COMMISSION OF THE EUROPEAN COMMUNITIES Directorate-General for Fisheries XIV

MAREA PROJECT

STOCKMED: "Stock units: Identification of distinct biological units

(stock units) for different fish and shellfish species

and among different GFCM-GSA"Specific Contract No 7 (SI2.642234)



FINAL REPORT

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Abstract

The STOCKMED project has tackled, according to a holistic perspective, the identification of the stock units and their boundaries for 19 species of commercial interest in the Mediterranean. The study is based on available scientific data and published literature, relative to any field that is considered key for the multidimensional identification of stocks. The challenges faced were therefore: 1) to critically review all the available data/information and select relevant descriptors for investigating the stock structure; 2) to develop a methodology to standardize and effectively integrate such highly heterogeneous kinds of data/information (moreover, in general collected for purposes others than stock identification). The strength of the proposed methodological framework relies on its explicit spatial formulation and the incorporation of experts' judgment at various steps of the process. To enable this, an original combination of tools of Geographic Information Systems (GIS)/spatial analysis and Multi-Criteria Decision Analysis (MCDA) has been proposed. The two techniques provide, when integrated, the methods to deal with large sets of multidisciplinary spatial information in a process able to reconcile as objectively as possible multiple perceptions of the stock units which may otherwise seem to be conflicting. Information gathered in the different WPs regarded the biological indicators estimated from scientific surveys, the biological parameters already available from scientific literature (e.g., growth, maturity, parasites), the genetic structure of population, and the patterns of the environmental factors (e.g., habitats and currents). Considering that a detailed knowledge of the population spatial distribution is essential to identify stock boundaries, a set of hypotheses was defined using the information of scientific surveys carried out within the Data Collection Framework funded by the European Union. A process of validation/ testing of these hypotheses through independent descriptors, which included considerations on the discriminating capacity of these descriptors for the different species, allowed to select the most plausible ones. Despite the difficulties in integrating with a rigorous analytical procedure different types of information (due to different spatial scales and time intervals), this methodology allowed to identify possible geographical configurations for the stocks of each species, and to assess their uncertainty. These results represent the first example in the Mediterranean of units of stocks distributions obtained thru a holisticstandardized approach and based on the available knowledge. For management purposes, the distribution of the stocks of each species was compared with information on the distribution of fishing fleets, in order to identify areas to be considered homogeneous for stock assessment and fisheries management. Given the quality of the available data, both in terms of discriminatory ability and spatial/temporal coverage, the distribution patterns obtained do not have the same degree of plausibility. While some appear coherent for data-rich species, (e.g., Merluccius merluccius), other distributions present a high degree of uncertainty (e.g., Eledone moschata). That notwithstanding, these distribution patterns represent a starting point to improve the knowledge of the distribution of the stocks in the Mediterranean. These proposed patterns would need to be validated through specific studies, including ad hoc collection of multidisciplinary set of data similarly to the approach adopted in other areas (ICES).

Furthermore, these stock configurations should be constantly updated according to the advancement of knowledge. In order to achieve this some gaps in knowledge need to be filled. In particular studies on modelling the retention and/or dispersion of eggs and larvae from the spawning grounds in relation to physical oceanographic processes were very poor. Eggs and larvae of some selected species, for which the ecology is well documented, could be represented as Lagrangian drifters released in the main spawning ground. Successively the simulated transport of the pelagic stages could be considered to identify connectivity mechanism among stock subunits and units. Another gap to be overcame is to improve the use of 'last generation' markers (SNPs), coupled with an extensive sampling scheme, which markedly increase the power of genetic data for stock identification.

Although the growing importance in the international literature, studies on microchemical composition and shape of otoliths still lack in the Mediterranean, as well as investigations on adults migrations and movements. Finally, a greater availability of standardized information on the spatial distribution will improve the accuracy in selecting data for stock assessment and the consequent adoption of appropriate management measures.

General objectives and background

Project objectives

The overall aim of the project is to investigate the presence of distinct biological units (stock units) for some target species in different GFCM-GSAs reviewing and integrating the available information through a multi-disciplinary approach. This approach is adopted because literature data show that inferring demographic stock boundaries using genetic data alone could lead to erroneous conclusions for fishery management purposes. It was recognised that investigation based on a single population characteristic can be insufficient to identify the occurrence of different stock units.

On the basis of life history traits and information coming from fisheries, biological research (e.g. biometry, parasites, physical tagging, multiple genetic markers, species mobility, characteristics and duration of embryo and larval phases, growth parameters, etc), hydrology and topography, this study aims at providing the updated scientific vision on stock boundaries in the Mediterranean of the following relevant species: European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), striped mullet (*M. surmuletus*), common Pandora (*Pagellus erythrinus*), common sole (*Solea solea*), horse mackerels (*Trachurus trachurus* and *T. mediterraneus*), anchovy (*Engraulis encrasicolus*), sardine (*Sardina pilchardus*), Norway lobster (*Nephropo norvegicus*), giant red shrimp (*Aristaeomorpha foliacea*), blue-and-red shrimp (*Aristeus antennatus*), deep-water rose shrimp (*Parapenaeus longirostris*), common octopus (*Octopus vulgaris*), broadtail shortfin squid (*Illex coindetii*), horned octopus (*Eledone cirrhosa*), musky octopus (*E. moschata*), blackmouth catshark (*Galeus melastomus*), and anglerfish-monk (*Lophius budegassa*).

The main objectives of the study are synthesized as follows:

- to undertake a multidisciplinary identification of distinct fishery/biological units (stock units) for the most relevant demersal and small pelagic species in the Mediterranean in order to contribute to the improvement of the quality and the reliability of their assessment;
- 2. to investigate the relationship between stock units and characteristics of the main fisheries to evaluate the spatial consistency of the current data collection system based on the GFCM-GSAs subdivision of the Mediterranean. In connection with this the project is also aimed at identify possible solutions for the different species and fisheries, taking into account the need to ensure the higher consistency with the main current stratifications for data gathering and statistics reporting;
- 3. to provide an inventory of gaps and suggestions for further investigations.

The area under investigation is that covered by FAO-GFCM GSAs in the northern sector of the Mediterranean (see table 1 and fig. 1).

FAO Area			1.1			1.2				1.3						2.2				2.1	3.1
37. Divisions																					
GSAs	1	2	3	5	6	7	8	9	10	11.1	11.2	12	13	14	15	16	19	20	18	17	22,23, 25

Table 1 - GSAs covered by this study.



Figure 1 -The current GSAs in the Mediterranean according to GFCM (2011).

Background

In the Mediterranean, stock assessment and fisheries management are highly oriented by the division in Geographical Sub-Areas (GSA) of the GFCM Area. The definition of GFCM-GSA was done on the basis of various criteria and analyses carried out in the first half of last decade (oceanographic, biological, fishery, congruency with FAO-GFCM capture statistics, etc.).

Fishery science has developed a number of methods for identifying fish stocks. Begg et al. (1999) reported that stock identification can be based upon several source of information such as : the interpretation of distribution and relative abundance and catch data, tag recoveries, meristics, morphometrics, scale and otoliths morphology and microchemistry, parasites, cytogenetics, protein electrophoresis (isoelectro focusing), immunogenetics, mitochondrial DNA, nuclear DNA, the elemental composition of otoliths, stable isotope measurements, otolith microstructure, and life-history parameters.

The recognition that there is not a single method that addresses the various assumptions of stock assessment and needs of fishery management has prompted a more holistic view of population structure that has called for multiple sources of demographic and genetic data (Pawson & Jennings, 1996; Begg & Waldman, 1999).

Furthermore, recent improvements in knowledge on stock structure of marine organisms challenge the traditional view of populations like geographically distinct units with homogeneous vital rates and reproductive isolation from adjacent units. More complex concepts such as metapopulations may be more applicable to many fishery resources with population structure organised in several sub-units with different level of connectivity (Stephenson, 1999; Kritzler & Sale, 2004).

A wider application of spatially explicit models in future stock assessments and management will require to clearly identify the stock components, evaluate the movement rates and determining the degree of reproductive isolation. Because spatial structure affects how populations respond to fisheries, incorporation of heterogeneous patterns and movement in stock assessment models should improve advice for fishery management (Cadrin & Secor, 2005).

According to ¹STECF, it is advisable to have a more refined and updated view on the different stock units in the Mediterranean and to verify whether the current GSAs classification matches with advisable assessment and management units of the main resources and fisheries. To this aim STECF suggests to consider also other supplementary information such as the distribution of commercial species, of their biological and genetic characteristics and the distribution of the fishing fleets by gear, also in relation to the oceanographic and sea bottom characteristics. Considering that a same fishery may harvest a quite high variety of resources and that fishing fleets may carry out different fisheries over the year, tradeoffs may be inevitable between the best classification of biologic stock units, the mobility and characteristics of the various fishing fleets, the consistency with sampling strata for data gathering and reporting of catch statistics (national, EU, GFCM, etc).

No specific studies were aimed to define the stock boundaries and structure for most of Mediterranean areas, including those encompassing the territorial sea of the European countries, for stock assessment and management purposes. Despite this uncertainty about stock units, GFCM has preliminary identified some critical areas were stock of main commercial species are considered as shared amongst EU or/and not EU countries (FAO, 2006).

An exercise of identification of putative stock units of European hake, red mullet and deep-water pink shrimp in the Mediterranean Sea, using simple correlation analysis (Pearson's correlation coefficient) of trends in trawl survey abundance was attempted by Cheilari and Rätz (2009).

This sparse and limited knowledge and the need of a more integrated and updated view of the different stock units and boundaries for the assessment and management of the main fisheries resources in the Mediterranean has prompted the activation of the Specific Contract No 7 (SI2.642234) the project "Stock units: Identification of distinct biological units (stock units) for different fish and shellfish species and among different GFCM-GSA; STOCKMED" within the MAREA framework (Contract MARE/2009/05/Lot 1). This also in view of checking whether the current GSAs classification matches with advisable assessment and management units of the main resources and fisheries.

The Specific Contract No 7 has been signed on December 18, 2012 for a duration of 18 months.

To carry out the STOCKMED project all the available information coming from scientific surveys (e.g. MEDITS, MEDIAS, SOLEMON, GRUND) and commercial catches from DCR and DCF were needed, as well as the results of MEDISEH project, the specific project 2 of the MAREA framework, on mapping of critical habitats of the main commercial species.

¹Report of the SGMED-08-02 Working Group on the Mediterranean Part II 21 25 APRIL 2008, ATHENS, GREECE as endorsed at the 28th Plenary meeting of the Scientific, Technical and Economic Committee for Fisheries (plen-08-02. 7-11 July 2008, Helsinki).

Bibliography

- Begg G.A., Waldman J.R. (1999) An holistic approach to fish stock identification. Fisheries Research 43: 35-44.
- Begg GA, Friedland KD and Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research, 43:1–8.
- Cadrin S.X., Secor S.H.2009. Accounting for Spatial Population Structure In Stock Assessment: Past, Present And Future. in: Beamish R.J. & Rothschild B.J. The Future of Fisheries Science in North America. Fish & Fisheries Series, volume 31: 405-426.
- Cheilari A., Rätz H-J. 2009. Review of possible stock units of European hake, red mullet and deepwater pink shrimp in the Mediterranean Sea by means of trends in survey abundance. STECF SG/ECA/RST/MED 09-01.
- FAO General Fisheries Commission for the Mediterranean/Commission générale des pêches pour la Méditerranée. 2006. Report of the ninth session of the Scientific Advisory Committee.
 Rome, 24–27 October 2006/Rapport de la neuvième session du Comité scientifique consultatif. Rome, 24-27 octobre 2006. FAO Fisheries Report/FAO Rapport sur les pêches. No. 814. Rome, FAO. 106p.
- Garoia, F., I. Guarniero, et al. (2007). "Comparative analysis of AFLPs and SSRs efficiency in resolving population genetic structure of Mediterranean Solea vulgaris." Molecular Ecology 16(7): 1377-1387.
- Guarniero, I, Garoia, F., et al. (2004). Genetic stock structure analysis revealed single population units in the shared stocks of Adriatic demersal species. Reports of the Working Group on Demersal Species, SAC – GFCM, Sub-Committee of Stock Assessment, Málaga, Spain, 6-7 May 2004, Doc. #18, 7 pp; http://www.faoadriamed.org/html/AvDocRes.asp.
- Kritzer J. P., Sale P. F. (2004) Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries, 5: 131–140.
- Levi D, Patti B, Lo Brutto S, Arculeo M, Parrinello N., & Rizzo P (2004). Genetic and morphometric variation of Mediterranean hake, Merluccius merluccius, in the Strait of Sicily (Central Mediterranean). Ital. J. Zool., 71, 165-170.
- Maggio, T., S. Lo Brutto, et al. (2009). "Microsatellite analysis of red mullet Mullus barbatus (Perciformes, Mullidae) reveals the isolation of the Adriatic Basin in the Mediterranean Sea." Ices Journal of Marine Science **66**(9): 1883-1891.
- Mattiucci S., P. Abaunza, L. Ramadori, G. Nascetti (2004) Genetic identification of Anisakis larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology 65, 495–510
- Pawson M. G., Jennings S. 1996. A critique of methods for stock identification in marine capture fisheries. Fisheries Research, 25: 203-217.
- Ramon, M. M. and J. A. Castro (1997). "Genetic variation in natural stocks of Sardina pilchardus (sardines) from the western Mediterranean Sea." Heredity 78: 520-528.
- Rolland, J. L., F. Bonhomme, et al. (2007). "Population structure of the common sole (Solea solea) in the Northeastern Atlantic and the Mediterranean Sea: revisiting the divide with EPIC markers." Marine Biology 151(1): 327-341.

- Stephenson, R.L. (1999) Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fisheries Research 43, 247–249.
- Turan, C. 2004. Stock identification of Mediterranean horse mackerel (Trachurus mediterraneus) using morphometric and meristic characters. e ICES Journal of Marine Science, 61: 774-781.
- Tinti, F., C. Di Nunno, et al. (2002). "Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the Adriatic and Ionian stocks of Sardina pilchardus." Marine Biotechnology 4(2): 163-172.

Team composition and workpackage leaders

Partners/Subcontractors involved in the project and the team composition are respectively reported in the tables 2a and 2b.

PARTNERS/SUBCONTRACTORS INVOLVED IN THE PROJECT									
PARTNER OR SUBCONTRACTOR NUMBER	SCIENTIFIC RESPONSIBLE	Affiliation	WPs/TASKS INVOLVED						
4	Fabio Fiorentino	CNR	WP0, WP1, WP2, WP3, WP4, WP5						
5	Maria Teresa Spedicato	COISPA	WP0, WP1, WP3, WP5						
	(MAREA Coordinator)								
5	Giuseppe Lembo	COISPA	WP1, WP3, W4, WP5						
S3	Enric Massuti	IEO	WP1, WP3, WP5						
1	Fausto Tinti	CoNISMa	WP1, WP2, WP3, WP5						
6	Stylianos Somarakis	HCMR	WP1, WP3, WP5						
4	Germana Garofalo	CNR	WP1, WP3, WP4, WP5						
2	Paolo Sartor	CIBM	WP1, WP3, WP5						
S5	Roberta Mifsud	MCFS	WP1, WP3, WP5						

Table 2a - Partners/Subcontractors involved in the project

Table 2b - Team composition and work package leaders with affiliation

SURNAME	NAME	TASK INVOLVEMENT	WP/TASK COORDINATION	PARTNER OR SUBCONTRACTOR
Fiorentino	Fabio	Project Coordinator	WP0	CNR
Spedicato	Maria Teresa	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	WP5	COISPA
Somarakis	Stylianos	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	WP3; Task 3.4	HCMR
Tsigenopoulos	Costas	2.1, 2.2, 2.3; & WP5	Task2.3	HCMR
Tinti	Fausto	2.1, 2.2, 2.3; & WP5	WP2; Task 2.1	CoNISMa

Sartor	Paolo	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		CIBM
Garofalo	Germana	4.1; 4.2; 4.3 & WP5	WP4; Task 4.3	CNR
Giannoulaki	Marianna	1.2, 1.3, & WP5		HCMR
Patti	Bernardo	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	Task 3.1	CNR
Colloca	Francesco	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	Task 1.2	CNR
Massutí	Enric	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	WP1, Task 1.5, 1.6	IEO
Quetglas	Antoni	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		IEO
Lefkaditou	Eugenia	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		HCMR
Kapiris	Kostas	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	Task 1.3	HCMR
Anastasopoulou	Katerina	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		HCMR
Russo	Tommaso	4.1; 4.2; 4.3 & WP5	Task 4.1	CoNISMA
Bitetto	Isabella	4.1; 4.2; 4.3 & WP5		COISPA
Carbonara	Pierluigi	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	Task 1.2	COISPA
Facchini	Maria Teresa	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5	Task 1.1	COISPA
Scarcella	Giuseppe	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		CNR
Valavanis	Vasilis	4.1 & WP5	Task 4.1	HCMR
Follesa	Maria Cristina	2.1, 2.2, 2.3; & WP5		CoNISMa
Lembo	Giuseppe	4.2, 4.3. WP5		COISPA
Kavadas	Stefanos	1.1, 1.3, 1.4, 3.1, 3.2, 3.3, 3.4, 4.1, 4.2, 4.3		HCMR

Mytilineou	Chryssi	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		HCMR
Cannas	Rita	2.1, 2.2, 2.3 & WP5	Task 2.2	CoNISMa
Sbrana	Mario	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4	Task 3.3	CIBM
Mannini	Alessandro	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4		CIBM
Bolognini	Luca	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		CNR
Grati	Fabio	1.1; 1.2; 1.3; 1.4; 3.1; 3.2; 3.3; 3.4 & WP5		CNR
Mifsud*	Roberta	1.1, 1.3; 1.4; 3.4; 4.2; 4.3; WP5		MCFS
Carlucci	Roberto	1.1; 1.2; 1.3; 1.4, 3.2		CoNISMa
Sion	Letizia	1.1; 1.2; 1.3; 1.4; 3.2		CoNISMa

Small changes or specifications have been done to the team composition. In particular the responsibility of the COISPA partnership has been specified (table 2a), the involvement of CoNISMa in the WPs 1 and 3 has been also specified, while the responsibility for the Ministry for Resources and Rural Affair, Fisheries Research Unit of Malta has been undertaken by Roberta Mifsud, in place of Leyla Knittweis who moved for a new job position. The specification of the involvement of MCFS in the task 1.1 was also added. In addition, Angelo Cau from CoNISMa was replaced by Maria Cristina Follesa. The coordination of the task 3.2 was assumed by Mario Sbrana from CIBM, because Paolo Sartor, from the same Institute, has been in the meanwhile committed with the coordination of the DRIFTMED project within the MAREA Consortium. Some misspecifications previously reported in the current table 2b have also been amended.

Project breakdown and deliverables

The project is split into 6 WPs and several tasks which cover different but complementary aspects. A brief and summarized description of WPs and Tasks is reported below with the indication of the project milestones and deliverables.

WP 0. General Coordination

WP 0 aims to ensure the following activities:

- 1) Preparation of a working document on the guidelines of the methods to be presented during the kick-off meeting;
- 2) Co-ordination of the Project Meetings;
- 3) Co-ordination of WP activities ensuring connectivity among WPs;
- 4) Preparation and submission of the required reports (Interim and Final Reports).

Four coordination meetings are foreseen:

- the first meeting scheduled on the *first month* of the project;

- the **second meeting** by *month 6* to update the group on the partial results achieved and to fine tune the selected approaches prior to the synthesis of the Interim report;

- the **third meeting**, on the *month 10* on the implementation of GIS-MCDA for the problem structuring and criteria/preferences elicitation;

- the **fourth and final meeting** on *month 16* to discuss the results achieved in the different WPs, finalising the work of WP5 and structuring the final report.

Deliverables

- D1 Guidelines of the approach and methods
- D2 Interim Report
- D3 Final Report

WP 1. Review and analyses of the existing biological information on stock units in the investigated areas (Coordinator E. Massuti)

WP1 aims at reviewing all the available biological information useful to identify stock units in the investigated areas. Specific objective of this WP is also the selection of relevant case studies for further investigations, while ensuring spatial coverage over various sub-regions in the Western, Central and Eastern Mediterranean. All the information produced in this WP is transferred to WP4 as geo-referenced data.

The work of this WP is subdivided in 4 tasks summarized as follows:

- Task 1.1. Analyses of trends of abundance indices from scientific surveys in different GSAs to highlight synchronisms in temporal patterns. Selection of relevant case studies and correlation matrices by GSAs for each species (Task coordinator M.T. Facchini).
- Task 1.2: Reviewing and analysis for the selected case studies of biological information on otoliths, biometry, spawning and recruitment patterns, growth performances and reporting as geo-referred information (task Coordinator P. Carbonara).
- Task 1.3 Reviewing and analysing for the selected case studies information on parasites, tagging, migration patterns, larval drift and reporting results as georeferred information (task Coordinator K. Kapiris).
- Task 1.4. Synthesis of the spatial pattern of the main biological information for the target species in the case study areas and identification of gaps in knowledge on biological aspects for stock units identification (task Coordinator E. Massuti).

Milestones:

M1.1 – Relevant data gathering from an *ad hoc* data call facilitated by DGMARE and from partners of the MAREA Consortium.

- M1.2 Correlation analyses and matrices by GSAs for each species.
- M1.3 Reporting results as geo-referred information for the case studies from task 1.2.
- M1.4 Reporting results as geo-referred information for the case studies from task 1.3.

Deliverables

D4 – Report on analysis of trends of abundance (trends of target species in different GSA and analyses of synchronic pattern, including correlation matrices).

D5 – Synopsis of the spatial pattern of the main biological information for the target species in the case study areas, including gaps identification of critical gaps in the available biological knowledge.

WP2. Reviewing existing Genetic Stock Structure Analysis (GSSA) data of target fishery resources and assessing spatial population connectivity in the Mediterranean GSAs. (Coordinator F. Tinti)

WP2 aims at:

1) critically reviewing the existing genetically-based knowledge on stock structure and stock unit identification and identify the existing critical gaps;

2) assessing spatial population connectivity and providing indicators/estimates of population genetic differentiation;

3) reviewing and updating, at methodological and technological level, the GSSA pipelines* based on the most advanced and highly-performing tools in fishery genetics.

The work of this WP is subdivided in 3 tasks summarized as follows:

- Task 2.1 Critical revision of data from GSSA of the target fish and crustacean Mediterranean fishery resources, reviewing and using adequate information of significant results obtained by EU-financed projects and assessing RTD critical gaps (Task Coordinator F. Tinti);
- Task 2.2 Assessment of spatial population connectivity of Mediterranean target fishery resources (Task Coordinator R. Cannas);
- Task 2.3 Methodological and technological updating of the GSSA pipelines based on the most advanced and highly-performing tools in fishery genetics (Task Coordinator C. Tsigenopoulos).

* The term "GSSA pipeline" would include sampling design, sampling procedure, markers selection, markers development, lab procedures, and tests, methods and models for data analysis plus anything else useful to address a genetically-based methodology with high-efficiency and accuracy for testing genetic homogeneity of a putative stock unit.

Milestones

M2.1 - Relevant data gathering from past projects and from partners of the MAREA Consortium.

Deliverables

D6 - Report and updated literature list on GSSA data of the target fishery Mediterranean resources.

D7 – Individual reporting species sheets with genetic estimates of population differentiation indicators/parameters.

D8 - Report on novel, highly-performing GSSA pipelines for fishery genetics.

The deliverables will form the content of a review on the status and advance of knowledge on spatial population connectivity and fishery genetics of the target Mediterranean fish and shellfish resources.

WP 3. Review and analyses of the geo-morphological, oceanographic and fishery spatial patterns as factors related to stock distribution (Coordinator S. Somarakis)

WP3 aims:

- to collate, review and comment as adequate information on the sea bottom topography, water circulation pattern at different spatial scale;
- to collate, review and comment as adequate information on nurseries and spawning grounds;
- to collate, review, and to comment as adequate information on past and current spatial pattern of the main fisheries in relation to the target species.

For each case study the main information on geo-morphological, oceanographic, sensitive habitats and fishery features along with their spatial patterns in the case studies areas will be made available for the synoptic analyses to be done in WP4. Knowledge gaps of hydrological factors, sea bottom features, and fisheries characteristic useful for stock unit identification will be examined.

Description of work

The work to be done in this WP is subdivided in 4 tasks summarized as follows.

- Task 3.1. Revision and analysis of the spatial pattern of geo-morphological and oceanographic features. In some selected case study retention or dispersion of eggs and larvae from the spawning grounds will be investigated using physical oceanographic models (task coordinator B. Patti).
- Task 3.2 Revision and analysis of the existing information on persistent nurseries and spawning areas of the target species in the investigated areas. (task coordinator F. Colloca).
- Task 3.3. Revision and analysis of the existing information on fishing grounds of target species in the case studies (task coordinator M. Sbrana).
- Task 3.4. Synthesis of knowledge related to the spatial pattern of physical breaks, critical areas, and fishery effort relevant for stock unit investigation and identification of the main knowledge gaps (task coordinator S. Somarakis).

Milestones:

M3.1 – Relevant data gathering from an *ad hoc* data call facilitated by DGMARE and from partners of the MAREA Consortium;

M3.2 – Gathering environmental information (e.g. hydrological factors, sea bottom features, etc..) at adequate spatial scale;

M3.3 - Reporting results as geo-referred information from MEDISEH project;

M3.4 – Identification of persistent nursery areas and spawning grounds for the species of the selected case study not supported by MEDISEH outputs;

M3.5 - Organizing information for the fishing pressure factors at adequate spatial scale.

Deliverables

D9 – Report geo-referred information on the geo-morphological and oceanographic information which can act as potential break factors for environmental driven demographic borders in the Mediterranean);

D10 – Report geo-referred information on the spatial distribution of essential fish habitats (persistent nursery and spawning areas) for the selected case studies;

D 11 - Report geo-referred information on the spatial and temporal distribution of fishing effort/grounds for the case studies;

D12 - Synopsis of the spatial pattern of hydrology, sea bottom features, essential fish habitat and fishing effort in the investigated case studies, including identification of knowledge gaps.

WP 4 Identification of stock units and stock boundaries by multi-criteria approach (Coordinator G. Garofalo)

The aim of WP4 is to provide a putative definition of stock structure and boundaries identified on the basis of multi-criteria approach. In particular this WP aims at:

1) developing a GIS-MCDA framework for stock unit identification based on multiple sources of data and knowledge;

2) applying the developed framework to selected case studies to reconcile possible conflicting signals from the analyses and outcomes of WPs 1-3.

Description of work

The work of this WP is subdivided in 3 tasks summarized as follows.

- Task 4.1 To organize outputs from WPs 1, 2, 3 and 4 in a GIS database possibly as implemented for the MEDISEH project (Task coordinator V. Valavanis).
- Task 4.2 Development of a framework for the application of GIS-MCDA to stock unit identification providing guidelines to:
 - 1) the selection of criteria to be used

2) the conversion of the collected information into explicitly spatial criteria, their normalization and mapping;

- 3) the assignment of a weight to each criterion;
- 4) the definition of the method of multi-criteria evaluation;
- 5) the performance of sensitivity analysis to determine robustness of results.

A literature review on different approaches for developing weights and evaluation rules ensures the construction of a consistent model with regard to the specific objective of stock unit identification (Task coordinator T. Russo).

Task 4.3 Application of the GIS-MCDA framework developed in Task 4.2 to the case studies for the identification of stock units and/or the detection of stock boundaries, reconciling possible conflicting signals from the analyses and outcomes from WPs 1-3. Reporting data and results of WP4 as geo-referred information and maps (Task Coordinator G. Garofalo).

Milestones:

M4.1 Structuring the geo-referenced data base;

M 4.2 - Literature review on different approaches for developing weights and evaluation rules of the GIS-MCDA;

M 4.3 Implementation of the GIS_MCDA framework scoring and comparing criteria and decision factors.

Deliverables

D13 – Geo-referenced data base.

D14 - Protocol of methodology to implement the GIS_MCDA framework (including identification of criteria, decision trees, weighing factors, etc..).

D15 – Implementation of the GIS_MCDA framework scoring and comparing criteria and decision factors with the preparation of a Synopsis of the stock units and the corresponding stock boundaries.

WP 5 Proposal of stock units, comparison with existing GSAs and suggestion for further researches (Coordinator M.T. Spedicato)

WP5 aims at harmonizing the results obtained by the different WPs (1, 2, 3 and 4) and preparing a synthesis of the main outcomes, establishing suitable relationships among the project results and the current configuration of GFCM GSAs.

This WP is based on the following main tasks:

1) comparing the existing GSAs with the outcomes related to the distribution of stock units as obtained from the GIS-MCDA and propose suitable areas for stock assessments;

2) identifying gaps both in data and knowledge and propose further investigations;

3) propose for the various stock units and fisheries the most adequate spatial scale and strata to carry out data gathering, and fisheries management.

Milestones:

M5.1 - Outcomes of the project meetings

M5.2 - Analysis and systematization of the information gathered in the WP1-4.

Deliverables

D.16 – Stock Units recommendations

This deliverable will contain recommendations about the identification of stock units in the case studies areas. In particular:

- GSA/species in which stock assessment must be done for units smaller than the whole GSA;
- GSA/species in which stock assessment must be done for units larger than a single GSA;
- acknowledgement of gaps and proposal for further investigation to validate the putative stock units identified and improve the identification of stock units in the Mediterranean areas not considered by the project;
- an evaluation of the effect of proposed change in spatial scale of stock units on the current FAO Divisions and GFCM GSAs.

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Executive summary

Stock identification is an interdisciplinary subject that involves the identification of self-sustaining components in natural populations. It is a central theme in fisheries science, being a prerequisite for stock assessment and accordingly for fishery resources management. Nevertheless, the population structure of commercial species and the geographic distributions of exploited stocks are uncertain in several marine areas including the Mediterranean. Consequently, the reliability of stock assessments and the effectiveness of fishery management can be severely limited for many fishery resources. The STOCKMED project is aimed to: i) undertake a multidisciplinary identification of distinct fishery/biological units (stock units) for the most relevant demersal and small pelagic species in the Mediterranean in order to improve the quality and reliability of their assessment; ii) investigate the relationship between the stock units, the characteristics of the main fisheries involved and the GFCM-GSAs system and the ongoing spatial stratification for the collection and analysis of fisheries data; iii) provide an inventory of knowledge gaps and suggestions for further investigations.

The area under investigation is that covered by FAO-GFCM GSAs in the sector of the Mediterranean, corresponding to GSAs 1, 5, 6, 7, 8, 9, 10, 11, 15, 16 17, 18, 19, 20, 22, 23, and 25. The project aims to identify the stock units of 19 target species: European hake (Merluccius merluccius), red mullet (Mullus barbatus), striped mullet (M. surmuletus), common Pandora (Pagellus erythrinus), common sole (Solea solea), horse mackerels (Trachurus trachurus and T. mediterraneus), anchovy (Engraulis encrasicolus), sardine (Sardina pilchardus), Norway lobster (Nephrops norvegicus), giant red shrimp (Aristaeomorpha foliacea), blue-and-red shrimp (Aristeus antennatus), deep-water rose shrimp (Parapenaeus longirostris), common octopus (Octopus vulgaris), broadtail shortfin squid (Illex coindetii), horned octopus (Eledone cirrhosa), musky octopus (E. moschata), blackmouthcatshark (Galeus melastomus), and anglerfish-monk (Lophius budeaassa). The general approach of the project is to combine in a novel holistic framework information from different domains, including abundance (total and by critical life stage), demography and lifehistory parameters, parasites, genetics, and environmental factors.. This multisource information was analysed using a holistic approach based on the integration of Geographic Information System (GIS) techniques with multi criteria analysis. Within this framework and in accordance with the STOCKMED proposal, four meetings were held during the course of the project. Due to the novelty of this approach, these meetings were essential to refine the methodology by discussion and the evaluation "in-progress" of the available data and the results obtained. A kick-off meeting was held in Rome (Italy) on 6-7 February 2013, in order to organize the work among the partners involved: timeframe for work and deliverables, the input data format as well as the GIS requirements. In addition, a preliminary framework of the methodological approach was presented and discussed. This work was held in parallel with the five work packages. A second meeting was held in Palermo (Italy) on 3-4 July 2013. Brief presentations on the progresses of work were made, emphasis was given on the difficulties and the problems encountered and extended presentations were made on the reviewing of available information. In order to fulfill the objective of the STOCKMED project, scientific surveys data were chosen to provide possible distribution patterns of stock. Furthermore, the methodological approach combining an original spatial analysis and multi-criteria decision analysis was presented and discussed. A third meeting took place in Rome (Italy) between 25-26 November 2013. During this third meeting, the revision of existing information per work package and associate tasks were

presented. The timeframe to follow in order to meet the project objectives and deliverables was discussed and agreed. The meeting focused on the evaluation of the analytical framework that was improved on the basis of discussion of the preliminary results. After the third meeting, due to the new adopted methodology, it was also decided to ask for a time extension of the project, without which the quality of the project output and deliverables could have been largely undermined. For these reasons, an extension of three months was granted the EU. by A fourth meeting was held in Rome between 15-17 July 2014 (Italy). During this fourth meeting, the work progress and the deliverables per work package and task were presented. Furthermore, the timeframe and the scheme to follow for the final report were discussed and agreed. The meeting focused on the presentation of the results obtained within the framework of WP1, WP2, and WP3. The stock spatial distribution pattern obtained for all the target species were presented and discussed. This was followed by a long discussion with regard to the improvement of the presentation of project results, including the most plausible stock spatial pattern.

Within the framework of the project, all (100%) of the deliverables foreseen have been produced. The Activities of WP1 and corresponding deliverables were subdivided in the following Tasks:

• Abundance trends analysis from surveys in different GSAs, Task 1.1 (deliverable 4).

• Review and analysis of biological information, Task 1.2 (deliverable 05).

- Review and analysis of parasites, tagging, migration and larval drift, Task 1.3 (deliverable 05).
- Synthesis of the spatial pattern of the main biological information for the target species in the

case study areas and identification of gaps in knowledge on biological aspects for stock unit identification, Task 1.4 (deliverable 5).

The main activities within WP1 involved a critical review of the data availability and feasibility (e.g. survey, fisheries and bibliography) concerning the biological domain. GFCM grids and MEDITS strata were considered to be the best option after a preliminary analysis aimed at identifying the more suitable geographical scale (statistical grids of GFCM, MEDITS strata & GFCM geographical sub-areas) was carried out in Task 1.1. Population structure indices were also investigated according to the planned procedures: mean fish weight, mean length diversity index and comparison of length frequency distributions from multi-variant analysis. The indices of growth and mortality, sex-ratio and reproduction (only females of species which spawning period coincides with MEDITS surveys) were also taken into account. With regard to Task.1.2, the compilation of information on recruitment, spawning periods and growth (VBGF) performance from the literature was collected. Among the biological parameters considered, the main information was derived by growth, size at first maturity and length of the spawning season. Literature data regarding these three parameters were collated for all 19 STOCKMED species. With regard to the growth, a total of 232 references were revised and 544 sets of parameters were compiled in Excel files. Statistical analysis of data was produced for 14 species (A. antennatus, A. foliacea, E. cirrhosa, E. encrasicolus, M.barbatus, M. merluccius, M. surmuletus, N. norvegicus, P. erythrinus, P. longirostris, S. pilchardus, S. vulgaris, T. mediterraneus and T. trachurus), and for 8 of them (A. antennatus, A. foliacea, M. barbatus, M. merluccius, P. erythrinus, S. vulgaris and T. trachurus), the presence of some geographical trend allowed for the computation of clustered maps, grouping GSAs by their Growth Performance Index. Concerning the length of first maturity (L50), a total of 186 references were revised and 379 sets of parameters were compiled in Excel files. In this case, statistical analysis of data was produced for 15 species: A. antennatus, A. foliacea, E. cirrhosa, E. encrasicolus, G. melastomus, I. coindetti, M. barbatus, M. Merluccius, M. surmuletus, N. norvegicus, O. vulgaris, P. erythrinus, P. longirostris, S. pilchardus, T. mediterraneus and T. trachurus), and for 5 of them (A. antennatus, E. encrasicolus, I. coindetti, M. merluccius and

M. surmuletus) the presence of some geographical trend allowed for the computation of clustered maps, grouping GSAs by their similar L50. Finally, with regard to the last parameter, the Length of spawning season, a total of 240 references were revised and 240 sets of parameters were compiled for STOCKMED analyses. Statistical analysis of length of spawning season data was performed for 15 species (A. antennatus, A. foliacea, E. cirrhosa, E. encrasicolus, E. moschata, I. coindetti, M. barbatus, M. merluccius, M. surmuletus, N. norvegicus, P. erythrinus, P. longirostris, S. pilchardus, T. mediterraneus and T. trachurus) but only for 1 of them (P. longirostris) could the geographical pattern be identified. Data regarding other biological parameters like morphometric and meristic and otoliths shape and biochemistry were very poor. The research done for all 19 STOCKMED species gave back only 4 references regarding the morphometric and meristic and 1 concerning the otoliths shape and biochemistry.

The available information on other parameters like parasites, tagging, migration and larval drift was also reviewed **(Task 1.3).** Concerning parasites, a total of 57 references (papers and reports) were revised for all 19 species. Information was only present for 15 out of the 19 species studied. Regarding the tagging, 5 references belonging to 4 species were revised while concerning migration and larval drift, data of 15 species (60 references were found) and 18 species (23 references) respectively were found. The main conclusions derived from the whole work done in WP1 **(Task 1.4)** were synthesized as follows:

• For the purposes of WP1, the MEDITS data were considered to be the most appropriate source of information among the available (i.e. other surveys and bibliography) and provided information at smaller geographical scale than the bibliography.

• Stock units/boundaries can differ depending on the species and the parameters considered.

• The smallest level of aggregation that bibliographic data allow is GSA, thus no conclusions can be drawn regarding smaller stocks units.

• Tagging, parasites, migration and larval drifting could provide reliable information on connectivity among areas, especially parasites, but data are so scarce that no statistical analysis can be driven.

• For biological parameters obtained from bibliography, the information was not so scarce, but it is not well standardised and, in some cases, no geographical trends were found and no clustering could be drawn.

The Activities of WP2 and corresponding deliverables were subdivided in the following Tasks:

• Critical revision of data from Genetic Stock Structure Analysis (GSSA) of the target fish and crustacean Mediterranean fishery resources. Task 2.1. (deliverable 6).

• Assessment of spatial population connectivity of Mediterranean target fishery resources. Task 2.2. (deliverable7).

• Methodological and technological update of the GSSA pipelines based on the most advanced and highly-performing tools in fishery genetics. Task 2.3. (deliverable 8).

Concerning **Task 2.1**, the STOCKMED genetic endnote reference master database was prepared, including 437 references. The STOCKMED GSSALitDB was generated by enquiring the most popular peer-reviewed reference databases (e.g. ISI Web of Sciences, Scopus), and it has been integrated with genetic data that were reported in non-peer reviewed papers, in-press publications and unpublished data according to the participants' knowledge. All 19 STOCKMED species were investigated. The species in which data are more abundant were: *Solea solea, Engraulis encrasicolus, Mullus barbatus, Trachurus trachurus, Sardina pilchardus, Merluccius*

merluccius and *Mullus surmuletus*, *Octopus vulgaris*, while *Galeus melastomus* (1 reference), *Eledone cirrhosa* and *Eledone moschata* (0 reference) were the less investigated. Furthermore, the GSSA data sets were ranked according to power indicators for potential success in stock unit identification in the Mediterranean. The 437 GSSA references have been scored according to 3 indicators of stock identification power whose metrics were combined in a simple algorithm. High power GSSA datasets pre-selection produced a selected set of 43 datasets regarding 14 target species for NE Atlantic & Mediterranean to be used by Task 2.2.

The main results of **Task 2.2** included a collection of 14 Species Sheets. Each Species Sheet contains all the relevant data along with genetic estimates of population differentiation indicators/parameters. No useful information on genetic structuring within the Mediterranean Sea was found for 5 species: *Eledone cirrhosa, E. moschata, Galeus melastomus, Illex coindetii* and *Lophius budegassa*.

Finally, 43 datasets providing useful information on population genetic differentiation within the Mediterranean Sea for the STOCKMED target species were found:

• 1 dataset is available for 5 species: Aristaeomorpha foliacea, Parapenaeus longirostris, Sardina pilchardus, Trachurus mediterraneus and T. trachurus;

• 2 datasets are available for 3 species: Aristeus antennatus, Nephrops norvegicus and Pagellus erythrinus;

• 3 datasets are available for 2 species: Merluccius merluccius and Octopus vulgaris;

• 4 and 5 datasets are available for Mullus surmuletus and M. barbatus, respectively;

- 6 datasets are available for Solea solea;
- 11 datasets are available for *Engraulis encrasicolus*.

The information contained in each dataset with regard to the Genetic marker, details of the sampling sites, main findings, and contribution to Genetic differentiation and stock structuring were analysed.

When multiple datasets were available for a given species, they were all analysed in detail and included in the species sheet. The identification of genetic differentiation and/or structuring was realised in three steps: i) Scrutiny of the dataset in terms of sampling size and design, genetic marker used, and/or data analysis performed, ii) Matrix of clustering transforming the genetic data into binary matrixes for each GSSA data sets, iii) Geo-visualization of clustering where population sample has been assigned to a specific Cluster according to the results of the literature.

The activities and results of **Task 2.3** consisted of reviewing the most advanced and highlyperforming methods, markers and computational tools used in GSSA, as well as identifying the main critical (i.e. technological and methodological) gaps in the existing GSSA pipelines for the identification of stock units. A cost-benefit analysis considering sampling, markers and technologies, analytical methods (including those not having any studies in genetics) was provided.

The main conclusion of the WP2 evidenced:

• Very few studies have been realized on the STOCKMED target species in the Mediterranean. Furthermore no raw data were available for checking or reanalysis of information;

• With exception of *T. trachurus*, any genetic studies were not integrated with other disciplines using a multidisciplinary approach for stock identification;

• For most of the species, the geographical coverage of genetic data was very poor and sporadic; not enough sampling and/or not enough loci have been analysed;

• Only few cases of broad and systematic sampling design were available (usually within the framework of International project funded by EU, e.g. *T. trachurus, S. solea* and *M. merluccius*).

• For some species, the data and/or the techniques used are very old and little informative;

• Only 3 cases of studies based on 'last-generation' markers (SNPs) were available to date (e.g. *E. encrasicolus, M. merluccius and S. solea*). When such a large number of markers are used, the power of genetic data in identifying differentiation and structuring increases markedly;

• Only adequate funding allows to collect adequate data and to give adequate answers to managers and fishers.

The Activities of WP3 and corresponding deliverables were subdivided in the following Tasks:

• Information on the sea bottom topography, water circulation pattern at different spatial scales; Task 3.1 (deliverable 9).

• Information on the Essential Fish Habitats (nurseries and spawning grounds); Task 3.2 Coordinator (deliverable 10).

• Information on past and current spatial pattern of the main fisheries/metiers in relation to the target species. Task 3.3 (deliverable 11).

• Synthesis of the spatial pattern of the main environmental (hydrological factors), EFH and fisheries features information and identification of gaps in knowledge, Task 3.4 (deliverable 12).

Regarding Task 3.1, the geomorphology of the Mediterranean basin, detailed bathymetry data of the entire Mediterranean and geo-referenced information limited to the western Mediterranean were collected. Moreover, the review of the available information on hydrological features and currents in each Mediterranean subarea (eastern, central and western Mediterranean) were used to infer potential connectivity between nursery and spawning areas and/or between adjacent GSAs. For this purpose, maps of sea surface geostrophic currents (speed and direction) obtained from the analysis of a satellite-derived sea level anomaly dataset and maps of average currents from 3-D hydrodynamic models (current for surface and deeper layers) were used. The review of larval stages and drift studies resulted in a weak potential inferences on connectivity through larval transport in the Mediterranean. Firstly, knowledge of the planktonic stages (e.g. larval durations, vertical distributions, ontogenetic changes in behavior and capabilities) is rudimentary, involving mainly anchovy and sardine, being nothing known for the most part of the demersal species. Secondly, although the general circulation patterns in the Mediterranean are currently well known, short-term and meso-scale variability might be high and the complex circulation of the shallow (on-shelf) areas is still poorly understood. This is especially true for the Mediterranean islands, such as the Balearic Islands (GSA 05), Corsica (GSA 08), Sardinia (GSA 11), Crete (GSA 23) and Cyprus (GSA 25), for which the hydrographic connection with 'mainland' GSAs is very unclear.

With regard to **Task 3.2**, the overview of MEDISEH results on nursery and spawning habitats distribution included 14 species. An additional analysis of MEDITS data for *Eledone moschata*, *Lophius budegassa*, *Octopus vulgaris*, *Solea solea* was also provided within the STOCKMED project. Concerning **Task 3.3**, a revision and analysis of the existing information on fishing grounds of target species was done. The fishing grounds identification were mainly based on the revision and analysis of information that already existed, such as: available reports, GFCM publications, VMS data, grey literature, peer-reviewed papers, unpublished data available to the Consortium, as well as expert knowledge. Published information on fishing grounds is scant and fragmentary, whereas the consortium was unable to collect any information for several GSAs (GSA2 – Alboran Island; GSA3 – Southern Alboran Sea; GSA 7 – Gulf of Lions; GSA 8 – Corsica; GSA 17 – Slovenian and Croatian side; GSA 22 – Turkish side). As decided during the 3rd STOCKMED meeting in November, maps of fishing grounds will not be used for the direct definition of stock boundaries but for the identification of fishery impact on stocks and the effects in assessments and management.

In the synthesis of **Task 3.4**, the cases in which persistent nursery and/or spawning sites defined in different (adjacent) GSAs are located in close proximity, implying the potential continuity of EFHs across the adjacent GSAs were underlined. For all "shelf" species (such as red mullet, pandora, anchovy and sardine) with adequate data to model EFHs, GSAs with proven 'hydrographic connectivity', namely GSA 06 & 07 and GSA 09 & GSA 10 in the Western Mediterranean and GSA 15 & GSA 16 and GSAs 17 & 18 in the Central Mediterranean, were proposed to belong to a stock unit. The lack of information from GSA 08 (Corsica) represents an impediment to evaluate the connectivity between island GSAs in the western Mediterranean (namely GSA 08 & GSA 11 [Sardinia]) with 'mainland' GSAs, such as GSA 09 & 10.

