution of the sea surface height field, as a proxy of the current circulation paths, we observed a progressive evolution from a generally cyclonic circulation at the Strait eastern exit, to a more stable anticyclonic structure of the western Alboran basin (compare panels of Figure 10). The former reflects the higher variability of the basin circulation in winter, with more variable Atlantic Jet intensity and instabilities in the resulting circulation in the rest of the basin. The latter is more typical of the more stable spring-summer season, with a wider and steadier Western Alboran Gyre, advecting over its northern rim a series of cyclonic eddies moving along the northern coast of the basin.

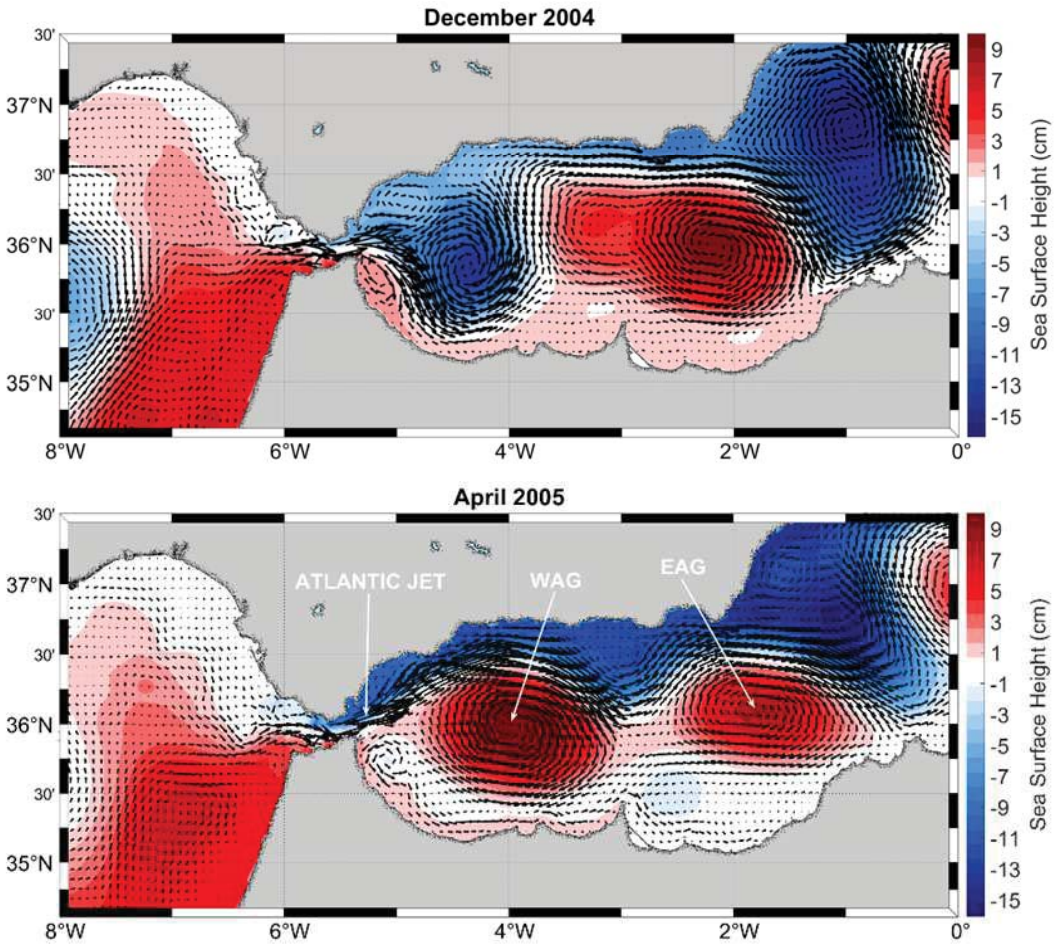

Figure 10. Monthly averages of sea surface height for December 2004 (upper panel) and April 2005 (lower panel). Mean current vectors are also depicted.