Considering the deep-water species (such as *Parapenaeus, Illex, Aristeus*), it could be assumed that GSA 05 could be grouped with GSA 06 & 7 and GSA 08 & GSA 11 could be considered together with GSA 09 & 10. In *Merluccius* and *Parapenaeus*, persistent EFH sites in southern GSA 19 (off eastern Sicily) seem to be extending from those in GSAs 15-16. Similarly, for deep water and medium pelagic species, persistent EFH sites in northern GSA 19 are in close proximity to those in GSA 18. However, the connectivity of northern GSA 20 and GSA 18 is not clear and needs further investigations. Under a parsimonious approach, GSAs 22 (Aegean) and 23 (Crete) could be considered together for deep-water species.

The Activities and corresponding deliverables of WP4 were subdivided in the following Tasks:

• Organise all data sets and output products from WP 1, 2 and 3 in a GIS database under a common geo-referenced system and common data format. Task 4.1. (deliverable 13).

• Development of a framework for the application of GIS-MCDA to stock unit identification Task 4.2 (deliverable 14).

• Application of the GIS-MCDA framework developed in Task 4.2 to the case studies for the identification of stock units and/or the detection of stock boundaries, reconciling possible conflicting signals from the analyses and outcomes from WPs 1-3. Reporting data and results of WP4 as geo-referred information and maps showing the provisional conclusion about stock structure (spatial based) on basis of GIS-MCDA. Task 4.3 (deliverable 15).

Concerning **task 4.1**, all datasets (in the form of shapefiles) and associated INSPIRE-formatted metadata files (in XML format), delivered by the different WPs, were zipped in individual files for each species and placed in a geodatabase for display and downloading through the project's Data Viewer. The Data Viewer is an enhancement of the related work carried out through the MEDISEH project and now the viewer includes all MEDISEH and STOCKMED datasets for viewing as well as downloading. The viewer itself is a customised Java applet application embedded in an HTML environment including the data display area and the data downloading area. The viewer may be accessed through the main MAREA website (http://www.mareaproject.net/) and becomes fully accessible after a user registration. The STOCKMED geodatabase is an ArcGIS 10.0 geodatabase located at the MAREA ftp site (under STOCKMED directory).

With regard to **task 4.2**, a methodological framework was developed to perform studies of stock identification in the Mediterranean by integrating multidisciplinary perspectives. The specific context is that of studies based on available scientific data, expert knowledge and published literature. This implies the necessity to collate and integrate highly heterogeneous types of data/information which may differ with respect to the goals, time and spatial scales of collection as well as with respect to their quality and accuracy. The strength of the proposed STOCKMED methodological framework relies on its explicit spatial formulation and the incorporation of experts' judgment at different steps of the process. To enable this, an original approach combining

Geographical Information Systems (GIS) tools of spatial analysis and Multi-Criteria Analysis (MCA) was proposed. In synthesis, the methodological framework develops in two main phases and uses two different spatial scales.

• Generating the alternative Hypotheses - In the first phase, different hypotheses of stock structure are identified at a spatial scale lower than the current management units (GSAs) applied in the Mediterranean, that is the GFCM 30' × 30' cells grid (GFCM Recommendation GFCM/33/2009/2). Six biological indicators provided by the MEDITS surveys (and computed within the WP1) are mapped into the GFCM grid and used to generate a finite number of different hypotheses of stock units through Constrained Clustering (CC), a semi-supervised learning algorithm, which guarantees that putative stock units occupy fully connected subareas of the Mediterranean Sea. As a matter of fact, the different indicators are characterized by a different relevance with respect to the objective of identifying the stock units, which need to be included into the evaluation. The Analytical Hierarchy Process (AHP) methodology is used to estimate weights of biological indicators according to the judgment of a panel of experts. Hence, the spatial constrained clustering is performed on the set of six indicators, using the weights vector in the variance/covariance matrix, for a number of clusters ranging from 2 to 20. After that, the Calinski-Harabasz (CH) index is computed to evaluate the effectiveness of choosing a given number of clusters, not known a priori. At the end of this phase, a set of scored alternatives representing Hypotheses of stock units are available.

• Selecting the most plausible alternative Hypotheses - In the second phase, all the thematic descriptors produced at the GSA scale, by the different WPs, such as genetics, parasites, growth, correlation of abundance trends and so on, are used to reinforce and/or validate the different hypotheses available.

This is accomplished by computing the Cohen's Kappa coefficient of agreement between each hypothesis and each thematic descriptor. In order to score the relative importance/impact of the different thematic layers, the approach known as Non-Structural Fuzzy Decision Support System (NSFDSS) has been implemented. NSFDSS applies fuzzy logic to model the ambiguity and imprecision of vague terms used by experts to express their preferences about criteria/thematic descriptors and/or evaluate the quality/quantity and coverage of the available information. One of the outputs of NSFDSS is a weights vector for the different thematic descriptors.

The Cohen's Kappa coefficients and the weights vector estimated through NSFDSS form the basis to construct the weighted decision matrix and, ultimately, calculate the Cohen's Kappa weighed mean which allows to rank the hypotheses. At the end of the process, the hypotheses that fall above the upper quintile of the mean Cohen's Kappa distribution are retained as candidate hypotheses of stock structure based on the best possible use of available multidisciplinary data. Finally, a sensitivity analysis is conducted to model uncertainty and judge the stability of the results. The Stochastic Multicriteria Acceptability Analysis (SMAA), based on Monte Carlo simulation, is selected as a method allowing to take into account, simultaneously, the uncertainty about the criteria and their weights. SMAA provides, for each alternative hypothesis, a vector of rank acceptability indices which measure the stability of the assignment of the alternative hypothesis to a given rank, and can be interpreted as the probability that the alternative appears in a given position in the rank order. The hypotheses that receive high acceptability for the highest ranks are the most relevant. The rank acceptability indices for each alternative hypothesis, which sum to unity, are finally aggregated in a Holistic Acceptability Index (HAI), measuring the overall acceptability of the alternative. The alternatives characterized by values of mean Cohen's Kappa above the upper quintile are retained as candidate hypotheses on stock structure. Acceptability analysis is applied to assess the robustness of the obtained ranking of hypotheses and to take a more informed decision. Alternatives with high acceptability for the best ranks and high Holistic Acceptability Index are the most plausible hypotheses.

Within **Task 4.3**, the developed methodological framework was applied to the 19 fish and shellfish target species of the project producing stock units and relative boundaries for each investigated species. The maps about most plausible hypotheses on stock structure for all species, together with main population parameters (abundance and demography) for each supposed stock units are presented.

The Activities of WP5, reported in deliverable 16, regard the following tasks:

• comparing the existing GSAs with the outcomes related to the distribution of stock units as obtained from the GIS-MCDA and propose suitable areas for joint stock assessments or areas where the assessment should be conducted at a local spatial scale;

• identifying gaps both in data and knowledge and propose further investigations to improve the identification of stock units in the Mediterranean, including the genetic characterisation of putative stock units already identified;

• propose for the various stock units and fisheries the most adequate spatial scale and strata to carry out data gathering, and fisheries management, while taking into account likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs.

The results gathered in the different WPs and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to work with, taking into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this configuration, in terms of stock assessment, data collection and fishery management.

Results established suitable relationships among the project results and the current configuration of GFCM GSAs, identifying areas in which conducting joint stock assessment is preferable or subunits are more appropriate for this objective. Stock assessment purposes are also linked to the proposition of a suitable spatial scale to gather data and for other broader utilizations related to the knowledge and management of fishery resources. The innovation from the STOCKMED results should however take into account the likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs.

WP5 also aimed to highlight the knowledge gaps identified for the different species in the different disciplines which have been the basis of the thematic descriptor. This was done in order to provide key information for recommending further investigations in line with the objective of stock unit identification. Indeed, this can be considered as moving objective strictly linked to the progress of knowledge in different fields, given that information in some fields are very scant.

The approach followed for the proposition of stock units by species investigated in STOCKMED was based on the following steps:

• scrutinize by species the information provided by the outcomes of WP4 as systematized in the Deliverable15 and select the most suitable configuration between the more probable identified in D15. The overall knowledge gathered during the project on the target species and thematic descriptors is a further support for selecting a trade-off configuration;

• using communication table comparing, by species, the areas in which new stock units have been identified with the current configuration of GSAs, taking into account the more relevant geographical benchmarks in the Mediterranean;

• identifying gap knowledge and suggest candidate field for further investigations on the basis of the outcome obtained by the survey conducted among the experts for weighing quality/quantity

and coverage of the available information complementary to the implementation of the Non-Structural Fuzzy Decision Support System analysis (NSFDSS).

The most probable distributions of the stock units obtained by framework produced in the STOCKMED with the contribution of all WPs involved represent the first example of stock structure in the Mediterranean according to a holistic approach. These results provide a guideline on areas to be considered homogeneous for the assessment and management of resources. Depending on the quality of the available data, both in terms of discriminatory ability and spatial/temporal coverage, the distribution patterns obtained do not all have the same degree of plausibility. For example while some species (e.g. Merluccius merluccius) show a high degree of acceptability others present a high degree of uncertainty (e.g. Eledone moschata). However, the distribution patterns obtained represent a starting point to improve the knowledge of the distribution of the stocks in the Mediterranean. These proposed patterns will be subject to re-evaluation according to the improvement of knowledge on stock properties. One of the main difficulties in this study was that in the majority of the cases the information used, was not derived from studies done with the specific aim of identifying stock boundaries. Therefore, the units of stock with relative boundaries provided by STOCKMED should be reassessed in the future in order to provide a specific collection of data similarly to methodologies proposed in other areas (ICES). In order to achieve this, the knowledge gaps need to be filled. In particular, modelling the retention and/or dispersion of eggs and larvae from the spawning ground in relation to physical oceanographic processes could represent a valid approach. For some selected species (for which the ecology is well documented), eggs and larvae will be represented as Lagrangian drifters released in the main spawning ground. Successively, the simulated transport of the pelagic stages will be considered to identify connectivity mechanism among stock subunits and units. Another gap to overcome is to improve the use of 'last generation' markers (SNPs) (e.g. Engraulis encrasicolus), coupled with an extensive sampling scheme realised within the framework of EU funded projects (e.g. Merluccius merluccius and Solea solea), which markedly increase the power of genetic data for stock identification. Even though the growing importance in the international literature, studies on micro-chemical composition and shape of otoliths are almost absent in the Mediterranean. Furthermore, investigation on adults migration and movements, which is a main topic in stock identification, is very poor and needs to be improved at Mediterranean scale. Finally, a major availability of standardised information on the spatial distribution could improve the accuracy in selecting data for stock assessment and the consequent adoption of appropriate management measures.

WP1. Review and analyses of the existing biological information on stock units in the investigated areas

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BACKGROUND

The effective definition of the different biological units is an essential knowledge for proper data acquisition and subsequent assessment of the state of resources and, consequently, for the formulation and implementation of knowledge based management measures. Numerous definitions of stock are available in literature, with diverse grade of emphasis to phenotypic/environmental, genetics or management aspects (Coyle, 1998). A useful operational definition of a stock in fisheries science was given by Begg et al. (1999), according to whom <u>"a stock is a semi-discrete group of specimens of the same species of fish with some definable attributes which are of interest to fishery managers"</u>. These attributes include spatial distribution, self renewing properties, size and demographic features, and reaction to environmental factors and fishery pressure.

Stock identification was classically based on knowledge of closed migration circuits among spawning, nursery and feeding areas and of geographic variation of phenotypic traits (Gushing, 1980; Coyle, 1997). With the improvement of genetic techniques in the last decades, the "stock concept" was refined to include a degree of reproductive isolation (Booke, 1999). Molecular genetic techniques appeared to be robust tools in conservation biology for identifying reproductive isolation between population, permitting delineation of management units, and allowing assessment of conservation priorities from an evolutionary perspective (Begg et al., 1999).

Fishery science has several methods for identifying fish stocks. Begg et al. (1999) reported that stock identification can be based upon: the interpretation of distribution and relative abundance and catch data, tag recoveries, meristics, morphometries, scale and otoliths morphology and microchemistry, parasites, cytogenetics, protein electrophoresis (isoelectro focusing), immunogenetics, mitochondrial DNA, nuclear DNA, the elemental composition of otoliths, stable isotope measurements, otolith microstructure, and life-history parameters. However, the recognition that there was no single method that addressed the various assumptions of stock assessment and needs of fishery management prompted a more holistic view of population structure that

called for multiple sources of demographic and genetic data (Pawson & Jennings, 1996; Begg & Waldman, 1999).

Furthermore, recent improvements in knowledge on stock structure of marine organisms challenge the traditional view of populations like geographically distinct units with homogeneous vital rates and isolation from adjacent resources. More complex concepts such as metapopulations may be more applicable to many fishery resources with population structure organized in several sub-units with different level of connectivity (Stephenson, 1999; Kritzler & Sale, 2004). A wider application of spatially explicit models in future stock assessments and management will require clearly identifying the stock components, evaluating the movement rates and determining the degree of reproductive isolation. Because spatial structure affects how populations respond to fisheries, incorporation of heterogeneous patterns and movement in stock assessment models should improve advice for fishery management (Cadrin & Secor, 2009).

Quetglas *et al.* (1998) used different marine disciplines such as geomorphology, ecology and fisheries, combined with the analysis of new data coming from official fishery statistics and scientific surveys concluded that Balearic Islands (GSA 5) should be maintained as an individualized area for assessment and management purposes in the western Mediterranean. Each of these methods relates to a different aspect of the stock definition and concept. For example, population parameters are useful to identify putative stocks at the practical level of fisheries management; physiological and behavioural characters are primarily used to follow adaptation of stocks to particular environments; while morphological characters (morphometrics, meristics, zonation in calcareous structures) provide data that are useful for the precise description and differentiation of stock (Ihssen *et al.* 1981).

Considering the Mediterranean, knowledge on stock units is limited and very rough stock boundaries of main commercial species are available for stock assessment and management purposes. Some recent papers on genetic approaches reported within-species significant genetic differences indicative of reproductively distinct units for some species within the Mediterranean (Tinti et al., 2002; Guarniero et al., 2004; Garoia et al., 2007; Ramon and Castro, 1997; Rolland et al., 2007; Maggio et al. 2009). Few papers used morphologic analyses to identify stock units (Turan, 2004), in some cases morphology was joined to micromolecular approaches (Levi et al., 2004). An interesting study on genetic identification of Anisakis larvae in hake of Mediterranean and Atlantic was due to Mattiucci et al. (2004). An exercise of identification of putative stock units of European hake, red mullet and deepwater pink shrimp in the Mediterranean Sea, using simple correlation analysis (Pearson's correlation coefficient) of trends in survey abundance was attempted by Cheilari and Rätz (2009).
In the Mediterranean, current stock assessment and fisheries management are highly oriented by the division in Geographical Sub-Areas (GSA) of the General Fisheries Commission for the Mediterranean (GFCM). The definition of GFCM-GSAs was done on the basis of various criteria and analysis carried out in the first half of last decade (oceanographic, biological, fishery, continuity of FAO-GFCM capture statistics, etc.). However, no specific studies were aimed to define the stock boundaries and structure for most of Mediterranean areas, including those encompassing the territorial sea of the European countries, for stock assessment and management purposes. Despite this uncertainty about stock units, GFCM has preliminary identified some critical areas were stock of main commercial species are considered as shared amongst EU or/and not EU countries (FAO, 2006).

According to Scientific, Technical and Economic Committee for Fisheries (STECF) opinion, it is advisable to have a more refined and updated view on the different stock units and to verify whether the current GSAs classification matches with advisable assessment and management units of the main resources and fisheries (STECF 2008). The knowledge of distribution of resources, of their biological and genetic characteristics and the distribution of the fishing fleets by gear, also in relation to the oceanographic and sea bottom characteristics, was also considered by STECF as potentially useful for the definition of more natural divisions based on this operational concept of stock. Considering that a same fishery may harvest a quite high variety of resources and that fishing fleets may carry out different fisheries over the year, tradeoffs may however be inevitable between the best classification of biologic stock units, the mobility and characteristics of the various fishing fleets, the consistency with sampling strata for data gathering and reporting of catch statistics (national, EU, GFCM, etc).

FAO Areas Division 37	1.1	1.2	1.3	2.2	2.1	3.1
GSAs	1, 2, 3, 5, 6	7	8, 9, 10, 11.1, 11.2, 12	13, 14, 15, 16, 19, 20, 18	17	22, 25

The study aimed to cover as adequate the following FAO-GFCM GSAs:

On the basis of life history traits as well as on information coming from fishery and biological research works (e.g. biometry, parasites, physical tagging, multiple genetic markers, species mobility, characteristics and duration of embryo and larval phases, growth parameters, etc) including also considerations on the hydrological factors and sea bottom features, this study will provide the updated scientific vision on the connectivity and stock boundaries in the Mediterranean of the following relevant species: European hake (*M. merluccius*), red mullet (*M. barbatus*), striped mullet (*M. surmuletus*), common Pandora (*P. erythrinus*), common sole (S. *solea*), horse mackerels (*Trachurus trachurus* and *Trachurus mediterraneus*), anchovy (*E. encrasicolus*), sardine

(S. pilchardus), Norway lobster (N. norvegicus), giant red shrimp (A. foliacea), blue-andred shrimp (A. antennatus), deepwater rose shrimp (P. longirostris), common octopus (O. vulgaris), broadtail shortfin squid (Illex coindetii), homed octopus (Eledone cirrhosa), musky octopus (Eledone moschata), blackmouth catshark (Galeus melastomus), and anglerfish-monk (Lophius budegassa).

OBJECTIVES

The main aim of WP1 was to "Review and analyses of the existing biological information on stock units in the investigated areas".

The specific objectives of this WP1 were:

- reviewing all the available biological information useful to identify stock units in the investigated areas, thus, it is centered on extracting, scrutinizing and analyzing all the biological information, other than genetic characterization, useful to the purpose

- selection of relevant case studies for further investigations, while ensuring spatial coverage over various sub-regions in the Western, Central and Eastern Mediterranean

All the information produced in this WP were returned to WP 4 as geo-referenced data.

DELIVERABLES AND MILESTONES FORESEEN BY THE PROJECT

All the deliverables and milestones foreseen by the project were achieved. The work of this WP is subdivided in 4 tasks summarized as follows:

• Task 1.1. Analyses of trends of abundance indices from scientific surveys in different GSAs to highlight synchronisms in temporal patterns. Selection of relevant case studies and correlation matrices by GSAs for each species (Task coordinator M.T. Facchini).

• Task 1.2: Reviewing and analysis for the selected case studies of biological information on otoliths, biometry, spawning and recruitment patterns, growth performances and reporting as geo-referred information (task Coordinator P. Carbonara).

• Task 1.3 Reviewing and analysing for the selected case studies information on parasites, tagging, migration patterns, larval drift and reporting results as geo-referred information (task Coordinator K. Kapiris).

• Task 1.4. Synthesis of the spatial pattern of the main biological information for the target species in the case study areas and identification of gaps in knowledge on biological aspects for stock units identification (task Coordinator E. Massuti).

Deliverables

D4 – Report on analysis of trends of abundance (trends of target species in different GSA and analyses of synchronic pattern, including correlation matrices).

D5 – Synopsis of the spatial pattern of the main biological information for the target species in the case study areas, including gaps identification of critical gaps in the available biological knowledge.

Milestones:

M1.1 – Relevant data gathering from an ad hoc data call facilitated by DGMARE and from partners of the MAREA Consortium.

M1.2 - Correlation analyses and matrices by GSAs for each species.

M1.3 - Reporting results as geo-referred information for the case studies from task 1.2.

M1.4 - Reporting results as geo-referred information for the case studies from task 1.3.

RESULTS ACHIEVED

Task 1.1 Analyses of trends of abundance indices from scientific surveys in different GSAs to highlight synchronisms in temporal patterns. Selection of relevant case studies and correlation matrices by GSAs for each species (Task coordinator M.T. Facchini).

"Milestone 1.1. Relevant data gathering from an ad hoc data call facilitated by DGMARE and from partners of the MAREA Consortium", consisted of the gathering of significant data to be used in the review and analyses of the biological parameters related with the 19 STOCKMED target species.

The existing data that could serve the purpose of WP1 was reviewed and their adequacy discussed. Generally speaking, available data come from three main sources: i) scientific surveys, i.e. MEDITS, MEDIAS, GRUND and SOLEMON surveys, ii) fisheries sampling data collected on board or at port and within the framework of the DCR and DCF and, iii) published information from peer-reviewed/non peer-reviewed journals, reports or working documents.

Semi-quantitative comparison of data sources

In order to compare the different data sources in a way as more standardized as possible and to be passed to the constrained clustering analysis in WP4, a semi-quantitative criterion was designed. A table of weights was constructed which provides a scoring for each data source and type of information (Table 1.1). Seven criteria were determined (i.e. geographical coverage, standardization of data, minimum spatial aggregation level, temporal coverage, species coverage, provision of biological data and data accessibility) and ranged as low (L), medium (M) or high (H) if the specific criterion was considered to poorly, intermediately or fully fulfil the needs of the abundance trend analysis. For each criterion and data type, a score of 1 was given if it was ranged as high, a score of 0.5 if it was considered medium and a score of 0 if it was low.

Criteria		Scientif	ic surveys		Fishe samp	eries oling	Bibliographic data
	MEDITS	MEDIAS	GRUND	SOLEMON	On board	Ăt port	
Geographical coverage	Н	M-H	М	L	М	М	M-L
Standardization data	Н	Н	М	Н	Μ	L	L
(Minimum) Spatial aggregation level	Н	L	Н	Н	L	L	L
Temporal coverage	Н	L-M	М	Μ	Μ	M-H	M-L
Species coverage	Н	М	Н	L	Η	Μ	L
Biological data (e.g. length, weight, sex, maturity)	Н	Н	Н	Н	М	М	M-L
Data accessibility	Н	Н	L	Н	Н	Η	Н
Points	7	4-5	4.5	3.5	4	3-3.5	1-2.5

Table 1.1. Semi-quantitative weighting criteria to compare the different data sources.

H: high (1); M: medium (0.5); L: low (0).

However, these sources of information differ regarding their geographical and temporal coverage, the degree of standardization, the minimum spatial aggregation level, the range of species covered, the collection of biological data as well as the accessibility to the data. For instance, regular annual **scientific surveys** like MEDITS or MEDIAS show a high degree of standardization and are ready accessible from the DCR. Further, they show a wide spatial coverage, i.e. producing information from the different Mediterranean basins that usually other national surveys like GRUND or SOLEMON do not offer. GRUND survey only covers Italian waters and SOLEMON is performed in part of GSA 17 in the Adriatic Sea.

The amount of GSAs covered by MEDITS is higher than those covered by MEDIAS surveys, i.e. these last surveys are not performed in GSA 9, 10 and 19; notwithstanding financial shortages have impinged the MEDIAS surveys to even reduce the surveyed surface in some GSAs. MEDIAS surveys, although high quality scientific surveys, were more recently initiated and as focused on small and medium sized pelagic species they do not provide information on most of the target species of the STOCKMED project. Further, the accessible MEDIAS data from DCR do not support a smaller spatial aggregation level other than the GSA. This was considered a main disadvantage as long as the spatial scale in which the analysis was produced was the GFCM 30'x30' in order to be able to detected potential stock units smaller than the GSAs. In order to fulfil the purpose of estimating indices of abundance and life history traits useful for the constrained clustering analysis as foreseen in WP4; information should be referred to the GFCM grid cells and the spatial coverage should be high, i.e. a very low percentage of cells is allowed to not carry any information.

The MEDITS surveys have a wide spatial coverage (i.e. all the European Mediterranean coast) and a wide temporal coverage (i.e. time series expanding from 1994 up to date) while collecting information on all of the target species of the project. The other available surveys mentioned above show a shorter temporal coverage, i.e. MEDIAS surveys since 2009 to 2013, SOLEMON from 2006 to 2012 and GRUND since 2008, and for instance SOLEMON samples only benthic species. Additionally, and as long as the MEDITS surveys are the longer available time series a set of routines for standard checks are available or data is not easily accessible, for example for GRUND survey and for some surveys the lower spatial aggregation that can be provided nowadays is at a GSA level, i.e. the MEDIAS surveys.

Regarding the information collected from **fisheries sampling** (on board and at port) provide a picture of the temporal variability of fisheries catches and may as well provide information on the temporal variability of the biological parameters. However this type of data could require higher efforts in the standardization an checks, the spatial and temporal coverage is somewhat limited (e.g. eastern Adriatic is not covered and in all the Mediterranean before 2009 the biological information of the priority species was only collected every 3 years and discards data were monitored only for trawlers), the aggregation coverage (by metier and at a GSA level) and the species coverage is also limited (only priority species depending on the landings in each GSA.

Regarding the information from the **bibliographic review**, temporal and geographical coverage tend to be low notwithstanding there are documents revising data from several years and different GSAs, for instance from MEDITS surveys. The standardisation of the data and the species coverage tends as well to be low. Thus bibliographical review would only be considered as a data source to be used in the absence of the other data sources.

For all these reasons, during the progress of the project it was discussed and agreed between the participants in the WP1 to base the analysis of *Task 1.1* solely on the data obtained from MEDITS surveys. The other available data sources mentioned above (i.e. other scientific surveys and fisheries sampling data) do not represent major additional advantages to fulfil the purpose of estimating indices of abundance and life history traits useful for the constrained clustering analysis as foreseen in WP4.

Nevertheless, we would like to draw the attention to the fact that although the abundance trends analysis and the biological indicators were based solely on MEDITS data, the other surveys mentioned above have been successfully used within the

framework of the MAREA project to produce specific and elaborated information. Specifically, MEDIAS surveys were used for the identification of nurseries and spawning grounds and for habitat modelling of small pelagic species also in areas without information (e.g. southern Mediterranean), within the MEDISEH project (MEDIterranean SEnsitive Habitats, 2013). GRUND and SOLEMON surveys have been used in the STOCKMED project to analyse nurseries and spawning grounds of demersal species (see Deliverable 10). The MEDITS surveys data have also been used in MEDISEH for detecting and mapping Essential Fish Habitats of demersal species, providing additional information also for species as *Trachurus*.

For the reasons explained above, with the aim of completing the specific objectives of *Task 1.1* while fulfilling the needs of WP4, it was considered that the analysis of the MEDITS data is the most efficient, reliable and robust dataset to be used. The other data sources would be of use although additional time and efforts should be devoted in order to check, standardize and combine the information from the different sources and might totally fall outside the scope of the present project. In relation to *Task 1.2* on the "*Review and analyse for the selected case studies of biological information on otoliths, biometry, and life history traits (spawning and recruitment patterns, abundance trends, growth performances)*" and *Task 1.3* on the "*Review and analyse for the selected case studies of for patterns, larval drift*", it became evident that no quantitative data from survey or fisheries sampling data was available, with the exception of some indicators (i.e. those referring to growth-mortality and reproduction in *Task 1.2*) for which MEDITS data would suffice. So, it was decided that only qualitative data from bibliography (not standardized) was going to be used as it was the only information available.

A specific Data Call from DGMARE was made available to the project participants through the Joint Research Center (JRC) from the European Commission. Data consisting of MEDITS database from 2002 to 2011 was checked using the R routine RoME (Bitetto et al., 2012). Several errors and inconsistencies were detected and corrected to before being used in the analysis. The temporal and spatial coverage of the MEDITS surveys is shown in Table 1.2 and Figure 1.1, together with the limits of the GSAs and of the GFCM statistical grid.

GSA	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	12
1																			
2																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
15																			
16																			
17																			
18																			
19																			
20																			
22+23																			
25																			

Table 1.2. Temporal coverage of MEDITS survey in each CGPM GSA: the grey cells indicate existence of data.



Figure 1.1. Spatial coverage of MEDITS trawls across the Mediterranean Sea.

The geographical scale

The definition of a suitable spatial scale in which to perform the revision and analysis of the biological parameters for the target species was considered a critical step for the progress of the project. Too large spatial units could reduce the accuracy of the stock boundaries, while too small spatial units could compromise the statistical robustness of the indicators.

Three different spatial scales were explored, i.e. General Fisheries Commission for the Mediterranean (GFCM) Geographical Sub-Areas (GSAs), Mediterranean trawl surveys (MEDITS) strata and GFCM Statistical Grid (Fig.1.2). The GFCM GSA scale is the spatial division in which stock assessment and fisheries management have being performed in the Mediterranean and Black Sea areas. MEDITS strata are the divisions that are used in the bottom trawl surveys to produce the outcomes of the surveys (MEDITS handbook, 2013). The Statistical grid subdivides the Mediterranean and Black Sea into rectangles of 30' x 30' and was recently proposed by GFCM (Recommendation **GFCM/33/2009/2**).



Figure 1.2. Geographical scales evaluated for the analysis of the biological parameters: GSA divisions (top), MEDITS strata (middle) and GFCM Statistical Grid (bottom). The GSAs names: 1 North Alboran Sea, 2 Alboran Island, 3 Morocco, 5 Balearic Islands, 6 North Mediterranean Spain, 7 Gulf of Lion, 8 Corsica, 9 North Thryrrhenian Sea, 10 South Thyrrhenian Sea, 11 Sardinia, 16 Strait of Sicily, 15 Malta, 17 North Adriatic, 18 South Adriatic, 19 Western Ionian, 20 Eastern Ionian, 22 Aegean, 23 Crete, 25 Cyprus Island.

The clustering techniques (CC, Constrained Clustering), which was selected in STOCKMED project as a suitable methodology to evaluate identify putative stock units (WP4), requires input data in homogeneous and consistent spatial units. Some preliminary analyses were performed at GSA scale. However, as GSAs are limited in most cases by lines perpendicular to the coast, depending on the geographical characteristics of each area, and the coast itself, they have different shapes and extensions. Therefore,

they are not homogeneous and the number of hauls for each GSA varies considerably, not accommodating the methodological approach of WP4.

Regarding MEDITS strata, some biological parameters were computed in this scale in order to explore this geographical approach. In particular, mean density (N/km²), biomass (kg/km²) and individual weight (kg), were computed for the case study species, i.e. *M. merluccius*, *M. barbatus*, *P. longirostris* and *S. solea* (*Annex 1. Prelimiary analyses*). Nevertheless, MEDITS strata were suffering from the same shortcomings than GFCM GSAs and were disregarded for the estimation of the biological parameters.

Conversely, the 30' x 30' statistical grid, proposed in 2009 by the GFCM, subdivides the Mediterranean and Black Sea into rectangles of homogeneous spatial divisions. The GFCM grid was considered the most appropriate geographical approach for the estimation of the biological indicators at a fine scale, and for further clustering in WP4. The GFCM grid units were considered small enough to be gradually aggregated in new larger areas in order to define new putative stock units. At the same time, a cell of the GFCM grid should include an adequate number of stations in the last years of the MEDITS survey, so that mean values for the biological indicators over the years can be calculated with statistical confidence.

A comparison of the standardized biomass of the case study species between the three geographical scales over the time series was produced (Table 1.3). The GSA and MEDITS strata scales suffer some sort of undesirable "smoothing" effect that could prevent the identification of critical areas with significantly high/low abundance. In particular, for all the case studies species the minimum values of the mean standardized biomass observed by GSA and by MEDITS strata (e.g. 1.24 kg/km² and 0.6 kg/km² for M. merluccius) are slightly higher than the minimum value observed in the cells of the GFCM grid (e.g. 0.14 kg/km²). Conversely, the maximum values of the mean standardized biomass observed by GSA and by MEDITS strata (e.g. 71.77 kg/km² and 171.13 kg/km² for *M. merluccius*) are lower than the maximum value observed in the GFCM cells (e.g. 357.55 kg/km² for *M. merluccius*). This situation occurs when in a GSA, a consistent number of hauls with very low biomass influence the average in the total area, which will be low as well, even if some specific hauls have significantly high biomass. On the contrary, when a consistent number of hauls has very high biomass the average in the total area will be high as well, even if in some specific hauls the biomass is significantly low.

Another advantage is that the units of the grid can be merged and assist the evaluation also under the hypothesis of stock units existing at smaller spatial scale than the current GSAs. However, the disadvantage is that not any unit in the grid can actually encompass the spatial distribution of all the life stages of a given species, especially when this distribution is depth related. Nevertheless, the GFCM grid was considered as the better available trade-off for the spatial scale to be used in the different steps of the analysis.

In summary, besides the already mentioned preliminary analysis that were produced at GSA or MEDITS strata scale, the 30'x30' grid cell was the selected spatial scale to perform the analysis of the biological indicators. Nevertheless, the GSAs were used to produce *"Milestone 1.2. Correlation analyses and matrices by GSAs for each species"* (see Results, section 3) and it was as well the spatial scale used for the parameters relying on bibliographic review as long as it was the most detailed spatial scale that could be retrieved.

		GSA		MEDITS	S strata	GFCM Sta	tistical grid
Species	Statistic	mean	sd	mean	sd	mean	sd
M. merluccius	Min	1.24	0.67	0.6	1.54	0.14	0.8
	Median	33.14	1.32	32.43	41.7	27.46	28.75
	Mean	27.56	1.73	40.87	82.63	36.69	45.17
	Max	71.77	5.62	171.13	710.85	357.55	840.39
M. barbatus	Min	10.54	0.97	2.03	5.82	0.01	0.04
	Median	31.02	3.75	13.9	45.41	11.23	20.17
	Mean	30.57	5.31	20.59	76.04	23.35	45.9
	Max	52.47	19.9	109.98	591.18	398.15	852.09
P. longirostris	Min	0.09	0.02	0.002	0.02	0.01	0.02
	Median	2.33	0.22	3.86	6.85	3.12	5.55
	Mean	4.67	0.35	5.12	10.21	8.01	9.64
	Max	17.41	1.35	20.19	50.005	86.04	74.36
S. vulgaris	Min	0.038	0.03	0.02	0.29	0.03	0.27
	Median	0.36	0.08	0.27	1.64	0.49	2.06
	Mean	0.9	0.16	0.84	4.03	1.16	3.5
	Max	4.55	0.71	7.85	36.07	13.3	51.98

Table 1.3. Comparison of the three spatial scale approaches for the case study species: summary statistics of the standardized biomass (kg/km²) over the time series.

Task 1.2 Reviewing and analysis for the selected case studies of biological information on otoliths, biometry, spawning and recruitment patterns, growth performances and reporting as geo-referred information (task Coordinator P. Carbonara).

The data from MEDITS surveys was used to compute the abundance trends over the last 10 years (i.e. from 2002 to 2011) for the 19 target species, in order to observe any analogies or dissimilarities in the abundances of adjacent areas. Two indicators of abundance were calculated, i.e. density in N/km² and biomass in kg/km². According to the bathymetric distribution of each species, the trends were calculated on the continental shelf and/or on the slope as follows:

- On the shelf, between 0 and 200 m: for *E. moscata*, *E. encrasicolus*, *I. coindetii*, *M. barbatus*, *M. surmuletus*, *O. vulgaris*, *P. erythrinus*, *S. pilcardus*, *S. solea* (*S. vulgaris*) and *T. mediterraneus*;
- On the slope, between 200 and 800 m: for *A. antennatus*, *A. foliacea*, *G. melastomus* and *N. norvegicus*;
- On the shelf and slope, between 0 and 800 m: for *E. cirrhosa*, *L. budegassa*, *M. merluccius*, *P. longirostris* and *T. trachurus*

The mean of the two abundance indexes mentioned above were calculated for each year and GSA, according to stratified sampling formulas (Souplet 1996), where the mean and variance are weighted by the proportion of area of the particular strata in each GSA (for the strata surface see Stratification Scheme table, Annex II MEDITS handbook 2013, version 7). The mean, variance and coefficient of variation (CV) over the whole time series was then computed (Fig. 1.3). *Abundance trends were used to compile a part of Deliverable 4.*



Figure 1.3. Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km2) and right, biomass (kg/km2).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km2) and right, biomass (kg/km2).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km2) and right, biomass (kg/km2).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km2) and right, biomass (kg/km2).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km²) and right, biomass (kg/km²).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km²) and right, biomass (kg/km²).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km²) and right, biomass (kg/km²).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km²) and right, biomass (kg/km²).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km²) and right, biomass (kg/km²).



Figure 1.3 (continued). Abundance trends (mean: black solid and CV: red dotted line) for the different species over the last 10 years by GSA: left, density (N/km2) and right, biomass (kg/km2).

The previously computed abundance trends from 2002 to 2011 (see Results, section 2) were standardized to the average over the time series for each species and in each GSA. Spearman's rank order correlation coefficient was then calculated to quantify the degree of correlation between the abundance trends in the various GSAs, following a similar to that applied by Cheilari and Rätz (2009). The correlation between each pair of GSAs was performed and the results were recorded in correlation matrices (Table 1.4).

The significance of the each correlation was estimated by means of the p-value of a onetailed test, i.e. testing the significance only in the direction of positive correlation between GSAs. Being the p-value the probability of finding the actual correlation rank estimate if the correlation coefficient was in fact zero (null hypothesis), a low p-value is taken as evidence that the null hypothesis can be 'rejected'. The time series of the standardized relative abundances in density (N/km²) and in biomass (kg/km²) in all the GSAs and the correlation matrices are reported for each species in *"Annex 2. Correlation matrices of abundance trends"*. Table 1.4. Sample correlation coefficient matrix of the time series of the relative abundance in biomass (kg/km2) for A. foliacea. Significant correlations are highlighted in yellow.

	gsa5	gsa6	gsa7	gsa8	gsa9	gsa10	gsa11	gsa15	gsa16	gsa17	gsa18	gsa19	gsa20	gsa22	gsa25
gsa5	1.00	0.46	NA	-0.67	0.50	0.60	0.10	0.80	-0.30	0.71	0.60	0.00	NA	NA	0.89
gsa6	0.46	1.00	-0.18	-0.26	0.51	0.28	0.03	-0.17	-0.79	-0.14	-0.10	-0.04	0.05	0.21	0.11
gsa7	NA	-0.18	1.00	-0.19	0.17	-0.41	0.41	0.41	0.29	0.36	-0.06	0.29	-0.71	-0.71	NA
gsa8	-0.67	-0.26	-0.19	1.00	-0.09	0.07	-0.05	-0.43	0.43	-0.37	-0.50	-0.27	0.71	0.35	-0.65
gsa9	0.50	0.51	0.17	-0.09	1.00	0.61	-0.03	-0.08	-0.16	0.13	-0.12	-0.10	-0.30	-0.30	0.15
gsa10	0.60	0.28	-0.41	0.07	0.61	1.00	-0.16	-0.42	0.05	-0.23	0.38	0.10	0.70	0.70	0.30
gsa11	0.10	0.03	0.41	-0.05	-0.03	-0.16	1.00	0.09	0.14	-0.01	0.37	0.59	-0.70	-0.80	-0.04
gsa15	0.80	-0.17	0.41	-0.43	-0.08	-0.42	0.09	1.00	0.14	0.10	0.09	-0.27	-0.80	-0.30	0.70
gsa16	-0.30	-0.79	0.29	0.43	-0.16	0.05	0.14	0.14	1.00	-0.23	0.16	0.02	0.10	0.10	-0.19
gsa17	0.71	-0.14	0.36	-0.37	0.13	-0.23	-0.01	0.10	-0.23	1.00	-0.01	0.35	-0.78	-0.78	0.64
gsa18	0.60	-0.10	-0.06	-0.50	-0.12	0.38	0.37	0.09	0.16	-0.01	1.00	0.68	-0.10	0.40	0.63
gsa19	0.00	-0.04	0.29	-0.27	-0.10	0.10	0.59	-0.27	0.02	0.35	0.68	1.00	-0.30	-0.50	0.30
gsa20	NA	0.05	-0.71	0.71	-0.30	0.70	-0.70	-0.80	0.10	-0.78	-0.10	-0.30	1.00	0.70	-1.00
gsa22	NA	0.21	-0.71	0.35	-0.30	0.70	-0.80	-0.30	0.10	-0.78	0.40	-0.50	0.70	1.00	0.50
gsa25	0.89	0.11	NA	-0.65	0.15	0.30	-0.04	0.70	-0.19	0.64	0.63	0.30	-1.00	0.50	1.00

Tables summarizing the most significant correlations in density and biomass that come out from the correlation matrices are shown (Table 1.5 and 1.6). The Spearman's correlation coefficient allowed the analysis of the GSAs showing the highest amount of species with significantly correlated time series as well as those species with highest amount of pairs of GSAs significantly correlated.

Referring to the biomass index, the pair of contiguous GSAs with highest amount of time series of species correlated was the Gulf of Lions (GSA 7) and Corsica (GSA 8) with 7 species significantly correlated. Two pairs of GSAs showed 6 species significantly correlated, i.e. Strait of Sicily (GSA 16) and Malta (GSA 15) and South Adriatic (GSA 18) and Western Ionian (GSA 19) while two additional pairs showed 5 species with significantly correlated time series, i.e. Northern Alboran Sea (GSA 1) and Northern Spain (GSA 6), Northern Spain (GSA 6) and Gulf of Lions (GSA 7). In relation to the density index, the pair of contiguous GSAs with highest amount of time series of species correlated was the Gulf of Lions (GSA 7) and Corsica (GSA 8) and South Adriatic (GSA 18) and West Ionian (GSA 19) with 7 species significantly correlated in both of them. South Tyrrhenian Sea (GSA 10) and Strait of Sicily (GSA 16) showed significant correlation in 5 species. Two pairs of GSAs showed 4 species significantly correlated, i.e. Ligurian and North Tyrrhenian Sea (GSA 9) and Gulf of Lions (GSA 7) and Western Ionian (GSA 19) and Suff of Lions (GSA 7) and Western Ionian (GSA 19) and Malta (GSA 15).

For *P. longirostris* a high amount of GSAs were significantly correlated in terms of biomass index, i.e. a total of 11 pairs of GSAs out of the 23 pairs of GSAs analyzed. It seems that the entire North-Western Mediterranean basin from North Alboran Sea (GSA 1) to South Tyrrhenian Sea (GSA 10), together with the South-Central Mediterranean up to Western Ionian (GSA 19) could belong to a shared stock for this species. Balearic Islands and Sardinia would remain as isolated stock units. However, in terms of density index this connection is not that clear and only 5 pairs of GSAs were significantly correlated.

Table 1.5. The most significant correlations in biomass between contiguous GSAs, number of observation (pairs of years), the coefficient of correlation for each species and the number of the species in which each correlation has been found. Only statistically significant correlation coefficients (p-value < 0.05) are shown.

Corre	lated	pairs of years	A. foliacea	A. antennatus	E. cirrhosa	E. moschata	E. encrasicolus	G. melastomus	I. coindetti	L. budegassa	M. merluccius	M. barbatus	M. surmuletus	N. norvegicus	O. vulgaris	P. erythrinus	P. longirostris	S. vulgaris	T. mediterraneus	T. trachurus	number of species showing significant correlation
1	2	5									0.97										1
1	5	5											1						0.9		2
1	6	10			0.72	0.59									0.73		0.79			0.56	5
5	6	5			0.9																1
5	11	5														0.9				1	2
6	7	10							0.65						0.7	0.72	0.7			0.92	5
6	8	9							0.64												1
6	11	10														0.78					1
7	8	9						0.7	0.59	0.76		0.6				0.8	0.77		0.8		7
7	9	10	0.6														0.56				1
8	9	9															0.77				1
9	10	10															0.78				2
9	11	10				0.87								0.59		0.56					3
10	11	10					0.72						0.6							0.56	3
10	16	10						0.7			0.89				0.58		0.67				4
15	16	10								0.75		0.6		0.76			0.58		0.62	0.62	6
15	19	10									0.81						0.72				2
16	19	10									0.59						0.81				2
17	18	10				0.73													0.67	0.84	3
18	19	10	0.7						0.89		0.65					0.77		0.65	0.82		6
18	20	5		0.8							0.9						0.9				3
19	20	5				0.9						0.9									2
20	22	5																	0.9		1
Num of si co	ber of GSAs v gnifica rrelati	pairs vith ant on	2	1	2	4	1	2	4	2	6	3	2	2	3	0	6	11	1	6	

Table 1.6. The most significant correlations in density between contiguous GSAs, number of observation (pairs of years), the coefficient of correlation for each species and the number of the species in which each correlation has been found. Only statistically significant correlation coefficients (p-value < 0.05) are shown.

Corre	lated	pairs of years	A. foliacea	A. antennatus	E. cirrhosa	E. moschata	E. encrasicolus	G. melastomus	I. coindetti	L. budegassa	M. merluccius	M. barbatus	M. surmuletus	N. norvegicus	0. vulgaris	P. erythrinus	P. longirostris	S. vulgaris	T. mediterraneus	T. trachurus	number of species showing significant correlation
1	2	5											0.87		0.9						2
1	5	5											0.9								1
1	6	10			0.72									0.85							2
5	6	5	0.87	0.9													0.83				3
5	11	5																			0
6	7	10							0.6							0.77				0.6	3
6	8	9							0.73												1
6	11	10									0.58					0.81					2
7	8	9							0.71	0.6	0.58	0.6				0.91	0.75		0.8		7
7	9	10						0.67			0.76						0.64		0.62		4
8	9	9															0.89				1
9	10	10											0.6		0.58	0.68					3
9	11	10			0.61									0.7							2
10	11	10				0.56															1
10	16	10						0.76	0.56		0.9		0.68								4
15	16	10						0.83	0.59	0.56				0.61							4
15	19	10					0.62													0.58	2
16	19	10								0.6											1
17	18	10					0.65			0.7											2
18	19	10																		0.82	1
18	20	5														0.77			0.9	0.83	3
19	20	5		0.82													1				2
20	22	5																			0
Num of si co	ber of GSAs gnific rrelat	f pairs with ant ion	1	2	2	1	2	3	5	4	4	1	4	3	2	0	5	5	0	3	

Correlation matrices between the temporal trends in biomass and abundance may highlight temporal synchronisms between adjacent GSAs (Table 1.7).

– Most similar GSAs were: Corsica (GSA 8) and Gulf of Lions (GSA 7), Southern Adriatic (GSA 18) and Western Ionian (GSA 19) and South Sicily (GSA 16) and Malta (GSA 15).

- Most dissimilar GSAs were: Aegean (GSA 22) and Eastern Ionian (GSA 20), Ligurian (GSA 9) and Corsica (GSA 8) and Balearic Islands (GSA 5) and Sardinia (GSA 11).

However, low proportion of species showing synchronisms is found in adjacent GSAs: highest amount of species with positive and significant correlation was 7 species out of the 19 STOCKMED target species (37% of the STOCKMED target species). Thus, from a biological point of view merging several GSAs in order to establish management boundaries seems not advisable. It might be useful to elaborate similar correlation matrices for the rest of the biological parameters in order to confirm this conclusions.

Finally, maps showing the amount of species showing significant correlation regarding biomass and density were produced (Fig. 1.4). The correlation matrices constituted the second milestone of the WorkPackage, "M.1.2. Correlation analyses and matrices by GSAs for each species" and were as well included in Deliverable 4.

Table 1.7. Correlation of abundance between GSAs. The number of correlated species is also reported

		Number of species correlated									
Corre GS	elated As	Biomass A	Abundance	Mean B&A							
7	8	7	7	7							
18	19	6	7	6.5							
15	16	6	5	5.5							
1	6	5	3	4							
6	7	5	3	4							
9	11	3	3	3							
15	19	2	4	3							
18	20	3	3	3							
7	9	1	4	2.5							
10	11	3	2	2.5							
10	16	4	1	2.5							
17	18	3	2	2.5							
1	2	1	2	1.5							
1	5	2	1	1.5							
5	6	1	2	1.5							
6	11	1	2	1.5							
9	10	2	1	1.5							
16	19	2	1	1.5							
19	20	2	1	1.5							
5	11	2	0	1							
8	9	1	1	1							
20	22	1	0	0.5							





Figure 1.4. GSA boundaries according to the number of species showing significant correlations in biomass (upper) and density trends (lower).

WP 1 focuses on the analysis of abundance, biomass indices and life history indicators in adjacent areas, under the assumption that similar metrics are identifying compatible units of putative stocks. A total of eleven biological indicators grouped in three categories were initially proposed to explore the spatial patterns of abundance, demography and life history traits. The time series of MEDITS surveys between 2002 and 2011 were used to compute the biological indicators.

Initially proposed indicators

The initially proposed indicators were the following:

Α.	Abundance
	1. Density (N/km ²)
	2. Biomass (kg/km ²)
В.	Demography
	3. Mean individual weight (MIW)
	Length-frequency diversity
	Pielou's Evenness index (J)
	5. Shannon index (H)
	6. Simpson index (S)
	7. Sex ratio
	Multivariate analysis of length-frequency
	8. Hierarchical clustering
<u> </u>	Life history traits
	Growth and mortality
	9. Z/k ratio
	Reproduction
	10. Percentage of spawners
	11. Mean length of spawners
	12. Median length of spawners

Table 1.8. Indicators from Medits scientific surveys

For the indicators suggested, the mean over the last ten years (2002-2011), the coefficient of variation (CV) and the inverse of the CV were computed at the selected spatial scale, i.e. CGPM grid cell, in order to explore their potential contribution to stock identification. The CV of the indices were taken into account as descriptors of the variability of that particular index over the time series, under the assumption that adjacent areas with similar variability are likely to belong to a more homogeneous entity. Conversely, the reciprocal of the coefficient of variation could be seen as a descriptor of the stability of the variable under investigation (higher is the metrics, more stable is the variable).

Besides indicators that are usually applied in terms of stock delimitation, several innovative indicators were tested for their potential use. These "innovative" indicators were diversity indices applied to length-frequency distributions and multivariate analysis, in particular, hierarchical clustering applied discriminate between similar or dissimilar length-frequency distributions.

Diversity indices are commonly used in ecology to measure similarities between communities in terms of the species composition, the amount of species and/or their relative abundance. In the present study, the diversity indices were proposed to identify similarities in the demographic structure of each species. Thus, traditional diversity indices were modified to accommodate them to compute length group diversity of each species. The focus will be in finding adjacent areas with similar estimates of the lengthfrequency diversity index rather than on the value itself. The length-frequency distribution diversity indices are calculated on the annual length frequency distributions (relative LFDs, as percentage) obtained summing all individuals measured within a spatial unit per year.

Multivariate data analysis (MVA) relies on a set of statistical methods aimed at examining the interaction among more than one variable to produce a particular outcome. In general, multivariate statistical methods attempt to explain the relative importance of the different variables to produce a particular outcome (e.g. discriminant analysis, multivariate regression), to merge similar variables into factors to turn the dataset into a more manageable one (e.g. factor analysis) or construct groups of observations according to similarities in the multiple variables so that the elements within a group are more similar among them than among the elements of other groups (e.g. cluster analysis). Multivariate analysis techniques are commonly used in biology to analyse differences in species composition and in ecology to analyse differences between communities or habitats. In this work, hierarchical cluster analysis (HC) is suggested for demographical purposes, in order to find similarities between the length frequency distributions (LFD) of adjacent cells, thus considering the length classes as different variables and the GFCM grid cells as observations.

Abundance

Abundance indicators estimate the size of a population or stock unit. Two abundance indicators were proposed in order to describe spatial patterns providing information on changes in abundance among neighbouring sites:

- Density (N/km²): is the amount of individuals by unit of space; this indicator does not discriminate between the size of the individuals thus a population or unit stock with e.g. 100 individuals of length 5 cm will have the same value than a population or unit stock constituted of 100 individuals of length 25 cm. Thus, can be affected by recruitment events.
- 2. Biomass (kg/km²): is the total weight of the individuals that are found in a space unit

Demography

Using demographical traits for stock boundary delimitation, assumes that a stock unit or population exhibits a particular length frequency distribution (LFD), with similar length ranges and probability of finding a particular length class, and that the stock unit can be defined according to it. The different proposed demographic indicators were:

- 3. Mean individual weight (MIW): is an indicator that synthesizes the structure of the population (Piet and Jennings, 2005). It is computed by dividing the total biomass by the total density. Only hauls with at least 30 measured individuals were considered in order to obtain robust estimates.
- 4. Pielou's eveness index (J): applied to LFDs, this indicator depends on the proportions, i.e. the relative distribution of individuals in the length class group, and on the number of groups in which the length classes of the LFD are grouped (e.g. 2 cm, 5 cm), which vary between species. The J index by cell (c), year (y) and haul (h) was estimated using the following formula:

$$J_{yhc} = \frac{-\sum p_i * \ln(p_i)}{\max(-\sum p_i * \ln(p_i))}$$

where p_i is the proportion of the individuals in the i-th length class group (e.g. 0-5 cm, 5-10 cm, etc). This parameter cannot be computed , When the LFD consists of only one length class the J index cannot be computed.

5. Shannon's diversity index (H): is commonly applied in ecology to measure the probability of finding an individual of a particular species within an ecosystem. The higher the amount of species and the more equally represented in terms of abundance, the higher is the H index and the diversity of the ecosystem. Applied to demography it measures the probability of finding an individual of a particular length class. We aim at finding areas with similar H index values, independently of whether the value is low or high. The H diversity index equals 0 when the LFD consists in only one group of length classes (e.g. in a specific GFCM cell, year and haul all the measured individuals are part of only one length class group between 0 and 5 cm).

$$\mathbf{H_{yhc}} = -\sum \mathbf{p_i} * \mathbf{ln}(\mathbf{p_i})$$

where p_i is the proportion of the individuals in the i-th length class group (e.g. 0-5 cm, 5-10 cm, etc...).

6. Simpson index (S): is based on the concept that the diversity of a system is higher when there is no dominance of any of the entities constituting the system. It measures the probability that two entities taken at random from a dataset of interest represent the same type of entity. Applied to demography it will measure the probability that two individuals taken at random come from the same length class.

$S_{yhc} = p_i^2$

where p_i is the proportion of the individuals in the i-th length class group (e.g. 0-5 cm, 5-10 cm, etc...).

7. Sex ratio (SR): provides information on the distribution of male and female individuals present in a population. It represents the proportion of females in a population and indicates the level of sex dominance (Adebiyi, 2013). Generally this is a peculiar trait of the population.

$$SR = \frac{\sum D_f}{\sum D_m + D_f}$$

where D_f is sum of the standardized number of females and Dm is the sum of the standardized number of males over the hauls of the pooled last 10 years of the MEDITS survey (2002-2011). Hauls with less than 50 measured and sexed individuals were not included in the analysis as they were considered not robust.

8. **Hierarchical clustering:** The hierarchical clustering is computed on the relative abundance (in %) in the i-th length class group (e.g. 0-5 cm, 5-10 cm, ...) by year and haul of every GFCM grid cell. At each step, the clusters are compared in terms of the average similarity of the observations (or grid cells, in our case) and the two clusters that are most similar are merged. The distance between two grid cells (a,b) is computed by means of Bray-Curtis dissimilarity of the form:

$$BC_{dist (a,b)} = \frac{\sum_{i=0}^{n-1} |p_{ai} - p_{bi}|}{\sum_{i=0}^{n-1} (p_{ai} + p_{bi})}$$

where pai and pbi are, respectively, the proportion of the individuals in the i-th length class group (e.g. 0-5 cm, 5-10 cm, ...) in the cell "a" and in the cell "b".

Life history traits

- 9. Z/k ratio: is a indicator between total mortality rate (Z), i.e. natural mortality and fishing, and the growth pattern (k). If k>Z, fishes have time to complete their growth and the length-distribution is towards L_{inf}. Conversely, if Z>k, fishes are caught when they are too young and many of them die before reaching more than a small fraction of L_{inf}. In the last case the length-frequency distribution moves towards the length at first capture lc rather than L_{inf}. The factors affecting the mean length I are not Z or k considered separately, but the ratio Z/k considered as unique factor (Gulland and Rosenberg, 1992). The Powell-Wetherall method (Powell, 1979) was applied in order to estimate of the Z/k parameter. Only the "robust" LFD are taken into account, i.e. LFD containing a number of individuals per km² greater than 1000.
- 10. **Percentage of spawners:** the proportion of matures represents the spawning potential of a species; in particular, the proportion of female spawners is of primary

importance with respect to egg production and subsequent recruitment. The proportion of matures females was calculated using the formula:

$$PM = 100 * \frac{\sum D_{f,mat}}{\sum D_{f,inmat}}$$

where D_{mat} is the density of mature females while D_{inmat} is the density of the inmature females.

The definition of mature individuals varied depending on the faunistic category as follows:

- fishes: maturity stage 3 for all the years of MEDITS data;
- decapods: maturity stage 2 before 2006, maturity stages 2c and 2d from 2007 onwards;
- cephalopods and selachians: maturity stage 3 before 2006, maturity stage 3a from 2007 onwards.

The differences among the years reflect the different specifications of the MEDITS protocol. Hauls with less than 50 measured and sexed individuals were not included in the analysis as they were considered not robust.

- 11. **Mean length of spawners:** it was computed only on females and synthesizes the LFDs of the spawners. It is a weighted mean by the standardized numbers of mature females in the length classes. This indicator was selected as proxy of size at first maturity. It is calculated as the average of the lengths observed. As long as the data are symmetrically distributed the mean is an unbiased central metric of the distribution. Hauls with less than 50 measured and sexed individuals were not included in the analysis as they were considered not robust.
- 12. **Median length of spawners:** the median length of spawners has been derived from a process analogous to that of the mean length of spawners. Considering the PDFs are not symmetrical but rather show some sort of skewness the median is expected to represent more precisely the central feature of a distribution. Hauls with less than 50 measured and sexed individuals were not included in the analysis as they were considered not robust.

Selection of the relevant descriptors

In the first phase of the project, four species were selected as case studies to test the methodological framework and identify possible critical aspects. The chosen case study species were three species with enough data, i.e. *Merluccius merluccius, Mullus*

barbatus and *Parapenaeus longirostris,* and one species for which data are scarce, i.e. *Solea vulgaris.*

Preliminary analysis on the case study species allowed the selection of the biological parameters that were considered more appropriate while avoiding redundancies between the information provided by each indicator. All the initially proposed indicators were estimated for the 4 case study species at a GFCM grid scale with standard errors, coefficient of variation and inverse of CV, except for Z/k parameter that was investigated only for *M. merluccius* and included in "Annex 3. Preliminary analysis on the case study species: maps of all the proposed biological indicators".

Studing the results of the four case study species and taking into account some theoretical considerations about the indicators some choices have been made for the further analysis. First of all, the two abundance parameters (i.e. density and biomass) revelead reduntant for most of the case study species. In particular, the dispersion index (r^2) between the two parameters showed values of 0.46, 0.79, 0.91 and 0.89 for *M. merluccius, M. barbatus, P. longirostris* and *S. vulgaris* respectively (Fig. 1.5). Thus, one of the two needed to be removed from the analysis. Biomass indices are generally less influenced by recruitment spikes due to the small contribution of recruits to the total biomass while density is strongly affected by recruitment spikes. Thus, biomass indicator was considered more robust and selected as a first choice while discarding density.

Further, a choice between CV and inverse of CV should be done as they mainly produce the same information. Due to the fact that the idea was to analyse the stability of the parameters in adjacent GFCM cells as a potential definitor of stock boundaries it was decided to use the inverse of the CV rather than the CV itself.

One important aspect is that the LFDs used in the calculation of the length-frequency diversity indices are affected very much by the peculiarity of the habitat. Due the spatial configuration of the habitat of one species, the different sizes may not be homogenously distributed on the grid. Therefore, the LFDs in some cells may cover the whole distribution for a given species and are fully representative of that species but in other grid cells it may not.

As things stand, the GFCM grid seems to be a spatial scale too detailed for the estimation of reliable diversity indices or for other indicators relying on LFDs such as Z/k, impinging difficulties in using this spatial scale to discriminate between entities. However, as already stated, the Constrained Cluster analysis needs to be feed with information in homogeneously distributed throughout the space. Thus, the indicators using LFDs, i.e. the diversity indices and Z/k were excluded from the analysis.

As regards sex ratio and reproduction, the calculation of variation and stability coefficients for the 4 case studies have been used only for qualitative evaluation of the
variables, but nor variation (CV) neither stability (inverse of CV) has been included in the constrained cluster analysis in WP4.

Regarding the mean and the median length of mature females, the median was selected instead of the mean as long as the median is a more robust being a good descriptor of the half of the probability distribution function both for symmetrically and non-symmetrically distributed data, up to a certain skewness.



Figure 1.5. Linear relationships and their dispersion indices between the two abundance indices proposed, i.e. density (N/km²) and biomass (kg/km²) for the case study species calculated by GFCM cell.

Finally selected indicators

The indicators finally chosen to be used in the constrained cluster analysis, calculated for each target species in each cell of the GFCM grid by means of the R routine and saved both in table form (.csv format) and in shape format, are the following (the names in bold are the denomination of the indicators in the files saved by the routine):

- Inverse of CV of Density (N/km²): InverseCV_Nkm2
- Biomass (kg/km²): Bkm2
- Individual mean weight (kg): individual_meanWeight
- Sex ratio as ratio of F to (F+M): SexRatio
- Percentage of mature females to all the females (excl. virgin):
 - Percentage_in_SpawningStage
- Median length of mature females (mm): *medianLength_in_SpawningStage*

An R routine was produced in order to support the analysis to be done in the WP1 by COISPA and in collaboration with IEO. The R_BIND_STOCKMED routine standardize MEDITS data from Access database, assign every haul with the corresponding GFCM grid cell to which it belongs, calculate the abundance trends by GSA and compute the biological indicators at diverse spatial aggregation levels: GSA, MEDITS stratum and GFCM grid for the target species. Moreover, the routine facilitates the production of the shape files for the selected indicators by GFCM cell to be used in the Constrained Clustering analysis (WP4). It is designed to perform different operations starting from raw MEDITS data, <u>one species at time</u>. The routine reads from each table the selected indicators to be passed to WP4; then, all the indicators are saved both in .csv tables and in shape file (.shp) to be passed to the constrained clustering analysis.

The selected parameters were estimated for each of the 19 STOCKMED target species and represented in maps (Fig. 1.6). The results show high heterogeneity in the spatial distribution of the different biological parameters and for the different species. It is difficult to establish boundaries within a single species and further, the boundaries will differ from one species to the other. Thus, the establishment of stock management units that would serve for all the species at a time does not seem reasonable. CV % of Density (N/km2) - MERL MER



Biomass (Kg/km2) - MERL MER



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for M.merluccius.

Mean weight (Kg) - MERL MER



Sex-ratio - MERL MER





Percentage of females in spawning stage - MERL MER



Median length in Spawning stage - MERL MER



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for M.merluccius.

CV % of Density (N/km2) - MULL BAR



Biomass (Kg/km2) - MULL BAR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M.barbatus*.

Mean weight (Kg) - MULL BAR



Sex-ratio - MULL BAR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M.barbatus*.





Median length in Spawning stage - MULL BAR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M.barbatus*.

CV % of Density (N/km2) - PAPE LON



Biomass (Kg/km2) - PAPE LON



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for P. longirostris.

Mean weight (Kg) - PAPE LON



Sex-ratio - PAPE LON



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for P. longirostris.

Percentage of females in spawning stage - PAPE LON



Median length in Spawning stage - PAPE LON



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for P. longirostris.

CV % of Density (N/km2) - SOLE VUL



Biomass (Kg/km2) - SOLE VUL



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for S. vulgaris.

Mean weight (Kg) - SOLE VUL



Sex-ratio - SOLE VUL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for S. vulgaris.

Percentage of females in spawning stage - SOLE VUL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for S. vulgaris.

Inverse CV % of Density (N/km2) - ARIS FOL



Biomass (Kg/km2) - ARIS FOL



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for A. foliacea.

Mean weight (Kg) - ARIS FOL



Sex-ratio - ARIS FOL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for A. foliacea.

Percentage of females in spawning stage - ARIS FOL



Median length in Spawning stage - ARIS FOL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for A. foliacea.

Inverse CV % of Density (N/km2) - ARIT ANT



Biomass (Kg/km2) - ARIT ANT



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for A. antennatus.

Mean weight (Kg) - ARIT ANT



Sex-ratio - ARIT ANT





Percentage of females in spawning stage - ARIT ANT



Median length in Spawning stage - ARIT ANT



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for A. antennatus.

Inverse CV % of Density (N/km2) - ELED CIR



Biomass (Kg/km2) - ELED CIR



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. cirrhosa*.

Mean weight (Kg) - ELED CIR



Sex-ratio - ELED CIR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. cirrhosa*.

Percentage of females in spawning stage - ELED CIR



Median length in Spawning stage - ELED CIR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for E. cirrhosa.

Inverse CV % of Density (N/km2) - ELED MOS



Biomass (Kg/km2) - ELED MOS



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. moschata*.

Mean weight (Kg) - ELED MOS



Sex-ratio - ELED MOS



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. moschata*.

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Percentage of females in spawning stage - ELED MOS



Median length in Spawning stage - ELED MOS



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. moschata*.

Inverse CV % of Density (N/km2) - ENGR ENC



Biomass (Kg/km2) - ENGR ENC



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for E. encrasicolus.

Mean weight (Kg) - ENGR ENC



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *E. encrasicolus*. .

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Inverse CV % of Density (N/km2) - GALU MEL



Biomass (Kg/km2) - GALU MEL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for G. melastomus.

Mean weight (Kg) - GALU MEL



Sex-ratio - GALU MEL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *G. melastomus*.

Percentage of females in spawning stage - GALU MEL



Median length in Spawning stage - GALU MEL



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *G. melastomus*.

Inverse CV % of Density (N/km2) - ILLE COI



Biomass (Kg/km2) - ILLE COI



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for I. coindetti.

Mean weight (Kg) - ILLE COI



Sex-ratio - ILLE COI



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for I. coindetti.





Median length in Spawning stage - ILLE COI



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *I. coindetti*.

Inverse CV % of Density (N/km2) - LOPH BUD



Biomass (Kg/km2) - LOPH BUD



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for L. budegassa.

Mean weight (Kg) - LOPH BUD



Sex-ratio - LOPH BUD



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for L. budegassa.
Percentage of females in spawning stage - LOPH BUD



Median length in Spawning stage - LOPH BUD



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for L. budegassa.

Inverse CV % of Density (N/km2) - MULL SUR



Biomass (Kg/km2) - MULL SUR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M. surmuletus*.

Mean weight (Kg) - MULL SUR



Sex-ratio - MULL SUR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M. surmuletus*.

Percentage of females in spawning stage - MULL SUR



Median length in Spawning stage - MULL SUR



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *M. surmuletus.*

Inverse CV % of Density (N/km2) - NEPR NOR



Biomass (Kg/km2) - NEPR NOR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for N. norvegicus.

Mean weight (Kg) - NEPR NOR



Sex-ratio - NEPR NOR



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for N. norvegicus.

Percentage of females in spawning stage - NEPR NOR



Median length in Spawning stage - NEPR NOR



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for N. norvegicus.

Inverse CV % of Density (N/km2) - OCTO VUL



Biomass (Kg/km2) - OCTO VUL



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for O. vulgaris.

Mean weight (Kg) - OCTO VUL



Sex-ratio - OCTO VUL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for O. vulgaris.

Percentage of females in spawning stage - OCTO VUL



Median length in Spawning stage - OCTO VUL



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *O. vulgaris*.

Inverse CV % of Density (N/km2) - PAGE ERY



Biomass (Kg/km2) - PAGE ERY



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *P. erythrinus*.

Mean weight (Kg) - PAGE ERY



Sex-ratio - PAGE ERY



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *P. erythrinus.*

Percentage of females in spawning stage - PAGE ERY



Median length in Spawning stage - PAGE ERY



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for P. erythrinus.





Biomass (Kg/km2) - SARD PIL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *S. pilchardus*.

Mean weight (Kg) - SARD PIL



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for S. pilchardus.

Inverse CV % of Density (N/km2) - TRAC MED



Biomass (Kg/km2) - TRAC MED



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for T. mediterraneus

Mean weight (Kg) - TRAC MED



Sex-ratio - TRAC MED



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for T. mediterraneus

Percentage of females in spawning stage - TRAC MED



Median length in Spawning stage - TRAC MED



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for T. mediterraneus

Inverse CV % of Density (N/km2) - TRAC TRA



Biomass (Kg/km2) - TRAC TRA



Figure 1.6 (continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for T. trachurus

Mean weight (Kg) - TRAC TRA



Sex-ratio - TRAC TRA



Figure 1.6(continued). Biological indicators from 10 years of MEDITS surveys (2002-2011) for *T. trachurus*

Regarding growth and maturity related parameters (i.e. growth, size at first maturity and spawning season) retrieved within *Task 1.2.* multivariant techniques were applied to analyse the data at GSA level. The influence of methodology, sex, latitude and longitude on each parameter was explored. In the event of presence of a geographical significant trend possible discontinuities were seeked which would be used to construct clusters of GSAs with similar characteristics. Significant clustering results were resumed on a geographic map, were the different clusters were plotted.

Growth

The differences of growth rate among diverse areas can be due to factors based on genetic characteristics or a plastic response to different environmental conditions or a combination of both. We found a high variability in the parameters analyzed, though in some situations a geographical pattern could be identified. In general, demersal species (hake, red mullet, common sole, common pandora, giant red shrimp and blue red shrimp) showed a geographic gradient of growth performance, differently from the pelagic and bentho-pelagic one (anchovy, sardine, Mediterranean horse mackerel, deep-water rose shrimp).

In the Mediterranean basin a west-east gradient was already highlighted for the red mullet and common pandora (Sonin et al., 2007; Somarakis and Machias, 2002), likewise in our analyses. This geographical difference was attributed to a "Levantine nanism", which is characterized by smaller body size of specimens in the Levantine basin compared with conspecifics in the western Mediterranean (Sonin et al., 2007). Besides red mullet and common pandora, in this study we found a decrease of the growth performance index (Φ') moving from for west to east also for hake, common sole, giant red shrimp and blue red shrimp (Fig. 6). These findings can be explained by a low productivity in the Levantine basin compared with the western Mediterranean, where chlorophyll concentration is higher in comparison to the est part (Moutin and Raimbault 2002). The average higher water temperature in the South-Eastern Mediterranean may be another explanation for Levantine nanism. Higher water temperature may cause more intensive metabolism in the southeastern population, resulting in earlier sexual maturity and deceleration of growth rate. Other environmental factor as salinity, density and food competition could be driving factors of dwarfism. Stergiou et al. (1997) also pointed out that the well-know phenomena of dwarfism characterizing benthic invertebrates species in the eastern Mediterranean basin might also be true for the marine fishes, mentioned also by Mytilineou et al. (2013) for Pagellus bogaraveo.

Regarding **horse mackerel** the data analysis show the increase of growth performance index (Φ') with a west – east gradient (Fig. 1.7). Abaúnza et al. (2008) found the same result comparing the age length key of 8 sampling sites from west to east (Alboran sea, Catalan coast, Balearic sea, Sardinia sea, Strait of Sicily, South Adriatic sea, Eastern

Ionian sea and Aegean sea). The high relative values of the lengths at age observed in Tyrrhenian and Ionian Sea, indicate that there might be distinct characteristics of these areas for their differential growth. These authors make the hypothesis of length-dependent migration from adjacent areas to explain the values observed in the Tyrrhenian and Ionian Seas. Indeed, having a large size is beneficial for migrating since this increases their metabolic efficiency and swimming capacity. Larger fish can migrate further and explore a larger area. This migration is supported by the general circulation in these areas of the Mediterranean Sea; indeed there are currents from Sicily and southwest Italy to the Tyrrhenian Sea and from the Adriatic Sea to the Ionian Sea. Abaúnza et al. (2003) suggest that the reason for the high relative values observed in the allometric parameter of the length–weight relationship in horse mackerel of Tyrrhenian and Ionian Sea is the coastal upwelling observed in the Eastern Ionian Sea (Theocharis et al., 1998), which would ensure higher food availability over the entire year.

Regarding sardine the data analysis does not show any significant diversity of growth performance index (Φ') between the GSAs. In a broad scale anchovy and sardine from the Mediterranean basin show a lower growth rate in comparison to the others area of the Atlantic (Silva et al., 2008; Morales-Nin and Pertierra 1990). This difference could be explained by the different environmental and biological factors in the region such as temperature, food available, primary productivity, competition and predation. For the anchovy, sea surface estimates of chlorophyll concentrations are good indicators of food availability (Basilone et al., 2004a; Martín et al., 2008), so the differences in the growth rate between Mediterranean and Atlantic could be explained by the different primary productivity. Moreover this finding are supported by genetic (Atarhouch et al., 2007) and reproductive characteristic (Ganias et al., 2004) in the sardine population. At the scale of Mediterranean Sea growth performance did not show any significant difference, probably because the differences in productivity are not so extreme or the migration pattern of this species contribute to mitigate the effect of the environmental influences on growth. Moreover crustaceans showed a high variability in growth because of the method applied. This makes us more cautious to the length-based methods and implies the need of various validation methods for crustaceans growth studies.



M. merluccius



M. barbatus

Figure 1.7. Map of the clusters obtained for the different species on the analysis of the growth parameters.



S. vulgaris



T. trachurus

Figure 1.7 (continued). Map of the clusters obtained for the different species on the analysis of the growth parameters.



P. erythrinus



A. foliacea

Figure 1.7 (continued). Map of the clusters obtained for the different species on the analysis of the growth parameters.



A. antennatus

Figure 1.7 (continued). Map of the clusters obtained for the different species on the analysis of the growth parameters.

Length at first maturity

In the bibliographical review and analysis performed regarding length at first maturity, the following clusters of different GSAs were detected in European hake (M. merluccius), European anchovy (E. encrasicolus), stripped red mulet (M. surmuletus), deep water rose shrimp (A. antennatus) and squid (I. coindetti) (Fig. 1.8). In particular, four clusters were identified for hake, i.e. one including GSAs 5, 6, 7 and 9; the second including GSA 4, 12, 13, 14; another one including GSAs 17, 18 and 10 and the fourth group consisting of GSAs 15, 19, 20, 22, 23 and 26. For European anchovy, four more clusters were detected, i.e. one consisting of GSAs 1, 6, 7 and 4; second cluster including GSAs 12, 13, 14; third cluster including N. Adriatic and the last cluster including GSA 15, 20 and 22. For stripped red mullet three clusters were detected: one group including GSA 5 and 12, second including 3 and 15 and a third group including GSAs 22 and 26. For deep water rose shrimp, three clusters were found: one including GSA 1, 4, 5, 6 and 11, a second cluster including GSA 7 and 9; a third cluster including GSAS 10, 19 and 20. Finally, for squid three clusters were observed, one consisting of a single GSA, i.e. GSA 9; second cluster made of GSAs 10, 16 and 18 and the last group integrated by GSAs 20, 22 and 23.

The differences of the length at first maturity between areas could be the results of different factors acting alone or in combination: the genetic base, the plastic response to different environmental conditions, some selective cause as the fishing pressure (Barot et al., 2004), the different estimation methods applied (ICES, 2008). In our analysis we found a high variability, and then it was difficult to recognize some geographical pattern. Our results indicated that for several species length at first maturity changed in different, sometimes very closely situated, areas. Such variability may be due to environmental differences (i.e. phenotypic variability), but the effect of sampling biases cannot be ruled out. In particular the estimation based on the macroscopically identified maturity stages could affect the estimation of the L₅₀ (ICES, 2008). This last aspect could be the reason why only few species showed a significant geographical trend (6 species out of the 19 analyzed) in our analysis (Fig. 1.8). Moreover, regarding the difficult of this kind of analysis, it is important to underline that for many species few data are available in literature regarding the length at first maturity.

In literature, this parameter was used to identify unit stock, but at larger geographical scale (i.e. Atlantic vs Mediterranean) (Abaúnza et al., 2008; Silva et al., 2006; Tsikliras et al., 2013). In sardine (Silva et al., 2006) and horse mackerel (Abaúnza et al., 2008) a significant latitude (north-south) decrease for the Atlantic sea is reported. In our context, the absences of this kind of trend could be due to the narrower latitudinal range.



Figure 1.8. Clusters obtained with the retrieved information regarding length at first maturity for the different species.

Length of the spawning season

The length of spawning season in our analysis revealed significant geographical trend only in one case, i.e. *P. longirostris* (Fig. 1.9). Four clusters were identified, one including the northern areas of the Western Mediterranean Sea (GSA 5, 6, 9 and 10) and South Adriatic (GSA 18); a second cluster including the Central Southern areas (GSA 12, 13 14 and 16); the third including Aegean Sea (GSA 22) and South Levant (GSA 26) and the last cluster being a single GSA (GSA 27). In general, spawning strategies, including the spawning duration, vary with geographical location (ICES 2005). Indeed some Authors report an established effect of latitude on the spawning duration for fishes (Longhurst and Pauly 1987), but in our analysis the spawning duration seems not differing significantly between the stocks inhabiting the Mediterranean regions. The absence of any latitudinal effect on the duration of spawning season could be attributed to the narrow latitudinal range of the Mediterranean Sea, especially in the western basin, as it has been hypothesized by Tsikliras et al. (2010).



Figure 1.9. Map of the clusters obtained regarding the spawning season for *P. longirostris*.

In general the reproduction aspects as the length at first maturity and length of spawning season seem less responsive than the growth parameters, but it is important to point out, in addition to the difficulties mentioned above, a more limited literature regarding the reproductive aspects (i.e. length at first maturity and length of spawning season) of the considered species.

Morphometric and meristic

The shape of a fish body is defined by an interaction between genetical and environmental factors especially during its early development stages. Morphometric variations with growth that may change between different locations may be useful for the discrimination of stock units. In fact, with this purpose, phenotypical characters may be as much, or even more useful than purely genetic ones because a gene flow of small magnitude may prevent the detection of significant genetic differences. However, consistent morphometric differences between locations may indicate a population separation, hence the existence of different stock units, because even if those differences are caused by environmental influence, an extensive mixing of individuals from different locations would make those differences undetectable. The potential capacity of populations to adapt and evolve as independent biological entities in different environmental conditions is restricted by the exchange of individuals between populations. A sufficient degree of isolation may result in notable phenotypic and genetic differentiation among fish populations within a species, which may be recognizable as a basis for separation and management of distinct populations.

The morphometric studies in the context of stock identification are carried out by a specific sampling and analysis program. In our context we analyzed few example of the stock discrimination based on morphometric data. Other approaches for stock identification, based on phenotypic characteristics of the fish body, have been put in practice. One of these is the analysis of meristic characters (Turan, 2004). However, these characters are considered less useful than the morphometric ones (Misra and Carscadden, 1987; Murta et al., 2008).

Murta et al. (2008) on base of morphometric study in the Mediterranean Sea, distinguish three significantly distinct groups of *Trachurus trachurus*: one included the Spanish areas in the Western Mediterranean (GSA 1, 5 and 6), a second group included the Tyrrhenian Sea (GSA 9, 10), Adriatic Sea (GSA 17 and 18), Sardinia seas (GSA 11), Sicily coast (GSA 16) and Western Ioninan (GSA 19) and the last one in the Eastern Mediterranean including Eastern Ionian Sea (GSA 20) and Aegean Sea (GSA 22), and a third one with locations in Balearic Islands (GSA 5), Alboran Sea (GSA 1) and Catalonia (GSA 6).

Kristoffersen and Magoulas (2008) for the anchovy body shape didn't found significantly differences between Aegean and Ionian groups. Six populations of *Nephrops norvegicus* (south coast of Portugal –Algarve- in the Atlantic and five areas in the Mediterranean: the Catalan Sea, the Ligurian Sea, the Tyrrhenian Sea, the Adriatic Sea and the Gulf of Euboikos) were compared by morphometric characters (Castro et al., 1998). There were no single pairs of populations showing complete separation, although the degree of overlap was different when different pairs were compared. The 3 populations in the West and Central Mediterranean, Catalan Sea, Ligurian Sea and Tyrrhenian Sea showed the highest levels of similarity. The population from the Atlantic showed greatest distances overall, followed by the population from the Euboikos Gulf, representing the Eastern extreme of the geographical range.

Morphologic differentiation among stocks of Mediterranean horse mackerel, *Trachurus mediterraneus*, throughout the Black, Marmara, Aegean and Eastern Mediterranean Seas (Turkish coast), was investigated using morphometric and meristic characters (Turan, 2004). Discriminant function analysis of both morphometric and meristic characters suggested that there is a restricted migration of mackerel among the adjacent seas. Overlapping of four Black sea samples on the discriminant space in morphometric and meristic characters suggested that there is one isolated population. The Marmara sea samples were the most isolated ones from all the others for both morphometric and meristic characters, which may indicate the existence of a distinguishable mackerel stock in the area. The sample from the Aegean Sea was grouped with one geographically close Eastern Mediterranean (Turkish coast) sample based on morphometrics, and separated from all other Mediterranean samples based on meristic characters, suggesting some degree of intermingling between these areas.

Otolith shape and microelement

Otolith shapes are species-specific and, in many cases, geographic variation in otolith shapes could be related to stock differences (Stransky, 2005, Stransky et al., 2008; Kristoffersen and Magoulas, 2008). Small-scale variation in otolith shapes of horse mackerel along the Portuguese coast has been investigated by Murta et al. (1996). They paved the way for large-scale studies on otolith shape variation and a possible use for stock identification. In the Mediterranean basin few studies were carried out on the geographical otolith shape variation.

For *Trachurus trachurus* multidimensional scaling of the average Fourier descriptors by area (Stransky et al., 2008), showed three distinct clusters of areas: a northern (Norway, Porcupine, Great Sole, German Bight, Britanny, Bay of Biscay), an Ibero - Mauritanian (Galicia, Aveiro, Setubal, Algarve, Alboran Sea, Balearic Islands, Gulf of Lions and Mauritania) and an eastern Mediterranean group (Tyrrhenian Sea, Sardinia seas, Adriatic Sea, Sicily, Ionian Sea and Aegean Sea).

Kristoffersen and Magoulas (2008) comparing the otolith shape of anchovy from Ionian and Aegean Sea didn't found any significant differences. Otoliths are predominantly composed of calcium and trace elements that are derived from the ambient waters inhabited by the fish (Campana et al., 1995). Because water bodies often differ in the concentrations of trace elements, stocks may often be distinguished by the chemical 'signatures' retained in otoliths (assayed using spectrometric or related techniques). A further advantage of this approach is that by analyzing selected portions of an otolith, the trace element signals can be associated with particular growth stages. This approach has potential to discriminate between stocks where an environmental signal is pronounced (e.g., where substantial reproductive interchange diminishes genetic differences). However, results from these techniques are often difficult to interpret because of the combined effects of physiological, ontogenetic, and environmental influences on the deposition of trace elements, not to mention a posteriori sampling problems with handling and elemental contamination (Begg and Waldman, 1999). For this topic there is a significant knowledge gap for the Mediterranean basin, indeed exceped for the hake no other papers were found in literature. Moreover for the hake the letterature analyzed show as the isotopes and microelemets was a usefull tool to discrimination the origin for a large geographical scale (e.g. Atlantic and Mediterranean), more knowledge, and sample and /or large set of element will be need to usage this studies in the stock discrimination at smaller scale level.

Task 1.3 Reviewing and analysing for the selected case studies information on parasites, tagging, migration patterns, larval drift and reporting results as geo-referred information (task Coordinator K. Kapiris).

Parasites

Data was compiled from bibliographic published information and the geographical location from where the data was retrieved (or the central position of the GSA) was recorded to produce maps of the presence of information regarding parasites. Information regarding parasites was found for fourteen out of the nineteen target species, i.e. *P. longirostris, N. norvegicus, O.vulgaris, E. encrasicolus, L. budegassa, E. cirrhosa, S. pilchardus, I. coindetti, M. merluccius, T. mediterraneus, P. erythrynus, M. barbatus, M. surmuletus* and *T. trachurus*. Maps of the parasites present in each species per phylum were produced (Fig. 1.9) as well as maps of the different species of parasites present in *E. encrasicolus* (Fig. 1.10), *M. merluccius* and *T. trachurus* (Fig. 1.11).

Parasites are a natural part of all ecosystems and play an important role in their functioning especially in shallow coastal areas where they represent a key component of the biodiversity. There is a plethora of papers describing the use of parasites to separate fish stocks (e.g. MacKenzie, 1983; Lester, 1990; Williams, MacKenzie *et al.* 1992; MacKenzie and Abaunz, 1998; Lester and MacKenzie, 2009). Parasites have been widely used as biological tags to provide information for fisheries management (Lester, 1990; Speare, 1995). Many of these studies began as surveys of parasites in a fish species and proceeded with statistical analysis of the whole data set.

The European anchovy (Engraulis encrasicolus) is a common paratenic host for Anisakidae in the Mediterranean, and concretely in GSA 6, 7, 9, 19 and 22 (Fig. 9). Many researchers have reported the presence of Anisakis spp. and Hysterothylacium aduncum third-stage larvae in *E. encrasicolus* in the Mediterranean Sea, with prevalence values of 26.6 to 77.0% (Anastasio et al., 2009; Rello et al., 2009; Angelucci et al., 2011; Mladineo et al., 2012). In GSA 6 (Catalan Sea) and in GSA 7 (Gulf of Lions, Anisakis larva type I and H. aduncum have been identified (Rello et al., 2009). H. aduncum is usually more commonly reported than Anisakis sp. in anchovy. Rello et al. (2009) studied four different fishing areas of the western Mediterranean Sea. In the Ligurian and North Tyrrenian Sea Anisakids sp. specimens were found in E. encrasicolus (Cavallero et al., 2012). H. aduncum was more prevalent than Anisakis sp. in all four areas, with prevalences of 68.9 and 3.9% for *H. aduncum* and *Anisakis sp.*, respectively, in the Gulf of Lion. These authors stated that prevalence of infected anchovies originating from different fishing areas could differ significantly, with higher prevalences estimated in the northwestern Mediterranean (Gulf of Lion and Ligurian Sea). Other studies carried out on different fish species have highlighted differences in anisakid prevalence in relation to the fishing area, probably linked to lower or higher abundances of the definitive hosts of these parasites (Mattiucci *et al.*, 2004; 2008). In the South and Central Tirrenian Sea (GSA 10) a molecular analysis to fully characterize the 35 detected larvae found in *E. encrasicolus* revealed 15 specimens of *Anisakis pegreffii*, 10 specimens of *Hysterothylacium aduncum*, and one hybrid genotype of *A. pegreffii* x *Anisakis simplex* (De Liberato *et al.*, 2013). In the whole area of the Aegean Sea some parasites have been found, as *Anisakis pegreffii*, *Hysterothylacium aduncum* and *Anisakis* simplex (Keser *et al.*, 2007; Chaligiannis *et al.*, 2012); Akmırza, 2013). Parasites belonging to Anisakideae were found also in the W. Ionian Sea (Campese, 2012).

There are many works on the parasite fauna in Merluccius merlucius in the Mediterranean, specifically in GSAs 1, 4, 5, 6, 7, 9, 10, 17, 20, 22, 23 and 25 (Fig. 9). The aim of the majority of the obtained studies in hake's parasites was to identify the presence of the parasites – mainly Anisakis sp. and other crustaceans- in M. merluccius. The parasitic copepods Clavella stellata and Lernaeocea lusci have been identified in M. merluccius individuals caught in South and Central Tyrrhenian Sea (Bonfiglio et al., 2010; Gaglio et al., 2011). According Mattiucci et al. (2004) the parasite Anisakis pergreffii and A. physeteris were found in hakes caught in Crete (GSA 23), Aegean Sea (GSA 23), Ionian Sea (GSA 20), Northern Adriatic Sea (GSA 17), Ligurian and Northern Tyrrhenian Sea (GSA 9), Balearic islands (GSA 5), off Malaga (GSA 1), off Alicante (GSA 6). That means that both parasites are the most prevalent in the central, the western and eastern basins of the Mediterranean Sea. The high abundances recorded for this species from the Mediterranean Sea can be explained by the occurrence of various dolphin species, such as the bottlenose dolphin Tursiops truncatus (Montagu, 1821), one of the main definitive hosts of A. pegreffii (Mattiucci et al., 2004). Anisakis simplex was found in specimens caught in the Aegean Sea (GSA 22) (Chaliggians et al., 2012), while A. typical was identified in hakes from the eastern part of the Mediterranean: off Cyprus (GSA 25), Crete (GSA 23), E. Ionian Sea (GSA 20) (Mattiucci et al., 2004). The nematode Hysterothylacium aduncum (Anisakidea) was identified in samples from the Aegean Sea (Chaligiannis et al., 2012). Valero et al. (2006) studied the parasitization by Anisakis spp. in European hake (Merluccius merluccius from the Atlantic off north-west Africa and the Mediterranean off southern Spain. Infection parameters differed: The fish from the Atlantic showing a prevalence of 87.97% and a mean intensity of 4.69, while, in those from the Mediterranean, these were 41.27% and 1.73, respectively. According to the Anisakis sp. fauna found in several samples, Mattiucci et al. (2004) suggest the existence of different stocks of *M. merluccius* since the migration of hake from Atlantic to Mediterranean waters appears not to occur.

Because of the benthic life and a wide range of prey items, red mullet (*Mullus barbatus*) (Fig. 1.9) are suitable hosts for a variety of helminth fish parasites transferred through the marine food web. For example Acanthocephala were found to parasite in red mullets in Sardinian waters (GSA 11) (Figus *et al.*, 2005), Apicomplexa, parasitic crustaceans and insects in fishes of the same species caught in the Catalan Sea (GSA 6) (Carreras-Aubets

et al., 2011). Nematods of the family Anisakidae were found: A. simplex in the Ligurian Sea (GSA 9) (Manfredi et al., 2000) and in the Aegean Sea (Chaligiannis et al., 2012), Contracaecum sp. and Hysterothylacium fabri in the Catalan Sea (Carreras-Aubets et al., 2011) and in Sardinian waters (Figus et al., 2005). Other nematods were found in the following sites: Ascarophis sp., Capillaria sp., Cuculanus sp. in the Catalan Sea (Carreras-Aubets et al., 2011) Echinocephalus sp. in the Sardinian waters (Figus et al., 2005), Cuculanus longicollis in the S. Adriatic Sea (GSA 18) (Jardas & Hristovski, 1985). Many platyelminths were found in the Catalan Sea (GSA 6), like Aponurus sp., Bothriocephalus sp., Derogenes latus, O.Trypanorhyncha larval, Opecoeloides furcatus, Phyllidostomum sp., Prosorhynchus sp. (Carreras-Aubets et al., 2011) and in Sardinian waters (GSA 11) (Aponurus loguncula, Eutetrarhynchus sp., Hemiuridae sp., Nybelinia lingualis, Opecoeloides furcatus, Proctotrma bacilliovatum, Phyllidostomum sp., Prosorhynchus crucibulum, Stephanostomun sp., Mongea ind.) (Figus et al., 2005). In the Northern Adriatic Sea (GSA 17) Derogenes latus was also identified (Paradižnik & Radujković 2007). There is a different abundance of the parasites found in each area ad their hospitality is influenced by the oceanographc condition, the pollution other secondary reasons. Carreras-Aubets et al. (2011) shown that nematodes have significant interactions with environmental impact.

The parasitic fauna of *Mullus surmuletus* has been studied mainly in the western part of the Mediterranean basin (Fig. 1.10). Parasitic copepods have been identified in striped red mullet in Gulf of Lion (GSA 7), in South Central Tirrenian Sea (GSA 10), in the Northern Adriatic Sea (GSA 17) (Klimpel et al., 2008). In the same areas isopods (Gnathidae) have been also found (Klimpel et al., 2008). A variety of Nematoda has been recognized, as Ascarophis valentine and Hysterothylacium aduncum in the Gulf of Lion (GSA 7) (Klimpel et al., 2008), Anisakis simplex in the Ligurian and Northern Tirrenian Sea (GSA 9) (Manfredi et al., 2000), Cucullanus longicollis and Hysterothylacium aduncum in the North and Central Tirrenian Sea (GSA 10) (Arculeo et al., 1997; Klimpel et al., 2008) Hysterothylacium fabri in Central Tirrenian Sea (GSA 10) (Arculeo et al., 1997), In the Sardinian waters (GSA 11) have been found Cucullanus sp., Hysterothylacium sp. and Raphidascaris sp. (Figus et al., 2005). The nematopod Hysterothylacium aduncum has been also found in the Northern Adriatic Sea (Klimpel et al., 2008), while Contracaecum (Jardas *clavatum* in the Southern Adriatic Sea & Hristovski, 1985). Platyhelminthes were found in individuals of *M. surmuletus* caught in: the gulfs of Lion and Marseille (GSA 7) like Derogenes varicus (Klimpel et al., 2008), Opecoeloides furcatus (Jousson & Bartoli, 2000; Klimpel et al., 2008) Tetraphyllidea spp. (Klimpel et al., 2008). Opecoeloides furcatus has been faund in Corsica (GSA 8), also (Jousson & Bartoli, 2000). The same parasite has been found in the striped mullets fished in the Gulf of Palermo (Arculeo et al., 1997; Jousson & Bartoli, 2000). The red mullets from the Sardinian waters (GSA 11) harboured the most platyelminth species in M. Eutetrarhynchus surmuletus: Aponurus laguncula, sp., Hemiuridae spp., Lasiotocus mulli, Nybelinia lingualis, Opecoeloides furcatus, Proctotrema bacilliovatum,

Prosorhynchus crucibulum, Scolex pleuronectis, Stephanostomun sp. (Figus *et al.*, 2005). In the Northern Adriatic Sea (GSA 17) *Opecoeloides furcatus* and *Timonia mediterranea* have been also identified (Klimpel et al., 2008). Klimpel *et al.* (2008) studied the parasites of *M. surmuletus* in the Mediterranean and North Sea. Many endemic parasites were found in the Mediterranean, while a bigger parasites number was found in the North Sea. According the authors the parasite fauna of striped red mullets in the Mediterranean is the consequence of recent parasite speciation and adaptation together with host-parasite co-evolution, possibly influenced by palaeogeographical and palaeoclimatic events.