As one of the most important features of the model employed in this study, tides affect notably the circulation in the Strait and consequently in the rest of the neighbor Alboran Sea. The main eastward current spans the Strait from high to low tide (ebb tide), while the westernmost side of the channel undergoes a current reversal from low to high (flood tide). Such current structure affects the whole column water, with higher eastward velocities at surface during ebb tide and stronger westward current at higher depths in flood tide. This scheme is strongly modulated at subinertial scale (from few to some tents of days) both by fortnightly tidal cycle (spring and neap tide alternation) and meteorological forcing (winds and atmospheric pressure gradients). Spring tide strengthens current and its tidal phases gradients, ultimately affecting the gyres structure within the basin, while neap tide generally weakens the Atlantic Jet providing more stable and less turbulent paths of the circulation in Alboran Sea (Figure 11).

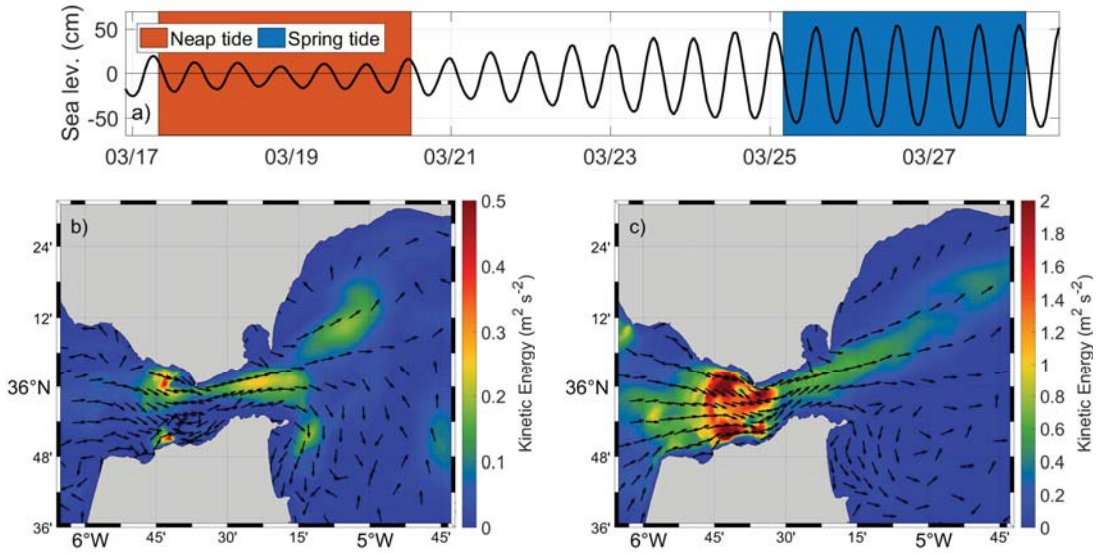

Figure 11. Averaged kinetic energy under neap (left) and spring (right) tide. The upper panel shows the sea level in Tarifa, with highlighted the averaged periods.

In turn, atmospheric gradients between the Alboran Sea and the Gulf of Cadiz, and more locally winds can affect the circulation at different depth range, depending of the nature of the phenomenon. Pressure affects the whole water column, provoking a weakening (strengthening) of the Atlantic inflow under high (low) pressures in Alboran, while wind affects current mostly at surface, generating a breaking effect on surface inflow under easterlies, and an accelerating impulse with westerlies. Subinertial modulation is generally only few tents of percentages of the tidal flow magnitude, but it can reach comparable effects when multiple forcings combine.

- ***Simulation of the dispersal patterns of Early Life Stages (eggs and larvae) – ELS of sardine*** *(Lead: Jesús García Lafuente, Jose Carlos Sánchez Garrido, Simone Sammartino and Irene Nadal from Physycal Oceanography Group of University of Málaga, Spain)*

The hydrodynamic connectivity of different sardine spawning areas (figure 12) of the Alboran Sea was investigated in the period Nov. 2003-Mar. 2004. The results were derived from the outputs of a high-resolution circulation model of the Alboran Sea (AS), which was then used as the basis of a Lagrangian Flow Network analysis. Special attention was paid to the possible exchange of ichtyoplankton between north and south coasts of the Alboran Sea.