Generally speaking, the parasites found in *Pagellus erythrinus* belonged to Arthropoda, Nematoda and Platyelminths (Fig. 1.10). A lot of parasites have been identified in the whole area of the Aegean Sea (GSA 22): the copepods *Clavellopsis fallax, Hatschekia sp, Lernaea sp.* (Akmirza, 2000) and the nematods *A. pegrefii, A. simplex* (Akmirza, 2000; Oktener *et al.*, 2009; Chaligiannis *et al.*, 2012). In the Corsica area (GSA 8) (Ternengo et al., 2009) found that the parasitic fauna in *P. erythrinus* was consisted from the parasite species: the acanthocephalan *Acanthocephaloides incrassatus*, the platyelminths *Holorchis pycnoporus, Lamellodiscus drummondi and* Tetraphyllidea larvae, the copepods *Alella pagelli* and *Caligus pagelli*, the isopods *Ceratothoa oestroides* and *Gnathia sp.*, the trematods *Macvicaria crassigula* and *Diphterostomum brusinae*, and *Chorycotyle chrisophrii*. In the Northern Adriatic Sea (GSA 17) the platyelminth *Aphanurus stossichi* (Paradižnik & Radujković 2007) and the nematopod *Ichthyonema (= Philometra) filiformis (Stossich, 1896)* were found, while in the South Adriatic Sea (GSA 18)

the platyelminth Hemiurus communis (Jardas & Hristovski 1985) and the nematopod

Philometra filiformis (Orecchia & Paggi, 1978) were identified. The nematode genus *Philometra* Costa, 1845, contains a large number of species parasitising different freshwater, brackish-water and marine fishes. The same nematode has been also found in the South and Central Tirrenian Sea (GSA 10) (Gaglio *et al.*, 2009). The platyelminth *Lamellodiscus* species has parasitized in the common pandora caught in the Gulf of Lions (GSA 7) (Desdevises et al., 2000; Desdevises, 2006).

The presence and abundance of single parasite groups between individuals found in various Mediterranean areas showed slight differences. In addition to this, a general tendency of a distinct parasitic fauna (taxonomical and in abundances) has not been observed yet.

Among other parasitic studies on Atlantic horse mackerel (*T. trachurus*) in the frame of the GSAs related to STOCKMED project, the following results have been obtained (Fig. 1.12):

In the Western part of the Mediterranean Sea, the following nematods have been identified in *T. trachurus* in the Northern Alboran Sea (GSA 1): *Anisakis pegreffii* (Mattiucci & Nascetti, 2008), *A. simplex* (Adroher *et al.*, 1996; Mattiucci & Nascetti, 2008), *A. physeteris* (Adroher *et al.*, 1996), *Hysterothylacium aduncum* and

Hysterothylacium sp. (Adroher *et al.*, 1996). In the Balearic Islands (GSA 5) the myxozoan *Alataspora solomoni* and the platyelminth *Prodistomum polonii* have been found (Campbell, 2005). The same myxozoan was found in the Northern Spain (GSA 6) also (Campbell, 2005). In the Gulf of Lion the myxozoan *Alatospora solomoni* and the platyelminthes *Hemiurus communis, Lasiotocus typicum, Monascus filiformis, Opechona bacillaris* have been identified (Campbell, 2000). The platyelminthes *Ectenurus lepidus* and *Paradiplectanotrema trachuri* were found in Corsica (GSA 8).

In the Central part of the Mediterranean, in the GSA 9 (Ligurian and Northern Tirrenian Sea) were found: the nematods *Anisakids sp., Anisakis physeteris, Anisakis pegreffii, Anisakis simplex* (Manfredi *et al.*, 2000; Mattiucci & Nascetti, 2008; Cavallero *et al.*, 2012). In Sardinia (GSA 11), in Sicilia (GSA 16), in Northern and Southern Adriatic Sea (GSA 17, 18) and in the Western Ionian Sea *Anisakis pegreffii* was also parasitized in *T. trachurus* (Mattiucci & Nascetti, 2008). In the last area the playelminth *Lecithochirium musculus* was identified in the Atlantic horse mackerel. Anisakidae larvae and another nematod, *Contracaecum fabri*, were found in the southern part of the Adriatic Sea (GSA 18) (Jardas & Hristovski 1985; Smrzlic *et al.*, 2012).

In the Eastern part of the Mediterranean, in the Eastern Ionian Sea a lot of different parasite infected *T. trachurus*: the copepods *Caligus elongates, Lernanthropus trachuri,* the isopod *Ceratothoa oestroides,* the brachyuran Argulus vittatus, the myxozoa *Alatospora solomoni* and *Myxobolus spinacurvatura,* and the platyelminths *Bathycreadium elongate* and *Paradiplectanotrema trachuri* (Campbell, 2000; 2005). In the Aegean Sea (GSA 22) the referred parasites are the copepod *Lernea sp* (Akmirza, 1998), the myxozoans *Alatospora solomoni* and *Goussia cruciata* (Lom & Dyková, 1992; Campbell, 2000) and the platyelminths *Ectenurus Lepidus, Monascus filiformis, Prodistomum polonii* (Keser *et al.*, 2007). The nematods found were: *Anisakis pegreffii* (Mattiucci & Nascetti 2008; Chaligiannis *et al.* 2012), *Anisakis spp.* (Tantanasi *et al.*, 2012; (Campbell, 2005) and *Hysterothylacium aduncum* (Keser *et al.*, 2007).

A lot of parasites' studies in *Trachurus mediterraneus* have also been occurred mainly in the eastern part of the Mediterranean basin (Fig. 9). In the Aegean Sea (GSA 22) the copepod *Lernaea sp.*, the isopod *Meinertia oestrides* (Akmirza, 1998) and the myxozoan *Goussia cruciata* (Lom & Dyková, 1992) have been infected *T. mediterraneus*. In the same area the nematods *Anisakis simplex*, *Anisakis sp.* and the platyelminths *Contraceacum aduncum, Ectenurus epidus, Haplocladus typicus, Lepocreadium pyriforme, Opechona bacillaris, Pseudaxine trachuri, Scolex pleuronectis, Teryestia laticollus* have been found (Akmirza, 1998). *Anisakis simplex* has been also found in the Northern Adriatic Sea (Jardas & Hristovski, 1985).

The parasitic fauna in *Sardina pichardus* consisted of some nematods like *Anisakis pegreffii*, found in the Aegean Sea (GSA 22) (Chaligiannis *et al.*, 2012) and
Hysterothylacium aduncum found in the North Alboran Sea (GSA 1) and in Northern Spain (GSA 6) (Rello *et al.*, 2009). The platyelminth *Aphanurus stossichi* was found to parasite *S. pilchardus* in the Northern Adriatic Sea (Paradižnik & Radujković 2007). Regarding parasites of *Lophius budegassa*, the nematode anisakid *Contracaecum clavatum* was found in N. Adriatic Sea (GSA 17) (Jardas & Hristovski, 1985) and the microsporidian *Tetramicia brevifilum* in Northern Spain (GSA 6) (Maíllo *et al.*, 1998).

Regarding *Parapenaeus longirostris*, the existent knowledge on parasites cannot be used for stock discrimination as long as one single species, the microsporidian *Ameson nelson* was found in the whole Mediterranean basin (Campillo & Comps, 1977; Loubes *et al.*, 1977). The cycliophoran *Symbion Pandora* was found in the mouthparts of *Nephrops norvegicus* in Ligurian and Northern Tyrrhenian Sea (GSA 9) (Zotto & Todaro, 2008).

In Illex coindetii (245 males, 190 females and 4 unsexed) sampled in the central part of the eastern Adriatic Sea (GSA 18), 2 larval helminths were isolated, i.e., larvae of Anisakis pegreffii, characterized by molecular tools at the species level, and plerocercoids of *Phyllobothrium* sp., with prevalence of 30.5% and 2.3%, respectively (Petric et al., 2011). Highly significant seasonal variation in diet consumption, congruent with seasonal variation in anisakid intensity, was observed, underlining the tight role of squid prey in the trophic transmission of parasite. Likewise, the highest helminth prevalence and intensity of infection was recorded in autumn, when the fish prey, mostly Maurolicus muelleri, comprised the greatest proportion of diet. This helped to assign the Adriatic broadtail shortfin squid not as a first, but as a second, paratenic host for the anisakid, unlike as suggested previously. The presence of larval A. pegreffii confirms its previously reported zoogeographical distribution in the Mediterranean and Adriatic Seas. Angelucci et al. (2011) has been found Anisakis sp. and Hysterothylacium spp. larvae parasites of in Illex coindetii individuals found in Sardinian waters (GSA 11). Serraca et al. (2013) found an Anisakidae larva in Illex coindetti caught in the Ligurian Sea (GSA 8). The myxozoan Aggregata spp. has been found to parasite Octopus vulgaris in Northern Spain (GSA 6) (Andree et al., 2012) and Northern Adriatic Sea (GSA 17) (Mladineo & Jozic', 2005). Ascaridoids of the genus Hysterothylacium have been reported from the digestive tract, and copepods of the genus Pennella have been reported from the gills of *E. cirrhosa* specimens caught in the north Tyrrhenian Sea (GSA 8) (Gestal *et al.*, 1999).





Figure 1.10. Maps of the parasites per phylum found in *Merluccius merluccius, Mullus barbatus.*





Figure 1.10 (continued). Maps of the parasites per phylum found in *Mullus surmuletus, Pagellus erythrinus.*





Figure 1.10 (continued). Maps of the parasites per phylum found in *Trachurus trachurus, Trachurus mediterraneus*.



Figure 1.10 (continued). Maps of the parasites per phylum found in Sardina pichardus.





Figure 1.11. Map of parasites found in *Engraulis encrasicholus*: Anisakis larva type I and Hysterothylacium aduncum.





Figure 1.12. Parasites found in *Merluccius merluccius*: Anisakis pegreffii and Anisakis physeteris.





Figure 1.13. Map of parasites found in Trachurus trachurus: Anisakis simplex, Anisakis pegreffii.



Figure 1.13. Map of parasites found in *Trachurus trachurus: Alatospora solomoni*.

The results from the review showed that regarding parasites the amount of works analysed by species ranges from 0 to 13 (Table 1.7). For E. encrasicolus, T. trachurus and P. erythrinus, the amount of analysed studies was above 10; for M. merluccius, M. barbatus and M. surmuletus the amount of published works were few (5 or 6) while for the rest of the target species the amount of analyzed references were even lower, i.e. \leq 3 references. There are three types of hosts according to the amount of species and Phylums present in their parasite fauna. A first group composed of three of the species (i.e. P. longirostris, N. norvegicus and O. vulgaris), show only one species of parasites belonging to one Phylum and they were never of the Phylum Nematoda (Table 1.7). A second group of species is formed by those with low amount of parasite species (≤ 6) but dominated by the the Phylum Nematoda, i.e. E. encrasicolus, L. budegassa, E. cirrhosa, S. pilchardus, I. coindetti and M. merluccius. Finally, there is a group in which the amount of species of the Phylum Nematoda again is higher than the species from anyother Phylum, however, the amount of total parasite species is high (>12 different species), i.e. T. mediterraneus, P. erythrinus, M. barbatus, M. surmuletus and T. trachurus. From the existing bibliography, for some of the species hosting Nematoda of the family Anisakidae (i.e. E. encrasicolus, M. merluccius and M. barbatus) it seems that the differing prevalence/intensity of the parasite species could be a good indicator for stock unit identification. However, for common pandora (P. erythrinus) remains uncertain, as a general tendency of a dissimilar parasitic fauna (taxonomical and in abundances) has not been observed yet for this species.

Species	N° references	Nematoda no Anisakidae	Nematoda Anisakidae	Platyhelminths	Arthropoda	Acantocefala	Apicomplexa	Microsporodian	Simbion	N species	N species not nematoda
P. longirostris	2	0	0	0	0	0	0	1	0	1	1
N. norvegicus	1	0	0	0	0	0	0	0	1	1	1
O. vulgaris	2	0	0	0	0	0	1	0	0	1	1
E. encrasicolus	13	0	2	0	0	0	0	0	0	2	0
L. budegassa	2	0	1	0	0	0	0	1	0	2	1
E. cirrhosa	1	0	1	1	0	0	0	0	0	2	1
S. pilchardus	3	0	2	0	1	0	0	0	0	3	1
I. coindetti	3	0	2	0	1	0	0	0	0	3	1
M. merluccius	6	1	3	2	0	0	0	0	0	6	2
T. mediterraneus	3	0	2	2	8	0	1	0	0	13	11
P. erythrynus	11	3	ND	6	8	1	0	0	0	18	15
M. barbatus	5	5	3	0	13	0	0	0	0	21	13
M.surmuletus	5	8	ND	0	13	0	0	0	0	21	13
T. trachurus	13	0	6	5	9	0	3	0	0	23	17

Table 1.9. Summary of the parasite found in the STOCKMED target species from bibliographic review.

Tagging

Tagging experiments are a potential pretty useful method for stock unit identification. However, the information available from this topic is sporadic and were only found for three out of the nineteen target species of the project, i.e. common sole (Solea solea), blue red shrimp (Aristeus antennatus), horned octopus (Octopus vulgaris) and European hake (Merluccius merluccius) (Fig. 1.14). Even for these three species the information was generally very few and scant, with one tagging experiments for each of the above mentioned species. In the case of common sole the tagging experiment was used to detect movements along the Adriatic Sea. For blue red shrimp and horned octopus the respective tagging experiment was used to analyse the range of movement, which in the case of the blue red shrimp was 10 miles in 1 month (in the Western Ionian, GSA 19) and in the case of horned octopus males and females showed significantly different distances of movement (males between 411 and 1200 m while females only around 20 m). Regarding European hake, tagging experiment was used to estimate growth parameters (von Bertalanffy k) which were found to be as double of the previously published values based on size frequency in the area.



Figure 1.14. Tagging studies in Aristeus antennatus, Merluccius merluccius, Octopus vulgaris and Solea vulgaris.

Migration

There is some information regarding **migration** for many of the STOCKMED target species, i.e. fifteen species which are *M. barbatus*, *M. surmuletus*, *P. erythrinus*, *Nephrops norvegicus*, *O. vulgaris*, *E. cirrhosa*, *I. coindetti*, *E. moschata*, *S. pilchardus*, *G. melastomus*, *A. antennatus*, *A. foliacea*, *M. merluccius*, and *P. longirostris*. Horizontal seasonal migrations have been described for some species for feeding and/or spawning as well as ontogenic migrations related to fish length. Moreover, vertical migrations in

the water column were described for two fish species, i.e. *M. merluccius* juveniles related to feeding and *S. pilchardus* due to light intensity, and for the decapod species and the cephalopods. Maps produced are included in Figure 1.15.

The **vertical distribution** of early life stages of STOCKMED target species in the Mediterranean is known for twelve out of the nineteen species. While crustaceans (*P. longirostris, A. antennatus and N. norvegicus*) larvae show a distribution in the upper water column (0-75 m), cephalopod (*I. coindetii, O. vulgaris* and *E. cirrhosa*) paralarvae tend to have a wide vertical distribution (found even at 650-750 m). Regarding the vertical distribution of ichthyoplankton, during the thermally stratified period, larvae of most of the shelf-dwelling species have a surface distribution above the thermocline (including *E. encrasicolus, T. mediterraneus, M. barbatus* and *M. surmuletus*). During the winter mixing period, larvae of the species like sardine (*S. pilchardus*) show a wide vertical distribution although concentrations are higher in the upper layers (above 50 m depth). For European hake (*M. merluccius),* larvae have been found to concentrate mainly between 60 and 80 m depth during winter while in summer they are always below the thermocline. For anchovy (*E. encrasicolus*) and sardine (*S. pilchardus*) larvae, daily vertical migrations have been described that might be linked to feeding purposes.

The extent of larval drift may be determined by the **larval (presumably planktonic) duration.** Such information is available in the Mediterranean for five of the nineteen STOCKMED target species, i.e. *E. encrasicolus* (40-60 days), *S. pilchardus* (50-120 days), *M. merluccius* (60-67 and 40 days), *M. surmuletus* (28-35 days) and *P. erythrinus* (40-49 days). However, it must be pointed out that the duration of the larval stage is dependent on growth rate and temperature which can be very variable in space and time.





Figure 1.15. Review of the migration patterns for different species within the Mediterranean Sea: Lophius budegassa (up), Merluccius merluccius (down).





Figure 1.15. Review of the migration patterns for different species within the Mediterranean Sea: *Galeus melastomus* (up) and *Parapenaeus longirostris* (down).

Larval drift

Regarding the advection/retention of larvae it has been demonstrated in the field and modeled in the Mediterranean for small pelagic fish, mainly European anchovy (E. encrasicolus) which is the dominant species in the ichthyoplankton during the stratification period. Advection has been described in the field to occur from the Gulf of Lions (GSA 7) to the Catalan coast (GSA 6); transport and retention along the Sicilian channel (GSA 16) has been described as well as retention areas in the Aegean Sea (GSA 22) (Fig. 1.16). Modeling studies analyzing the advection of eggs and larvae using current fields from hydrodynamic models have been started for European anchovy in the Gulf of Lions (GSA 7) and the Catalan Sea (GSA 6), in the Alboran Sea (GSA 1) and for small pelagic (both anchovy and sardine) in the Adriatic (GSA 17 and 18) (Fig. 15). Studies modeling the larval advection of fish early life stages, generally treat the early life stages like passive particles transported by currents simulated by 3-D hydrodynamic models and often ignoring major biological aspects (e.g. feeding, mortality), or sometimes testing the effect of some specific factors (e.g. egg buoyancy, vertical migration behavior, temperature-dependent growth and location of spawning sites).

It might be that that the larvae of other species with similar (surface) vertical distributions (like *Mullus, Trachurus, Pagellus*) may also be transported between these adjacent GSAs.



Figure 1.16. Review of the larval drift information regarding Engraulis encrasicolus and Sardina pichardus.

Results obtained by Task 1.3 were presented during the third STOCKMED meeting held in Rome (Italy) on 25-26 November 2013..

Considering the 19 target species there were great differences in the parasites studies with the highest contribution for *Trachurus trachurus* and *Merluccius merluccius*, whereas *Sardina pilchardus*, *Trachurus mediterraneus*, and *Engraulis encrasicolus* presented very few studies.

The most known invaders in the studied species were: Nematoda, Anisakidae, the most abundant family, Platyelminthes and Myxozoa.

During the discussion it was noted that at least in the case of hake and horse mackerel the parasite data could give an important contribute in the identification of stock according to a multi criteria approach (MCA).

It was also proposed that correlation matrices were built for those species and indexes where there was enough information.

Finally, since the relationship between host and parasite often are species-specific was suggested to take into account parasites at species level.

The procedure of parasite cluster analysis (Figure 1.17) has involved three steps:

- Preparation of the presence-absence (qualitative) data matrices by samples (GSAs) and variables (parasite species);
- Choice of a metrics to measure similarities or distance between each pair of elements;
- iii) Choice of an algorithm allowing the construction of a dendrogram or hierarchic structure from the similarity matrix
- iv) Forcing " a posteriori" the cluster to join the MCA approach



Figure 1.17 Stages in a multivariates analysis based on similarity coefficients (from Clarke and Warwick, 1994).

1) Preparation of the data matrices

The first step was to prepare a database on parasite data gathered from literature by species and geographical areas. Database structure included a number of fields in which to return the parasite species observed (at the level of species, genus, or larval stage), the host species, the geographical area covered with an indication of

geographical coordinates, the GSA reference. In Table 1.10 is shown a subset of the parasite database.

Parasites species	Name of fishes/decapods infected	Area	Area (geographical coordinates)	GSA
Hysterothylacium sp.	Eledone cirrhosa	N. Tyrrhenian Sea	42°41'N 10°23'E	9
Pennella sp.	Eledone cirrhosa	N. Tyrrhenian Sea	42°41'N 10°23'E	9
Anisakis sp.	Engraulis encrasicholus	Tyrrhenian Sea	43°00'-42°0'N 10°00'-11°3'E	9
Anisakis pegreffii	Engraulis encrasicholus	Aegean Sea	39°N 25°E	22
Hysterothylacium aduncum	Engraulis encrasicholus	Western Anatolia	40°17'N 26°41'E	22
Anisakis larva type I	Engraulis encrasicholus	Catalonia	40°48'N 1°32'E	6
Hysterothylacium aduncum	Engraulis encrasicholus	Catalonia	40°48'N 1°32'E	6
Anisakis larva type I	Engraulis encrasicholus	Gulf of Lion	42°53'N 4°57'E	7
Hysterothylacium aduncum	Engraulis encrasicholus	Gulf of Lion	42°53'N 4°57'E	7
Anisakis larva type I	Engraulis encrasicholus	Ligurian Sea	43°12'N 09°55'E	9
Hysterothylacium aduncum	Engraulis encrasicholus	Ligurian Sea	43°12'N 09°55'E	9
Anisakis larva type I	Engraulis encrasicholus	Catalonia	40°48'N 1°32'E	6
Hysterothylacium aduncum	Engraulis encrasicholus	Catalonia	40°48'N 1°32'E	6
Anisakis larva type I	Engraulis encrasicholus	Gulf of Lion	42°53'N 4°57'E	7
Hysterothylacium aduncum	Engraulis encrasicholus	Gulf of Lion	42°53'N 4°57'E	7
Anisakids sp.	Engraulis encrasicholus	Tyrrhenian Sea	43°00'-42°0'N 10°00'-11°3'E	9
Anisakis larva type I	Engraulis encrasicholus	Ligurian Sea	43°12'N 09°55'E	9
Hysterothylacium aduncum	Engraulis encrasicholus	Ligurian Sea	43°12'N 09°55'E	9
Anisakis pegreffii	Engraulis encrasicholus	Aegean Sea	39°N 25°E	22
Anisakis simplex	Engraulis encrasicholus	Aegean Sea		22
Hysterothylacium aduncum	Engraulis encrasicholus	Western Anatolia	40°17'N 26°41'E	22
Anisakis sp.	Engraulis encrasicholus	W. Ionian Sea		19
Chromidina elegans	Illex coindetti	Naples	40°50'N 14°16'E	10
Chromidina coronata	Illex coindetti	Naples		10
A. simplex	Illex coindetti	N. Tyrrhenian Sea	42°39'N 10°58'E	9
Pennella sp.	Illex coindetti	N. Tyrrhenian Sea	42°39'N 10°58'E	9

Table 1.10. Database structure about parasite data gathered from literature by species and geographical areas.

Unfortunately, in many cases, literature references reported parasite at "Genera" level and, moreover, without a quantification in terms of numbers (e.g. number of fishes infected on total numbers fishes observed). So the available data were mainly of qualitative type and low taxonomic resolution. Furthermore, in many cases, due to absence of target studies there were many GSAs not covered by enough data. Finally, only for 14 out 19 target species was possible to obtain information.

Table 1.11 shows clearly that only for two species we have obtained enough data to carry out a multivariates analysis. The choosen species were European hake (*Merluccius merluccius*) and Atlantic horse mackerel (*Trachurus trachurus*).

		Total number of parasites species by area and target species																			
Target species/GSAs	1	4	5	6	7	8	9	10	11	12	16	17	18	19	20	<mark>21</mark>	22	23	25	28	Total GSAs recognized
Eledone cirrhosa							2														1
Engraulis encrasicholus				2	2		4							1			3				5
Illex coindetti							2	2													2
Lophius budegassa				1									1								2
Merluccius merluccius	2		2	2	1		2	1		3		1			3	1	3	3	1	1	14
Mullus barbatus		1		18			1		13	1		1	1				1				8
Mullus surmuletus		1			7	1	1	6	15	1		7	1								9
Nephrops norvegicus							1														1
Octopus vulgaris				1								1									2
Pagellus erythrinus					1	1		1		5		1	2				5				7
Sardina pilchardus	1			1						2		1					1				5
Solea solea										1											1
Trachurus mediterraneus												2					13				2
Trachurus trachurus	4		2	1	5	2	5		1	1	1	3	2	1	8		12			1	15
	7	2	4	26	16	4	18	10	29	14	1	17	7	2	11	1	38	3	1	2	
	Total number of parasite species observed per GSAs																				

Table 1.11. Parasite species numbers by target species and GSAs obtained from literature.

Due to the qualitative nature of the data gathered an absence/presence approach was adopted.

So, the second step was to create a binary matrix of parasite species and GSAs by target species. We added to the matrix only the GSA in which at least one of the parasite species was observed. Table 1.12 shows the binary matrix for European hake (*M. merluccius*).

	Merluccius merluccius													
Parasite Species	GSA1	GSA5	GSA6	GSA7	GSA9	GSA10	GSA12	GSA17	GSA20	GSA21	GSA22	GSA23	GSA25	GSA28
Acanthocephaloides propinquus	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Anisakis pegreffii	1	1	1	0	1	0	0	1	1	1	1	1	0	0
Anisakis physeteris	1	1	1	0	1	0	0	0	1	0	0	1	0	0
Anisakis simplex	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Anisakis typica	0	0	0	0	0	0	0	0	1	0	0	1	1	0
Clavella stellata	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hysterothylacium sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Lernaeocera lusci	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Neobrachiella insidiosa	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Neobrachiella merlucci	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 1.12. Binary matrix of parasites species hosted by European hake in the different GSAs.

2) Choice of a metrics to measure similarities or distance between binary data

In biology, the presence /absence data are very common and in many surveys all the variables are of this type. Therefore in literature shows many specific coefficients for variables of binary type (i.e. simple matching, Put index, Jaccard index etc). In absence /presence matrix the distances are computed on the basis of contingency tables with two ways:



According to Podani (2007):

a is the number of variables present in both samples to be compared (joint presence or positive correlation);

b is the number of variables present in the sample 1 and absent in sample 2;

c is the number of variables present in the sample 2 and absent in sample 1 (so that b + c is the number of discordances);

d represents the number of variables absent from both objects, but present in at least one object in the sample (no joint, double zero or negative correlation).

Obviously a + b + c + d = n, that is the number of variables that describe the sample is given by the overall total. The totals represent the number of variables present and absent in each object.

When choosing a coefficient presence / absence a crucial problem is the fact that we should consider whether or not the d (double zero) value. Since in many areas the absence of parasite should depend moreover by the absence of specific works the results using Jaccard approach (whose not consider double zero informative) were more reliable. In fact, this index takes into account only the double presence number as source of similarity. It constitutes a simple and quite good similarity index which represents the probability that a variable randomly chosen between the set of variables which appear in at least one of the objects being compared is present in both samples. It is, therefore, a conditional probability of the value of which is included in a potential interval [0,1].

The Jaccard index (JAC) is define as:

In Table 1.13 are shown percentage Jaccard similarity matrices obtained for *M. merluccius* and *T. trachurus*.

$$JAC = \frac{a}{a+b+c} (0 \le JAC \le 1)$$

Similarity ((0 to 100)													
		1		1								1		
	GSA1	GSA5	GSA6	GSA7	GSA9	GSA10	GSA12	GSA17	GSA20	GSA21	GSA22	GSA23	GSA25	GSA28
GSA1														
GSA5	100													
GSA6	100	100												
GSA7	0	0	0											
GSA9	100	100	100	0										
GSA10	0	0	0	0	0									
GSA12	0	0	0	33.333	0	0								
GSA17	50	50	50	0	50	0	0							
GSA20	66.667	66.667	66.667	0	66.667	0	0	33.333						
GSA21	50	50	50	0	50	0	0	100	33.333					
GSA22	25	25	25	0	25	0	0	33.333	20	33.333				
GSA23	66.667	66.667	66.667	0	66.667	0	0	33.333	100	33.333	2	0		
GSA25	0	0	0	0	0	0	0	0	33.333	0	(0 33.33	3	
GSA28	0	0	0	0	0	0	0	0	0	0	(0 ()	0

Similarity (0 to	0 100)														
	GSA1	GSA5	GSA6	GSA7	GSA8	GSA9	GSA11	GSA12	GSA16	GSA17	GSA18	GSA19	GSA20	GSA22	GSA28
GSA1															
GSA5	0														
GSA6	0	50													
GSA7	0	16.667	20												
GSA8	0	0	0	0											
GSA9	33.333	0	0	0	0										
GSA11	25	0	0	0	0	25									
GSA12	0	0	0	0	0	0	0								
GSA16	25	0	0	0	0	25	i 100	0							
GSA17	16.667	0	0	0	0	16.667	33.333	0	33.333						
GSA18	16.667	0	0	0	0	16.667	33.333	0	33.333	20					
GSA19	0	0	0	0	0	0	0	0	0	0	33.333				
GSA20	0	11.111	12.5	8.3333	11.111	0	0	12.5	0	0	0	0			
GSA22	25	18.182	9.0909	14.286	8.3333	25	9.0909	0	9.0909	7.6923	7.6923	0	5.5556		
GSA28	0	0	0	0	0	0	0	0	0	0	0	0	0	0)

Table 1.13. Percentage Jaccard indeces matrices for M. merluccius (upper) and T. trachurus (downer)

3) Choice of an algorithm allowing the construction of a dendrogram or hierarchic structure from the similarity matrix.

There are other cluster procedures, but those generating dendrograms are the most common ones in the ecological literature and the easiest to interpret. There are several algorithms based on different clustering criteria. According Sneath & Sokal (1973) the commonest are single linkage clustering, complete linkage clustering, UPGMA, UPGMC, WPGMA, WPGMC, etc. The parasite cluster analysis was computed using an UPGMA (Unweighted Pair Group Method with Arithmetic Mean, Sokal and Michener, 1958; Rohlf, 1963).) UPGMA can be defined as a simple agglomerative (bottom-up) hierarchical clustering method. It is one of the most popular methods in ecology for the classification of sampling units on the basis of their pairwise similarities in relevant descriptor variables. The method is a mix between the complete linkage and the single linkage in an attempt to compensate the disadvantages of a single strategy using the advantages of the other. In practice, single link clustering has a tendency to produce chains of linked samples, with each successive stage just adding another single sample onto a large group. Complete linkage will tend to have the opposite effect, with an emphasis on small clusters at the early stages. Groupaveraging, on the other hand, is often found empirically to strike a balance in which moderate number of medium-sized clusters are produced, and only grouped together at later stage (Clarke and Warwick, 1994). The UPGMA algorithm constructs a rooted tree (dendrogram) that reflects the structure present in a pairwise similarity matrix (or a dissimilarity matrix). At each step, the nearest two clusters are combined into a higher-level cluster. The distance between any two clusters A and B is taken to be the

average of all distances between pairs of objects "x" in A and "y" in B, that is, the mean distance between elements of each cluster:

$$\frac{1}{|\mathcal{A}| \cdot |\mathcal{B}|} \sum_{x \in \mathcal{A}} \sum_{y \in \mathcal{B}} d(x, y)$$

The threshold level for grouping the different GSAs was choose at a very low percentage value (similarity index <5%) in order to reduce the number of cluster (Figure 1.18)



4) Forcing " a posteriori" the groups to joint better the MCA approach

Cluster analysis showed a little bit "patched" mosaic of clusters, in which putative stock units defined by parasites presence are largely intersecting each other and one stock can be divided in non-contiguous subunits separated by other stocks. Since these outputs substantially disagree with the STOCKMED framework, in which we assumed that each stock unit is separated by the others and composed by fully connected cells/areas, for some cluster groups, an "a posteriori" rearrangement was applied. The analysis was carried out by imposing a contiguity constraint in order to obtain a output layer more compatible with the Multi Criteria Analyses approach (Table 1.14)

M. m	nerluccius - Jaccard t	reshold 5%
GSAs	Original cluster	MCA cluster
GSA1	d	f
GSA5	d	f
GSA6	d	f
GSA9	d	f
GSA17	d	е
GSA20	d	е
GSA21	d	е
GSA22	d	е
GSA23	d	е
GSA25	d	е
GSA7	с	d
GSA12	С	С
GSA10	a	а
GSA28	b	b
T. trac	<i>churus</i> - Jaccard t	reshold 2%
GSAs	Original cluster	MCA cluster
GSA1	b	b
GSA9	b	b
GSA11	b	b
GSA16	b	b
GSA17	b	b
GSA18	b	b
GSA19	b	b
GSA22	b	а
GSA5	С	b
GSA6	С	b
GSA7	С	b
GSA8	С	b
GSA12	С	b
GSA20	С	а
GSA28	а	С

Table 1.14 – Cluster adopted to carry out the MCA approach

Task 1.4 Synthesis of the spatial pattern of the main biological information for the target species in the case study areas and identification of gaps in knowledge on biological aspects for stock units identification

A quantitative method to summarise the degree of spatial coverage for every parameter in each Mediterranean basin was constructed. The Mediterranean Sea was divided in three basins, i.e Western, Central and Eastern, according to the GFCM division (GFCM, 2009). For every species and basin, a grade was given to every parameter according to the proportion of GSAs for which published information existed on that parameter. The spatial coverage of a parameter within a basin was graded as high (H) if more than 50% of GSAs in that basin had at least one source of information for that parameter; a score of medium (M) if the amount of GSAs was between 25 and 50% and a grade of low (L) if the amount of GSAs with information was less or equal to 25%. Finally, a score of 1 was given for the parameters having a "H" grade, 0.5 for those graded as "M" and 0 for those graded as "L". Tables summarizing this analysis were prepared, one for the fishes, a second for the cephalopods and a third one for the decapods.

The degree of coverage varied greatly between species and parameters. The spatial coverage of growth and reproduction studies was much wider than the spatial coverage of the rest of the indicators, i.e. meristic and morphometry, otoliths shape and biochemistry, parasites, tagging/migration or larval drifting. For instance, the spatial coverage of growth information was generally high for European hake (Merluccius merluccius), red mullet (Mullus barbatus) or common pandora (Pagellus erythrinus), with at least two of the three basins graded as high, i.e. > 50% of the GSAs containing information on growth. L₅₀ and spawning season studies have as well a general wide spatial coverage with differing intensities between species and basins but still high in comparison with the other indicators. Conversely, data regarding parasites was few and scant. Although there were some information regarding parasites for fourteen out of the nineteen target species of the STOCKMED project (i.e. Engraulis encrasicolus, Merluccius merluccius, Lophius budegassa, Mullus barbatus, M. surmuletus, Trachurus trachurus, T. mediterraneus, Parapenaeus longirostris, Nephrops norvegicus, Illex coindetti, Octopus vulgaris and Eledone cirrhosa) for most of them the spatial coverage was graded with low, i.e. < 25% of the GSAs had some information regarding the parasites. The information regarding parasites was totally absent for five of the nineteen species, i.e. Solea vulgaris, Galeus melastomus, Eledone moschata, Aristeus antennatus and Aristeomorpha foliacea. For a few species (i.e. M. merluccius, M. surmuletus and T. trachurus) the information regarding parasites was graded as medium or high in some basins.

In general terms, for the cephalopods, i.e. *E. cirrhosa*, *E. moschata*, *I. coindetti* and *O. vulgaris*, the information was especially poor many of the revised parameters, with many areas for which the data was totally absent or while for others tended to be low

or medium at the most . On the opposite, there were some species, such as European hake (*M. merluccius*), striped red mullet (*M. surmuletus*) or red mullet (*M.barbatus*, with a rather good spatial coverage for many of the analyzed parameters;).

Although the **MEDITS scientific survey** data were considered the most suitable and standardized among the available data sets to accomplish *Task 1.1*, two shortcomings were identified. The first one related to the geographical coverage that is limited to the-EU GSAs (see Deliverable 04, section 2.1). Seasonality of the surveys which are conducted in spring is the other limitation of MEDITS survey data as long as seasonal variations in the biological parameters, as well as in biomass and density, cannot be assessed. This is especially important for short life-span species like cephalopods.

Regarding the **bibliographic review** performed **within** *Task* **1.2** the main gaps encountered were the following:

In the Mediterranean Sea, there is knowledge regarding growth and reproduction for most of the STOCKMED target species although the degree of spatial coverage varies depending on the species and basin. For the other parameters reviewed in Task 1.2, i.e. meristic/morphometry, otoliths shape and otoliths biochemistry, the knowledge in the Mediterranean is pretty scarce and limited to some species and areas. In particular, the growth and reproduction studies are more abundant for fishes (osteichtyes) and rather more scant for crustaceans, cephalopods and elasmobranches. Methods to study growth in fish are mainly size frequency data, periodic markings on hard structures (e.g. scales and otoliths in fish; cartilage or vertebrae in elasmobranches; statolith or gladius for cephalopods) or direct observation of growth from tagging-recapture data. The different methods provide significantly different estimates of growth and should be used with caution in order to ensure comparability among areas. For cephalopods and crustaceans, the estimation of growth parameters is mainly based on size frequency data and tagging as long as other direct methods are still under investigation in order to find out standardised and suitable methods to be routinely used (Sifner 2008; Hartnoll 2001). For all the cephalopod species included in the project the information regarding growth and reproduction is very scant. The generally short life span of cephalopods impinges the need of producing biological information on a short temporal scale than annual scientific surveys. The proper temporal scale should be analysed but a monthly or bimonthly basis might be adequate. Additional resources and efforts needs to be devoted to overcome this gap of knowledge. For some fish species the knowledge on growth and reproduction is dramatically scarce throughout the Mediterranean. Thus, there is a need to produce studies on growth and reproduction in most of the Mediterranean for the following species: angler fish (Lophius budegassa), common sole (Solea vulgaris) and blackmouth catshark (Galeus melastomus). Further, for some fish and crustaceans although the lack the spatial coverage on growth and

reproduction would be advisable to be increased in some basins: European hake (*Merluccius merluccius*) in the Eastern Mediterranean, striped red mullet (*Mullus surmuletus*) in the Central Mediterranean, common pandora (*Pagellus erythrinus*) in the Western Mediterranean, Atlantic and Mediterranean horse mackerel (*Trachurus trachurus* and *Trachurus mediterraneus*) in the Eastern basin, European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) in the Eastern basin, giant red shrimp (*Aristaeomorpha foliacea*) in the Western and the Eastern basins, red shrimp (*Aristeus antennatus*) in the Eastern Mediterranean and Norway lobster (*Nephrops norvergicus*) in the Eastern basin.

However, the low abundance of some of these species in certain GSAs may make the production of biological information unfeasible. These may be the case of giant red shrimp (*Aristaeomorpha foliacea*) in many Western Mediterranean GSAs or blackmouth catshark (*Galeus melastomus*) in many Central and Eastern Mediterranean GSAs (see Deliverable 4, section 2.2).

There are some studies regarding meristic/morphometry and otoliths shape for Atlantic horse mackerel (*Trachurus trachurus*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and European anchovy (*Engraulis encrasicolus*) and meristic/morphometry studies on Norway lobster (*Nephrops norvegicus*). For the rest the information regarding meristic/morphometry and otoliths shape and biochemistry is totally absent. There is a need to produce studies on meristic/morphometry, otoliths shape and biochemistry for most of the species in the Mediterranean basin.

Regarding the **bibliographic review** performed **within** *Task* **1.3** the main gaps encountered were the following:

There are general papers on the **parasite** fauna of the STOCKMED fishes, decapods and cephalopods. However, most of these published papers are related with the biology of the parasite or of the host rather than with establishing baselines for fisheries management. A paper with a poor choice of parasites for stock delineation can contain useful biological information but the analysis should not be used to claim stock identification. Regarding tagging, there are only very scanty studies of the target species of STOCKMED project in the Mediterranean basin. Many gaps exist in this sector in order to extract results connecting the tagging technique and stock identification. There is a need for: funding of tagging projects in the whole Mediterranean basin, the improvement of the tagging methods, the encouragement of exchanging ideas amongst the various scientist by means of workshops, practical training courses for fish tagging, the improvement of user guidelines (establishment of theoretical approaches and assumptions), clarification and quantification of underlying assumptions in quantitative application of tagging data (e.g. about tagging mortality, tag shedding, mixing of tagged and untagged populations) and the development of data integration between different data sources (i.e. tags, scientific surveys, fisheries and environmental data) through Geographical Information Systems (GIS).

Contrarily to what happens in other European areas, the knowledge about **migration** of most of the target species in STOCKMED project is very scant. Sampling bias, incomplete coverage of the stock and misinterpretation of the data may easily occur obscuring the knowledge of the general migration patterns of the target species. Traditional mark–recapture experiments with bottom-dwelling species may provide more acc describe the distribution of fishing fleets more accurately than the true extent of fish dispersion. Studies of fishes have therefore lagged in understanding individual variation in migratory behavior, and consequences for population subdivision and convergence on the breeding and non-breeding grounds. Several of the methods presented so far, i.e. length-based methods like modal progression analysis and catch curve analysis, are often insufficient and difficult to apply for highly migratory or schooling fish stocks. Migration may bias fish stock assessments and introduce complex problems regarding shared stocks between countries. The stock identification is a prerequisite for intelligent management of shared stocks and it needs to be based on knowledge of the migration routes of the stock.

Regarding **larval drift**, data concerning larval development of fishes and decapods is scarce indicating the need for further studies. There is a need for species-specific field, laboratory and accurate modeling (i.e. based on accurate circulation models) studies concerning the planktonic stages of Mediterranean species. Moreover, the larval stage for most Mediterranean species is still poorly known, and some aspects of early

life history like behavior, food consumption or natural mortality have not yet been explicitly incorporated into bio-physical models.

Summarizing, a big discussion regarding the methods used in stock identification exists. According to some author, the phenotypic methods do not provide evidence for separate breeding populations but they can be more appropriate tools for defining stocks than genetic studies. This is because the small amount of interchange between populations which is necessary to maintain genetic homogeneity might be unimportant by fishery-management standards. No single stock definition can incorporate all factors (environmental, biological, and political) and the working definition has to change with the management aims. Therefore, a comprehensive stock identification study should include several methods which relate to different aspects of the stock concept and provide definitions appropriate to scientists, fishers and managers. The abundance and occurrence of the parasites directly relates to the distribution, migration patterns and population biology of their hosts. Apart from parasites, tagging data generally provide broad-scale stock identification information, but may be inadequate for determining more complex multi-stock structures, unless greater emphasis is placed on obtaining more thorough recapture information than is typical. The utility of stock identification techniques should be considered on a caseby-case basis depending also on the degree of resolution required. The process of defining fish stocks is essential for effective fisheries management, and will continue to undergo refinement as new tools and technologies are developed. Although, a precise determination of stock identification remains a major challenge, and will not necessarily be sufficient if fisheries occur on mixed stocks, our best opportunity at identification between these mixed catches relies on examining all available stock identification information using the most up-to-date technologies when logistically feasible. Future research must aim to extent the approaches (parasites, life history characteristics, tagging, migrations, larval drift, otolith microstructure, etc.) already applied to other fishes, and achieve the integration of the whole set of methods used in a multiple stock identification approach to maximize the likelihood of correctly defining stocks over the complete distribution area of most of the STOCKMED target species in the Mediterranean.

DIFFICULTIES ENCOUNTERED AND REMEDIAL ACTIONS

The development of the WorkPackage 1 faced several difficulties that were overcome in the most favourable way for the project interests and in the most feasible manner in terms of available time and resources.

The main aim of WP1 was to revise and analyse the biological information that could serve for stock boundary delimitation in WP4. The different sources of data that could contribute to the WP progress are scientific surveys, fisheries sampling data and bibliographic data. These sources of data differ in terms length of the time series (temporal coverage), geographical scale (or minimum spatial aggregation level), degree of standardization of the data, geographical coverage, species coverage, accessibility and existence of biological data. In order to help the decision of how to merge all these information, a semi-quantitative criteria was created (see *Results Achieved, section 4.1 Data gathering and checking*). Each data source was ranked from 0 to 1 for each of the evaluating criteria. It was decide to use the MEDITS database whenever possible.

Nevertheles, it was found that the MEDITS database provided by the JCR of the EU Commission after a Specific Data Call, showed some errors and inconsistencies. A standard tool (RoME) that was previously designed for error and inconsistencies detection was used. Error and inconsistences correction was produced, whenever it was possible.

Another difficulty was the selection of the geographical scale for conducting the analysis. Three geographical scales were proposed, i.e. GFCM GSAs, MEDITS strata and GFCM grid. Several preliminary analyses were conducted in each of the geographical scales to choose the most appropriate between them. It was decided to work with the GFCM grid for two reasons. First of all, as the smallest scale it was the only one being able to detect stock units smaller than the actual geographical unit used for stock assessment, i.e. the GSA. On the other hand, the analysis to be produced within WP4 using the outcomes of WP1 (and others) needed homogenously distributed space units and the GFCM grid was the only one accomplishing this requirement.

The amount of species that are target in the project (nineteen) is a huge amount of species to be analysed in a short period of time, owing to the fact that the STOCKMED project spands for 18 months (plus 3 months extension) and that the methodological approach needed to be defined during the project. Two actions were taken to solve this problem. On the one hand, four species were selected as case studies, with the aim to test the different methodological approaches initially suggested only in these four species. Three case study species were species for which there is plenty information and that show a rather high abundance through the Mediterranean, i.e. *M. merluccius, M. barbatus* and *A. antennatus,* and one spcies for which the abundance is scarce, i.e. *S. vulgaris.* On the other hand, an R routine

(R_BIND_STOCKMED) was produced by COISPA in collaboration with IEO in order to standardize the outcomes of the analysis and produce the same outputs for all 19 target species.

The inexistence of regular scientific surveys collecting information on some of the parameters within the Mediterranean was another difficulty. The revision of the existing bibliographic information from peer-reviewed publications and from grey literature, i.e. reports and working documents, was performed in order to overcome this shortcoming.

REFERENCES

Abaúnza P, Gordo L, Karlou-Riga C, Murta A, Eltink ATGW, Garcia Santamaria MT, Zimmermann C, Hammer C, Lucio P, Iversen SA, Molloy J, Gallo E. 2003. Growth and reproduction of horse mackerel, *Trachurus trachurus* (Carangidae). Rev. Fish. Biol. Fisher., 13:27–61

Abaúnza P., Gordo L.S., Garcia Santamaria M.T., Iversen S.A., Murta A.G., Gallo E., 2008. Life history parameters as basis for the initial recognition of stock management units in horse mackerel (*Trachurus trachurus*) Fisheries Research 89: 167–180

Adebiyi F.A. 2013. The sex ratio, gonadosomatic index, stages of gonadal development and fecundity of sompat grunt *Pomadasys jubelini* (Cuvier, 1830). Pakistan J. Zool., 45(1): 41-46

Adroher, F., A. Valero, et al. 1996. Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada (Spain). Parasitology research, 82(3): 253-256

Aguzzi, J., N. Bahamon, et al. 2009. The influence of light availability and predatory behavior of the decapod crustacean *Nephrops norvegicus* on the activity rhythms of continental margin prey decapods. Mar. Ecol., 30(3): 366-375

Akmirza, A. 1998. The parasite fauna of horse mackerel. III. National Symposium of Aquatic Products (10–12 June 1998), Erzurum

Akmirza, A. 2000. Seasonal distribution of parasites detected in fish belonging to the Sparidae family found near Gökçeada. Türkiye Parazitoloji Dergisi 24(4): 435-441

Akmirza, A. 2013. Parasitic cestodes of fish in the waters off Gökçeada, North Aegean Sea. J. Black Sea/Mediterranean Environment, 19 (2): 178-184

Anastasio, A., R. Marrone, et al. 2009. Valutazione della qualita` igienico-sanitaria del pesce azzurro pescato nel golfo di Napoli. http://www.orsacampania.it/wpcontent/uploads/2009/12/OpusoloAnisakis.pdf

Andree K, Gairin I, et al. 2012. Observations from the Mediterranean Coast of Spain of *Aggregata* spp. (Protista: Apicomplexa), a Parasite of *Octopus vulgaris*. World Aquaculture Society Congress

Angelucci G, Meloni M, et al. 2011. Study on the presence of *Anisakis* sp. and *Hysterothylacium* sp. larvae in teleosts and cephalopods sampled from waters off Sardinia. J. Food. Prot., 74: 1769-1775

Arculeo M, Hristosvki N, et al. 1997. Helminth infestation of three fishes (Serranus scriba, Mullus surmuletus, Scorpaena porcus) from a coastal seaground in the Gulf of Palermo (Tyrrhenian Sea). Italian Journal of Zoology, 64(3): 283-286

Atarhouch T, Rami M, Naciri M, Dakkak A. 2007. Genetic population structure of sardine (*Sardina pichardus*) off Morocco detected with intron polymorphism (EPIC-PCR). Mar. Biol., 3, 521 – 528

Barot S, Heino M, O Brien L, Dieckman U. 2004. Estimation reaction norm for age and size at maturation when age at first reproduction is unknown. Evolutionary Ecology Research 6, 659-678

Basilone G, Guisande C, Patti B, Mazzola S, Cuttitta A, Bonanno A, Kallianiotis A. 2004. Linking habitat conditions and growth in the European anchovy (*Engraulis encrasicolus*). Fish. Res., 68: 9-19

Begg GA, Friedland KD, Pearce JB. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fish. Res., 43:1-8

Begg GA, Waldman JR. 1999. An holistic approach to fidi stock identification. Fish. Res., 43: 35-44

Bitetto I, Facchini MT, Spedicato MT. 2012. RoME (version 1.2): R code to perform multiple and cross checks on MEDITS Survey data (TA, TB and TC files)

Bonfiglio R, Gaglio G, et al. 2010. On the presence of Clavella stellata (Copepoda: Lernaeopodidae, Kroyer 1838) in the European hake (*Merluccius merluccius*). Rapport de la Commission Internationale de la Mer Mediterranee, 39

Booke HE. 1999. The stock concept revisited: perspectives cm its history in fisheries. Fish. Res., 43: 9-11

Cadrin SX, Secor SH. 2009. Accounting for Spatial Population Structure In Stock Assessment: Past, Present And Future, in: Beamish RJ. & Rothschild B.J. The Future of Fisheries Science in North America. Fish & Fisheries Series, 31: 405-426

Campana SE, Gagné JA, McLaren JW. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. 122: 115-120

Campbell N. 2000. Population studies of horse mackerel (*Trachurus trachurus*, L.) and herring (*Clupea harengus* L.) using parasites as biological tags. Ph.D. Thesis.

Campbell N. 2005. The myxosporean parasitofauna of the Atlantic horse mackerel, *Trachurus trachurus* (L.) in the North-East Atlantic Ocean and Mediterranean Sea. Acta Parasitologica, 50(2): 97-101

Campese E. 2012. Caratterizzazione morfologica e distribuzione di Nematodi parassiti del genere Anisakis in *Scomber japonicus, Engraulis encrasicolus* e *Trachurus* sp., University of Bari. Ph. D. Thesis: 87.

Campillo A, Comps M. 1977. Observation en Mediterranee de la microsporidie *Ameson* (Nosema) *nelsoni* (Sprague, 1950), un parasite de la crevette *Parapenaeus longirostris* Lucas. Revue des Travaux de l'Institut des Peches Maritimes, 41(3): 213-215

Carreras-Aubets M, Montero FE, et al. 2011. Parasites and hystopathology of *Mullus barbatus* and *Citharus linguatula* (Pisces) from two sites in the NW Mediterranean with different degrees of pollution. Sci. Mar., 75(2): 369-378

Castro M, Gancho P, Henriques P. 1998. Comparison of several populations of Norway lobster, *Nephrops norvegicus* (L.), from the Mediterranean and adjacent Atlantic. A biometrics study. Sci. Mar., 62 (Supl. 1): 71-79

Cavallero S, Ligas A, et al. 2012. Molecular identification of *Anisakis* spp. from fishes collected in the Tyrrhenian Sea (NW Mediterranean). Veterinary parasitology, 187(3): 563-566

Chaligiannis I, Lalle M, et al. 2012. Anisakidae infection in fish of the Aegean Sea. Veterinary parasitology, 184(2): 362-366

Cheilari A, Rătz HJ. 2009. Review of possible stock units of European hake, red mullet and deep-water pink shrimp in the Mediterranean Sea by means of trends in survey abundance. STECF SG/ECA/RST/MED 09-01

Coyle T. 1998. Stock identification and fisheries management: the importance of using several methods in a stock identification study. In Takino Stock: definino and manrurin shared resources. Edited by O.A. Hancock. Australian Society for Fishery Biology, Sydney pp. 173-182

De Liberato C, Bossu T, et al. 2013. Presence of Anisakid Larvae in the European Anchovy, *Engraulis encrasicolus,* Fished Off the Tyrrhenian Coast of Central Italy. Journal of Food Protection, 76(9): 1643-1648

Desdevises Y. 2006. Determinants of parasite species richness on small taxonomical and geographical scales: *Lamellodiscus monogeneans* of northwestern Mediterranean sparid fish. Journal of helminthology, 80(03): 235-241

Desdevises Y., Jovelin R, et al. 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp. (Monogenea, Diplectanidae) parasitising *Pagellus* (Sparidae, Teleostei) in the North Mediterranean Sea: species divergence and coevolutionary interactions. International Journal for Parasitology, 30(6): 741-746

FAO General Fisheries Commission for the Mediterranean/Commission générale des pêches pour la Méditerranée. 2006. Report of the ninth session of the Scientific Advisory Committee. Rome, 24-27 October 2006. № 814, Rome, FAO, 106 pp

Figus V, D'Amico V, et al. 2005. Prevalence, intensity and abundance of Helminth parasites in *Mullus barbatus* L. and *M. surmuletus* L. from the south coast of Sardinia, Italy. Biologia Marina Mediterranea, 12(1): 675-677

Gaglio G, Bottari T, et al. 2011. Prevalence of *Clavella stellata* (Copepoda: Lernaeopodidae) (Kroyer 1838) in the European hake (*Merluccius merluccius*) (L. 1758), South Tyrrhenian Sea (Central Mediterranean). Journal of Applied Ichthyology, 27(1): 136-138

Ganias K, Somarakis S, Machias A, Theodorou A. 2004. Pattern of oocyte development and batch fecundity in the Mediterranean sardine. Fish. Res., 67: 13 – 23

Garoia F, Guarniero I, et al. 2007. Comparative analysis of AFLPs and SSRs efficiency in resolving population genetic structure of Mediterranean Solea vulgaris. Mol. Ecol., 16(7): 1377-1387

Gestal, C., P. Belcari, et al. 1999. Parasites of cephalopods in the northern Tyrrhenian Sea (western Mediterranean): new host records and host specificity. Sci. Mar., 63(1): 39-43

Guarniero I, Garoia F, et al. 2004. Genetic stock structure analysis revealed single population units in the shared stocks of Adriatic demersal species. Reports of the Working Group on Demersal Species, SAC - GFCM, Sub-Committee of Stock Assessment, Málaga, Spain, 6-7 May 2004, Doc. #18,7 pp; http://www.faoadriamed.org/html/AvDocRes.asp

Guijaro B, Massuti E, Moranta J, Cartes J. 2009. Short spatio-temporal variations in the population dynamics and biology of the deep-water rose shrimp *Parapenaeus longirostris* (Decapoda: Crustacea) in the western Mediterranean. Sci. Mar., 73(1): 183-197

Guijarro B, Massutí E, Moranta J, Díaz P. 2008. Population dynamics of the red shrimp *Aristeus antennatus* in the Balearic Islands (western Mediterranean): Short spatio-temporal differences and influence of environmental factors. J. Mar. Syst. 71: 385-402

Gulland JA, Rosenberg AA. 1992. A review of length-based approaches to assessing fish stocks, Fisheries Technical Paper. FAO Corporate Document Repository. URL: <u>http://www.fao.org/DOCREP/003/T0535E/T0535E00.HTM</u>

Gushing DH. 1980. Marine Ecology and Fisheries. University Press, Cambridge: 278 pp

ICES, 2005. Spawning and life history information for North Atlantic cod stocks. ICES Coop Res Rep 274:1–152

ICES, 2008. Report of Workshop on Maturity Ogive Estimation for Stock Assessment (WKMOG). 3 June 2008, Lisbon, Portugal: 68 pp.

Ihssen P, Booke H, et al. 1981. Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences, 38(12): 1838-1855

Jardas I, Hristovski N. 1985. A new contribution to the knowledge of helminth parasite fauna of fishes from the channels between the mid-Dalmatian Islands, Adriatic Sea. Acta Adriatica, 26: 145-164

Jousson O, Bartoli P. 2000. The life cycle of Opecoeloides columbellae (Pagenstecher, 1863) n. comb.(Digenea, Opecoelidae): evidence from molecules and morphology. International Journal for Parasitology, 30(6): 747-760

Keser, R., R. Bray, et al. 2007. Helminth parasites of digestive tract of some teleost fish caught in the Dardanelles at Çanakkale, Turkey. Helminthologia, 44(4): 217-221

Klimpel S, Kleinertz S, et al. 2008. Distribution of parasites from red mullets (*Mullus surmuletus* L., Mullidae) in the North Sea and Mediterranean Sea. Bulletin of Fish Biology, 10: 25-38

Kristoffersen JB, Magoulas A. 2008. Population structure of anchovy *Engraulis encrasicolus* L. in the Mediterranean Sea inferred from multiple methods. Fish. Res., 91: 187–195

Kritzer JP, Sale PF. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Jush and Fisheries, 5: 131-140

Lester R. 1990. Reappraisal of the use of parasites for fish stock identification. Marine and Freshwater Research, 41(6): 855-864

Lester R, MacKenzie K. 2009. The use and abuse of parasites as stock markers for fish. Fish. Res., 97(1): 1-2

Levi D, Patti B, Lo Brutto S, Arculeo M, Parrinello N, Rizzo P. 2004. Genetic and morphometric variation of Mediterranean hake, *Merluccius merluccius*, in the Strat of Sicily (Central Mediterranean). Ital. J. Zool., 71: 165-170

Lom J, Dykova I. 1992. Protozoan Parasites of Fishes. Elsevier Academic Press: 316

Longhurst AR, Pauly D. 1987. Ecology of tropical oceans. Academic Press, San Diego

Loubes C, Maurand J, et al. 1977. Observations ultrastructurales sur *Ameson nelsoni* (Sprague, 1950) microsporidie parasite de la crevette *Parapenaeus longirostris* Lucas. Consequences taxonomiques. Revue des Travaux de l'Institut des Peches Maritimes, 41(3): 217-222

MacKenzie K. 1983. Parasites as biological tags in fish population studies. Advances in Applied Biology, 1983

MacKenzie K, Abaúnza P. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fish. Res., 38(1): 45-56

Maggio T, Lo Brutto S, et al. 2009. Microsatellite analysis of red mullet *Mullus barbatus* (Perciformes, Mullidae) reveals the isolation of the Adriatic Basin in the Mediterranean Sea. ICES J. Mar. Sci., 66 (9): 1883-1891

Maíllo PA, Amigó JM, et al. 1998. *Tetramicra brevifilum* (Matthews & Matthews, 1980) (Microsporida: Tetramicriidae) in a new fish host, *Lophius budegassa* (Spinola, 1807) in Spain. Parasitology research, 84(3): 208-212

Manfredi M, Crosa G, et al. 2000. Distribution of *Anisakis simplex* in fish caught in the Ligurian Sea. Parasitology research, 86(7): 551-553

Martin P, Bahamon N, Sabates A, Maynou F, Sánchez P, Demestre M. 2008. European anchovy (*Engraulis encrasicolus*) landings and environmental conditions on the Catalan coast (NW Mediterranean) during 2000-2005. Hydrobiologia, 612:185-199

Mattiucci S, Abaúnza P, Ramadori L, Nascetti G. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. J. Fish. Biol., 65: 495-510

Mattiucci S, Farina V, Campbell N, Mackenzie K, Ramos P, Pinto AL, Abaúnza P, Nascetti G. 2008. *Anisakis* spp. Larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: their genetic identification and use as biological tags for host stock identification. Fish. Res., 89: 146–151

Mattiucci S., Nascetti G. 2008. Advances and trends in the molecular systematics of Anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Advances in Parasitology, 66: 47-148

MEDITS. 2013. International bottom trawl survey in the Mediterranean (Medits). Instruction manual. Version 7. Ifremer, Nantes. 60 p.

Misra RK, Carscadden JE. 1987. A multivariate analysis of morphometrics to detect differences in populations of capelin (*Mallotus villosus*). J. Cons. Int. Expl. Mer., 43: 99–106

Mladineo I, Šimat V, et al. 2012. Molecular identification and population dynamic of *Anisakis pegreffii* (Nematoda: Anisakidae Dujardin, 1845) isolated from the European anchovy (*Engraulis encrasicolus* L.) in the Adriatic Sea. International journal of food microbiology, 157(2): 224-229

Morales-Nin B, Pertierra P. 1990 Growth rates of the anchovy *Engraulis encrasicolus* and the sardine *Sardina pichardus* in the Northwestern Mediterranean Sea. Mar. Biol.,107: 349-356

Moutin T, Raimbault P. 2002. Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise). J Mar Syst 33-34:273–288

Murta AG, Pinto AL, Abaúnza P. 2008. Stock identification of horse mackerel (*Trachurus trachurus*) through the analysis of body shape. Fish. Res., 89: 152–158

Murta AG, Borges MF, Silveiro ML. 1996. Morphological variations in the sagitta otoliths of horse mackerel (*Trachurus trachurus*) in Portuguese waters (Div. IXa). ICES C.M. 1996/H:27, 8 pp.

Mytilineou Ch., Tsagarakis K., Bekas P., Anastasopoulou A., Kavadas S., Machias A., Haralabous J., Smith C.J., Petrakis G., Dokos J., Kapandagakis A. 2013. Spatial distribution and life-history aspects of blackspot seabream *Pagellus bogaraveo* (Osteichthyes: Sparidae). J. Fish. Biol., 83: 1551–1575

Öktener A, Torcu Koç H, et al. 2009. Fish/Parasites interrelationships in Turkey: a review. Ittiopatologia, 6: 121-139

Orecchia P, Paggi L. 1978. Aspetti di sistematica e di ecologia degli elminti parassiti di pesci marini studiati presso l'Istituto di Parassitologia dell'Universita di Roma. Parassitologia, 20: 73-89

Paradižnik V, Radujković B. 2007. Digenea trematodes in fish of the North Adriatic Sea. Acta Adriatica, 48(2): 115-129

Pawson MG, Jennings S. 1996. A critique of methods for stock identification in marine capture fisheries. Fish. Res., 25: 203-217

Petric M, Mladineo I, et al. 2011. Insight into the short-finned squid *Illex coindetii* (Cephalopoda: Ommastrephidae) feeding ecology: is there a link between helminth parasites and food composition? The Journal of parasitology, 97(1): 55-62

Powell DG. 1979. Estimation of mortality and growth parameters from the length frequency of a catch. Rapp.P.-v.Réun.CIEM, 175:167-169

Quetglas A, Alemany F, *et al.* 199). Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). Fish. Res., 36(2): 237-249

Ramon MM, Castro JA. 1997. Genetic variation in natural stocks of *Sardina pichardus* (sardines) from the western Mediterranean Sea. Heredity, 78: 520-528

Rello FJ, Adroher FJ, et al. 2009. The fishing area as a possible indicator of the infection by anisakids in anchovies (*Engraulis encrasicolus*) from southwestern Europe. International journal of food microbiology, 129(3): 277-281

Rolland JL, Bonhomme F, et al. 2007. Population structure of the common sole (*Solea solea*) in the Northeastern Atlantic and the Mediterranean Sea: revisiting the divide with EPIC markers. Mar. Biol., 151(1): 327-341

Serracca L, Cencetti E, et al. 2013. Survey on the presence of Anisakis and Hysterothylacium larvae in fishes and squids caught in Ligurian Sea. Veterinary parasitology, 196(3): 547-551

Silva A, Santos MB, Caneco B, Pestana G, Porteiro C, Carrera P, Stratoudakis Y. 2006. Temporal and geographic variability of sardine maturity at length in the north- eastern Atlantic and the western Mediterranean. ICES J Mar Sci, 63: 663–676

Silva A, Carrerab P, Massé J, Uriarte A, Santos MB, Oliveira PB, Soares E, Porteiro C, Stratoudakis Y. 2008. Geographic variability of sardine growth across the northeastern Atlantic and the Mediterranean Sea. Fish. Res., 90 (1–3): 56–69

Smrzlić, I. V., D. Valić, et al. 2012. Molecular characterisation of Anisakidae larvae from fish in Adriatic Sea. Parasitology research, 111(6): 2385-2391

Somarakis S, Machias A. 2002. Age, growth and bathymetric distribution of red pandora (*Pagellus erythrinus*) on the Cretan shelf (eastern Mediterranean). Journal of the marine biological association of the United Kingdom, 82: 149-160

Sonin O., Spanier E., Levi D., Patti B., Rizzo P. et al. 2007. Nanism (dwarfism) in fish: a comparison between red mullet *Mullus barbatus* from the southeastern and the central Mediterranean. Marine Ecology Progress Series, 343: 21-228.

Souplet A. 1996. Calculation of abundance indices and length frequencies in the MEDITS survey. In: J. A. Bertrand et al. (eds), Campagne internationale du chalutage démersal en Méditerraneé. Campagne 1995. EU Final Report, Vol. III.

STECF 2008. Report of the SGMED-08-02 Working Group on the Mediterranean Part II21 25 APRIL 2008, ATHENS, GREECE as endorsed at the 28th Plenary meeting of the Scientific, Technical and Economic Committee for Fisheries (plen-08-02. 7-11 July 2008, Helsinki)

Stephenson RL. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fish. Res., 43: 247-249

Stergiou KI, Christou ED, Georgopoulos D, Zenetos A, Souvermezoglou C. 1997. The Hellenic Seas: physics, chemistry, biology and fisheries. Oceanography and marine Biology. Annual Review, 35:415-538

Stransky C, Murta AG, Schlickeisen J, Zimmermann C. 2008. Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean. Fish. Res., 89: 159–166

Stransky C. 2005. Geographic variation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the North Atlantic based on otolith shape analysis. ICES J. Mar. Sci., 62: 1691–1698

Tantanasi J, Diakou A, Tamvakis A, Batjakas IE. 2012. *Anisakis* spp. burden in *Trachurus trachurus*. Helminthologia 49(1): 16 – 20

Ternengo, S., C. Levron, et al. 2009. Site influence in parasite distribution from fishes of the Bonifacio Strait Marine Reserve (Corsica Island, Mediterranean Sea). Parasitology research 104(6): 1279-1287

Theocharis A, Gacic M, Kontoyiannis H. 1998. Physical and dynamical processes in the coastal and shelf areas of the Mediterranean. In: The Sea, 11, Robinson AR & Brink KH. John Wiley and Sons, Inc., ISBN 0-471-11545-2

Tinti F, Di Nunno C, et al. 2002. Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the Adriatic and Ionian stocks of *Sardina pichardus*. Marine Biotechnology, 4(2): 163-172

Tsikliras AC, Antonopoulou E, Stergiou KI. 2010. Spawning period of Mediterranean marine fishes. Rev Fish Biol Fisheries 20: 499–538

Tsikliras A, Koutrakis ET. 2013. Growth and reproduction of European sardine, *Sardina pichardus* (Pisces: Clupeidae), in northeastern Mediterranean. Cah Biol Mar, 54 (3): 365-374

Turan C. 2004. Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. ICES J. Mar. Sci., 61: 774-781

Valero A, López-Cuello MM, et al. 2006. *Anisakis* spp. in European hake, *Merluccius merluccius* (L.) from the Atlantic off north-west Africa and the Mediterranean off southern Spain. Acta Parasitologica 51(3): 209-212

Zotto, M. and M. Todaro. 2008. Cycliophora. Biologia Marina Mediterranea, 15: 177-180

WP2. Reviewing existing Genetic Stock Structure Analysis (GSSA) data of target fishery resources and assessing spatial population connectivity in the Mediterranean GSAs.

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Note: The WP2 final report is presented according to task breakdown. The data gathered and mined are dating to 31 July 2014.

BACKGROUND

Genetic stock structure analysis (GSSA) is a basic tool for understanding the dynamics of fish populations and a necessary step towards sustainable management. GSSA can provide information on population expansions, bottlenecks or gene flow leading to estimations on the impact of population interactions with environmental factors or human activities. There is already a series of reports and books dedicated to the techniques developed and currently in use for the identification and traceability of seafood products and could further allow both assignment to species, but also to stock or population of origin (see ICES Reports of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 2009 & 2013 and "Stock Identification Methods: Applications in Fishery Science", Second Edition, Elsevier Inc.). In brief there are five main groups of techniques: 1) Morphological trait markers, 2) Non-genetic analysis of soft tissues, 3) Otoliths: shape analysis, microstructure and microchemistry, 4) Genetic analysis of associated organisms, and 5) Genetic markers (which will be detailed below).

At the population level study and the identification of regional/stock origin, the genetic research so far has demonstrated that many marine organisms are separated into more or less genetically distinct populations, which allow for genetic traceability (for a review see Reiss et al. 2009). The golden standard for inferring population structure is the identification of genetic markers that can reflect the genetic structure in the species. Common types of genetic markers are allozymes, mtDNA, AFLPs, microsatellites, or single nucleotide polymorphisms (SNPs). In modern era, the technological advances of sequencing technology have opened up numerous new possibilities of massive analyses to species that are not classical genetic model species (e.g. human, mouse, fly, etc.) and can lead to discovery of hundreds to thousands markers with a fairly small budget. Microsatellites are widely-used polymorphic
markers driving population structure due to their multi-allelic nature that greatly assists the monitoring of population structure. The main bottleneck has always been the amount of molecular markers and their distribution in the genome. Restricted genomic sampling leads to erroneous conclusions. The revolutionary advance of nextgeneration sequencing (NGS) coupled with traditional methods of genotyping led to tremendous progress in genetic analyses. Various options of genotyping by sequencing (GBS) provide the possibility to fast, accurate and massively genotype hundreds of thousands of SNPs in multiple individuals and populations. Genome-wide analyses can lead to identification of adaptive loci, loci that resulted from hybridization, etc. However, new methods require not only additional pipeline and computational steps, but also great improvements in the widely-used genetic analyses packages that cannot easily incorporate the magnitude of markers usually obtained in NGS experiments.

Next-generation sequencing has changed the way we conduct genetic and genomic research, and nowadays we are facing a "democratization" of high-throughput sequencing being also accessible to the wider research community. Virtually now any researcher, in his own lab or with assistance from a service provider, can conduct whole-genome sequencing, transcriptomics and population genomics studies. Past difficulties are gradually being solved as new bioinformatics tools and algorithms are developed, sequencing reads become longer and more accurate, benchtop sequencers and sequencing-service-providers have more accessible prices, and NGS is more and more adopted by the research community. What is the next challenge is to get access to large samplings and samples are considered now 'the new commodity' (http://massgenomics.org/). Tissue samples for high-quality DNA, and soon RNA, from rare species and/or difficult to reach and sample localities are increasingly valuable.

OBJECTIVES

According to the STOCKMED proposal the WP2 aims to provide an updated base of genetic information and data on the target species and to assess the stock structure and to identify stock units in the Mediterranean based on existing data and information. In detail, for each target species, WP2

- 1) critically reviews the existing genetically-based knowledge on stock structure and stock unit identification and identify the existing critical gaps;
- assess spatial population connectivity and providing indicators/estimates of population genetic differentiation for assessment of the number and boundaries of stock units.

In addition, WP2 aims to improve methodological knowledge by

3) reviewing and updating the Genetic Stock Structure Analysis (GSSA) pipelines based on the most advanced and highly-performing tools in fishery genetics of the Mediterranean target fishery species and stocks.

DELIVERABLES AND MILESTONES FORESEEN BY THE PROJECT

- According to the approved proposal and in-progress decisions assumed during the Stockmed meetings we delivered
- D6 Report and updated literature list on GSSA data of the target fishery Mediterranean resources at Month 9 (Responsible CONISMA-UNIBO)
- D7 Individual reporting species sheets with genetic estimates of population differentiation indicators/parameters at Month 9 (Responsible CONISMA-UNICA);
- D8 Report on novel, highly-performing GSSA pipelines for fishery genetics, scheduled at Month 12 and delayed at Month 18 (Responsible HCMR).
- A single Milestone 2.1 was identified in the proposal (Relevant data gathering from past projects and from partners of the MAREA Consortium). It was passed by acquiring unpublished genetic information from the Small or Medium-Sized Research Project "The Structure of Fish Populations and Traceability of Fish and Fish Products (FishPopTrace)", EU-FP7 KBBE1 COGE 212399 on hake Merluccius merluccius and sole Solea solea.

RESULTS ACHIEVED

The results achieved in the WP2 are presented according to task breakdown.

Task 2.1 Critical revision of data from GSSA of the target fish and crustacean Mediterranean fishery resources, reviewing and using adequate information of significant results obtained by EU-financed projects and assessing RTD critical gaps (Task Coordinator F. Tinti)

Task 2.1 (Leader CONISMA-UNIBO) provided a comprehensive literature data base of the genetically-based knowledge (STOCKMEDGSSlitDB) available for the 19 target species has been generated and built in Endnote X5 version as well as in free-access Mendeley version (Deliverable 6). The Endnote or Mendeley versions of the reference database can be released in the StockMed/MAREA web site.

The STOCKMED GSSLitDB has been generated by enquiring the most popular peerreviewed reference databases (e.g. ISI Web of Sciences, Scopus) using the taxonomic and common species names as key-words for the literature search (e.g. *Solea solea*, common sole) and by selecting the references i) reporting genetically-based data, ii) for the Mediterranean and NE Atlantic populations and iii) published as research paper, short note, primer note, review. The reference list has been integrated with genetic data that were reported in non-peer reviewed papers, in-press publications and unpublished data as the participants' knew.

The **Primary Reference List** of the STOCKMED GSSLitDB included 437 references, of which 96 reported population genetic studies suitable for the STOCKMED purposes (**Primary Reference List POPGEN**). The most investigated species were the bony fish *Solea solea, Engraulis encrasicolus, Mullus barbatus, Trachurus trachurus, Sardina pilchardus, Merluccius merluccius* and *Mullus surmuletus* and the cephalopod *Octopus vulgaris* with at least 6 references. The less investigated species were the shark *Galeus melastomus* (1 reference) and the cephalopods *Eledone cirrhosa* and *Eledone moschata* (0 reference).

Since Task 2.2 and D6 aimed at critically review the existing knowledge on stock structure and stock unit identification (henceforth, for the Genetic Stock Structure Analysis, GSSA), from the Primary Reference List, a **Primary Dataset List** was generated by identifying single dataset according to the genetic marker type used in the genetic study (1 = allozyme and similar enzymatic markers; 2 = mtDNA markers, nuDNA intronic markers; 3 = codominant nuDNA markers as SNPs, SSRs, and dominant AFLP). According to this, the STOCKMED Dataset list included 109 datasets in which predominated those generated by mtDNA and nuDNA intronic markers (type 2: 54) while the number of datasets generated with the other two marker types were similar (type 1: 39; type 3: 39).

A **Primary Ranked Dataset List** was further generated by ranking the 109 datasets according to three hierarchical criteria implemented in the following algorithm:

Score = (E*M)^S

- 1) criterion **S** = **Detection of Population Structure** (score: 1 = not significant population structure; 2 = significant population structure)
- criterion E = Geographical Coverage, namely the number of GSAs sampled (score: 1 point for each GSA covered in the Mediterranean; 1 point for each ICES zone covered in the NE Atlantic; 0 point, this will exclude references dealing with other types of investigations, e.g. primer notes)
- criterion M = Marker type (score: 1 = allozyme and similar enzymatic markers; 2 = mtDNA markers, nuDNA intronic markers; 3 = codominant nuDNA markers as SNPs, SSRs, and dominant AFLP).

The scores calculated provided a qualitative-quantitative assessment of the GSSA datasets in order to provide useful data to define stock unit in the fishery target resources. Such a qualitative and quantitative assessment is dependent primarily from the detection of population structure (criterion S) given the type of marker used (criterion M) and the number of GSAs sampled and analysed (criterion E).

From the Primary Ranked Dataset List two selected databases were created: the **Selected Dataset List NEA-MED** included 57 datasets targeting North-East Atlantic ICES zones and Mediterranean GSAs and the **Selected Dataset List MED** with 44 datasets only targeting Mediterranean GSAs. The latter database represented the final list of datasets candidate to be used in the multi-approach definition of stock units within the STOCKMED WP4. These GSSA datasets are detailed in the following table according to target species and with the associated mean number of GSAs sampled and analysed.

Target Species	GSSA Data sets	Mean number of GSAs analysed
Aristeomorpha foliacea	1	5
Aristeus antennatus	2	6
Eledone cirrhosa	-	-
Eledone moschata	-	-
Engraulis encrasicolus	11	7
Galeus melastomus	-	-
Illex coindetii	-	-
Lophius budegassa	-	-
Merluccius merluccius	3	13
Mullus barbatus	5	4
Mullus surmuletus	4	4
Nephrops norvegicus	2	6
Octopus vulgaris	3	4

Table 2.1 GSSA dataset by target species and GSAs.

Pagellus erythrinus	2	3
Parapeneaus longirostris	1	6
Sardina pilchardus	1	11
Solea solea	6	8
Trachurus mediterraneus	1	4
Trachurus trachurus	1	4

The quality of the GSSA datasets in terms of i) taxonomic/ecological coverage for the target fishery resources in the Mediterranean, ii) geographical (number of GSAs) coverage and iii) performance of marker types in providing stock structure discrimination is reported in the following table.

Target species <u>without</u> data	Counts
Crustaceans	0/4
Cephalopods	3/4
Cartilaginous fish	1/1
Bony fish (pelagic)	0/4
Bony fish (demersal)	1/6
Total	5/19
E= Number of GSAs	Counts
Mean	6,4
Max	19
Min	2
Mean per species	6,1
M = Marker type	Counts
1 = allozyme and similar enzymatic markers	12 (27,3%)
2 = mtDNA markers, nuDNA intronic markers	19 (43,1%)
3 = codominant nuDNA markers (SNPs, SSRs, AFLP)	13 (27,3%)

Table 2.2. Quality of GSSA dataset according to different criteria.

The species that displayed the greatest number of GSSA data sets were anchovy, hake, red mullet and common sole among bony fishes and the octopus among cephalopods.

Five species did not display GSSA datasets (1 teleost, 3 cephalopods, 1 cartilaginous fish) and these represented critical gaps in the StockMed WP2 framework. The number of GSAs sampled and analysed was highly variant across species with GSSA datasets. The mean number of GSAs sampled was relatively high and this is promising for retrieving data and information suitable for the StockMed main objective. It is relevant to note that only about 27% of the GSSA datasets were generated using high-resolution (type 3) markers. This can be considered as a technological critical gap to be filled in the future research works.

Task 2.2 Assessment of spatial population connectivity of Mediterranean target fishery resources (Task Coordinator R. Cannas)

Task 2.2 (Leader CONISMA-UNICA) led to the building up the collection of the 39 species-specific Genetic Stock Structure Analysis (the STOCKMED Genetic Stock Structure Analysis Dataset Species Sheets; STOCKMED GSSA SpS; Deliverable 7) obtained for 14 species for which in Task 2.1 information on genetic data on structuring or population differentiation was retrieved. Each STOCKMED GSSA SpS reported a detailed form with the genetic information available in the selected STOCKMED GSSA dataset and showed up the estimates of population differentiation/structure and indicators/parameters. Each STOCKMED GSSA SpS reported available information concerning

- the genetic markers used in the lab work to assess genetic differentiation .

- the area investigated as well as the number of specimens analysed and, if available, latitude and longitude (as in the original papers). The FAO area and GFCM GSA for each sampling site is also indicated.

- the main results of the genetic paper, related to the identification of genetic differentiation genetic and/or structuring of population samples either within the Mediterranean Sea or between the Mediterranean Sea and other areas (principally the adjacent north eastern Atlantic Sea). To facilitate the visualization of the genetic differentiation and stock structuring, the spatial population genetic data of Mediterranean target fishery resources has been also illustrated through

1) the geo-mapping visualization in the Google Earth map on the GFCM Geographical SubAreas (GSAs) grid;

2) according to the WP4 requirements and as agreed with in the 2nd StockMed

Meeting held in Palermo (3-4 July 2013), transformed binary matrixes in which each population sample has been assigned to a specific Cluster (score 1) according to the results reported in the literature. In some cases, clusters have been further divided in sub-clusters to better illustrate more complex patterns of genetic differentiation emerging from the data.

The whole process of identifying genetic differentiation and structuring has been addressed for each STOCKMED GSSA SpS at least twice, in parallel through the independent work of experts' participant to the WP2. The double check for each STOCKMED GSSA SpS was necessary to testing the congruency of interpretations, especially for datasets when feeble genetic differentiation or very complex patterns of genetic structure emerged from the data. When multiple datasets were available for a given species, all of them were included in the STOCKMED GSSA SpS in the same order as in the STOCKMED GSSA Db. Table 2.3. Summary description of GSSA by species.

Target Species	GSSA SpS / Genetic structure: summary description
Aristeomorpha foliacea	SM004 allowed the detection of a weak genetic structuring within the Mediterranean even if it was neither supported coherently by tests and analysis nor by other GSSA based on different markers.
Aristeus antennatus	SM225 (includes SM015) strongly supports the occurrence of genetically differentiated clusters. Feeble population substructuring occurs within the Western Mediterranean area. However, evidence of genetic structuring are not confirmed by other GSSAs of the same authors analysing the same sites with different markers and/or different sampling sizes SM008/009) or are in contrast with other GSSAs that reported a substantial genetic homogeneity within the Mediterranean Sea.
Engraulis encrasicolus	Eleven different GSSAs (SM016/017/023/024/032/034/035/041/235/236/237) did not revealed coherently differentiated genetic clusters because results were not always congruent among GSSAs using different and/or similar markers. The main discrepancies concerned several comparisons whose differentiation is or is not significant depending on GSSA. These results were in contrast with those of SM226 that reported a substantial genetic homogeneity within the Mediterranean Sea.
Merluccius merluccius	 SM060 and SM062 revealed low differentiation and provided low support to the identification of genetic clusters within the Mediterranean. SM073 strongly supported the existence of well-differentiated clusters across all the Mediterranean and with respect to the NE Atlantic. These results were in contrast with those of SM063, that reported a substantial genetic homogeneity within the Mediterranean Sea and they are not completely congruent among them in identifying the main clusters within the Mediterranean Sea; however, experts considered the results of SM073, based on many more loci, as the most strongly supported.
Mullus barbatus	Five different GSSAs (SM082 /084 /089 /090 /091) provided low differentiation and low support to the identification of genetic clusters within the Mediterranean. These results were not congruent among GSSAs in identifying the main clusters within the Mediterranean Sea and they were in contrast with those of SM074-075 and 094, that reported a substantial genetic

	homogeneity within the Mediterranean Sea.
Mullus surmuletus	Four different GSSAs (SM082 /109 /240 /241) coherently provided medium-low support to the identification of genetic clusters but they are in contrast with SM097 /106 /110 that reported a substantial genetic homogeneity within the Mediterranean.
Nephrops norvegicus	Two different GSSAs (SM113 /115) coherently provided low differentiation and low support to the identification of genetic clusters within the Mediterranean. All GSSAs highlighted the absence of a clear geographical pattern of genetic differentiation among the populations studied.
Octopus vulgaris	Two different GSSAs (SM121 /123) provided medium support to the identification of genetic structure and clusters. SM122 provided instead high support to the identification of genetic clusters. GSSAs were complementary each other because of they analyse different areas. These results were discordant with those of SM128 that reported lack of significant differentiation among populations.
Pagellus erythrinus	Two different GSSAs (SM228 /229) provided medium support to the occurrence of very weak but significant genetic differentiation between populations off the coasts of Tunisia and Lybia. These results were discordant with those of SM132 that reported lack of significant differentiation among populations in the Aegean Sea.
Parapeneaus longirostris	SM0140 (included SM139) provided medium support to the occurrence of 4 distinct genetic clusters in the Mediterranean. Other studies on population genetics not available.
Sardina pilchardus	SM0151 provided medium support to the occurrence of 5 distinct genetic clusters (2 in the Mediterranean). These results were in contrast with other studies (SM150, 156-157, 231) pointed out the occurrence of a substantial homogeneity within the Mediterranean Sea, when analysing samples east of the Alboran front.
Solea solea	Six different GSSAs (SM0158 /175 /177 /181 /182 /183) were coherent in identifying the occurrence of genetic differentiated stocks within the Mediterranean Sea. They were complementary each other since they analyse different GSAs. In particular, SM182 included most of the GSA analysed by other GSSAs and hence summarized the results of all GSSAs.

Trachurus mediterraneus	SM202 revealed the occurrence of genetic differentiated clusters between the eastern Mediterranean and the Black Sea. A substantial genetic homogeneity is described by paper SM200, which encompasses a wider sampling area (Western Mediterranean, Ionian and Aegean Sea).
Trachurus trachurus	SM224 revealed the occurrence of genetic differentiated populations between the eastern Mediterranean and the Black Sea. Other papers (GSSA_SM211-212-218-219), investigating a wider area, describe a substantial genetic homogeneity among Mediterranean populations.

Task 2.3 Methodological and technological updating of the GSSA pipelines based on the most advanced and highly-performing tools in fishery genetics (Task Coordinator C. Tsigenopoulos)

Task 2.3 (Leader HCMR) provided an overview and recommendations on the use of GSSA pipelines (namely a procedure involving experimental and analytical designs, techniques and technologies, markers and tools for data analysis) in the specific field and research framework of the identification of stock units in the Mediterranean fishery resources (Deliverable 8).

In the first place, Task 2.3 has explored and summarized "GSSA know-how" highlighting best practices on

- samples and sampling design: size, number of GSSAs sampled and localities, tissue preservation for current and future genetic use;

- tools and markers: number, type (neutral, non-neutral and potentially under selection), maternally inherited, genetic informativeness and resolution power with a special focus on the comparison between the two most powerful type of markers in the GSSA (Microsatellites vs SNPs)

- analytical tools and methods, with special discussion on the future use of Genotyping-by Sequencing (GBS) techniques.

In the second place, Task 2.3 assessed gaps in the existing GSSA pipelines for the identification of stock units by critically reviewing available GSSAs targeting the Mediterranean fishery resources of high-priority under a technological and methodological magnifying glass identifying the major needs of an interdisciplinary analysis approach taking into account the influence of species life history traits and environmental characteristics to genetic stock identification and of an adequate funding to collect adequate data and to give adequate answers to managers and fishermen.

In general, it was emerged there is relatively little information that may potentially discriminate between major evolutionary units and current management units in the StockMed species. Accordingly, population genetic studies on a smaller geographical scale should be encouraged for species like *E. encrasicolus, M. merluccius* and *S. solea* for which there are already big progress achieved through the application of adequate markers and techniques. Furthermore, a prerequisite for obtaining robust results, which can be later used for defining management units, is the temporal sampling of spawning aggregations. Future studies should particularly focus on elucidating whether there is genetic differentiation among spawning aggregations and if these are stable in time (across generations) and thus represent independent units. In that objective, archived material in the form of ethanol preserved tissue and historical otoliths should be available in many areas for these types of investigations.

Because divergence time among neighbouring populations is expected to be short in some species (e.g. *Mullus surmuletus* and *M. barbatus* which have a long pelagic stage), migration rates are expected to be high and effective population sizes relatively large. The application of genetic

markers subject to selection may prove valuable as genetic markers for Genetic stock identification (GSI) in conjunction with presumed neutral markers. One note of caution is that natural selection may alter allele frequencies within a cohort, so tests for temporal stability from egg-to-adult should be conducted (see also ICES 2009 & 2013).

We should have in mind that it is quite likely that there are differences in patterns of population structure for different species managed in the same areas. For instance, some species exhibit temporally stable and highly significant population structure in areas where other, seemingly ecologically similar, species do not (see also ICES 2009 & 2013). The realization of these interspecific differences is of great importance in multispecies approaches when focusing on area specific management.

Various types of hard (e.g. highly significant temporally stable DNA based evidence) and soft evidence (e.g. differences in morphology, growth patterns) for population structuring is available, and a framework would ideally encompass both types of information. At the same time, however, the framework should rank the various types of evidence in an effort to evaluate where management decisions are required.

The term 'seascape genetics' first proposed by Galindo et al. (2006) as an analogy to 'landscape genetics' (Manel et al. 2003) to indicate an approach that employs the physical, chemical, and biological features of the habitat to explain the observed patterns of spatial genetic structure. Seascape genetics is expected to become a very fertile ground of research, which can bridge the gap between ecology and population genetics. Although seascape genetics approaches have so far emphasized the relationship between the physical environment and dispersal, we foresee that with more elaborate suitability maps and a broader array of predictor variables which will likely also take into account known aspects of the life history of the target species it will be possible to include adaptive responses among the effects investigated. This multimarker, multilayer, and, ideally, multispecies approach will likely help identify areas of particular ecological significance and hence play a potentially pivotal role in marine spatial planning (e.g., the design of marine protected areas) (see in Mariani & Bekkevold 2014).

DIFFICULTIES ENCOUNTERED AND REMEDIAL ACTIONS

During the realization of this WP significant difficulties were not found.

REFERENCES

ICES (2006) Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM). ICES, Copenhagen, 59 pp.

ICES (2009) Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 1–3 April 2009, Sopot, Poland. ICES CM 2009/MCC:03. 74 pp.

ICES (2013) Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM), 7-9 May 2013, Reykjavik, Iceland. ICES CM 2013/SSGHIE:11. 52 pp.

Galindo, H., Olson, D., Palumbi, S., 2006. Seascape genetics: a coupled oceanographic-genetic model predicts population structure of Caribbean corals. Curr. Biol. 16, 1622-1626.

Manel, S., Schwartz, M.K., Luikart, G., Taberlet, P., 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol. Evol. 18, 189-197.

Mariani S. & Bekkevold D. (2014) The Nuclear Genome: Neutral and Adaptive Markers in Fisheries Science. In "Stock Identification Methods: Applications in Fishery Science" (Second Edition), pp.297-328. Edited by Steven X. Cadrin, Lisa A. Kerr and Stefano Mariani, Elsevier Inc.

Reiss, H., Hoarau, G., Dickey-Collas, M., and Wolff, W.J. (2009). Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries 10, 361-395.

WP3. Review and analyses of the geo-morphological, oceanographic and fishery spatial patterns as factors related to stock distribution

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BACKGROUND

WP3 aimed at the collection and review of all available environmental data (e.g. hydrological factors, sea bottom features) relevant for species distribution and spatial connectivity between life stages of each target species as well as data on fisheries and fishery grounds of the STOCKMED target species.

OBJECTIVES

The objectives of WP3 were:

- To collate, to review, to scrutinize and to comment as adequate information on the sea bottom topography, water circulation pattern at different spatial scales;
- To collate, to review, to scrutinize and to comment as adequate information on the Essential Fish Habitats (nurseries and spawning grounds);
- To collate, to review, to scrutinize and to comment as adequate information on past and current spatial pattern of the main fisheries/métiers in relation to the target species.

The ultimate goal was to provide input for the synoptic analyses of WP4. A last objective was to identify the gaps in knowledge concerning environmental (hydrological factors and sea bottom features) and fisheries exploitation patterns useful for stock unit definition.

DELIVERABLES AND MILESTONES FORESEEN BY THE PROJECT

The work in WP3 was subdivided in four tasks each of which was associated with a corresponding deliverable:

Task 3.1 Revision and analysis of the spatial pattern of geo-morphological and oceanographic features of the Mediterranean in order to identify the existence of environmentally-driven potential demographic breaks (Task coordinator: B. Patti)

Associated deliverable:

Deliverable 09

Report geo-referred information on the geo-morphological and oceanographic information which can act as potential break factors for environmental driven demographic borders in the Mediterranean)

Task 3.2 Revision and analysis of the existing information on the position of the persistent nurseries and spawning areas of the target species in the investigated areas. Part of this work was supported by the results obtained in the MEDISEH project, while new analysis were carried out to include all species covered by the STOCKMED project, namely *Eledone moschata, Lophius budegassa, Octopus vulgaris, Solea solea* (Task coordinator: F. Colloca)

Associated deliverable:

Deliverable 10

Report geo-referred information on the spatial distribution of essential fish habitats - persistent nursery and spawning areas - for the case studies

Task 3.3 Revision and analysis of the existing information on fishing grounds of target species in the case studies (Task coordinator: M. Sbrana)

Associated deliverable:

Deliverable 11

Report geo-referred information on the spatial and temporal distribution of fishing effort/grounds for the case studies

Task 3.4 Synthesis of knowledge related to the spatial pattern of physical breaks, critical areas, and fishery effort relevant for stock unit investigation and identification of the main knowledge gaps (Task coordinator: S. Somarakis)

Associated deliverable:

Deliverable 11

Synopsis of the spatial pattern of hydrology, sea bottom features, essential fish habitat and fishing effort in the investigated case studies, including identification of knowledge gaps

All deliverables have been finalized and are available on the MAREA ftp.