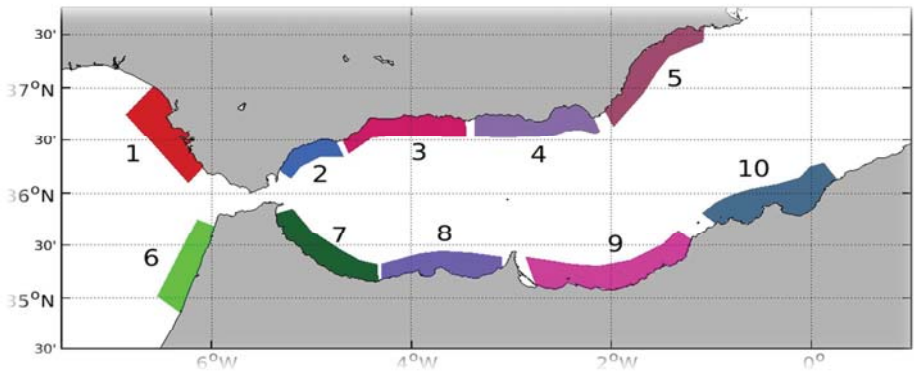

Figure 12. Ten boxes identified as spawning areas by the experts on sardine biology.

We found that the probability for connectivity between north and south spawning areas was of the order of 60‰, with the north-to-south flux of biological material being somewhat more likely than that from south-to-north. We also found a non-null input of particles form the Atlantic spawning regions to the Mediterranean regions. This west-to-east flux of material took place fundamentally through the southern margin of the Strait of Gibraltar and the AS. Curiously, the two Atlantic regions of the network were not connected with each other in any of the simulations. This probabilities in units per thousand are summarized in figure 13.

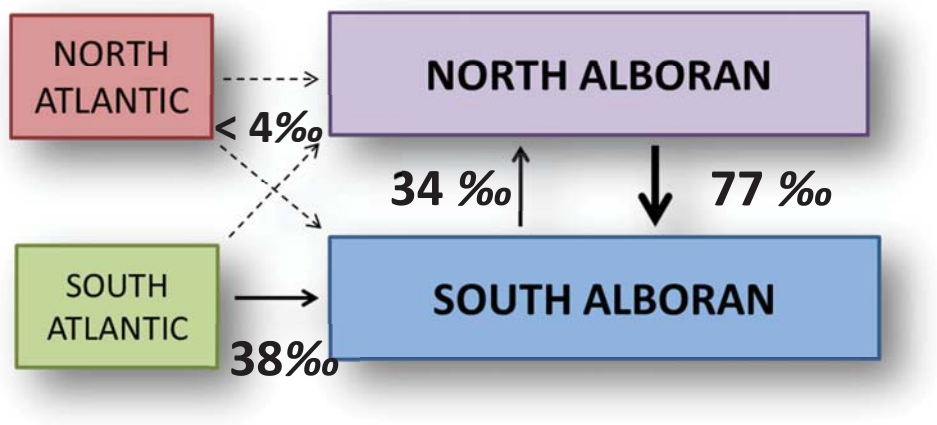

Figure 13. Summary of the probabilities of connection of particles spawned.

Additional runs showed that these results are sensitive to the depth at which sardine larvae were transported by the currents. While the overall pattern of connectivity did not change dramatically with depth, the probability for connection between the areas of the network increased substantially as larvae were located at greater depth (at least up to 25 m depth). Connectivity patterns resulted also rather sensitive to the time release of the particles (i.e. larvae), suggesting that the mesoscale configuration of the flow (and perhaps also other shorter-scale processes) affect greatly the exchange of particles among spawning areas. Because of the mesoscale configuration of the AS circulation is subject to substantial interannual variability, the analysis of additional years seems necessary to provide more statistically confident connectivity patterns of the AS.

Next steps:

Based on the sensitivity analysis, the group agreed that additional runs have to be made to obtain more reliable statistics of sardine connectivity metrics. These runs will include more scenarios (i.e. other years) of the Alboran Sea surface circulation, covering at least a two-gyre and single-gyre configuration, for which we will simulate at least one additional year.