The milestones of WP3 are listed below:

M3.1 — Relevant data gathering from ad hoc data calls and from partners of the MAREA consortium

M3.2 — Gathering environmental information (e.g. hydrological factors, sea bottom features, etc.) at adequate spatial scale.

M3.3 — Reporting results as geo-referred information from MEDISEH project.

M3.4 — Identification of persistent nursery areas and spawning grounds for the species of the selected case study not supported by MEDISEH outputs.

M3.5 — Organizing information for the fishing pressure factors at adequate spatial scale

RESULTS ACHIEVED

Task 3.1 Revision and analysis of the spatial pattern of geo-morphological and oceanographic features. In some selected case study retention or dispersion of eggs and larvae from the spawning grounds will be investigated using physical oceanographic models (task coordinator B. Patti).

Regarding the geo-morphology of the Mediterranean basin, detailed bathymetry data were already available and mapped for the entire Mediterranean Sea. Geo-referenced information on substrate types available from the EUSeamap project (<u>http://jncc.defra.gov.uk/euseamap</u>) were also collected but they were limited to the western Mediterranean basin. Thus they were not so useful for further analyses.

Regarding hydrological features and current patterns the following products have been provided:

- A detailed and up-to-date review of available information on hydrological features and currents in each Mediterranean subarea (eastern, central and western Mediterranean) based on bibliographic reviews.
- Maps of sea surface geostrophic currents (speed and direction) obtained from the analysis of altimetric satellite data (from AVISO). Specifically, maps of average surface currents for 2002-2012 on a seasonal basis (January-March, April-June, July-September and November-December)

- Maps of gyres (from AVISO altimetric satellite data, seasonal climatological averages, 2002-2012)
- Maps of mesoscale thermal fronts (analysis of SST AVHRR satellite images, seasonal climatological averages, 2002-2012)
- Maps of average currents from 3-D hydrodynamic models (currents for surface, 15m, and deeper layers, 200-300m, 1987-2007). This information was made available from Pinardi et al. (2013) and was finally used to define geo-referenced hydrological landscapes of the Mediterranean basin (based on surface and intermediate circulation patterns) to be used for the analyses of WP4.

Task 3.1 was successful in providing a suite of geo-referenced data on oceanographic features of the Mediterranean Sea. The rational for providing this information was to infer potential connectivity between nursery and spawning areas and/or between adjacent GSAs. For example, the identification of a persistent current system flowing from a particular GSA to an adjacent GSA is a strong indication that meroplanktonic larvae of the STOCKMED species could be transported from the former to the latter. In that case, one should start considering to potentially merge these two GSAs for assessment and management purposes. On the contrary, a strong shelf-slope front in one GSA could act as a barrier to the advection of larvae off-shore, thus contributing to the integrity of the stock in the particular GSA.

The results of the analysis made in Deliverable 09 as well as the review of larval stages (e.g. larval durations, vertical distributions etc) and drift studies for the STOCKMED species (presented in Deliverable 05) demonstrated that such inferences (connectivity through larval transport) are not straightforward in the Mediterranean, for two main reasons:

- Knowledge of the planktonic stages of the STOCKMED species (e.g. larval durations, vertical distributions, ontogenetic changes in behavior and capabilities) is rudimentary, involving mainly anchovy and sardine. Related site-intensive field studies as well as modelling exercises are still in their infancy (see Deliverable 05). Almost nothing is known for most demersal species (especially crustaceans and cephalopods), which represents an impediment to our evaluation of potential drift patterns and survival probabilities.
- It must be noted here that, at least for shelf dwelling species like small pelagics, advection of eggs and larvae from shallow spawning grounds to deep oligotrophic basins (such as most Mediterranean deep basins) is most likely to result in severe starvation mortality for the larvae. Hence, both transport and survival must be considered in studies of stock connectivity through larval advection.

Although the general circulation patterns in the Mediterranean are currently well known (especially the overall cyclonic circulation in all basins), short-term and meso-scale variability might be high and the complex circulation of the shallow (on-shelf) areas is still poorly understood. Such variability and complexity is not captured by averaged current patterns and hydrographic features calculated in Deliverable 09. This is especially true for Mediterranean islands, such as the Balearic islands (GSA 05), Corsica (GSA 08), Sardinia (GSA 09), Crete (GSA 23) and Cyprus (GSA 25), for which the hydrographic connection with 'mainland' GSAs is very unclear.

For the purposes of STOCKMED, we can reach to the following general conclusion:

Under the general cyclonic circulation pattern, certain permanent surface currents flow parallel to the coast in many areas, usually meandering around the shelf break. Such currents, like the Northern Current (NC) in the NW Mediterranean, the Atlantic Ionian stream (AIS) in the Sicilian channel, the Western Adriatic current (WAC) in the Adriatic and the Black Sea Water current in the North Aegean, are very important for GSAs with relatively large continental shelves acting as barriers to the advection of larvae of epipelagic and shelf-dwelling species in offshore, oligotrophic waters (they consist strong thermohaline fronts between coastal and offshore waters).

These currents can transport eggs and larvae from one GSA to an adjacent GSA, as has been shown for small pelagic fish in the NW Mediterranean and the Adriatic (see review in Deliverable 05). The advection of larvae of epipelagic and shelf dwelling species by the Northern current from GSA 07 (Gulf of Lions) to GSA 06 (Catalan Sea) is likely to result in their high survival (and subsequent recruitment) in the latter area as the shelf waters of GSA 06 are also very productive.

The same is true for larvae transported from GSA 17 to GSA 18 (for example the Gulf of Manfredonia is a well-known nursery area of small pelagic late larvae and juveniles, originating mainly from GSA 17).

Finally, in the Sicilian Channel larvae may be transported with high survival probabilities from GSA 16 (South of Sicily) to GSA 15 (Malta Island) where a prominent retention area for ichthyoplankton has been identified (see, in particular for anchovy, the recent study by Palatella et al. [2014] who performed numerical simulations of Lagrangian transport of eggs and larvae and estimated success rates in the connectivity between spawning and nursery areas).

With current knowledge, hydrographic connectivity through larval transport (and subsequent increased probability of survival) can only be assumed between **GSA 06 & 07**, between **GSA 17 & 18** and, at a smaller geographical scale, between **GSA 16 & 15**.

Task 3.2 Revision and analysis of the existing information on persistent nurseries and spawning areas of the target species in the investigated areas. (task coordinator F. Colloca).

Essential fish habitats (EFHs)

Concerning the task 3.2:

- An overview of MEDISEH results (14 species) with presentation of maps of persistent nursery and spawning habitats were provided
- Additional spatial analyses of MEDITS data for the species *Eledone moschata*, *Lophius budegassa*, *Octopus vulgaris*, and *Solea solea* were carried out using the same approach as in MEDISEH.

Regarding revision and analysis of the existing information on the position of the persistent nurseries and spawning areas of the target species in the investigated areas, results of the MAREA MEDISEH project were acquired for most of the target species. New analysis have been carried out for the target species not included in the MAREA MEDISEH project (*Eledone moschata, Lophius*)

budegassa, Octopus vulgaris, and Solea solea). The identification of nurseries and spawning grounds for these species was based on data collected during the MEDITS survey from 1994 to 2011. Observed survey indices for juveniles and spawners (possibly using also the GFCM grid as spatial scale for the analyses) are going to be analysed using the same standardized procedure as in MEDISEH. The four species showed however low and highly variable survey abundance and occurrence, reducing the possibility to model the spatial distribution of recruits and spawners in most of the areas.

All the maps of distribution of nurseries and spawning grounds, produced during the MEDISEH project, are available under the MEDISEH GIS-web. All the maps of distribution of the most persistent nurseries and adults/spawners aggregation areas used for the purposes of STOCKMED are provided in the Deliverable 10.

Task 3.3 Revision and analysis of the existing information on fishing grounds of target species in the case studies (task coordinator M. Sbrana)

Within the short timescale of the STOCKMED project, an effort was made to collect and review all available information on the spatial and temporal distribution of fishing effort/grounds in relation to the STOCKMED target species. The main aim was to provide, where possible, geo-referred information that could be useful for the analyses of WP4.

The fishing grounds identification task (Task 3.3) had to be mainly based on the revision and analysis of information that already existed. The plan was to use available reports, GFCM publications, grey literature, peer-reviewed papers, unpublished data available to the Consortium, as well as expert knowledge, to improve our understanding on the extent of the main fishing grounds. The bibliographic search revealed that in many Geographic Sub-Areas there is very few published information and, in many cases, *ad hoc* maps were produced based on the knowledge of researchers and supported by information provided by the fishermen. VMS data were also used to assess the spatial distribution of fishing effort and to identify the horizontal extend of fishing grounds.

For the GSAs surrounding Greece, a new methodology was devised to disentangle speciesspecific potential fishing grounds, by combining predictions of statistical habitat models (probability of species presence) with VMS-derived estimates of the fishing effort. However, given that habitat modeling was largely dominated by data derived from surveys at sea (pooled over a large annual time period, not taking into account seasonal changes in species and/or fishing effort distribution), this new approach should be considered with caution, until tested with more precise data and methods (e.g. methods combining logbook with VMS data).

Finally, a new approach has been developed to provide estimates of potential fishing areas of the vessels registered in the EU ports, according to the Fleet Register of the European Union. The estimation of the fishing grounds is based on specific criteria which include:

- The bathymetry for each gear type and fleet segment
- a spatial activity index for registered vessels at the fishing ports

- additional spatial information at National and EU level (closed areas, Marine Protected Areas etc.)
- the assumption that fishermen prefer to operate in areas which are more productive (CHL)
- distance from coast calculated in nautical miles and expressed by the minimum distance of each fishing rectangle's centoid from the coastline

The results obtained with this new method were in line with available information, especially for vessels smaller than 24 m.

Task 3.4 Synthesis of knowledge related to the spatial pattern of physical breaks, critical areas, and fishery effort relevant for stock unit investigation and identification of the main knowledge gaps (task coordinator S. Somarakis).

In the task 3.4, the cases in which persistent nursery and/or spawning sites defined in different (adjacent) GSAs are located in close proximity, implying the potential continuity of EFHs across the adjacent GSAs, were underlined.

Based on proximity of EFHs, we came up with propositions for GSA groupings (Table 3.1). In our approach, we assumed that GSAs with continuous or nearby EFHs (therefore assigned to the same group) have an increased probability of being inhabited by the same stock.

Table 3.1. Propositions for GSA groupings (different colors) based on the continuity or proximity of EFHs (recurrent nursery or spawning grounds) of STOCKMED species. The groupings were based on the scrutiny of corresponding maps presented in Deliverable 10 and imply an increased probability that the corresponding GSAs are inhabited by the same stock.

	GSA																
Species	1	5	6	7	8	9	10	11	15	16	17	18	19	20	22	23	25
Merluccius merluccius													*				
Galeus melastomus																	
Pagellus erythrinus																	
Parapenaeus													*				
longirostris																	
Aristeus antennatus																	
Aristeomorpha foliacea																	
Nephrops norvegicus																	
Illex coindetii																	
Eledone cirrhosa																	
Sardina pilchardus																	
Engraulis encrasicolus																	

Trachurus							**	**		
mediterraneus										
Trachurus trachurus							**	**		
Scomber scombrus							**	**		
Scomber colias							**	**		

* southern part of GSA 19 (off eastern Sicily) could be grouped with GSAs 15 &16 and northern part with GSA 18

** for medium pelagics and possibly other species, the northern part of GSAs 19 & 20 could be grouped with GSA 18.

General conclusions, comments and recommendations regarding Table 1 are summarized below:

- For all species with adequate data to model EFHs, GSAs with proven 'hydrographic connectivity', namely GSA 06 & 07 in the NW Mediterranean and GSAs 17 & 18 in the Adriatic appear in the same group.
- For all species with adequate data to model EFHs, GSA 09 & GSA 10 are grouped together.
- For all species with adequate data to model EFHs, GSA 15 & GSA 16 are grouped together.
- With regard to pelagic and shelf dwelling species, GSA 1 is most likely separated from GSAs 5 & 6. This can be attributed to the presence of the Almera-Oran front (a known biogeographic boundary) and is supported by a recent modeling study on anchovy. This does not seem to apply for deep water species (such as *Parapenaeus, Illex, Aristeus*).
- Under a 'precautionary' (parsimonious) approach, GSA 05 could be grouped with GSA 06 & 7 for deep water species.
- The lack of information from GSA 08 (Corsica) represents an impediment to evaluating the connectivity between island GSAs in the western Mediterranean (namely GSA 08 & GSA 11 [Sardinia]) with 'mainland' GSAs, such as GSA 09 & 10. Under a 'precautionary' approach, GSA 08 & GSA 11 could be considered together with GSA 09 & 10 (i.e. assuming that they are inhabited by the same stocks) at least for deep water and medium pelagic species.
- In *Merluccius* and *Parapenaeus*, persistent EFH sites in southern GSA 19 (off eastern Sicily) seem to be extending from those in GSAs 15-16. Similarly, for deep water and medium pelagic species, persistent EFH sites in northern GSA 19 are in close proximity to those in GSA 18. In that sense, the line separating GSA 15-16 and GSA 19 could be moved more northerly (between the Italian peninsula and Sicily) for defining stock boundaries.
- The connectivity of northern GSA 20 and GSA 18 is not clear and warrants further investigations.
- Under a precautionary approach, GSA 22 (Aegean) and 23 (Crete) could be considered together for deep water species.

DIFFICULTIES ENCOUNTERED AND REMEDIAL ACTIONS

Definition of essential fish habitats

Problems encountered in defining EFHs are summarized in Table 3.2.

Table 3.2.	Conclusions	from	the	scrutiny	of	analyses	and	maps	presented	in	Deliverable	10	(essential
habitats).													

Species	Problems and gaps in defining EFHs
Merluccius merluccius	During MEDITS, the catch rate of mature hake in most GSAs is generally very low and gonads are mostly inactive. This represents an impediment to our understanding of the extent and continuity of hake spawning grounds in the Mediterranean
	and their link to defined nursery grounds.
Solea solea	MEDITS data not suitable for defining the EFH of sole in most of the areas except GSA 17 and 11. In this latter GSA, only aggregation areas of spawners were identified. The distribution and connection of sole EFHs have only been identified in GSA 17 based on the SOLEMON beam trawl survey.
Mullus barbatus	The timing of the MEDITS survey does not match the recruitment period of <i>M. barbatus.</i> This represents an impediment to defining the extent and continuity of nursery grounds and their connection with identified spawning grounds.
Mullus surmuletus	During MEDITS, the catch rate of recruits and mature striped red mullet is generally very low with the exception of the Strait of Sicily, Sardinia and the Aegean Sea. The survey does not help to define the whole extend of stripped red mullet EFHs in the Mediterranean.
Pagellus erythrinus	The timing of the MEDITS survey does not match the recruitment period of <i>P. erythrinus.</i> This represents an impediment to defining the extent and continuity of nursery grounds and their connection with spawning grounds.
Aristeus antennatus	The youngest specimens of the species are scant and mainly live on bottoms deeper than 800 m, i.e. beyond the depth range sampled by the MEDITS survey. This represents an impediment to defining the extent and continuity of nursery grounds and their connection with spawning grounds.
Additional STOCKMED species: Eledone moschata, Lophius budegassa, Octopus vulgaris, Solea solea	The four species exhibit high spatial variability in abundance and frequency of occurrence during the MEDITS survey. This hindered spatial modelling in most GSAs, thus limiting the usefulness of MEDITS data to understanding the spatial connectivity between nurseries and spawning areas.

Fishing grounds

The work done in Task 3.3 identified major information gaps which, currently, represent an impediment to identify species-specific spatial fishing patterns and facilitate the identification of stock boundaries. Except of the major problem of assigning fishing effort to target species, published

information on fishing grounds is scant and fragmentary whereas the Consortium was unable to collect any information at all for several GSAs:

GSA2 – Alboran Island GSA3 – Southern Alboran Sea GSA 7 – Gulf of Lions GSA 8 – Corsica GSA 17 – Slovenian and Croatian side GSA 22 – Turkish side

In conclusion, during the activities carried out in Task 3.3, a great effort was made to produce maps useful for the activities of WP4. Results showed that, for most of the target species, data are heterogeneous and fragmentary, and only in few cases was it possible to produce exhaustive maps of the fishing grounds. Therefore, the new approach based on the use of the EU Fleet Register to estimate potential fishing grounds seemed to be the most suitable. The results obtained with the new method were in line with available information, especially for vessels smaller than 24m. For larger vessels, mainly located in the Gulf of Lions, in Sicily (Mazara del Vallo) and the Adriatic Sea, VMS data were the most appropriate source of information, as this part of the fleet operates far from the home ports, performing trips of many days.

As agreed at the meeting held in Rome on November 2013, maps of fishing grounds will not be used for the direct definition of stock boundaries.

REFERENCES

- Pinardi, N., Zavatarelli, M., Adani, M., Coppini, G., Fratianni, C., Oddo, P., Tonani, M., Lyubartsev, V., Dobricic, S., Bonaduce, A., 2013. Mediterranean Sea large-scale low-frequency ocean variability and water mass formation rates from 1987 to 2007: a retrospective analysis. Progress in Oceanography. DOI: 10.1016/j.pocean.2013.11.003.
- Palatella, L., F. Bignami, F. Falcini, G. Lacorata, A. S. Lanotte, and R. Santoleri, 2014. Lagrangian simulations and interannual variability of anchovy egg and larva dispersal in the in the Sicily Channel, J. Geophys. Res. Oceans, 119, 1306–1323, doi:10.1002/2013JC009384.

WP4. Identification of stock units and stock boundaries by multi-criteria approach.

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BACKGROUND

In the last decades, a variety of methods from different disciplines have been developed and promoted as proper ways to deal stock identification. At the same time, there has been a growing recognition, in fisheries sciences, that no single method is able, alone, to address the multidimensional nature of stock, the various assumptions of stock assessment and the istances of fishery management. An integrated approach is needed to deal with this problem in an effective way, incorporating different criteria, disciplines, methods and expert knowledge (Pawson & Jennings, 1996; Begg & Waldman, 1999; Cadrin et al., 2005; Cadrin et al., 2014).

A general agreement exists about the advantages of conducting stock structure investigations with an interdisciplinary approach from the onset (Abaunza et al., 2014; Higgins et al., 2010; Sala Bozano et al., 2009; Sala Bozano & Mariani, 2011). This methodology allows collection of multiple stock descriptors on the same individuals, hence providing the opportunity for a comprehensive quantification of population structure in a common statistical framework, and without the limitations associated with comparisons among different sets of data collected at different times and often with different purposes.

In practice, this approach is rarely feasible on a large scale and for the variety of species of commercial interest. A significant contribution toward improving the knowledge on stock structure of most species is therefore expected from re-analysis of existing data/information in a holistic multidisciplinary perspective. 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 involves:

1) a comprehensive multidisciplinary review of available information,

2) interdisciplinary analysis for synthetic conclusions, and

3) practical considerations of monitoring, assessment, and management.

The challenge to face is therefore:

1) to critically review available data/information and select relevant descriptors for investigating stock structure;

2) to develop a method to standardize and effectively integrate such highly heterogeneous types of data/information.

The complexity of these tasks is rooted in the variety of goals, time and spatial scales of collection of historical datasets and published research studies as well as in their likely different quality and accuracy. Indeed, the review of updated literature showed the scarcity of consolidated methods based on rigorous formal approach to identify stock units. This point seems to be critical and underlines the need to develop formal methods able i) to deal with explicit spatial formulation of large sets of multidisciplinary data, ii) to incorporate experts' knowledge iii) to reconcile multiple perceptions of stock units which may be conflicting, and finally iv) to pursue the objective of reducing the well-known subjectivity in defining stock units.

OBJECTIVES

According to the STOCKMED proposal the WP4 aims to provide a putative definition of stock structure and boundaries identified on the basis of multi-criteria approach. In particular the WP aims at:

1) developing a GIS-MCDA framework for stock unit identification based on multiple sources of data and knowledge (sea bottom topography, current pattern, morphology and meristics, biological traits, parasite occurrence, migration routes, genetics and species distribution, essential fish habitats, etc..).

2) applying the developed framework to selected case studies to reconcile possible conflicting signals from the analyses and outcomes from WPs 1-3.

In addition, WP4 aims to organize the most significant outputs in terms of maps, delivered by WP 1, 2, 3 and 4, in a GIS database under a common geo-referenced system and common data format. In particular, it is decided to use the same platform (Data Viewer) developed for the MEDISEH project.

DELIVERABLES AND MILESTONES FORESEEN BY THE PROJECT

All Deliverables due by WP4 were completed and made available on the MAREA ftp:

D13 – Geo-referenced data base (Responsible HCMR).

D14 - Protocol of methodology to implement the GIS_MCDA framework (including identification of criteria, decision trees, weighing factors, etc..) (Responsible CoNISMa - "Tor Vergata" University of Rome).

D15 – Implementation of the GIS_MCDA framework scoring and comparing criteria and decision factors with the preparation of a synopsis of the stock units and the corresponding stock boundaries (Responsible IAMC-CNR).

The expected Milestones have been reached, as documented by the released Deliverables:

M 4.1 Structuring the geo-referenced data base;

M 4.2 - Literature review on different approaches for developing weights and evaluation rules of the GIS-MCDA;

M 4.3 Implementation of the GIS-MCDA framework scoring and comparing criteria and decision factors.

RESULTS ACHIEVED

Task 4.1 Organize all data sets and output products from WP 1, 2 and 3 in a GIS database under a common geo-reference system and common data format (Leader: HCMR)

All datasets (in the form of shapefiles) and associated INSPIRE-formatted metadata files (in XML format), delivered by the different WPs, were zipped in individual files for each species and placed in a geodatabase for display and downloading through the project's Data Viewer (Valavanis et al., 2013).

The Data Viewer is an enhancement of the related work carried out through the MEDISEH project and now the viewer includes all MEDISEH and STOCKMED datasets for viewing as well as downloading.

The viewer itself is a customized Java applet application embedded in an HTML environment including the data display area and the data downloading area. The viewer may be accessed through the main MAREA website (http://www.mareaproject.net/) and becomes fully accessible after a user registration.

The STOCKMED geodatabase is an ArcGIS 10.0 geodatabase located at the MAREA ftp site (under STOCKMED directory).

Some examples of the STOCKMED enhancements of the Data Viewer follow (Fig. 4.1, Fig. 4.2).



Fig. 4.1. Biological descriptors in the format of polygon shapefile at GFCM statistical rectangle level.



Fig. 4.2. Clusters of correlation of biomass trends in the format of polygon shapefile at GSA level.

Shapefles and Metadata (zip)							
Best une right olick -> Save Link Ar							
	Diological			Correlatio	n Trends		
SPECIES SHAPEFILE AND METADATA (zip)	Descriptors	Biomass	Density	Growth	Parasites	Reproduction	Genetics
Glorit red string (Aristeeonorpha toliacea)	Senitor.	APPERCALARMAN	ABUALamatu	ARTECOMMENT		ANTANINENAN	second and
Dive and red styling (Anisteus antennatus)	Disk106			ARTANDEDH			ARTANTan
Hirned octopus (Elevisee alivitosa)	BLASKIR.	Sector sector sector	BLESS BARRIER	1			1
Musky octopus (Eledone moschata)	B.EDMOS	BAUM/Sconess					
European anchovy (Engraufis encreat/colirs)	DOPEN					ENGENERATION	BORDSON
Backmouth catchers (Galeus melastomics)	GALLMEL	GALIMELINAMENT	GALLMELmonty				
Broadial squit (illex canalett)	LLEGO	\$1500mment	ALPCONTARY.			ALL RECOMMENDERS	
Back belled angler (Lophius budegassa)	LOPHELE	LOPER, DECEMBER	LOP B. Compto				
European hake (Merclacolas merclacolas)	HERARE.	MERLIETUNINELL	MELMERMONT	MERLMERLING	HERAMPHICS.	UERLASTrans.60	MERLINE IN
Red mulet (Mullins barbatus)	MALEAR	MALLEARDINGST		MALDARIE-			MALDARD
Styped red malet (Mullus surmaietus)	MANSIN	MALLIL PROPERTY.	MARLEY BOWNEY			MALLERMONT	maintener
Norway lobster (Neptrops norvegicas)	agennoe	IEFG2CHigman	1800 Committee				Deposition
Common octopub (Octopus volgaris)	SSIGNAL	OCTO/GLASSINGS	OCTOM Asternativ				COLUMN AND
Common pandora (Pagellus endivirus)	EAGERY	Inclusion incoments	excessionerste	BAGERS VORDARD			
Deep water pirk streng (Parapenaeus Iongirostris)	WB.OI	RAISS CRAMMERS	Endling reasoning			RADIA CORRECTION	PARTICULAR DE
European pilchard (Santina pilchardys)	SARCEL	Water and a second	SASSAL annats	-		Construction and	SALENTAR
Common sole (Soliva vulgarris)	SCARVIA.	5		SOLULAR DESCRIPTION OF			SCLOAL M
Modberranean horse macherel (Trachurus mediterraneus)	TRACMED	TRACHICOMMUNIC	Installighter the				
Alignity house machinesi I Tracharas Interferenti	TRACTRA	TALCIPADEMANT	TRACTICA departs	THAT TRANSMENT	TRACTROOM.		

Fig. 4.3. The table of STOCKMED shapefile data and associated metadata (all in zip format for individual species) located at the data downloading area of the Data Viewer

Task 4.2 Development of a framework for the application of GIS-MCDA to stock unit identification (Leader: CoNISMa - "Tor Vergata" University of Rome)

The STOCKMED methodological framework

A methodological framework was developed to perform studies of stock identification in the Mediterranean by integrating multidisciplinary perspectives. The specific context is that of studies based on available scientific data, expert knowledge and published literature. This implies the necessity to collate and integrate highly heterogeneous types of data/information which may differ with respect to the goals, time and spatial scales of collection as well as with respect to their quality and accuracy. The strength of the proposed STOCKMED methodological framework relies on its explicit spatial formulation and the incorporation of experts' judgment at different steps of the process. To enable this, an original approach combining Geographical Information Systems (GIS), tools of spatial analysis and Multi-Criteria Analysis (MCA) (Figueira et al., 2005; Moffett and Sarkar 2006; Greene et al., 2011) was proposed.

In synthesis, the methodological framework develops in two main phases and uses two different spatial scales (Fig. 4.4).

Generating the alternative Hypotheses

In the first phase, different hypotheses of stock structure are identified at a spatial scale lower than the current management units (GSAs) applied in the Mediterranean, that is the GFCM $30' \times 30'$ cells

grid (GFCM Recommendation GFCM/33/2009/2). Six biological indicators (Tab. 4.1) provided by the MEDITS surveys (and computed within the WP1) are mapped into the GFCM grid and used to generate a finite number of different hypotheses of stock units through Constrained Clustering (CC), a semi-supervised learning algorithm, which guarantees that putative stock units occupy fully connected subareas of the Mediterranean Sea. As a matter of fact, the different indicators are characterized by a different relevance with respect to the objective of identifying the stock units, which need to be included into the evaluation. The Analytical Hierarchy Process (AHP) methodology, originally developed by Saaty (1980; 2008), is used to estimate weights of biological indicators



Figure 4.4. Analytical framework of the decision problem aimed at identifying stock units of target species in the Mediterranean

according to the judgment of a panel of experts (see paragraph below). Hence, the spatial constrained clustering is performed on the set of six indicators, using the weights vector in the variance/covariance matrix, for a number of clusters ranging from 2 to 20. After that, the Calinski-Harabasz (CH) index (Calinski & Harabasz, 1974) is computed to evaluate the effectiveness of choosing a given number of clusters, not known *a priori*. At the end of this phase, a set of scored *alternatives* representing Hypotheses of stock units are available.

DATA Domain	Criteria I level	Criteria II level	Indicators USE	Scale
	Population parameters from MEDITS surveys	Abundance Demography Life history traits	1.Biomass indexPhase 22.Inverse of CV of Density-3.Mean Fish Weight-4.Sex ratio-5.% of spawnersalternative6.Median length of spawnershypotheses of stock units	GFCM grid
ological data	Population parameters from literature review Abundance	Growth parameters Maturity parameters	 Growth performance index Lm50% Spawning season Correlation of Density Phase 3 trends 	
Bic	trends (from MEDITS surveys)	Biomass	5. Correlation of Biomass trends To corroborate/validat e alternative	GSA
	Genetics		6. Parasites infestation hypotheses 7. Genetic structure 1	
	EFH and connectivity		8. Location of persistent nursery and spawning areas and hydrographic connectivity	
nmental lata	Oceanographic systems	Surface layer	9. Structures of the mean To circulation -Surface layer corroborate/validat e alternative	High spatial
Enviro		Deep layer	10. Structures of the mean hypotheses circulation - Deep layer	resoluti on

 Table 4.1. List of selected criteria and indicators. The use of the criteria within the different phases of the framework is reported.

				WP5	
Fisheries	data	Fishing grounds	Modelled spatial distribution of fishing capacity	- To compare patterns of stock units and spatial distribution of fishing grounds	High spatial resolu tion

The Analytical Hierarchy Process (AHP)

The Analytical Hierarchy Process was applied to elicit the preferences of a panel of experts on the individual weight to be assigned to each criterion and indicator of the biological domain (Population parameters from MEDITS surveys) involved in the Constrained Clustering. In short the method works through different steps:

- 1. Identification of criteria and indicators (identification of the hierarchy);
- 2. Questionnaire with pairwise comparisons in order to collect preferences of a certain number of experts about the criteria and the indicators;
- 3. Transformation of pairwise comparisons in the questionnaire and calculation of weights vector for the indicators and the criteria by means of the principal eigenvector method (Saaty, 2003).
- 4. Calculation of composite weight for each indicator, multiplying the weight of each indicators by the weight of the corresponding criteria (Saaty, 2008);
- 5. Group decision making (synthesis of the prioritization performed for the different experts).

An R routine was developed to support the survey elaboration (Bitetto et al., 2013).

On the basis of the defined hierarchy (Fig. 4.5) experts' preferences were prioritized. According to these preferences (Tab. 4.2), the life history traits was the most representative criterion for the identification of putative stock units (55%), followed by demography (27%).



Figure 4.5 Hierarchy of criteria pertaining to the biological domain of the population parameters from MEDITS surveys

Table 4.2. (Criteria	weights	estimated	through	AHP
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Criteria	Aggregated_preferences
LIFE HISTORY TRAITS	0.55
DEMOGRAPHY	0.27
ABUNDANCE	0.15

Consistently, the corresponding indicators (percentage of spawners and mean length of females spawners) are the ones characterized by greater score (20 and 28%), followed by sex ratio (11%) and mean individual weight (13%). On the basis of the results of the present analysis, seems that the abundance criterion has been considered weaker compared to the other criteria involved. The consistency index was smaller than 10% for all the questionnaires, probably due to the small number of questions for which this index is computable (3 questions) not allowing great inconsistencies to be put in the answers. The final output to be used to weight the single indicators in the constrained clustering is shown in Table 4.3.

Indicators	Aggregated_preferences
MEDIAN LENGTH OF SPAWNERS	0.280
% of SPAWNERS	0.200
MEAN INDIVIDUAL WEIGHT	0.130
SEX RATIO	0.110
INVERSE CV OF DENSITY	0.071
BIOMASS INDEX	0.067

Table 4.3. Indicators weights estimated through AHP

Selecting the most plausible alternative Hypotheses

In the second phase, all the thematic descriptors produced at the GSA scale, by the different WPs, such as genetics, parasites, growth, correlation of abundance trends and so on, are used to reinforce and/or validate the different hypotheses available. This is accomplished by computing the Cohen's Kappa coefficient of agreement (Cohen, 1960) between each hypothesis and each thematic descriptor. In order to score the relative importance/impact of the different thematic layers, the approach known as Non-Structural Fuzzy Decision Support System (NSFDSS) (Chen, 1998; Tam et al., 2006) has been implemented (see paragraph below). NSFDSS applies fuzzy logic to model the ambiguity and imprecision of vague terms used by experts to express their preferences about criteria/thematic descriptors and/or evaluate the quality/quantity and coverage of the available information. One of the outputs of NSFDSS is a weights vector for the different thematic descriptors (see paragraph below). The Cohen's Kappa coefficients and the weights vector estimated through NSFDSS form the basis to construct the weighted decision matrix and, ultimately, calculate the Cohen's Kappa weighed mean which allows to rank the hypotheses. At the end of the process, the hypotheses that fall above the upper quintile of the mean Cohen's Kappa distribution are retained as candidate hypotheses of stock structure based on the best possible use of available multidisciplinary data.

The Non-Structural Fuzzy Decision Support System (NSFDSS)

As already said, the Non-Structural Fuzzy Decision Support System methodology was applied to weight appropriately the different criteria/thematic descriptors, taking into account the quality/quantity and coverage of the available information.

To rank the expert preferences, a 2 phases process was applied (Fig. 4.6). This was deemed necessary given the complexity of the problem. In the first phase experts evaluated the available information for each combination of thematic descriptor and species.

The results were used for twofold objectives and obtained in two phases which were complementary in a sequential order:

- 1) phase 1: for estimating a standardized matrix, which assisted both the compilation of the questionnaires in the successive phase 2, when the thematic descriptors were ranked with associated scores, and to feed WP5 for the knowledge gap analysis;
- 2) phase 2: estimating a standardized vector with associated scores to rank the species over thematic descriptors for the successive phase represented by the Non-Structural Fuzzy Decision Support System analysis.

NSFDSS is an MCDA tool, belonging to the methods of deterministic preference modeling. It is used for ranking a set of possible decisions on the basis of agreed decision factors. In our case the goal was to discriminate stock units on the basis of thematic descriptor weights. Decision factors were the species, each with its specific background of information (spatial coverage, temporal coverage, number of papers scrutinized, expert evaluation of global quality), associated to each thematic descriptor.



Figure 4.6 The two phases process of Non-Structural Fuzzy Decision Support System (NSFDSS) implementation

Nineteen decision factors (species) were used in the analysis and 7 alternatives represented by the thematic descriptors were tested. Finally 45 pairwise combinations were tested for each species, for a total of 855 pairwise combinations for each expert. For obtaining the aggregated preference over all the experts a geometric weighted mean over 15 experts was computed using a routine developed in R environment. In Table 4.4, the aggregated vector of the preferences estimated by geometric mean and standardized is reported.

Decision	Aggregated preference	Aggregated preference standardized
Genetics	0.741	0.215
Critical area and connectivity	0.704	0.205
Growth parameters	0.598	0.174
Reproduction L50	0.398	0.116
Oceanographic system surface	0.268	0.078
Parasites distribution	0.194	0.056
Abundance trends biomass	0.156	0.045
Reproduction spawning season	0.154	0.045
Abundance trends density	0.146	0.042
Oceanographic system deep	0.084	0.024

Table 4.4. Aggregated preference vector estimated by NSFDSS.

The Stochastic Multicriteria Acceptability Analysis (SMAA)

In the final step of the methodological framework, a sensitivity analysis is conducted to model uncertainty and judge the stability of the results. The Stochastic Multicriteria Acceptability Analysis (SMAA) (Butler et al., 1997), based on Monte Carlo simulation, is selected as a method allowing to take into account, simultaneously, the uncertainty about the criteria and their weights. SMAA provides, for each alternative hypothesis, a vector of rank acceptability indices which measure the stability of the assignment of the alternative hypothesis to a given rank, and can be interpreted as the probability that the alternative appears in a given position in the rank order (Tervonen et al., 2009). The hypotheses that receive high acceptability for the highest ranks are the most relevant. The rank acceptability indices for each alternative hypothesis, which sum to unity, are finally aggregated in a Holistic Acceptability Index (HAI), measuring the overall acceptability of the alternative.

As said before, the alternatives characterized by values of mean Cohen's Kappa above the upper quintile are retained as candidate hypotheses on stock structure. Acceptability analysis is applied to assess the robustness of the obtained ranking of hypotheses and to take a more informed decision. Alternatives with high acceptability for the best ranks and high Holistic Acceptability Index are the most plausible hypotheses.
Task 4.3 Application of the GIS-MCDA framework developed in Task 4.2 to the case studies for the identification of stock units and/or the detection of stock boundaries, reconciling possible conflicting signals from the analyses and outcomes from WPs 1-3. Reporting data and results of WP4 as geo-referred information and maps showing the provisional conclusion about stock structure (spatial based) on basis of GIS_MCDA (Leader: IAMC-CNR)

Within Task 4.3, the developed methodological framework was applied to the 19 fish and shellfish target species of the project.

In the following, the different steps and the outputs of a complete run of the STOCKMED framework are presented using the Hake case study for explicative purposes. The full results (maps and graphic outputs) for all 19 species are reported in the Deliverable 15, while only the discussion is included in this report, with indications about most plausible hypotheses of stock structure for all species.

The European hake case study

- 1. The first operation is to construct a grid map of the potential distribution of the species, at GFCM statistical rectangle level, wherein all cells are fully connected as required by the Constrained Clustering (see map below). This is realized considering the bathymetric range of the species, known from literature, and its actual distribution obtained from MEDITS data.
- 2. The CC is performed on the selected biological indicators, using the weights provided by AHP, for a number of clusters ranging from 2 to 20. The Calinski-Harabasz index (CH) is computed for each clustering and plotted against the number of clusters. The higher the index CH, more effective is the clustering in terms of ratio between within-group similarity and between-group differences.









3. The 19 maps corresponding to each clustering are produced (the maps corresponding to 4 and 7 clusters respectively, are reported as example). They represent the alternative hypotheses of stock structure generated by the CC algorithm. Each map is scored with the corresponding value of the CH index.





Merluccius merluccius - Num of Stocks = 7



4. The thematic layers available for the species are produced as geo-referenced map











- 5. The pairwise spatial agreement between each alternative hypothesis and each thematic layer is calculated using the Cohen's Kappa statistic
- 6. The Cohen's Kappa coefficients and the vector of weights estimated through NSFDSS, form the basis to construct the Decision Matrix

					CRITERIA				
		Gr	L ₅₀	Gen	Par	EFH	СТ _{ві}	CT _{DI}	Weighed mean
V	Veights	0.1740	0.1160	0.215	0.056	0.2050	0.0450	0.0420	Cohens' Kappa
	2	0.0156	0.1402	0.1325	0.0579	0.0785	0.0947	0.0000	0.0833
	3	0.0964	0.1402	0.1538	0.3613	0.0785	0.0947	0.0000	0.1251
	4	0.1117	0.1551	0.5575	0.1701	0.1117	0.0947	0.0000	0.2274
Δ	5	0.1597	0.4128	0.5950	0.2774	0.3701	0.0923	0.0000	0.3507
÷.	6	0.4015	0.5576	0.4406	0.0975	0.4586	0.1318	0.1424	0.3994
Ŧ	7	0.6656	0.3683	0.2212	0.0932	0.1144	0.1318	0.0485	0.2846
÷.	8	0.2492	0.2454	0.0926	0.0928	0.1144	0.1318	0.0519	0.1506
R	9	0.2430	0.2456	0.1231	0.0793	0.1139	0.1086	0.0603	0.1553
N	10	0.2406	0.2504	0.1053	0.0692	0.1139	0.1086	0.0603	0.1503
Δ	11	0.2284	0.2270	0.0895	0.0599	0.1009	0.1086	0.0603	0.1369
т	12	0.1671	0.1347	0.0895	0.0573	0.0887	0.1086	0.0603	0.1087
÷	13	0.1019	0.1023	0.1008	0.0318	0.0454	0.1086	0.0539	0.0815
v	14	0.0979	0.1047	0.0849	0.0311	0.0405	0.1086	0.0524	0.0757
F	15	0.0929	0.0958	0.0627	0.0307	0.0377	0.0982	0.0537	0.0667
s	16	0.1162	0.0962	0.0584	0.0279	0.0546	0.0982	0.0537	0.0743
Ŭ	17	0.1179	0.0952	0.0545	0.0253	0.0546	0.0982	0.0537	0.0733
	18	0.0754	0.0662	0.0553	0.0086	0.0532	0.0900	0.0537	0.0591
	19	0.0714	0.0632	0.0518	0.0086	0.0500	0.0739	0.0558	0.0554
	20	0.0680	0.0633	0.0397	0.0132	0.0500	0.0739	0.0558	0.0520

7. The Cohen's Kappa coefficients is plotted against the number of clusters. The Cohen's Kappa coefficients are calculated as pairwise spatial agreement between each alternative hypothesis (with a given number of clusters) and each thematic layer. Besides, the weighed mean Cohen's Kappa is plotted against the number of clusters. For a given hypothesis, it is calculated by averaging the Cohen's Kappa coefficients across the criteria, weighted by their relative importance estimated through NSFDSS



Merluccius merluccius - Cohen's Kappa coefficients for each layer

Merluccius merluccius - Cohen's Kappa weighed mean



8. The graph reporting the Cohen's Kappa vs the CH index is plotted. It plots the position of each hypothesis in the 2D space described by the weighed mean Cohen's Kappa and the CH index. The threshold corresponding to the upper quintile of the Cohen's Kappa distribution is drawn in the graph. The hypotheses which have mean Cohen's Kappa greater than the threshold are highlighted and selected as the most probable hypotheses of stock structure



Merluccius merluccius - Cohen's Kappa vs CH index

9. Sensitivity analysis is conducted to model uncertainty and to judge the stability of the results. The heat map of the matrix of the Rank Acceptability Indices (RAI) is plotted. It originates from the 2D display of the values of the RAI matrix in which hypotheses are on the rows and the rank in the columns. In the map, the pixels are colored in proportion to the acceptability that a given hypothesis appear in a given position of the rank order.



Merluccius merluccius - Rank Acceptability indices

10. Finally the distribution of the individual hypotheses in the multidimensional space formed by the three indices - Cohen's Kappa coefficient, Calinski-Harabatz index, Holistic Acceptability

Index - is plotted. The graph allows to investigate the degree of concordance among the different indices in ranking the alternative hypotheses



Merluccius merluccius - Combined Indexes

- 11. The best ranked hypotheses on stock structure according to the holistic approach are finally presented. For each hypothesis different graphic outputs are produced and organized in individual sheets:
 - a) the map of the selected hypotheses on stock structure. The number of identified putative stocks and the value of the Holistic Acceptability Index of the hypothesis is reported
 - b) a table reporting the basic statistics median, first quartile Q1, third quartile Q3 of the biological indicators which characterize the individual clusters
 - c) a spider graph displaying the median values (on a min-max scale) of the biological parameters
 - d) a plot in which the logarithm of the median BI is plotted for all the clusterings produced by CC, ordered in a descent manner. A line identifying the quantile of order 0.05 of the median BI distribution is superimposed, as well as the points identifying the median BI of the individual clusters of the selected hypothesis. The graph allows to inspect where the median BI of individual clusters is located with respect to the overall distribution of values and to detect eventual clusters showing very low biomass value



Merluccius merluccius - Num of Stocks = 4 - HAI = 0.72





	CV%_DI (N/km2)			E	31 (kg/km2	2) W (kg) Sr				%SF			MeL_SS (mm)					
Cluster	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
1	0.004	0.005	0.007	11.570	24.530	43.210	0.060	0.117	0.206	0.462	0.526	0.595	0.001	0.034	0.083	359.063	385.932	414.814
2	0.007	0.009	0.012	22.131	35.475	59.344	0.057	0.102	0.184	0.467	0.531	0.590	0.000	0.010	0.047	306.208	348.913	380.869
3	0.006	0.007	0.007	2.368	2.680	3.098	0.177	0.183	0.194	0.528	0.545	0.549	0.000	0.000	0.000	324.659	327.014	328.952
4	0.005	0.007	0.009	3.766	11.042	16.400	0.101	0.118	0.141	0.678	0.714	0.735	0.022	0.035	0.081	327.900	339.538	348.585









	CV%_DI (N/km2)			BI (kg/km2)			W (kg)			Sr				%SF		MeL_SS (mm)			
Cluster	Q1	Median	Q,3	Q1	Median	Q,3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	
1	0.004	0.005	0.007	11.570	24.530	43.210	0.060	0.117	0.206	0.462	0.526	0.595	0.001	0.034	0.083	359.063	385.932	414.814	
2	0.008	0.010	0.012	23.540	37.815	54.108	0.040	0.062	0.129	0.404	0.464	0.517	0.030	0.070	0.115	300.961	340.000	375.360	
3	0.006	0.007	0.007	2.368	2.680	3.098	0.177	0.183	0.194	0.528	0.545	0.549	0.000	0.000	0.000	324.659	327.014	328.952	
4	0.005	0.007	0.009	3.766	11.042	16.400	0.101	0.118	0.141	0.678	0.714	0.735	0.022	0.035	0.081	327.900	339,538	348.585	
5	0.007	0.009	0.012	21.363	32.068	63.193	0.088	0.139	0.216	0.523	0.557	0.615	0.000	0.001	0.010	311.262	349.785	381.554	









	CV%_DI (N/km2)			B	I (kg/km2	.m2) W (kg) Sr %SF					MeL_SS (mm)							
Cluster	Q1	Mediar	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
1	0.005	0.006	0.009	10.960	19.910	32.070	0.034	0.069	0.126	0.522	0.569	0.623	0.000	0.072	0.197	376.241	392.545	408.000
2	0.008	0.010	0.012	23.540	37.815	54.108	0.040	0.062	0.129	0.404	0.464	0.517	0.030	0.070	0.115	300.961	340.000	375.360
3	0.006	0.007	0.007	2.368	2.680	3.098	0.177	0.183	0.194	0.528	0.545	0.549	0.000	0.000	0.000	324.659	327.014	328.952
4	0.005	0.007	0.009	3.766	11.042	16.400	0.101	0.118	0.141	0.678	0.714	0.735	0.022	0.035	0.081	327.900	339.538	348.585
5	0.007	0.009	0.012	21.363	32.068	63.193	0.088	0.139	0.216	0.523	0.557	0.615	0.000	0.001	0.010	311.262	349.785	381.554
6	0.004	0.005	0.006	12.249	27.945	46.810	0.077	0.142	0.222	0.449	0.498	0.577	0.003	0.030	0.066	351.216	383.045	429.486







	C\	/%_DI (N/kn	n2}	BI (kg/km 2)			W (kg)			Sr				%SF		MeL_SS (mm)			
Cluster	Q1	Median	Q3	Q1	Median	Q3	Q.1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3	
1	0.004	0.005	0.005	6.049	14.568	19.593	0.061	0.094	0.147	0.560	0.602	0.638	0.082	0.200	0.282	382.500	398.000	430.566	
2	0.008	0.010	0.012	23.540	37.815	54.108	0.040	0.062	0.129	0.404	0.464	0.517	0.030	0.070	0.115	300.961	340.000	375.360	
3	0.006	0.007	0.007	2.368	2.680	3.098	0.177	0.183	0.194	0.528	0.545	0.549	0.000	0.000	0.000	324.659	327.014	328.952	
4	0.005	0.007	0.009	3.766	11.042	16.400	0.101	0.118	0.141	0.678	0.714	0.735	0.022	0.035	0.081	327.900	339.538	348.585	
5	0.007	0.009	0.012	21.363	32.068	63.193	880.0	0.139	0.216	0.523	0.557	0.615	0.000	0.001	0.010	311.262	349.785	381.554	
6	0.004	0.005	0.005	12.249	27.945	46.810	0.077	0.142	0.222	0.449	0.498	0.577	0.003	0.030	0.056	351.216	383.045	429.486	
7	0.006	0.007	0.011	15.293	29.310	43.000	0.029	0.056	0.086	0.473	0.544	0.579	0.000	0.016	0.076	361.506	387.882	398.625	

Discussion and recommendations

As results of the application of the STOCKMED methodological framework, the most plausible Hypotheses of stock structure of 19 fish and shellfish species of fishery interest in the Mediterranean have been identified. In some cases, there is agreement between the ranking based on the mean Cohen's Kappa and the acceptability analysis, suggesting that a single hypothesis is highly favored. In other cases, the discrimination of the alternatives provided by both mean Cohen's Kappa and acceptability analysis is too weak to express a single preference.

It must be pointed out that despite the framework has been conceived to be applicable even in poor-data situations, the reliability of results is strongly dependent from adequate data availability within the appropriate spatial scale.

Merluccius merluccius

The stock structure hypotheses for hake were generated through CC executed using the complete set of the MEDITS biological parameters (all 6 indicators – Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females). The Calinski-Harabasz index presents a relative maximum at 5 clusters. The hypotheses were evaluated in terms of 7 independent criteria (Genetics, Parasites, EFH and connectivity, Growth, L50, Density trends, Biomass trends). The distribution of the mean Cohen's Kappa indicates the "6 stock units" as the configuration with the best agreement followed by the configurations with 5, 7 and 4 units. The acceptability analysis reinforces these results. Indeed the hypotheses with 6 units (HAI= 0.95), 7 units (HAI=0.90) and 5 units (HAI=0.85) present high acceptability indices for the best ranks and are taken as candidates for the best hypothesis of stock structure. In particular, the "6 stock units" is considered the most plausible stock structure hypothesis based on currently available knowledge.

Mullus barbatus

The Calinski-Harabasz index, measuring the performance of the CC executed using six biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females), shows a decreasing trend from 2 clusters to 6 clusters with a weak relative maximum at 7 clusters. The mean Cohen's Kappa, based on four layers of information (Genetics, Growth, Biomass trends and Oceanographic systems–surface), shows a peak at 3 clusters. The other configurations located above the upper quintile of the distribution are those with 2, 4, and 5 clusters. According to the acceptability analysis, all these candidate hypotheses receive some degree of acceptability for the highest ranks. In particular, considering the first rank acceptability index, the 3 clusters configuration appears more plausible than the 4 clusters configuration even if its HAI is lower (0.60 against 0.81). Taking into account the high number of descriptors used in the holistic approach, 6 biological indicators and 4 thematic layers of information, the results for red mullet are considered plausible.

Mullus surmuletus

In the case of *M.surmuletus*, the CC was performed on only three biological indicators (Biomass index, CV % of density, mean fish weight). The plot of the Calinski-Harabasz index shows a progressive decreasing trend with a weak relative maximum at 11 clusters. The graph of the mean Cohen's Kappa, evaluated on five layers of information (Genetics, L₅₀, Biomass trends, Density trends and Oceanographic systems–surface), shows a plateau between the configurations with 5 and 8 clusters, being the last the highest value. Considering the hypotheses falling above the upper quintile (5, 6, 7, and 8 units), the acceptability analysis suggests that the two best ranked hypotheses are the "6 stock units" (HAI=0.80) and the "8 stock units" (HAI=0.79). However the "6 stock units" also presents the highest first rank acceptability index. Based on currently available knowledge, the results for *M.surmuletus* are considered plausible.

Pagellus erythrinus

The stock structure hypotheses for common Pandora were generated through CC performed on six indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females). The Calinski-Harabasz index indicates 3 as the optimal number of clusters. The curve obtained from averaging the Cohen's Kappa coefficients across five layers of information (Growth, EFH and connectivity, Biomass trends, Density trends and Oceanographic systems–surface),) results very flat with a weak maximum at 5 clusters. The other configurations above the upper quintile of the Cohen's Kappa distribution are those with 4, 7 and 8 units. The acceptability analysis shows that the candidate hypotheses have comparable acceptability indices for the first ranks, which is also indicated by their HAI ranging between 0.72 and 0.79. Taking into account the high number of descriptors used in the holistic approach, 6 biological indicators and 5 thematic layers of information, the results for common Pandora are considered plausible.

Solea solea

Concerning *Solea solea,* the CC was performed on only two biological indicators (Biomass index, CV % of density). The Calinski-Harabasz index shows two clear peaks at 3 and 7 clusters, respectively. The graph of the mean Cohen's Kappa, evaluated on three layers of information (Genetics, Growth and Oceanographic systems—surface), shows a plateau between 5 and 7 clusters. The upper quintile includes the configurations with 5, 6, 7, and 9 units but according to the acceptability analysis only the hypothesis with 5 clusters receive a high acceptability index for the first rank. The Holistic Acceptability Index suggests that a "5 stock units" is the configurations range between 0.72 (9 clusters) and 0.84 (7 clusters). However considering that, overall, the analysis relies on only 5 descriptors, the final results are considered unreliable.

Lophius budegassa

Overall, the stock structure identification of *L.budegassa* was based on three biological indicators (Biomass index, CV % of density, mean fish weight) and two layers of information (Biomass trends and Density trends). The Calinski-Harabasz index has a relative maximum at 6 clusters, whereas the mean Cohen's Kappa shows the highest values at 4 and 5 clusters. One other hypothesis included in the upper quintile is the 10 clusters configuration. According to the acceptability analysis, the only hypothesis that attains an acceptability for the first rank is the "4 stock units" configuration (HAI=1). The configurations with 5 and 10 clusters respectively, attain acceptability for the second rank but the 10 clusters configuration has the highest HAI (0.92 against 0.83). According to these results the "4 stock units" configuration represents the best hypothesis of stock structure. However, considering that the analysis was based on few strata of information (3 biological indicators and 2 thematic layers of information), the proposed stock structure should be considered as provisional.

Galeus melastomus

For *G.melastomus*, the full set of biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) was available to perform CC. The Calinski-Harabasz index exhibits a relative maximum at 7 clusters. Also the mean Cohen's Kappa, evaluated on three layers of information (Growth, EFH and connectivity, Oceanographic systems–surface), shows a peak at 7 clusters. The upper quintile of the distribution includes also the configurations with 8, 12 and 13 units. The results of acceptability analysis provides again strong support for the "7 stock units" hypothesis. It receives the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.94), being the HAI of the other candidate hypotheses comprised between 0.64 and 0.81. The "7 stock units" hypothesis is hence chosen as the best stock structure of *G. melastomus* in the Mediterranean. The results (based on a total of 9 descriptors) are considered plausible.

Trachurus trachurus

The stock structure hypotheses for the Atlantic horse mackerel were generated through CC performed on five biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females). The Calinski-Harabasz index shows a decreasing trend from 2 clusters onward. The graph of the mean Cohen's Kappa, evaluated on five layers of information (Growth, Density trends, Biomass trends, Parasites, EFH and connectivity, Oceanographic systems–surface), shows a rather flat portion including configurations with 5, 6 and 7 clusters. Besides these configurations, also the 8 units hypothesis falls in the upper quintile of the Cohen's Kappa distribution. Although the configuration with 5 stock units is ranked first according to the mean Cohen's Kappa, the acceptability analysis suggests that the "8 stock units" is the configuration with the highest acceptability index for the first rank as well as with the highest Holistic Acceptability Index (HAI=0.95). The other candidates hypotheses received a HAI ranging between 0.80 and 0.84. Taking into account the high number of descriptors used in the holistic approach, 5 biological

indicators and 5 thematic layers of information, the results for the Atlantic horse mackerel are considered plausible.

Trachurus mediterraneus

In the case of Mediterranean horse mackerel, five biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females) were used to perform CC. The Calinski-Harabasz index shows a decreasing trend from 2 clusters onward. The mean Cohen's Kappa, evaluated on four layers of information (Density trends, Biomass trends, Parasites, EFH and connectivity, Oceanographic systems–surface), shows a peak at 8 clusters. The upper quintile of the distribution includes also the configurations with 6, 7, and 10 units. The results of acceptability analysis provides again strong support to the "8 stock units" both in terms of acceptability index for the first rank than of Holistic Acceptability Index (HAI=0.96). The HAI exhibited from the other candidate hypotheses ranges between 0.80 (10 clusters) and 0.83 (6 clusters). Hence the configuration with 8 clusters, characterized by both the highest Cohen's Kappa and the highest level of acceptability, is chosen as the best stock structure hypothesis for the Mediterranean horse mackerel in the Mediterranean. The results, based on 5 biological indicators and 4 thematic layers of information, are considered plausible.

Engraulis encrasicholous

In the case of *E.encrasicholous*, the CC was performed on only three biological indicators (Biomass index, CV % of density, mean fish weight). The Calinski-Harabasz shows a pattern with different relative maxima at 4, 7 and 9 clusters. On the other hand the mean Cohen's Kappa, evaluated on four layers of information (Genetics, L50, EFH and connectivity, Oceanographic systems–surface), shows a clear peak at 5 clusters and a weak relative maximum at 9 clusters. Overall the candidate hypotheses falling in the upper quintile of the distribution are the configurations with 5, 6, 7 and 9 clusters. According to the acceptability analysis, the two best ranked hypotheses are the "5 stock units" and "6 stock units", both in terms of acceptability index for the first rank than of Holistic Acceptability Index (HAI= 0.87 and HAI= 0.90 respectively). The results, based on 3 biological indicators and 4 thematic layers of information, are considered plausible.

Sardina pilchardus

The stock structure hypotheses for *S.pilchardus* were generated through CC using only three biological indicators (Biomass index, CV % of density, mean fish weight). The Calinski-Harabasz index shows a progressive decreasing pattern with two relative peaks, corresponding to 4 and 9 clusters. Also the mean Cohen's Kappa, evaluated on four layers of information (Genetics, Density trends, EFH and connectivity, Oceanographic systems–surface), shows two relative peaks at 4 and 8 clusters respectively. Besides, also the configurations with 5 and 6 clusters fall in the upper quintile of the

distribution. Considering the results of the acceptability analysis, the "4 stock units" configuration, which has the highest mean Cohen's Kappa, is the second best ranked hypothesis in terms of HAI (HAI= 0.89). On the other hand, the "8 stock units" configuration receives the highest acceptability for the first rank and the highest Holistic Acceptability Index (HAI=0.92). The two configurations are taken as candidates for the best hypothesis of stock structure of *S.pilchardus*. Based on currently available information, the results are considered plausible.

Parapenaeus longirostris

The full set of MEDITS biological parameters (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) was used in the CC to generate stock structure hypotheses of Deep-water pink shrimp. The Calinski-Harabasz index shows a peak at 3 clusters and a progressive decreasing trend onward, with an inflexion point at 5 clusters. The mean Cohen's Kappa, evaluated on five layers of information criteria (Genetics, EFH and connectivity, Spawning season, Density trends, Biomass trends), results rather flat in the region from 3 to 9 clusters. The configurations with 5, 6, 7, and 8 units are within the upper quintile of the distribution with the "5 stock units" configuration exhibiting the highest mean Cohen's Kappa. According to results of acceptability analysis, the four candidate configurations are comparable in terms of HAI (5 clusters, HAI= 0.83; 6 clusters, HAI=0.81; 7 clusters, HAI=0.84; 8 clusters, HAI=0.84). Based on the overall results, the "5 stock units" is considered the best hypothesis of stock structure of Deep-water pink shrimp. Taking into account the high number of descriptors used in the holistic approach, 6 biological indicators and 5 thematic layers of information, the results are considered plausible.

Nephrops norvegicus

Concerning *N.Norvegicus*, the CC was performed on six biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) and the Cohen's Kappa coefficients averaged across four layers of information (Genetics, EFH and connectivity, Density trends, Biomass trends). The Calinski-Harabasz index shows a progressive decreasing trend with an inflection point at 6 clusters. The mean Cohen's Kappa suggests that the "7 stock units" configuration has the best agreement with the three criteria. The other configurations within the upper quintile are those with 5, 6 and 8 units. According to the acceptability analysis there is weak discrimination between the candidate hypotheses in terms of acceptability Index (HAI=0.79) whereas the "7 stock units" has the lower HAI (HAI= 0.66). Based on current information, the "7 stock units" is taken as best ranked hypothesis even if the other configurations deserve high consideration as well. The results are considered plausible, based on currently available information.

Aristeus antennatus

The stock structure hypotheses for *A.antennatus* were generated through CC performed on six indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females). The Calinski-Harabasz index shows a decreasing trend with a peak at 4 clusters and other minor peaks at 6 and 8 clusters. The mean Cohen's Kappa, evaluated on five layers of information (Genetics, EFH and connectivity, L50, Growth, Density trends), identifies the "4 stock units" configuration as the hypothesis with the best level of agreement. The upper quintile includes also the configurations with 5, 6 and 8 units. The results of acceptability analysis provide support for these candidate hypotheses as they are the only hypotheses which obtain an acceptability for the first rank (besides the 7 clusters configuration). However, they are comparable in terms of HAI (4 clusters, HAI= 0.87; 5 clusters, HAI= 0.88; 6 clusters, HAI=0.85; 8 clusters, HAI=0.82). According to the currently available information the configuration with 4, 5 and 6 are taken as candidates for the best hypothesis of stock structure. Taking into account the high number of descriptors used in the holistic approach, 6 biological indicators and 5 thematic layers of information, the results are considered plausible.

Aristaeomorpha foliacea

Overall, the stock structure identification of *A.foliacea* was based on six biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) and five layers of information (Genetics, EFH and connectivity, Growth, Biomass trends). The Calinski-Harabasz index presents relative maxima at 4 and 6 clusters, whereas the mean Cohen's Kappa shows a peak at "4 stock units" configuration. The other configurations included within the upper quintile are those with 3 and 5 units. The results of acceptability analysis provides strong support for the "4 stock units" hypothesis. It receives the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.84). According to this results the configuration with 4 clusters represents the best hypothesis of stock structure. Taking into account the high number of descriptors used in the holistic approach, 6 biological indicators and 5 thematic layers of information, the results are considered plausible.

Illex coindetii

In the case of Broadtail shortfin squid, the full set of biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) was available to perform CC. The Calinski-Harabasz index shows a progressive decreasing trend with relative maxima at 4 and 8 clusters. Also the mean Cohen's Kappa, evaluated on four layers of information (EFH and connectivity, Biomass trends, Density trends, L50), exhibits a peak at 4 clusters. The upper quintile of the distribution includes also the configurations with 7, 8 and 9 units. The results of acceptability analysis provides strong support for the "8 stock units" hypothesis. It receives the highest acceptability index for the first rank as well as the highest Holistic Acceptability

Index (HAI=0.94). The "4 stock units" receives the highest acceptability index for the second rank as well as a HAI=0.85. The results for the Broadtail shortfin squid are considered reliable, based on currently available information.

Octopus vulgaris

Concerning *O.vulgaris*, the CC was performed on only two biological indicators (Biomass index, CV % of density). The Calinski-Harabasz index, peaks at 3 clusters and then shows a progressive decreasing trend. Also the mean Cohen's Kappa, evaluated on three layers of information (Genetics, Density trends, Biomass trends), exhibits a sharp peak at "3 stock units" configuration. According to acceptability analysis, again the "3 stock units" is ranked first in terms of both the acceptability index for the first rank and the HAI (0.93). It is worth noting that also the configuration with 4 clusters receives a high HAI (0.90) and is ranked first in terms of acceptability index for the second rank. Despite, overall, the analysis relies on only 5 descriptors, the high concordance about the "3 stock units" configuration among the different indices, allows to consider the results plausible.

Eledone cirrhosa

Overall, the stock structure identification of *E.cirrhosa* was based on six biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) and two layers of information (Biomass trends, EFH and connectivity). The Calinski-Harabasz index shows a relative maximum at 4 clusters. The graph of the mean Cohen's Kappa results rather flat from 3 to 7 clusters, being 7 the maximum. According to the acceptability analysis the "6 stock units" receives the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.93), whereas the "7 stock units" configuration shows a HAI=0.87. The "6 stock units" hypothesis is hence chosen as the best stock structure of *E.cirrhosa* in the Mediterranean based on currently available information. Based on currently available information, the results are considered plausible.

Eledone moschata

In the case of *E.moschata*, only two biological indicators were available for perfoming CC. The Calinski-Harabasz index shows a relative maximum at 4 clusters and then a progressive decreasing trend. The Cohen's Kappa, evaluated on only one layer of information (Biomass trends), results rather flat from 4 to 11 clusters. Due to the paucity of data, the analysis was not further continued.

DIFFICULTIES ENCOUNTERED AND REMEDIAL ACTIONS

The methodological approach proposed in STOCKMED project, integrating GIS and MCA methods provides an adequate suite of techniques to deal with multidimensional dimension of stock identification. However, due to the originality of the methodology, some initial insights and ideas were changed and refined also based on results provided by the other WPs. Indeed, a feedback process on the performance of the methodological framework has been followed during the entire duration of the project and guided the production of revised versions of the methodological protocol.

Also, delays occurred in the planned activities of WP4 due to delays in data availability. As an example, the consolidated picture of the complete set of information provided by the different WPs, and the final decision about their use in the holistic framework, was ultimately reached during the 3rd meeting.

During the 4th meeting, wherein results for all species were presented, some criticism were raised about unreliability of results for some species, and particularly about the method for weighing thematic descriptors. It was proposed, in that occasion, the Non-Structural Fuzzy Decision Support System (NSFDSS) as a method for weighing more appropriately the different criteria/thematic descriptors, taking into account the quality/quantity and coverage of the available information. Since the meeting, the remaining time was used to develop NSFDSS, as well as for fine tuning and any further development of the methodology.

Finally, the results of the analysis were constrained, for some species, by the limited availability of data. To obtain more robust and reliable results would require considerable more quantitative data at the appropriate spatial scale. Nevertheless, the methodology developed in the project proved to be fully operational even in those cases with limited data availability.

In order to provide a simple assessment of robustness of the identified stock units by species, a semi-quantitative approach was used. Three indices, representative of quantity and quality of information, were considered: the number of MEDITS indicators used in CC, the standardized index of knowledge used in MCDA, and the Cohen's Kappa (see Deliverable 16 for details). Each index was scored using a five levels scale. Hence, for each species, the scores over the three indices were summed to obtain a vector representing a robustness index of the identified stock units by species. This vector of total scores for all the species was divided in quantiles. The quantiles were finally used to rank the reliability of the identified stock according to the following degrees:

- •score less than the first quantile = unreliable in red,
- •score between the upper limit of the first quantile and the median = uncertain in yellow,
- •score equal or higher than the median = reliable in green.

The reliability of the identified stock units by different species is reported in the table 4.5.

Table 4.5. Semi-quantitative robustness rank of the identified stock units by species (reliable in green, uncertain in yellow, and unreliable in red).

Species	semi- quantitative robustness rank	Species	semi- quantitative robustness rank
Merluccius merluccius		Sardina pilchardus	
Mullus barbatus		Parapenaeus longirostris	
Mullus surmuletus		Nephrops norvegicus	
Pagellus erythrinus		Aristeus antennatus	
Solea solea		Aristaeomorpha foliacea	
Lophius budegassa		Illex coindetii	
Galeus melastomus		Octopus vulgaris	
Trachurus trachurus		Eledone cirrhosa	
Trachurus mediterraneus		Eledone moschata	
Engraulis encrasicolus			

REFERENCES

Abaunza, P., Murta, A.G., Stransky, C., 2014. Sampling for interdisciplinary analysis. In: Cadrin, S.X., Kerr, L.A., Mariani, S. (Eds.), Stock Identification Methods. Applications in Fishery Science (Second ed). Elsevier Academic Press.

Begg G.A., Waldman J.R. (1999) An holistic approach to fish stock identification. Fisheries Research 43: 35-44.

Bitetto I., Spedicato M.T., Carbonara P., Facchini M.T., and G. Lembo, 2013. R package. Multi-Criteria Analysis with two-levels Analytic Hierarchy Process (AHP), COISPA.

Cadrin S.X., Friedland K.D., Waldman J.R. (Eds.), 2005. Stock Identification Methods Applications in Fishery Science Elsevier Academic Press, 735 pp.

Cadrin S.X., Kerr L.A., Mariani S., 2014. Interdisciplinary Evaluation of Spatial Population Structure for Definition of Fishery Management Units. In: Cadrin S.X., Kerr L.A., Mariani S., (Eds) Stock Identification Methods (Second Ed.) - Applications in Fishery Science. Academic Press, 535-552.

Butler J., Dia J., Dyer J., 1997. Simulation techniques for the sensitivity analysis of multi-criteria decision models. European Journal of Operational Research 103 (3), 531–545.

Calinski R.B., Harabasz J., 1974. A dendrite method for cluster analysis. Communications in Statistics, Vol. 3, pages 1-27.

Chen S.Y., 1998. Engineering fuzzy set theory and application. State Security Industry Press, Beijing.

Cohen J., 1960. A coefficient of agreement for nominal scales. Educational and Psychological Measurement, 20, 37–46, 1960.

Figueira J., Greco S., Ehrgott M. (eds), 2005. Multiple Criteria Decision Analysis. State of the Art Survey. Springer: New York.

Greene R., Devillers R., Luther J.E., Eddy B.G., 2011. GIS-Based Multiple-Criteria Decision Analysis. Geography Compass 5/6:412-432.

Higgins R.M., Danilowicz B.S., Balbuena J.A., Danielsdottir A.K., Geffen A.J., Meijer W.G., Modin J., Montero F.E., Pampoulie C., Perdiguero-Alonso D., Schreiber A., Stefansson, M.O., Wilson B., 2010. Multi-disciplinary fingerprints reveal the harvest location of cod Gadus morhua in the northeast Atlantic. Mar. Ecol. Prog. Ser. 404, 197e206.

Moffett A. and S. Sarkar, 2006. Incorporating multiple criteria into the design of conservation area networks: a mini review with recommendations. Diversity and Distributions 12:125–137.

Pawson M.G., Jennings S., 1996. A critique of methods for stock identification in marine capture fisheries. Fisheries Research, 25: 203-2 17.

Saaty T.L., 1980. The Analytical Hierarchy Process. RWS Publications, Pittsburg, Pennsylvania.

Saaty T.L., 2003. Decision-making with the AHP: Why is the principal eigenvector necessary. European Journal of Operational Research 145, 85–91.

Saaty T. L., 2008. Decision making with the analytic hierarchy process. Int. J. Services Sciences, Vol. 1, No. 1.

Sala-Bozano M., Mariani S., 2011. Life history variation in a marine teleost across a heterogeneous seascape. Estuar. Coast. Shelf Sci. 92, 555-563.

Sala-Bozano M., Ketmaier V., Mariani S., 2009. Contrasting signals from multiple markers illuminate population connectivity in a marine fish. Mol. Ecol. 18, 4811-4826.

Tam C.M, Tong T.K.L., Chiu G.W.C., 2006. Comparing non-structural fuzzy decision support system and analytical hierarchy process in decision-making for construction problems. European Journal of Operational Research, 174(2): 1317-1324.

Tervonen T., Figueira J.R., Lahdelma R., Dias J.A., Salminen P., 2009. A stochastic method for robustness analysis in sorting problems. European Journal of Operational Research, 192:236–242.

Valavanis V., Martin C., Nikolopoulou S., Tugores P., Garofalo G., De Felice A., Gristina M., Belluscio A., Telesca L., G.Fabi, Grati F., Frascheti S., Colloca F., Giannoulaki M., Papadopoulou N, Markantonatou V., 2013. GIS rendering: GIS viewer and geo-reference database. In: Mediterranean Sensitive Habitats (MEDISEH) Final Report, DG MARE Specific Contract SI2.600741, Heraklion (Greece).

WP5. Proposal of stock units, comparison with existing GSAs and suggestion for further researches (Coordinator M.T. Spedicato)

BACKGROUND

STOCKMED project aims at identifying stock units and related boundaries for a group of demersal and small pelagic species which are considered important fishery resources in the Mediterranean Sea.

The results gathered in the different WPs and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to work with, taking into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this configuration, in terms of stock assessment, data collection and fishery management.

This deliverable thus aims at establishing suitable relationships among the project results and the current configuration of GFCM GSAs, identifying areas in which conducting joint stock assessment is preferable or sub-units are more appropriate for this objective. Stock assessment purposes are also linked to the proposition of a suitable spatial scale to gather data and for other broader utilizations related to the knowledge and management of fishery resources. The innovation from the STOCKMED results should however take into account the likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs.

A further relevant objective of this deliverable is to highlight the knowledge gaps identified for the different species in the different disciplines which have been the basis of the thematic descriptor. This in order to provide key information for recommending further investigations in line with the objective of stock unit identification. Indeed this can be considered a moving objective strictly linked to the progress of knowledge in different fields, given that information in some fields are very scant.

OBJECTIVES

The WP5 is aimed to following tasks:

- compare the existing GSAs with the outcomes related to the distribution of stock units as obtained from the GIS-MCDA and propose suitable areas for joint stock assessments or areas where the assessment should be conducted at a local spatial scale. Task 5.1;
- identify gaps both in data and knowledge and propose further investigations to improve the identification of stock units in the Mediterranean, including the genetic characterisation of putative stock units already identified. Task 5.2;

propose for the various stock units and fisheries the most adequate spatial scale and strata to carry out data gathering, and fisheries management, while taking into account likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs Task 5.3.

DELIVERABLES AND MILESTONES FORESEEN BY THE PROJECT

The Milestones of this WP are:

M5.1 - Outcomes of the project meetings

M5.2 - Analysis and systematization of the information gathered in the WP1-4.

The Deliverable of this WP is:

D.16 – Stock Units recommendations

- this deliverable will contain recommendations about the identification of stock units in the case studies areas. In particular:;
- GSA/species in which stock assessment must be done for units smaller than the whole GSA;
- GSA/species in which stock assessment must be done for units larger than a single GSA;
- acknowledgement of gaps and proposal for further investigation to validate the putative stock units identified and improve the identification of stock units in the Mediterranean areas not considered by the project.
- an evaluation of the effect of proposed change in spatial scale of stock units on the current FAO Divisions and GFCM GSAs.

All the milestones and the deliverable of WP were achieved

RESULTS ACHIEVED

The approach followed for the proposition of stock units by species investigated in STOCKMED was based on the following steps.

a) Scrutinize by species the information provided by the outcomes of WP4 as systematized in the Deliverable15 and select the most suitable configuration between the more probable identified in D15. In principle only configurations with the higher score both in terms of agreement between thematic layers (Cohen's Kappa coefficient) and overall acceptability of the alternative (Holistic Acceptability Index) were taken into account. Between the selected options the better performing was finally chosen taking into account the overall knowledge gathered during the project on the target species, on the thematic descriptors and on the intensity of fishing effort (by fleet segment) as a further support for selecting the more likely trade-off configuration.

b) Using communication table which are comparing, by species, the areas where new stock units have been identified with the current configuration of GFCM GSAs, taking into account the more relevant geographical benchmarks in the Mediterranean.

c) Identifying gap knowledge as appeared during the development of the project and suggest candidate fields for further investigations on the basis of the outcome obtained by the survey conducted among the experts for weighing quality/quantity and coverage of the available information. This survey was complementary to the implementation of the Non-Structural Fuzzy Decision Support System analysis (NSFDSS) developed in WP4.

d) Provide recommendations finalized to the carrying out of joint stock assessment.

In the following paragraphs the results by species are reported, while at the end of this chapter a synoptic table synthesizing the communication among the proposed stock unit and the GFCM GSAs is reported.

Merluccius merluccius

Two hypotheses among those considered more likely in WP4 have been selected: the 6 units (Holistic Acceptability Index= 0.95) and the 7 units (HAI=0.90) hypothesis. Both were remarkably robust because based upon 6 biological indicators and 7 thematic layers. However the first one was also characterized by a higher Cohen's Kappa coefficient (0.4). Thus the 6-units hypothesis is selected. According to this configuration there are some border zones, i.e. the Gulf of Lions, the Gulf of Lakonikos along the Peloponnesus, and the area western to Adalia (Turkey) where some intrusions from the neighbour GSAs are observed, possibly as a result of the influence of some thematic descriptor (in these cases especially genetics and growth, see D15 for details). Regarding North Adriatic, instead, it seems that the selected configuration is more driven by the combination of the indicators used in the constrained clustering, because, on the basis of the current knowledge on the species distribution, the cluster identified on the north is very likely the northernmost propagation of the stock unit identified in the rest of the Adriatic. Taking into account these considerations and the fact that along the Cote d'Azur, as well as along the Peloponnesus and western to Adalia trawling, which is the fishery more impacting the stock is practically absent (see D11 for details, in particular the chapter "New approach for the estimation of fishing grounds"), the joining to the main neighbour areas is suggested, according to the following table in which the two units of the North Adriatic are joined.

Mullus barbatus

Two hypotheses among those considered more likely in WP4 have been selected: the 3 units (Holistic Acceptability Index= 0.6) and the 4 units (HAI=0.81) hypothesis. Both were robust because based upon 6 biological indicators and 4 thematic layers. However the first one was also characterized by a higher Cohen's Kappa coefficient (0.6) and higher ranked in the quintile distribution. Thus the 3-units hypothesis is selected. According to this configuration there are some border zones, i.e. the southernmost side of the Adriatic Sea (GFCM GSA18) and to a lesser extend a very small area in the border between the GSAs 22 and 24 where some clusters from the neighbour GSAs expand, possibly as a local effect of the combination of the indicators used in the constrained clustering and the thematic descriptors related to genetics and

growth. Considering the distribution of the fishing effort in GSA18 (trawling 12-24 and small scale) and in GSA 22 the joining to the main neighbour areas is suggested.

Mullus surmuletus

Two hypotheses among those considered more likely in WP4 have been selected: the 6 units (Holistic Acceptability Index= 0.8) and the 8 units (HAI=0.79) hypothesis. Both were based upon 3 biological indicators from the survey (the inverse of CV of density index, the biomass index and the mean weight) and 5 thematic descriptors (Genetics, L50, Biomass trends, Density trends and Oceanographic systemssurface) with scattered information among the GFCM GSAs. The 8 units hypothesis had the higher Cohen's Kappa coefficient, though the 6 units hypothesis was rather equivalent in terms of ranks in the quintile distribution and had the first rank acceptability index. In addition, it appeared less affected by possible spurious signs in the constrained clustering process. Thus the 6-units hypothesis is selected. According to this configuration there are some border zones, i.e. a statistical rectangles in the northernmost side of Sardinia (GFCM GSA11), a small area in the Peloponnesus between GSA20 and GSA22 and another very small area between the GSAs 22 and 24, where rectangles from the clusters of the neighbour GSAs are present. Another area which seems differentiated inside GSA22 is the Gulf of Thessaloniki. These situations are possibly spurious signs in the constrained clustering process, given that the thematic layers are not bringing such kind of signs. Thus the two units identified in the Aegean were joined.

Pagellus erythrinus

For this species both Cohen's Kappa coefficient and Holistic Acceptability Index were rather similar between the hypotheses with 4 and 5 clusters, and thus related configuration of stock units were very similar too. Both were based upon a sound pool of indicators, 6 biological ones from the trawl survey and 5 thematic descriptors (Growth, EFH and connectivity, Biomass trends, Density trends and Oceanographic systems–surface). The 4 units (Holistic Acceptability Index= 0.72) configuration has been selected. According to this configuration there are some statistical rectangles that from the cluster of GSA18 expand to GSA17, however, considering the correlation of biomass and density trends, the GSAs 18 and 19 were more similar. In addition, given the local behaviour of the small scale fisheries affecting this kind of species it seems reasonable to assign these statistical rectangles to the GSA17 in the new configuration of stock units.

Solea solea

Regarding common sole the results obtained were considered unreliable in accordance with the outcomes in D15. This is especially because the trawl survey data were poorly informative for this species and thus the constrained clustering returned a very fragmented picture.

Lophius budegassa

According with the results reported in D15 the two configurations with higher probability were the 4 stock units configuration which gained a HAI=1 and the 10 clusters configuration with a HAI=0.92. However the Cohen's Kappa coefficient of the 4 stock units configuration was the higher and this configuration was also in the first rank of acceptability. However, considering that the analysis was based on few strata of information (3 biological indicators, i.e. inverse of density CV, biomass index and mean weight which were

considered less powerful by the expert panel) and 2 thematic layers, the proposed stock structure should be considered as provisional. Thus the communication table is not provided.

Galeus melastomus

For this species the results reported in D15 were converging on a 7 stock units configuration. This result was also considered robust because based upon the full set of biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) and three layers of thematic layers (Growth, EFH and connectivity, Oceanographic systems-surface). According to this configuration there are some a statistical rectangles that from the GSA7 expand into the GSA9 in the border zone of the Ligurian sea. Considering the higher fishing intensity in the Gulf of Lions from fleet segments LOA12-24 and LOA24-50 compared to the fishing intensity in the Ligurian Sea, as reported in D11 (chapter "New approach for the estimation of the fishing grounds") and taking into account that the species is mainly caught from trawlers, these rectangles were joined to the cluster of GSA9. Few rectangles from the cluster of GSA9 were falling both into the cluster of GSA8 (north and east side of Corsica) and offshore the north east side of Sardinia (GSA11). In this case, considering the very low fishing intensity in these areas, the rectangles were assigned to the cluster of GSA11 and GSA8. Similar considerations were developed also for the rectangles in the border of GSA 19 and 18, which were associated in the EFH and connectivity thematic descriptor, and for the eastern side of the Mediterranean for the GSA20, 22 and 23, as well as between GSA 22 and 24. The clusters in the border between GSA25 and 24 were considered associated to GSA24, given that, according to the outcomes from D11, in Cyprus offshore trawling is practically absent.

Trachurus trachurus

Two hypotheses among those considered more likely in WP4 have been selected: the 5 units (Holistic Acceptability Index= 0.8) and the 8 units (HAI=0.95) hypotheses. Both were based upon 5 biological indicators from the trawl survey and 6 thematic descriptors, thus results were considered robust. The 5 units hypothesis had the higher Cohen's Kappa coefficient, though the 8 units hypothesis was in the first rank of acceptability. However the 5 stock units was selected, especially taking into account the results of the thematic descriptor on parasite which is contrasting with a higher fragmentation of stock units and is more in accordance with the species pelagic behaviour. Thus the 5-units hypothesis is selected. According to this configuration there are only two border zones in which rectangles from a cluster extend in another one. Few rectangles from GSA18 expand into GSA17, which were associated to GSA17 considering that the fishery of small pelagics is more intense in the north and central Adriatic. In addition, just one rectangle from the GSA9 cluster expands in GSA7, as well as in the border between GSA20 and 22-23. A similar situation was observed also in the eastern Mediterranean for one rectangle that from GSA22 and 23 expands into the GSA 24 and was associated to this GSA.

Trachurus mediterraneus

For the Mediterranean horse mackerel, results from WP4 highlighted that the configuration with 8 clusters was characterized by both the highest Cohen's Kappa and the highest level of acceptability (HAI=0.96), thus it has been chosen as the best stock structure hypothesis for the Mediterranean horse mackerel. The results, based on 5 biological indicators and 4 thematic layers of information, are considered reliable. This 8-units hypothesis is selected. According to this configuration one rectangle from the cluster of GSA9 expands in the Gulf of Lions and was associated to the GSA7. In the case of southern Adriatic, some rectangles from the cluster of GSA19 expand in the southernmost side of GSA18, which was for the major part clustered with GSA17. Following the signal of three thematic descriptors, these rectangles were associated to GSA17. In this are, a small inner cluster could be interpreted as a spurious signal, probably

due to the effect of the survey indicators. In the eastern Mediterranean, one rectangle belonging to the cluster of Aegean Sea on the border between GSAs 20 and 22 was associated to the GSA 20, while some rectangles in the border between GSA22 and GSA23 and belonging to the cluster of GSA23 were assigned to GSA22.

Engraulis encrasicholous

In the case of anchovy two hypotheses have been selected from the results of WP4, the "5 stock units" and "6 stock units" because gained acceptability index for the first rank of the Holistic Acceptability Index (HAI= 0.87 and HAI= 0.90 respectively). However the mean Cohen's Kappa, evaluated on four layers of information, shows a clear peak at 5 clusters. Thus the five stock units was selected as more probable option. Results are considered fairly reliable because based on 3 biological indicators from the trawl survey and 4 thematic layers of information (Genetics, L50, EFH and connectivity, Oceanographic systemssurface). In order to compare this configuration with the current GSAs, few rectangles from the cluster of GSA8 expanding in GSA9 should be associated to this GSA and similarly between GSAs 9 and 10 (rectangles belonging to the cluster of GSA9 and expanding in GSA should be associated to this GSA). In the GSA 18 the rectangles belonging to the cluster of GSAs 19-20 should instead be associated with GSA17. Besides consideration on the distribution of fishing effort in the area, this attribution can be supported by the information from the thematic descriptors of EFH and connectivity and Surface Oceanographic System. It seems that in this case the pattern of the clusters from the constrained clustering was fairly driven by the abundance indices and mean weight that were the only indicators available from the trawl survey for this species. Analogous considerations hold for the GSA22, where, in the northernmost part, a cluster is separated from the rest of GSA. However, information from the thematic descriptor of Genetics and L50 could support the hypothesis of a unique cluster in the Aegean Sea.

Sardina pilchardus

In the case of sardine two hypotheses have been selected from the results of WP4, the "4 stock units" and "8 stock units" because gained acceptability index for the first rank of Holistic Acceptability Index (HAI= 0.89 and HAI= 0.92 respectively). Both hypotheses also had an equivalent mean Cohen's Kappa coefficient. As for anchovy, results can be considered fairly reliable because based on 3 biological indicators (inverse of CV of density, biomass and mean weight) and 4 thematic layers of information (Correlation of Density Index, Genetics, EFH and connectivity, Oceanographic systems-surface). The pattern based on 4 stock units seems plausible because better separated stock units of the central western side of the Mediterranean from those of the central eastern side, that seems quite plausible on the basis of the four thematic descriptors and the displacement of fishing effort in some areas (for example Ionian compared to central-northern Adriatic). The hypothesis of 4 stock units was thus selected, though this hypothesis needs to be further corroborated in the future. In order to compare this configuration with the current GSAs, few rectangles from the cluster of GSA9 expanding in GSA7 should be associated to GSA7 and few rectangles in GSA19 belonging to the cluster of GSA18 should instead be associated with the cluster of GSA19. This is also supported by the knowledge on the displacement of fishing effort in these contiguous areas, because the fleet from GSA18 often operates in the GSA17 or along the borders of the two GSAs. In addition, few rectangles in GSA 23 belonging to the cluster of GSAs 20-22 should be instead associated with those of the eastern side of GSA23. This is also in line with the knowledge on the main fishing grounds and fishing intensity of the purse seine fishery (LOA: 12-24 m) in the region. The proposed association between the identified stock units and the FAO GFCM GSAs is reported in the table 3.9.

Parapenaeus longirostris

For the deep water rose shrimp, the configuration with 5 clusters was considered the best candidate. This configuration was characterized by both the highest Cohen's Kappa and a higher level of acceptability (HAI=0.83). The results, based on 6 biological indicators and 5 thematic layers of information, are considered reliable. In order to compare this configuration with the current GSAs, few rectangles in the GSA9 belonging to the cluster of GSA10 should be instead attributed to the GSA9 and few rectangles belonging to the cluster of GSA17 and expanding in the GSA18 should instead be associated with GSA18. Similar considerations hold in the GSA24, where few rectangles belonging to the cluster of GSA24.

Nephrops norvegicus

As regards *N. norvegicus*, two hypotheses were selected from the results of WP4 and further analysed for a last choice. The 7 stock units and the 8 stock units. The former had the higher Cohen's Kappa coefficient, while the latter had the highest HAI (0.79). Both are quite informative, thus given the better accordance between Cohen's Kappa and Calinski-Harabasz indices for the 7 units option, this has been selected. Results are considered reliable because based on 6 biological indicators and 4 thematic layers. In order to compare this configuration with the current GSAs, few rectangles in the GSA7 belonging to the cluster of GSA9 should be instead attributed to the GSA7, few rectangles in the GSA19 belonging to the cluster of GSA15 should instead be associated with cluster of GSA18. The same is for 2 rectangles in GSA23 which are belonging to the cluster of GSA24 and should be instead associated with the cluster to which GSA23 is belonging. Some rectangles of the GSA23 cluster expanding into GSA22 should be considered part of GSA22.

Aristeus antennatus

Regarding blue and red shrimp configurations with 4, 5 and 6 and 8 units had comparable holistic acceptability indices (4 clusters, HAI= 0.87; 5 clusters, HAI= 0.88; 6 clusters, HAI=0.85; 8 clusters, HAI=0.82) though the hypothesis of 4 units had also the higher value of mean Cohen's Kappa, coefficient. Results are considered reliable as based on 6 biological indicators and 5 thematic layers. In order to compare this configuration with the current GSAs, few rectangles in the GSA7 belonging to the cluster of GSA6 should be instead attributed to the cluster of GSA8 (and other GSAs), one rectangle of GSA20 belonging to the cluster of GSA22 should instead be associated with cluster of GSA20. The same is for few rectangles in GSA24 which are belonging to the cluster of GSA22 and should be instead associated with the cluster to which GSA25 is belonging.

Aristaeomorpha foliacea

According to this results the configuration with 4 clusters represents the best hypothesis of stock structure as obtained in WP4, as it is based on the highest mean Cohen's Kappa and the higher Holistic Acceptability Index (HAI=0.84). Results are considered reliable as based on 6 biological indicators and 4 thematic layers. The spatial pattern of stock units appears however rather fragmented in the eastern side. In order to compare this configuration with the current GSAs, some rectangles in the GSA9 belonging to the cluster of GSA10 should be instead attributed to the cluster of GSA7 (and other GSAs), some rectangles in the GSA23 belonging to the cluster of GSA20 should instead be associated with cluster of GSA23. In the Aegean Sea the situation seems more patchy. Probably this is because GSA22 is more a transition area, because the species seems not or poorly present in the northern part. For this reason it is proposed to consider the GSA22 as belonging to the same cluster of GSA23, 24 and 25.

Illex coindetii

In the case of Broadtail shortfin squid two hypotheses were selected from WP4: the 8 stock units with the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.94) and the 4 stock units with an HAI=0.85 and the higher mean Cohen's Kappa. This hypothesis was selected. The results were considered reliable as based on 6 biological indicators and 4 thematic layers. In order to compare this configuration with the current GSAs, two rectangles in the GSA19 belonging to the cluster of GSA18 should be instead attributed to the cluster of GSA19 (and other GSAs), some rectangles in the GSA20 belonging to the cluster of GSA23 should instead be associated with cluster of GSA20, while few rectangles in GSA24 belonging to the cluster of GSA22 should be associated to GSA24.

Octopus vulgaris

Concerning common octopus two hypotheses were formulated in WP4, the 4 clusters that had a high HAI (0.90) and the "3 stock units" configuration characterized by the higher mean Cohen's Kappa and ranked first in terms of both acceptability index for the first rank and HAI (0.93). The analysis was based on two biological indicators (Biomass index, CV % of density) and three thematic descriptors. Given the high level of concordance between alternative hypothesis the 3 stock units one was selected. In order to compare this configuration with the current GSAs, few rectangles belonging to the cluster of GSA10 and expanding into the cluster of GSA9 were associated to GSA9 and similarly between GSA23 and 20.

Eledone cirrhosa

Two hypotheses were considered more probable from the outcomes of WP4, the "7 stock units" which had the maximum of mean Cohen's Kappa coefficient though the accordance between constrained cluster outputs and thematic descriptor was rather flat from 3 to 7 clusters. The "6 stock units" received the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.93). The results, based on 6 biological indicators and on two 2 thematic layers (correlation of density trends and EFH and connectivity) of information only, can be considered fairly reliable. In order to compare this configuration with the current GSAs, one rectangle belonging to the cluster of GSA9 and expanding into the cluster of GSA7 was associated to GSA7, and similarly between GSA9 and 10. In the Eastern Ionian sea some rectangles from GSA19 expanding into GSA18 were associated to GSA18 and similarly between GSAs 22 and 20 and GSAs 23 and 22.

Eledone moschata

For this species information were insufficient to estimate any pattern of stock units.

FAO SUB- AREA	FAO Statistical Division	GFCM GSAs	Hake	Red mullet	Stripped mullet	Common pandora	Common Sole	Black mouthed dogfish	Atlantic horse mackerel	Mediterran ean horse mackerel	Anchovy	Deep water rose shrimp	Norway lobster	Blue and red shrimp	Giant red shrimp	Broadtail shortfin squid	
	Balearic (Division 37.1.1)	Northern Alboran Sea Alboran Island Southern Alboran Sea Algeria Balearic Island	1 2 3 4 5														
Mediterranea n (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Sardinia (west) Gulf of Lions	11.1 7														
	Sardinia (Division 37.1.3)	Corsica Island Ligurian and North Tyrrhenian Sea South Tyrrhenian Sea Sardinia (east) Northern Tunisia	8 9 10 11.2 12														
	Adriatic (Division	Northern Adriatic	17														
Central Mediterranea n (Subarea	lonian	Gulf of Hammamet Gulf of Gabes Malta Island South of Sicily Southern Adriatic Sea	13 14 15 16														
37.2)	(Division 37.2.2)	Southern Adriatic Sea Western Ionian Sea Eastern Ionian Sea Southern Ionian Sea	18(east) 19 20 21														
Eastern	Aegean (Division	Aegean Sea Crete Island	22 23														
Mediterranea n (Subarea 37.3)	Levant (Division 37.3.2)	North Levant Cyprus Island South Levant Levant	24 25 26 27														
Stock units				6	3	6	4	5	7	5	8	5	5	8	4	4	4
Scale Stock units				2	2 3	8 4	5	6	7	no info	absence	-					

Table 5.1.Synoptic table of the identified stock units compared with GFCM GSAs.