- **Simulation of blackspot seabream ELS** *(Lead: Jesús García Lafuente, Jose Carlos Sánchez Garrido, Simone Sammartino and Irene Nadal from Physycal Oceanography Group of University of Málaga, Spain)*

Blackspot seabream early life stages (ELS) dispersal has been simulated applying a Lagrangian Particle Tracking Algorithm to the outputs of the hydrodynamic model described above. Three release areas and ten landing areas have been used to assess the dynamic connectivity of this species from their spawning regions to their potential recruiting zones (Figure 14).

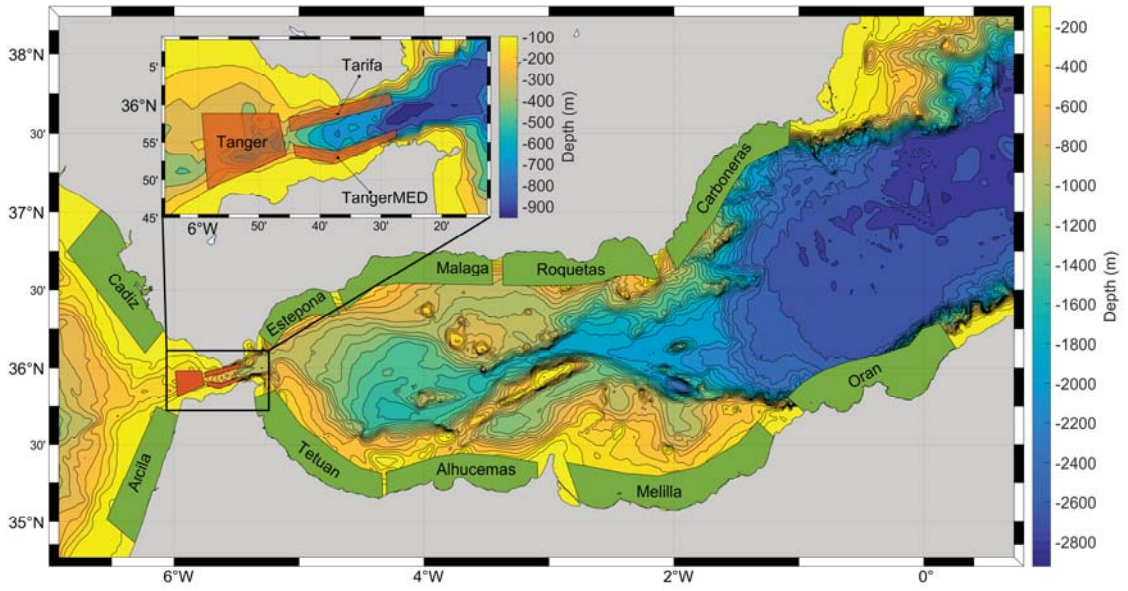

Figure 14. Three release (i.e. spawning) areas in orange and ten arrival (i.e. recruitment) areas in green.

Five different depths of release have been considered (1m, 12m, 25m, 52m and 81m). A total of 480 runs have been executed, with the aim of evaluating the temporal and spatial variability of the connectivity patterns caused by tides, which prevails at semidiurnal/diurnal time-scale, and meteorological forcing (subinertial variability), which is mainly due to atmospheric pressure variations over the Mediterranean basin and predominates at few-days time scale. Tidal modulation has been assessed by releasing particles under four different tidal conditions (phases): high, ebb, low and flood tide. Subinertial modulation has been investigated by repeating these conditions under spring and neap tide. In order to obtain robust estimation of the different forcings effect, four replicas of both subsets of conditions have been carried out.

The northern coast of the Strait is strongly connected with the northern margin of the Alboran Sea, thanks to the straight connection of the Atlantic Jet (AJ) and the northern rim of the Western Alboran Gyre (WAG), which flows swiftly along the northern margin of the basin (Figure 15). Spread of ELS in the Estepona coastal area is especially enhanced by spring tides, which promote the broadening of the cyclonic eddies generating along the northern rim of the WAG. The rest of the northern coast is mostly reached under neap tides, which spread particles in a less extent, and allow them to reach farthest distances. Under spring tides the higher spread of the AJ at its exit also promotes the formation of a southern coastal gyre which feeds the southern coast directly, and gives origin to a much quicker and more efficient connectivity of the northern Strait to the African coast. In this framework, then, ELS that reach the easternmost portion of the southern coast proceed from west. Conversely, under neap tides, the connection along the southern coast is mostly westwards, where ELS moving there proceed mostly from east. In both cases, times for ELS spreading along the southern coast are long, especially for the second mechanism, occurring under the action of weaker currents.

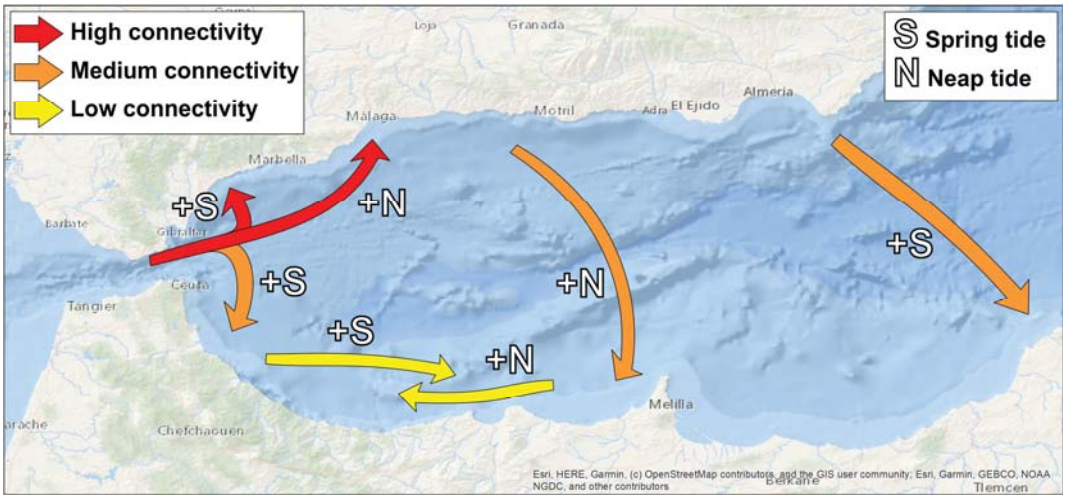

Figure 15. Sketch of the main connectivity paths from northern coast of the Strait.

The southern coast of the Strait is mostly connected to the southern African coast (Figure 16). The main mechanism is the rather permanent coastal gyre forming at the very southern exit of the Strait. Almost regardless the tidal intensity modulation, ELS are swiftly advected southwards and flow along the whole African coast featuring eastward connectivity. Spring tides give origin to the only case of northward connectivity in the basin. When the coastal gyre is especially wide and strong (under spring tides) the probability that the most external portion of it detaches and starts flowing north-eastwards is quite high. ELS, initially destined to the African coast have the chance of being spread along the northern coast. When it 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.

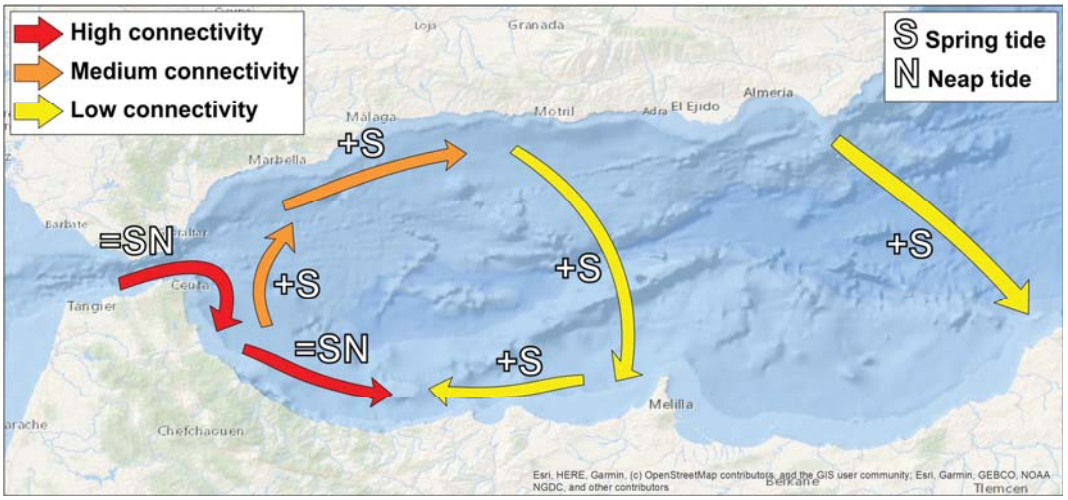

Figure 16. Sketch of the main connectivity paths from southern coast of the Strait.

Although the mean patterns afore described so far can be reasonably assumed as valid for most of situations, it is clear that depth and subinertial variability, especially of meteorological nature, can easily turn the tables. Generally speaking, depth fosters the farthest connectivity. The higher the depth, the weaker and especially the more stable the current. This helps to limit the ELS scattering along longer pathways and hence enhance the connection among farther sites. This can be observed for all the three release areas.

All these sources of variability likely overcome the expected interannual variability of the Strait of Gibraltar – Alboran Sea circulation, especially if we take into account that winter – spring period shows the highest degree of unpredictability of the system. It is very likely that the expected discrepancy between the results coming from the repetition of this study over further years, and the ones obtained by this one, could be masked by the own system variability.

- **Discussion on modelling design for hake**

As the next step after sardine and blackspot seabream distribution are completed, simulations on hake's ELS dispersal will be conducted by the team of physical oceanography of the University of Málaga. The experimental strategy for this species was discussed. The workshop finally concluded that, in principle, the same spawning areas as sardine will be used while hake larvae will be initially dispersed between 60-90 m depth, which will be the starting point of releasing from early April and the end of May for a period of 40 day (30 and 50 days could be also tested).

4. Organized discussion and synthesis about the integration and implications of the results of the different techniques and for different species (M. Hidalgo)

Discussions held during the two previous sessions are summarized below as general conclusions and the main decisions are structured by discipline. Furthermore the workplan for the next semester has been included within the section for each discipline above.

GENERAL CONCLUSIONS AND WORK PLAN

Some techniques have completed the analyses**:** ***i)*** hake parasites**,** ***ii)*** fisheries information for hake (pending considering Algerian landings), and ***iii)*** hydrodynamic simulations for blackspot seabream, while all the others are in progress. The reasons are varied, but most commonly due to the FAO bureaucratic burden in the procedures of purchasing laboratory materials and formalizing contracts.

Regarding sampling process, with the exception of scarcity of samples in the north Alboran for blackspot seabream, minimal limitations observed in some ports for the other two species do not prevent reaching the objectives of the project.

While some of the preliminary results for hake (parasites and fisheries data) suggest certain demographic segregation between north and south, final interpretation need to be done with all analyses completed providing complementary information of connectivity processes and segregation at different spatial scales.

GENETICS

Sardine: 12 microsatellite loci have been selected for configuration of multiplex PCR. Genotyping and allele scoring will be initiated in IEO once all the formalities for the purchase of material will be finalized by FAO.

Hake: 6 Microsatellites loci and SNPs are ready to go to genotyping and allele scoring, pending to finalize the purchase procedures by FAO.

Blackspot seabream: Markers are ready as in the other two species. All fish available will be analyzed but potential limitations due to scarcity of samples in some Spanish port must be overcome.

One day of extra sampling by a Spanish fishing vessel in Tarifa area may be needed to comply with the necessary statistical robustness of the analysis, although due to governmental funding restrictions in the second half of 2019 these samples are not guaranteed .

MERISTICS

Sardine and Hake: While branchial arch to count gill rakers were available, not all vertebrate photos were correctly taken to count the vertebrae. The group agreed to circumvent this limitation as follows: i) eliminate the invalid photos and develop the analyses per port without these photos, ii) if the final number of samples per port is too low affecting the robustness of the statistical analyses, pool the samples with a neighboring port.

To homogenize reading criteria, meristics team met together in a workshop whereby counts were done for three readers independently.

Pending work would be to finalize the countings of few left samples and develop the statistical analyses.

BODY MORPHOMETRICS

Sardine and Hake: Since there were some pictures in which landmarks were not possible to identify the group recommends a two-fold approach: 1) identify to landmarks in a reasonable number of photos to develop the statistical analyses, 2) in case this is not possible, remove these landmarks.

Pending work relates to develop the statistical analyses once the landmark limitation is solved.

OTOLITHS SHAPE

Sardine and Hake: After a training performed at the University of Malaga, otolith shape analyses with the software OTOLAB and after a preliminary analysis the group recommended to follow some criteria to select the metrics i) adimensionality, ii) respond to the project objectives (variability vs. mean values), and iii) no-correlated metrics before performing the discrimination analyses.

Pending work relates to develop the statistical analyses. The group recommended to work independently in the otolith shape and in the microchemistry since each technique gives information at different temporal and spatial scale and, therefore the results obtained may not be comparable.

OTOLITHS MICROCHEMISTRY AND ISOTOPES

Sardine: Analyses have been already performed. Although some preliminary and promising analysis have been performed with data with few locations the group recommend to develop the complete set of analyses to interpret the data. The group also recommended to go beyond analyses attempting to respond the segregation of different areas (CCA and PCA), and analyse the probabilities of natal homing combining information of core and edge of the otolith.

Hake: Analyses to be performed between October and November 2019.

Blackspot seabream: Analyses to be performed between December 2019 or early 2020.

Pending work relates to develop the statistical analyses for each species.

Isotopes (C and O) in the carbonate (edge and core) are still pending

PARASITES

Sardine: Since no infection was detected in the individuals collected for the project, additional samples (of individuals larger than 19 cm) are kindly requested from the acoustic surveys to be developed by the end 2019 and in 2020.

Hake: Preliminary results show a primary segregation Atlantic-Mediterranean populations, and most likely a potential segregation of population subunits between north and south Alboran Sea (i.e. adult fish do not share feeding grounds; persistent-multiannual indicator). These results are consistent with those obtained when analyzing the CPUEs over the whole Alboran Sea.

To get a more statistically robust pattern, more samples are requested. Additional samples from Málaga were analyzed the days after the mid-term meeting while those from Cadiz and Morocco are kindly requested. Final statistical analyses will be performed once additional samples are analyzed.

FISHERIES AND SURVEY DATA

Sardine: First results of the CPUE suggest a homogeneous pattern a the whole Alboran scale. Pending analyses will use information from size distributions (from surveys) as well as information from Algeria (landings and surveys data).

Hake: First set of analyses have been completed revealing primary north-south differences in the CPUE, and a secondary pattern east-west. Information obtained from the scientific bottom trawl surveys are consistent with a potential connectivity processes between north and south Alboran shaping the recruitment.

Landings and length distributions from Algeria will be considered in a new set of analyses.

HYDRODYNAMICS

Sardine: The oceanographic simulations has been completed for one year suggesting a higher connectivity pattern from north to south than from south to north. However, the group agreed that this pattern needs to be contrasted with other three years of different oceanographic scenarios in the Alboran Sea.

Hake: The same years used for sardine simulations will be used for hake. A protocol for hake simulations will be agreed in terms of: depth, timing, and pelagic larval durations. The areas of releasing will be the same as those selected for sardine since, to date, there is not any information on the spatial distribution of hake spawners.

Blackspot seabream: The oceanographic simulations on seabream are considered as finished. The main sources of variation on the final arrival of particles (i.e. early life

stage) is the location of releasing in the Strait of Gibraltar along with the tidal and subinertial dynamics.

5. Any other matters

- ***Introduction of other similar initiatives in the Mediterranean***

Copemed II Fishery Expert, introduced the main elements of a similar project which can be complementary to TransBoran, It is called MED_UNITS project, has been launched in 2018, it is funded by the EC and executed by a consortium of 9 institutions from Italy, France, Spain and Greece and leaded by CoNISMa (Italy).

This project aims to study all Mediterranean Geographical Sub-Areas (GSAs) (from 1 to 27) and addresses the following list of priority species: European hake (*Merluccius merluccius),*red mullet (*Mullus barbatus*), deep water rose shrimp (*Parapenaeus longirostris*), giant red shrimp (*Aristaeomorpha foliacea*), blue and red shrimp (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*). It intends to have a duration of 24 months, from, January 2019 to December 2020. Its main objectives of MED_UNITS can be summarized as follows:

1. Identify biogeographical boundaries and population structure of priority species.

2. Investigate genetic distribution among and within sub-basins

3. Identify and delineate stocks using the 'state of the art' approach i.e. the application of multiple approaches, to the same biological samples, to achieve an interdisciplinary perspective and consensus (Cadrin et al., 2013).

4. Identify and delimit the more important fishing grounds with associated main origin of the operating fleet to define spatial units for fisheries management.

5. Combine the information on fish stock boundaries and delimitation of spatial units for fisheries management.

6. Perform stock assessments based on the updated list of stock units for the examined priority species as emanating from the present study and compare with previous assessments.

The expected outputs of the MED_UNITS project are:

- A pilot genetic study and comprehensive genetic studies at Mediterranean Sea scale.
- Otolith shape and microchemistry characterization of European hake and red mullet.
- Maps on the fishing grounds and maps combining fishing grounds with the spatial distribution of the target species.
- Maps of the delineated population units of the target species on the basis of biological
  information (genetic and otolith results).
- Maps combining population and management units identifying areas of potential joint
  management at sub-regional and regional level.
- A SWOT (Strength, Weakness, Opportunity, Threats) analysis evidencing the advantages/disadvantages of the various methods and techniques used.
- Report on the stock assessments with the new stock configurations for the 6 target species of the study.
- Scenario modelled to explore the potential of developing adaptive spatial fisheries management.

To accomplish these objectives in a whole Mediterranean scale the consortium has requested collaboration in the collection of samples from the GSAs 3, 4, 12, 13, 14 of the species targeted by MED_UNITS. This collaboration would be on a voluntary basis, but the consortium would provide detailed protocols of the samples manipulation and expendable laboratory material if needed.

Participants welcome this new initiative and agreed to share the outcomes of the respective projects. Concerning the provision of samples, the group decided to consult with their institutions and formulate a reply to MED-UNITS on a case by case basis.

6. Closure of the meeting

Participants agreed on the main conclusions as reported here and arranged to finalize the text of the report on-line in the following weeks.

The WK agreed to undertake a final meeting once the remaining analysis are performed tentatively in the first semester of 2020.

By thanking the '*Subdelegación del Gobierno*' for their excellent venue and the participants for their valuable contributions, CopeMed Fishery expert closed the meeting.

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Appendix II

Agenda

Monday 22nd July 9:00 to 18:00

1. Background and objectives of the workshop *(CopeMed II)*

2. Overview of the status of samples and the sampling process *(M. Hidalgo)*

3. Current advances by the different methods used (*by the tasks coordinators)*

3.1. Genetic markers

- *Blackspot Seabream (A. Cariani)*
- *European hake (K. Mokhtar-Jamaï)*
- *Sardine (C. Johnstone, M. Pérez).*

3.2. Morphometry and meristics.

- *Body morphometry (T. Filali)*
- *Vertebra and gill-rakers (F. Wahbi)*
- *Otoliths (A Gamour, S. Khemiri)*

3.3. Elemental composition.

- *Microchemistry analysis of otoliths (S. Khemiri)*

Tuesday 23rd July 9:00 to 18:00

3.4. Parasites as natural markers.

- *Analysis of Anisakis spp. in hake (S. Mattiucci., M Feki)*

3.5. Analysis of fishery data and life history traits

- *Spatiotemporal variation of fishery patterns, demographic indices and life history traits of European hake, Merluccius merluccius, in the Alboran sea (H. Eyaagoubi)*
- *DFA (dynamic factor analysis) based on CPUEs for sardine in Morocco and Spain (M. Hidalgo)*

3.6. Hydrodynamic modelling of the Strait of Gibraltar *(Physical Oceanography Group, University of Málaga)*

- The model.
- Simulation of the dispersal patterns of Early Life Stages (eggs and larvae) – ELS of sardine (*J. C. Sánchez*).
- Simulation of blackspot seabream ELS (*S. Sammartino*).
- Discussion for modelling designed on hake.

Wednesday 24th July 9:00 to 14:00

4. Organized discussion and synthesis about the integration and implications of the results of the different techniques and for different species (*M. Hidalgo*).

5. Program of work for the next moths
6. Any other matters
    - Introduction of other similar initiatives in the Mediterranean
7. Closure of the meeting

Appendix II

Provisional List of Participants

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