The results gathered in the different WPs of the STOCKMED project and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to work with, taking into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this configuration, in terms of stock assessment, data collection and fishery management.

Only for two species (*Eledone moschata* and *Solea solea*) results were considered not enough robust for proposing new stock units, while for *Lophius budegassa* the results from the stock unit identification process in D15 were considered still provisional. However, not for all the species the same number of descriptors was available and thematic layers differed in terms of information by species. In addition, in some cases, although the thematic layer was available, the spatial coverage in terms of GSAs was poor and this could have affected, to a certain extent, the analyses.

Knowledge gaps and recommendations

Regarding the gap knowledge, available information was scant for cephalopods, black-mouthed dogfish and blackbellied angler, while if the thematic layers are considered (accounting for all the investigated species), genetics, oceanographic systems and parasites had a lower availability of information, whilst the thematic layers linked to the trawl survey data were better represented. The outputs of the MEDISEH project were very useful for the Essential fish habitat and connectivity layer.

However, besides the remarkable gaps of information emerged for genetics (especially study with significant spatial coverage and fine spatial scale representation) and parasite, the lack of knowledge in different key disciplines was experienced throughout the project. In particular, for the following topics the available information useful for stock identification and definition of stock boundaries was very scant or absent:

- otolith shape and chemical composition;
- analyses of hard structures like vertebrae and spines;
- larval drift associated with the pattern of the currents;
- spatial movements and habitat use at different life stages and species;
- spatial and temporal pattern of oceanographic fronts.

Further, information on the spatial localization of fishing effort is in general poorly informative on the true composition by species of the catches or of the main targets.

Another gap is represented by vast areas of the Mediterranean, in particular on the southern borders, where the information is too scant or sparse for a comprehensive analysis. This implies that the view gathered by the project is mainly localized in the European region of the Mediterranean.

All these considerations entail that the view we gathered should be regarded as a "work in progress" linked to the current level of knowledge, but that can be easily updated along with the improvements of the level of information. Indeed, a methodological framework has been developed that can be adapted to new gathered data.

Given the background knowledge used in the identification of stock units, these should be mainly regarded as homogeneous biological entities. In general, the process of stock units identification resulted in a smaller number of entities compared to the current GFCM GSAs frame and thus with the aggregation of units in bigger areas for all the stocks. In many situations it was necessary to incorporate some GFCM statistical rectangle in one GSA or another, in order to reconcile the current spatial segmentation of the Mediterranean with the stock units identified. This adaptation process was in general limited and supported by considerations related to the information conveyed in the thematic descriptors as well as from the fishing effort spatial intensity as derived from the Deliverable 11.

The aggregation of the current GFCM GSAs in larger areas for stock assessment purposes recalls for a better harmonization of the Data Collection also between different Member States, in terms, for example, of sampling certain métier and period, or gathering and make available a better knowledge on the spatial distribution of the fleet and, in turn, of the intensity and type of fishing effort deployed in certain areas.

A further aspect is related to the necessity of using, developing or adapting stock assessment and forecast tools that allow to take into account the different components of the pressure in terms of fishing mortality and effort by fleet and gear. If the identified stock units will be adopted this is particularly essential, especially for designing management measures calibrated for the different fleets and gears that are affecting the sustainable harvest of target species.

DIFFICULTIES ENCOUNTERED AND REMEDIAL ACTIONS

No particular difficulties were found in this WP activities.

KNOWLEDGE GAPS AND RESEARCH NEEDS, specifying priorities

The work done in WP1, WP2 and WP3 consisted in the revision and analysis of information, mainly concerning biology, genetic and fishery of the 19 target species, available across the Mediterranean. This work provided the basis for extensive reporting of GAPs of knowledge.

Main findings by WP are listed below together with suggested priorities

WP1 - biological information from scientific surveys and literature

Scientific surveys are the most adequate platform to produce standardized and comparable data throughout the Mediterranean. However, there is a lack of availability of information regarding regular scientific surveys of non-EU GSAs. Moreover, the annual periodicity of the scientific surveys is not adequate to tackle inter-annual variations in the biological
parameters (e.g. MEDITS surveys are conducted in spring, MEDIAS in summer). <u>It would be</u> advisable ensuring wide spatial coverage in all EU GSAs and ensuring long temporal series

- Concerning the biological parameters from bibliographic review, the degree of the overall spatial coverage of studies varies greatly between species and parameters. Overall, growth and reproduction studies provide a spatial coverage much wider than the spatial coverage provided by studies reporting on other indicators, i.e. meristic and morphometric, otoliths shape and biochemistry, parasites, tagging/migration or larval drifting. <u>Additional resources and efforts needs to be devoted to overcome this gap of knowledge</u>
- Knowledge regarding growth and reproduction are more abundant for fishes (osteichtyes) and rather poor for crustaceans, cephalopods and elasmobranches. Moreover, the different methods used to estimate the growth parameters provide significantly different estimates of growth and should be used with caution in order to ensure comparability among areas. <u>There is a need to increase the validation and the intercalibration of approaches</u>
- Knowledge in the Mediterranean regarding meristic/morphometry, otolith shape and otolith micro-chemistry is pretty scarce and limited to some species and areas, being available for 4 out of the 19 target species of the project. <u>There is a need to produce</u> <u>comparative studies on meristic/morphometry, otoliths shape and biochemistry for most of</u> <u>the species in the Mediterranean basin, which can be useful in stock identification</u>
- Most of literature on **parasites** is related with the biology of the parasites or of the host species rather than with establishing baselines for fisheries management. <u>Specific studies</u> <u>aimed to use parasites for stock unity identification should be addressed</u>
- There are very scanty studies regarding the tagging/migration of the target species of STOCKMED project in the Mediterranean basin. The general knowledge is very limited because sampling bias, incomplete coverage of the stock and misinterpretation of the data may easily occur
- Regarding larval drift, data concerning larval development of fishes and decapods are scarce. The bathymetric distribution of larval stage for most Mediterranean species is still poorly known, and some aspects of early life history like behavior, food consumption or natural mortality have not yet been explicitly incorporated into bio-physical models. With regards to modeling, whereas it represents an interesting tool for understanding the importance of various factors on larval dispersal as well as connectivity among populations, it can still not offer a totally realistic picture of the process of larval drift and survival. There is a need of coupling accurate circulation models with species-specific field and laboratory studies concerning the planktonic stages of Mediterranean species

WP2 - Genetic Stock Structure Analysis (GSSA) data

- Main limitation of any literature review is that no raw data was available for checking or reanalysis of data. <u>Sample and data repositories should be established within a statistically</u> <u>rigorous framework</u>
- Most of the research investigations (65 studies out of 109) carried out on the 19 target fishery resources are inconclusive for providing GSSA data suitable for assessing and defining

stock units and stock boundaries (data and/or the techniques used are very old and little informative). The 44 datasets providing GSSA data suitable for stock definition were related to 14 target fishery resources and were unequally distributed among demersal and pelagic bony fish with respect to the other taxonomic groups (cartilaginous fish, crustaceans and cephalopods). There is a need to increase the use of 'last-generation' markers (SNPs). When such a large number of markers is used, the resolutive power of genetic data in identifying population differentiation and structuring, increases markedly

- For most of the species the geographical coverage of genetic data is very poor and sporadic; not enough sampling and/or not enough loci have been analysed. <u>Additional resources and</u> <u>efforts needs to be devoted for broad and systematic sampling design</u>
- There is a general lack of research investigations integrating genetic studies with other disciplines for pursuing a multidisciplinary approach to stock identification. <u>There is the need</u> to strengthen cross-disciplinary approaches and analyses

WP3 - geo-morphological, oceanographic and fishery spatial patterns

- The current knowledge on the ecology of meroplanktonic stages (ontogenetic changes, vertical distributions and migrations, growth and mortality) of STOCKMED species is very limited and studies focusing on population integrity through larval transport are very scant. Hence, the inference of **connectivity between spawning and nursery areas** and/or between adjacent GSAs is very difficult, if possible. <u>The development of individual-based fish models coupled with hydrodynamic (and possibly biogeochemical) models (currently implemented in many areas around the Mediterranean) will represent an advancement towards that <u>direction</u></u>
- The very limited knowledge on species- and métier-disaggregated fishing grounds in the Mediterranean Sea calls for data and relevant studies that will focus on assigning fishing effort of fisheries/métiers to respective target species. Data from VMS and log-books are the most appropriate for this purpose. There is a need to develop standardized approaches to their collection and analysis. Furthermore, an easy accessibility to these data should be ensured.

OVERALL DISCUSSION AND CONCLUDING REMARKS

STOCKMED project aims at identifying stock units and related boundaries for a group of demersal and small pelagic species which are main target species for Mediterranean fisheries.

The study is based on available data concerning all those domains that are recognized in the literature as determinants for multidimensional identification of stocks. Since these data derive from studies carried out for several different purposes(e.g. analysis of growth, maturity, spatial distribution, etc.), it was necessary to develop an appropriate methodology that allowed for data standardization and analysis. The developed methodology has allowed devising a framework that

combines spatial analysis and Multi Criteria Decision Analysis, incorporating geo-referred indicators derived from both biological (e.g. abundance, growth, maturity and parasite) and environmental (e.g. habitats and currents) domain. Considering that detailed knowledge about the spatial distribution of populations are essential to identify stock boundaries, the hypothesis on the initial stock configuration to be tested with the MCDA were defined using the information of scientific surveys carried out within the Data Collection Framework funded by the European Union. The results of the analysis depend on both the number and the quality of the indicators used. Despite the difficulties of integrating in a rigorous analytical procedure different types of information (due to different spatial scales and time intervals), the methodology developed allowed to identify possible geographical configurations of the stocks of each species, including the assessment of their uncertainty. These configurations represent the first example of the distribution of units of stocks in the Mediterranean according to a holistic - standardized approach and based on the current knowledge available. For management purposes, the distribution of the stocks of each species was compared with available information on the distribution of fishing fleets. This was used to identify areas to be considered homogeneous for stock assessment and fisheries management. Depending on the quality of the available data, both in terms of discriminatory ability and spatial/temporal coverage, the distribution patterns obtained do not all have the same degree of plausibility. While these appear consistent for rich data species, (e.g. Merluccius merluccius) for others they present a high degree of uncertainty (e.g. Eledone moschata). The results gathered in the different WPs and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to work with, taking into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this configuration, in terms of stock assessment, data collection and fishery management.

Areas in which conducting joint stock assessment is preferable or areas where sub-units are more appropriate were identified. Stock assessment purposes are also linked to the proposition of a suitable spatial scale to gather data and for other broader utilizations related to the knowledge and management of fishery resources. The innovation from the STOCKMED results should however take into account the likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs.

Detailed results were produced for each investigated species. Despite differences according the diverse species were found, an overall pattern can be observed with stock units reflecting the division in basin of the Mediterranean. In general actual GSAs were grouped in larger areas. A synopsis of number of stock units identified in the investigated GSA are reported in the following table.

Target Species	Number of putative stocks
Merluccius merluccius	5

Mullus barbatus	3
Mullus surmuletus	5
Pagellus erythrinus	4
Solea solea	Not reliable
Lophius budegassa	Not reliable
Galeus melastomus	7
Trachurus trachurus	5
Trachurus mediterraneus	7
Engraulis encrasicolus	4
Sardina pilchardus	4
Parapenaeus longirostris	5
Nephrops norvegicus	7
Aristeus antennatus	4
Aristaeomorpha foliacea	4
Illex coindetii	4
Octopus vulgaris	3 (plausible)
Eledone cirrhosa	6 (plausible)
Eledone moschata	Not reliable

However in some case the new stock boundaries split portion of the current GSA in sectors belonging to different stock units.

In any case, the distribution patterns obtained represent a starting point to improve the knowledge of the distribution of the stocks in the Mediterranean. These proposed patterns would need to be validated through specific studies or the collection of supplementary/independent information. These proposed patterns will be subjected to re-evaluation according to the improvement of knowledge on stock properties.. Therefore the units of stock with relative boundaries provided by STOCKMED should be reevaluated in the future in order to provide a specific collection of data similarly to methodologies proposed in other areas (ICES).

One of the limits in STOCKMED project was that information covering the southern countries of the Mediterranean was poor and data are at a scale not comparable with that collected by scientific surveys off the coast of the European countries. In order to achieve this the knowledge gaps need to be filled.

The scientific cooperation with the African and Middle East countries is relevant and effort should be done to fund a data collection similar to the DCF funded by EU.

Considering the main gaps in knowledge on stock structure of fishery resources in the Mediterranean, several disciplines need to be supported. In particular modelling the retention and/or dispersion of eggs and larvae from the spawning grounds in relation to physical oceanographic processes could provide supplementary important data. For the eggs and larvae of some selected species, for which the ecology is well documented, will be represented as Lagrangian drifters released in the main spawning ground. Successively the simulated transport of the pelagic stages will be considered to identify connectivity

mechanism among stock subunits and units. Another gap to be overcame is to improve the use of 'last generation' markers (SNPs) (e.g. Engraulis encrasicolus), coupled with an extensive sampling scheme realized within the framework of EU funded projects (e.g. Merluccius merluccius and Solea solea), which markedly increase the power of genetic data for stock identification. Although the growing importance in the international literature, studies on micro-chemical composition and shape of otoliths are almost absent in the Mediterranean. Furthermore investigation on adults migration and movements, which is a main topic in stock identification, is very poor and needs to be improved at Mediterranean scale. Finally a major availability of standardized information on the spatial distribution could improve the accuracy in selecting data for stock assessment and the consequent adoption of appropriate management measures.

In some cases the available information was insufficient for stock unit identification. In other ones the available information was not geo-referenced at a fine spatial scale that resulted in a simplification of the spatial structure. In addition, some fields of knowledge are quite completely not covered, like for example animal movement and habitat use, that are of crucial importance for stock identification and definition of stock boundaries. Further, information on the spatial localization of fishing effort is in general poorly informative on the true composition by species of the catches or of the main targets.

The aggregation of the current GFCM GSAs in larger areas for stock assessment purposes recalls for a better harmonization of the Data Collection also between different Member States, in terms, for example, of sampling certain métier and period, or gathering and make available a better knowledge on the spatial distribution of the fleet and, in turn, of the intensity and type of fishing effort deployed in certain areas.

A further aspect is related to the necessity of using, developing or adapting stock assessment and forecast tools that allow to take into account the different components of the pressure in terms of fishing mortality and effort by fleet and gear. If the identified stock units will be adopted this is particularly essential, especially for designing management measures calibrated for the different fleets and gears that are affecting the sustainable harvest of target species.

ANNEX I - Meeting MINUTES

Annex 1.1	Report of the Kick-Off Meeting	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED kickoff meeting /
Annex 1.2	Report of the 2nd Meeting	Marea /Specific Projects /Specific Project 7 STOCKMED /2th STOCKMED meeting /
Annex 1.3	Report of the 3rd Meeting	Marea /Specific Projects /Specific Project 7 STOCKMED /3th STOCKMED meeting /
Annex 1.4	Report of the 4th Meeting	Marea /Specific Projects /Specific Project 7 STOCKMED /4th STOCKMED meeting /

ANNEX II - DELIVERABLES

WP0

D1	Guidelines of the approach and methods	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 01
D2	Interim report	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 02
D3	Final report	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 03

D4	Report on analysis of trends of abundance (trends of target species in different GSA and analyses of synchronic pattern, including correlation matrices)	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 04
D5	Synopsis of the spatial pattern of the main biological information for the target species in the case study areas, including gaps identification of critical gaps in the available biological knowledge	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 05

M1.1	Relevant data gathering from an ad hoc data call facilitated by DGMARE and from partners of the MAREA Consortium	successfully achieved
M1.2	Correlation analyses and matrices by GSAs for each species	successfully achieved
M1.3	Reporting results as geo-referred information for the case studies from task 1.2	successfully achieved
M1.4	Reporting results as geo-referred information for the case studies from task 1.3	successfully achieved

D6	Report and updated literature list on GSSA data of the target fishery Mediterranean resources	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 06
D7	Individual reporting species sheets with genetic estimates of population differentiation indicators/parameters	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 07
D8	Report on novel, highly-performing GSSA pipelines for fishery genetics	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 08

M2.1	Relevant data gathering from past projects and from partners of the MAREA Consortium	successfully achieved
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D9	Report geo-referred information on the geo-morphological and oceanographic information which can act as potential break factors for environmental driven demographic borders in the Mediterranean)	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 09
D10	Report geo-referred information on the spatial distribution of essential fish habitats (persistent nursery and spawning areas) for the selected case studies	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 10
D11	Report geo-referred information on the spatial and temporal distribution of fishing effort/grounds for the case studies	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 11
D12	Synopsis of the spatial pattern of hydrology, sea bottom features, essential fish habitat and fishing effort in the investigated case studies, including identification of knowledge gaps	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 12

M3.1	Relevant data gathering from an ad hoc data call facilitated by DGMARE and from partners of the MAREA Consortium	successfully achieved
M3.2	Gathering environmental information (e.g. hydrological factors, sea bottom features, etc) at adequate spatial scale	successfully achieved
M3.3	Reporting results as geo-referred information from MEDISEH project	successfully achieved
M3.4	Identification of persistent nursery areas and spawning grounds for the species of the selected case study not supported by MEDISEH outputs	successfully achieved
M3.5	Organizing information for the fishing pressure factors at adequate spatial scale	successfully achieved

D13	Geo-referenced data base	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 13
D14	Protocol of methodology to implement the GIS_MCDA framework (including identification of criteria, decision trees, weighing factors, etc)	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 14
D15	Implementation of the GIS_MCDA framework scoring and comparing criteria and decision factors with the preparation of a Synopsis of the stock units and the corresponding stock boundaries	Marea /Specific Projects /Specific Project 7 STOCKMED /STOCKMED Deliverables /Deliverable 15

M4.1	Structuring the geo-referenced data base	successfully achieved
M4.2	Literature review on different approaches for developing weights and evaluation rules of the GIS- MCDA	successfully achieved
M4.3	Implementation of the GIS_MCDA framework scoring and comparing criteria and decision factors	successfully achieved

WP5

		Marea /Specific Projects /Specific Project 7
D1C	Stock Unite recommendations	STOCKMED /STOCKMED Deliverables
	Slock Units recommendations	/Deliverable 16

M5.1	Outcomes of the project meetings	successfully achieved
M5.2	Analysis and systematization of the information gathered in the WP1-4	successfully achieved

ANNEX III - DELIVERABLE 16



MAREA Framework program STOCKMED: STOCK units in the MEDiterranean

WP5 – Proposal of stock units, comparison with existing GSAs and suggestion for further researches

Stock Units recommendations

DELIVERABLE 16

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Summary

STOCKMED project aims at identifying stock units and related boundaries for a group of demersal and small pelagic species which are considered important fishery resources in the Mediterranean Sea.

The results gathered in the different Work Packages (WPs) of the STOCKMED project and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to evaluate, taking also into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this current configuration, in terms of stock assessment, data collection and fishery management.

The approach followed for the proposition of stock units by species investigated in STOCKMED was based on the following steps:

- a) scrutinize by species the information provided by the outcomes of WP4 and select the most suitable configuration between those identified in D15;
- b) apply a semi-quantitative method to evaluate the robustness of the identified stock units by species;
- c) using cross-cutting tables which compare, by species, the areas where new stock units have been identified with the current configuration of GFCM GSAs, taking into account the more relevant geographical benchmarks in the Mediterranean.

The objectives of this deliverable are in addition:

- d) to identify knowledge gaps;
- e) to provide recommendations.

For one species (*Eledone moschata*) results not sufficient to progress in the first step of the analysis, i.e. identify potential alternative configuration to be scored. For other 4 species (*L. budegassa, S. pilchardus, O. vulgaris* and *E. cirrhosa*) results were considered not enough robust for proposing new reliable stock units overall the Mediterranean. However, not for all the species the same number of descriptors was available and thematic layers differed in terms of information by species. In addition, in some cases, although the thematic layer was available, the spatial coverage in terms of GSAs was poor and this could have affected, to a certain extent, the analyses.

Finally the achieved results were related to the identification of stock units for 14 species, as reported in the following synoptic table, where the proposed stock units are compared with the current GFCM GSAs.

Regarding the knowledge gaps, available information was scant for cephalopods, black-mouthed dogfish and blackbellied angler, while if the thematic layers are considered (accounting for all the investigated species), genetics, oceanographic systems and parasites had a lower availability of information, whilst the thematic layers linked to the trawl survey data were better documented. The outputs of the MEDISEH project were very useful for the Essential fish habitat and connectivity layer.

However, besides the remarkable gaps of information emerged for genetics (especially study with significant spatial coverage and fine spatial scale representation) and parasites, the lack of knowledge in different key disciplines was experienced throughout the project. In particular, for the following topics the

available information useful for stock identification and for definition of stock boundaries was very scant or absent:

- otolith shape and chemical composition;
- analyses of hard structures like vertebrae and spines;
- larval drift associated with the pattern of the currents;
- spatial movements and habitat use at different life stages and species;
- spatial and temporal pattern of oceanographic fronts.

Furthermore, information on the spatial localization of fishing effort is in general poorly informative on the true composition by species of the catches or of the main target resources.

Another gap is represented by vast areas of the Mediterranean, in particular on the southern borders, where the information is too scant or sparse for a comprehensive analysis. This implies that the view gathered by this project is mainly localized in the European region of the Mediterranean.

All these considerations entail that the view we gathered should be regarded as a "work in progress" linked to the current level of knowledge, but that can be easily updated along with the improvements of the level of information. Indeed, a methodological framework has been developed that can be adapted to new gathered data.

Given the background knowledge used in the identification of stock units, these should be mainly regarded as homogeneous biological entities. In general, the process of stock units identification resulted in a smaller number of entities compared to the current GFCM GSAs frame and thus with the aggregation of units in bigger areas for all the stocks. In many situations it was necessary to incorporate some GFCM statistical rectangle in one GSA or another, in order to reconcile the current spatial segmentation of the Mediterranean with the stock units identified. This adaptation process was in general limited and supported by considerations related to the information conveyed in the thematic descriptors as well as from the fishing effort spatial intensity as derived from the Deliverable 11.

The aggregation of the current GFCM GSAs in larger areas for stock assessment purposes recalls for a better harmonization of the Data Collection also between different Member States, with the task of sampling allocation reflecting the stock distribution and the relative catch among the relevant GSAs. In addition, this should be realized in terms, for example, of sampling certain métier and period, or gathering and make available a better knowledge of the spatial distribution of the fleet and, in turn, of the intensity and type of fishing effort deployed in certain areas. Data like VMS and AIS would be very important to these purposes.

If the new stock units will be adopted It is also suggested to apply a gradual approach during the stock assessment process, for example conducting the assessment at the spatial scale used so far and then expanding the evaluation to the new stock units in order to make comparison and highlight possible improvements.

A further aspect is related to the necessity of using, developing or adapting stock assessment and forecast tools that allow to take into account the different components of the pressure in terms of fishing mortality and effort by fleet and gear. If the identified stock units will be adopted the partitioning of fishing mortality

among fleets and gears is crucial, especially for designing management measures calibrated for the different fleets and gears that are affecting the sustainable harvest of target stocks.

				Hake	Red	Stripped	Common	Common	Black	Atlantic	Mediterran	Anchovy	Deep water	Norway	Blue and	Giant red	Broadtail
FAO SUB- FAO Statistic	FAO Statistical	GECM GSAs			mullet	mullet	pandora	Sole	mouthed	horse	ean horse		rose shrimp	lobster	red	shrimp	shortfin
AREA	Division								dogfish	mackerel	l mackerel				shrimp		squid
		Northern Alboran Sea	1														
		Alboran Island	2														
	Balearic		2														
	(Division	Southern Alboran Sea	3														
	37.1.1)	Algeria	4														
		Balearic Island	5														
Western		Northern Spain	6														
wiediterranea		Sardinia (west)	11.1														
37 1)	Gulf of Lions	Culf of Lions															
57.17	37 1 2)	Guil of Lions	· '														
		Corsica Island	8														
	Sardinia	Ligurian and North	9														
	(Division 37.1.3)	Tyrrhenian Sea															
		South Tyrrhenian Sea	10														
		Sardinia (east)	11.2														
	Adriatic	Northern Tunisia	12														
	(Division	Northern Adriatic	17														
		Gulf of Hammamet	13														
C • • •		Gulf of Gabes	14														
Central		Malta Island	15														
n (Subarea	Ionian	South of Sicily	16														
37.2)	(Division	Southern Adriatic Sea	18														
	37.2.2)	Southern Adriatic Sea	18(east)														
		Western Ionian Sea	19														
		Eastern Ionian Sea	20														
	A	Southern Ionian Sea	21														
Fastara	Aegean (Division	Aegean Sea Croto Island	22														
Mediterranea		North Levant	23														
n (Subarea	Levant	Cyprus Island	24														
37.3)	(Division	South Levant	26														
	37.3.2)	Levant	27														
Stock units	-			6	3	6	4	5	7	5	8	5	5	8	4	4	4
		Scale]					
		Stock units	1	2	3	4	5	6	7	no info	absence]					

1.Introduction

STOCKMED project aims at identifying stock units and related boundaries for a group of demersal and small pelagic species which are considered important fishery resources in the Mediterranean Sea.

The results gathered in the different Work Packages (WPs) of the STOCKMED project and the methodological approach followed in WP4 allowed to achieve a set of preferential stock configurations to evaluate, taking also into account other external factors, such as the spatial distribution of fishing grounds and the current configuration of the GSAs to verify how the outputs from WP4 could impact this current configuration, in terms of stock assessment, data collection and fishery management.

This deliverable 16 thus aims at establishing suitable relationships among the project results and the current configuration of GFCM GSAs, identifying areas in which joint stock assessment is preferable or, in contrast, if and where sub-units are more appropriate for this objective. Stock assessment purposes are also linked to the proposition of a suitable spatial scale to gather data for other broader utilizations related to the knowledge and management of fishery resources. The innovation from the STOCKMED results should however take into account the likely trade-offs among the different spatial scales and sorting out relations with the FAO Divisions and GFCM GSAs.

A further relevant objective of this deliverable is to highlight the knowledge gaps identified for the different species in the different disciplines used as basis to map thematic descriptor. This in order to provide key information for recommending further investigations in line with the objective of stock unit identification. Indeed, this can be considered a moving objective strictly linked to the progress of knowledge in different fields, given that information on some scientific aspects are still very scant.

2. The method applied

The approach followed for the proposition of stock units by target species in STOCKMED was based on the following steps.

- a) Scrutinize by species the information provided by the outcomes of WP4 as systematized in the Deliverable15 and select the most suitable configuration between those more probable as identified in D15. As a rule, only configurations with the higher score both in terms of agreement between thematic layers (Cohen's Kappa coefficient) and overall acceptability of the alternatives (Holistic Acceptability Index) were taken into account. Between the selected options the one better performing in terms of accordance with the overall knowledge gathered during the project on the target species, thematic descriptors and displacement/intensity of fishing effort (possibly by fleet), was finally chosen as the more likely trade-off configuration.
- b) Apply a semi-quantitative method to evaluate the robustness of the identified stock units by species, using the information from D15. The method is based on the following steps (see also table 2.1 below):
 - 1. scoring the information quality/quantity according to the following range: very low (0.2), low (0.4), medium (0.6), high (0.8) and very high (1);
 - 2. after a categorization process, apply the above scale to three key levels of the analysis:
 - the number of indicators used in the constrained clustering process,
 - the standardised index of the information/knowledge coverage obtained in the first step of the MCDA (see table 4.1 of this Deliverable),
 - the Cohen's Kappa coefficient;
 - 3. for each species sum the final score over the three levels of the analysis and divide the obtained vector in quantiles, then use the quantiles to rank the reliability of the identified stock according to the following degrees:
 - score less than the first quantile=unreliable,
 - score between the upper limit of the first quantile and the median=uncertain,
 - score equal or higher than the median=reliable.
- c) Using cross-cutting tables to compare by species, the areas where new stock units have been identified with the current configuration of GFCM GSAs, taking into account the more relevant geographical benchmarks in the Mediterranean.
- d) Identifying gap knowledge as appeared during the development of the project and suggest candidate fields for further investigations on the basis of the outcome obtained by the survey conducted among the experts for weighing quality/quantity and coverage of the available information. This survey was complementary to the implementation of the Non-Structural Fuzzy Decision Support System analysis (NSFDSS) developed in WP4.
- e) Provide recommendations finalized to the carrying out of joint stock assessment.

Tab. 2.1 – Semi-quantitative evaluation of robustness of the identified stock units before comparing these results with the current GSAs boundaries. The number of indicators from MEDITS survey per species, the value of the index used to support the NSFDSS, the Cohen's kappa coefficient, the range of the qualiquantitative score and the final rank by species are reported, as well as the score range and statistics used in the analysis.

		va indio	value of the used indicator/coefficient		value of the used indicator/coefficie		qualitative attribute score		score			
Target species	Number of identified stocks	N. of MEDITS indicators	NSFDSS related index	Cohens' Kappa	N. of MEDITS indicators	NSFDSS related index	Cohens' Kappa	N. of MEDITS indicators	NSFDSS related index	Cohens' Kappa	Sum of scores (RI=Robustness Index)	semi- quantitative robustness rank
M.merluccius	6	6	0.084	0.399	VH	VH	М	1	1	0.6	2.6	reliable
M. barbatus	3	6	0.081	0.581	VH	VH	VH	1	1	1	3	reliable
M. surmuletus	6	3	0.063	0.463	L	Н	н	0.4	0.8	0.8	2	reliable
P. erythrinus	4	6	0.048	0.260	VH	М	VL	1	0.6	0.2	1.8	uncertain
S. solea	5	2	0.067	0.520	VL	Н	Н	0.2	0.8	0.8	1.8	uncertain
L. budegassa	4	3	0.018	0.301	L	VL	VL	0.4	0.2	0.2	0.8	unreliable
G. melastomus	7	6	0.025	0.416	VH	VL	Н	1	0.2	0.8	2	reliable
T. trachurus	5	5	0.067	0.356	Н	Н	L	0.8	1	0.4	2.2	reliable
T. mediterraneus	8	5	0.061	0.470	Н	М	Н	0.8	0.6	0.8	2.2	reliable
E. encrasicolus	5	3	0.069	0.535	L	Н	VH	0.4	0.8	1	2.2	reliable
S. pilchardus	4	3	0.051	0.315	L	М	L	0.4	0.6	0.4	1.4	unreliable
P. longirostris	5	6	0.043	0.413	VH	L	М	1	0.4	0.6	2	reliable
N. norvegicus	7	6	0.054	0.376	VH	М	М	1	0.6	0.6	2.2	reliable
A. antennatus	4	6	0.060	0.327	VH	М	L	1	0.6	0.4	2	reliable
A. foliacea	4	6	0.077	0.354	VH	VH	L L	1	1	0.4	2.4	reliable
I.coindetii	4	6	0.034	0.375	VH	L	М	1	0.4	0.6	2	reliable
O. vulgaris	3	2	0.036	0.556	VL	L	VH	0.2	0.4	1	1.6	unreliable
E. cirrhosa	6	6	0.034	0.205	VH	L	VL	1	0.4	0.2	1.6	unreliable
E. moschata	6	2	0.027	NA	VL	VL		0.2	0.2		0.4	unreliable
	1			1		I					I I	
	1,2=Very Lo	w <=0.27=	=VL		<=0.3=VL		0.2				quantiles	
	3= LOW	>0.27 a	na <=0.43 L	>0.3 and	<=0.3557L		0.4			statistics	median 2	
score range	4=Medium	>0.43 a	nd <=0.61 M	>0.3557 a	and <=0.4129	M	0.6				1	1.70
	5=High	>0.61 a	nd <=0.69 H	>0.4129 a	and <=0.5199	н	0.8				3 ^{ra}	2.2
	6 Very High	>0.69 V	Н	>0.5199 \	/H		1				4	3

3. Scrutinize the results from WP4 and propose the most suitable configuration for stock units

Merluccius merluccius

Two hypotheses among those considered more likely in WP4 have been selected: the 6 units (Holistic Acceptability Index= 0.95) and the 5 units (HAI=0.90) hypothesis. Both were remarkably robust because based upon 6 biological indicators and 7 thematic layers. However the first one was also characterized by a higher Cohen's Kappa coefficient (0.4). According to the semi-quantitative robustness index (RI=2.6) this configuration (6 units) was ranked as reliable, thus it was selected and reported in the figure 3.1



Merluccius merluccius - Num of stocks = 6 - HAI = 0.95

Fig. 3.1 – European hake. Configuration of six stock units with the overlay of the GFCM GSAs.

In this configuration few zones, i.e. the Gulf of Lions and the coast offshore northern Spain, the Gulf of Lakonikos along the Peloponnesus, and the area western to Adalia (Turkey) presented a slight mixture of elements belonging to two different contiguous clusters from neighbour GSAs, possibly as a result of the influence of some thematic descriptors (in these cases probably genetics, EFH and connectivity and growth, see D15 for details). Regarding North Adriatic, instead, it seems that the selected configuration is more driven by the combination of the indicators used in the constrained clustering, because, on the basis of the current knowledge on the species distribution, the cluster identified on the north is very likely the northernmost propagation of the stock unit identified in the rest of the Adriatic. Taking into account these considerations and the fact that along the Cote d'Azur, as well as along the Peloponnesus and western to Adalia trawling, which is the fishery more impacting the stock, is practically absent (see D11 for details, in particular the chapter "New approach for the estimation of fishing grounds"), the joining of the intermixed elements to the main neighbour areas is suggested, according to the following table 3.1, in which the two units of the North Adriatic are joined, while the Gulf of Lion and the northernmost side of north Spain were

associated to the same cluster as GSAs 1 and 5. It should be also taken into account that in GSAs 6 and 7, as well as in GSA17 hake is also considered a shared stock by GFCM. Finally the trade-off for the most suitable configuration is based on 5 stock units (tab. 3.1).

Tab. 3.1 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for hake (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Hake
		Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
	Balearic (Division 37.1.1)	Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
		Ligurian and North	<u>م</u>	
	Sardinia (Division 37.1.3)	Tyrrhenian Sea	9	
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
Mediterranean	Ionian (Division 27.2.2)	South of Sicily	16	
(Subarea 37.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aggaan (Division 27.2.1)	Aegean Sea	22	
Festern	Aegean (Division 57.5.1)	Crete Island	23	
Eastern		North Levant	24	
(Subarea 37.3)	Levant (Division 37 3 2)	Cyprus Island	25	
	Levant (Division 37.3.2)	South Levant	26	
		Levant	27	

Mullus barbatus

Two hypotheses among those considered more likely in WP4 have been selected: the 3 units (Holistic Acceptability Index= 0.6) and the 4 units (HAI=0.81) hypothesis. Both were robust because based upon 6 biological indicators and 4 thematic layers. However the first one was also characterized by a higher Cohen's Kappa coefficient (0.6) and higher ranked in the quintile distribution. According to the semi-quantitative robustness index (RI=3) this configuration was ranked as reliable. Thus the 3-units hypothesis is selected and reported in the figure 3.2



Mullus barbatus - Num of stocks = 3 - HAI = 0.6

Fig. 3.2 – Red mullet. Configuration of three stock units with the overlay of the GFCM GSAs.

According to this configuration there are some border zones, i.e. the southernmost side of the Adriatic Sea (GFCM GSA18) and to a lesser extend a very small area in the border between the GSAs 22 and 24 where some elements of thecluster from the neighbour GSA expand, possibly as a local effect of the combination of the indicators used in the constrained clustering and the thematic descriptors related to genetics and growth. Considering the distribution of the fishing effort in GSA18 (trawling 12-24 and small scale) and in GSA 22 the joining of such elements to the main neighbour areas is suggested. Thus GSA18 is globally aggregated with the cluster of GSAs 17, 20, 22 and 23, while 2 rectangles of GSA22 are aggregated to GSA 24, according to the following table 3.2.

Tab. 3.2 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for red mullet (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical		STOCKMED red]
	Division	GFCIVI USAS	mullet	

		Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
	Balearic (Division	Algeria	4	
	37.1.1)	Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
	Sardinia (Division	Ligurian and North Tyrrhenian Sea	9	
	37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)	Ionian (Division	South of Sicily	16	
(5050100 57.2)	37.2.2)	Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division	Aegean Sea	22	_
Factorn	37.3.1)	Crete Island	23	
Eastern Mediterranean (Subarea 37.3)		North Levant	24	
	Levant (Division	Cyprus Island	25	
	37.3.2)	South Levant	26	
		Levant	27	

Mullus surmuletus

Two hypotheses among those considered more likely in WP4 have been selected: the 6 units (Holistic Acceptability Index= 0.8) and the 8 units (HAI=0.79) hypothesis, although even the hypotheses based on 5 and 7 units had almost equivalent HAI and Cohen's Kappa coefficient. All were based upon 3 biological indicators from the survey (the inverse of CV of density index, the biomass index and the mean weight) and 5 thematic descriptors (Genetics, L50, Biomass trends, Density trends and Oceanographic systems–surface) with scattered information among the GFCM GSAs. The 8 units hypothesis had the higher Cohen's Kappa coefficient, though the 6 units hypothesis was rather equivalent in terms of ranks in the quintile distribution and had the first rank acceptability index. In addition, it appeared less affected by possible spurious signs in the constrained clustering process, probably as consequence of the number of biological

indicator. According to the semi-quantitative robustness index (RI=2) the 6-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.3.



Mullus surmuletus - Num of stocks = 6 - HAI = 0.8

Fig. 3.3 – Stripped mullet. Configuration of six stock units with the overlay of the GFCM GSAs.

According to this configuration there are some border zones, i.e. a statistical rectangles in the northernmost side of Sardinia (GFCM GSA11.2), a small area in the Peloponnesus between GSA20 and GSA22 and another very small area between the GSAs 22 and 24, where very few rectangles from the clusters of the neighbour GSAs are present. Another area which seems differentiated inside GSA22 is the Gulf of Thessaloniki. These situations are possibly spurious signs in the constrained clustering process where only 3 biological indicators could be considered. In addition, the thematic layers were not bringing such kind of signs. Thus the two units identified in the GSA 22 of the Aegean Sea were joined and finally 5 units identified (tab. 3.3).

Tab. 3.3 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for stripped mullet (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Stripped mullet
Western Mediterranean (Subarea 37.1)	Balearic (Division 37.1.1)	Northern Alboran Sea Alboran Island Southern Alboran Sea Algeria Balearic Island	1 2 3 4 5	

		Northern Spain	6	
		Sardinia (west)	11.1	
	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
		Ligurian and North Tyrrhenian Sea	9	
	Sardinia (Division 37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
Mediterranean	Ionian (Division 37.2.2)	South of Sicily	16	
(Subarea 37.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division 37 3 1)	Aegean Sea	22	
Factors		Crete Island	23	
Mediterranean		North Levant	24	
(Subarea 37.3)	Levant (Division 37 3 2)	Cyprus Island	25	
		South Levant	26	
		Levant	27	

Pagellus erythrinus

For this species both Cohen's Kappa coefficient and Holistic Acceptability Index were rather similar between the hypotheses with 4 and 5 clusters, as well as under the hypothesis of 7 and 8 clusters. As a consequence, the configurations with 4 and 5 clusters were very similar each other, and likewise those with 7 and 8 clusters. All were based upon a sound pool of indicators, 6 biological ones from the trawl survey and 5 thematic descriptors (Growth, EFH and connectivity, Biomass trends, Density trends and Oceanographic systems–surface). Given the value of Cohen's Kappa coefficient, the semi-quantitative robustness index (RI=1.8) was in between the upper limit of the 1st quantile and the median value, therefore the results were considered uncertain. The 4 units (Holistic Acceptability Index= 0.72) configuration seems the more acceptable and it is reported in the figure 3.4.

Pagellus crythrinus - Num of stocks = 4 - HAI = 0.72



Fig. 3.4 – Common pandora. Configuration of four stock units with the overlay of the GFCM GSAs.

According to this configuration there are some statistical rectangles that from the cluster of GSA18 expand to GSA17, however, considering the correlation of biomass and density trends, the GSAs 18 and 19 were more similar. In addition, given the local behaviour of the small scale fisheries affecting this kind of species it seems reasonable to assign these statistical rectangles to the GSA17 in the new configuration of stock units (see table 3.4). In addition, 2 rectangles in the GSA9, belonging to the cluster of GSA7 were aggregated to the GSA9.

Tab. 3.4 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for common pandora (shaded areas=no information available). For this species results are considered uncertain.

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED common pandora
Western		Northern Alboran Sea	1	
Mediterranean		Alboran Island	2	
(Subarea 37.1)	Deleguis (Division	Southern Alboran Sea	3	
	Balearic (Division	Algeria	4	
	57.1.17	Balearic Island	5	
		Northern Spain	6	
		Sardinia (west)	11.1	
	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
	Sardinia (Division	Corsica Island	8	

	37.1.3)	Ligurian and North Tyrrhenian Sea	9	
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
	lonian (Division 37.2.2)	Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)		South of Sicily	16	
(5050100 57.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
Eastern Mediterranean (Subarea 37.3)	Aegean (Division	Aegean Sea	22	
	37.3.1)	Crete Island	23	
	Levant (Division 37.3.2)	North Levant	24	
		Cyprus Island	25	
		South Levant	26	
		Levant	27	

Solea solea

For this species Cohen's Kappa coefficient was identical for 5, 6 and 7 clusters. The Holistic Acceptability Index suggests that 5-stock units is the configuration with the highest level of acceptability (HAI=0.94). However, the analysis relies only on two biological indicators (Biomass index, CV % of density) and three thematic descriptors (Genetics, Growth and Oceanographic systems–surface). The semi-quantitative robustness index (RI=1.8) was in between the upper limit of the 1st quantile and the median value, therefore the results were considered uncertain. The 5-stock units configuration seems the more acceptable and it is reported in fig. 3.5.

According to this configuration there are some rectangles that from the cluster of GSA20 expand to GSA23, however, these differences seem very likely due to the poor number of indicators from MEDITS survey available for the constrained clustering analysis. Hence, those rectangles were aggregated to the cluster of GSA23. In addition, few rectangles belonging to the cluster of GSA23 were present into the area of GSA24 and were assumed as belonging to GSA24, on the basis of the results from genetics studies. Finally, it is worth mentioning that, given the results from genetics studies, the east side of GSA18 should be considered as a separate stock unit from the rest of the GSA.

Solea solea - Num of stocks = 5 - HAI = 0.94



Fig. 3.5 – Common sole. Configuration of four stock units with the overlay of the GFCM GSAs.

Tab. 3.5 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for common
sole (shaded areas=no information available). For this species results are considered uncertain.

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Common sole
	Balearic (Division 37.1.1)	Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
		Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
	Sardinia (Division 37.1.3)	Corsica Island	8	
		Ligurian and North Tyrrhenian Sea	9	
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
Central	Adriatic (Division 37.2.1)	Northern Adriatic	17	
Mediterranean	Ionian (Division 37.2.2)	Gulf of Hammamet	13	
(Subared 37.2)		Gulf of Gabes	14	

		Malta Island	15	
		South of Sicily	16	
		Southern Adriatic Sea	18	
		Southern Adriatic Sea	18(east)	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
Eastern Mediterranean (Subarea 37.3)	Aegean (Division 37.3.1)	Aegean Sea	22	
		Crete Island	23	
	Levant (Division 37.3.2)	North Levant	24	
		Cyprus Island	25	
		South Levant	26	
		Levant	27	

Lophius budegassa

According to the results reported in D15 the two configurations with higher probability were the 4 stock units configuration which gained a HAI=1 and the 10 clusters configuration with a HAI=0.92. However the Cohen's Kappa coefficient of the 4 stock units configuration (fig. 3.6) was the higher and this configuration was also in the first rank of acceptability. Nevertheless, considering that the analysis was based on few strata of information (3 biological indicators, i.e. inverse of density CV, biomass index and mean weight which were considered less powerful by the expert panel) and 2 thematic layers, the proposed stock structure should be considered unreliable, as the semi-quantitative robustness index (RI=0.8) was lower than the upper limit of the 1st quantile. Thus the cross-cutting table is not provided.



Lophius budegassa - Num of stocks = 4 - HAI = 1

Fig. 3.6 – Blackbellied angler. Configuration of four stock units with the overlay of the GFCM GSAs.

Galeus melastomus

For this species the results reported in D15 were converging on a 7 stock units configuration. This result was also considered robust because based upon the full set of biological indicators (Biomass index, CV % of density, mean fish weight, sex ratio, % of spawning females, median length of spawning females) and three layers of thematic layers (Growth, EFH and connectivity, Oceanographic systems–surface). According to the semi-quantitative robustness index (RI=2) the 6-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.7.



Galeus melastomus - Num of stocks = 7 - HAI = 0.94

Fig. 3.7 – Black-mouthed dogfish. Configuration of four stock units with the overlay of the GFCM GSAs.

According to this configuration there are some elements that from the cluster of GSA7 expand into that of the GSA9, in the border zone of the Ligurian sea. Considering the higher fishing intensity in the Gulf of Lions from fleet segments LOA12-24 and LOA24-50 compared to the fishing intensity in the Ligurian Sea, as reported in D11 (chapter "New approach for the estimation of the fishing grounds") and taking into account that the species is mainly caught from trawlers, these rectangles were joined to the cluster of GSA9. Similarly, few rectangles from the cluster of GSA9 were falling both into the cluster of GSA8 (north and east side of Corsica) and offshore the north east side of Sardinia (GSA11). In these cases, considering the very low fishing intensity in north east Sardinia and east Corsica, the rectangles were assigned to the cluster aggregating GSA11 and GSA8. Similar considerations were developed also for the rectangles in the border between GSA 19 and 18, which were deemed connected on the basis of the EFH and connectivity thematic descriptor. Also few elements of GSA20 in GSA22 and GSA23 were aggregated to these GSAs. Similarly few elements belonging to the cluster of GSA24 but located in GSA22 were aggregated to this GSA. The clusters in the border between GSA25 and 24 were considered associated to GSA24, given that,

according to the outcomes from D11, in Cyprus offshore trawling is practically absent. This configuration of stock units is reported in the table 3.6.

Tab. 3.6 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for Blackmouthed dogfish (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Black-mouthed dogfish
	Balearic (Division 37.1.1)	Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
		Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
	Sardinia (Division 37.1.3)	Ligurian and North Tyrrhenian Sea	9	
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
	Ionian (Division 37.2.2)	Gulf of Gabes	14	
Central		Malta Island	15	
Mediterranean		South of Sicily	16	
(Subarea 37.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
Eastern Mediterranean (Subarea 37.3)	Aegean (Division 37.3.1)	Aegean Sea	22	
		Crete Island	23	
	Levant (Division 37.3.2)	North Levant	24	
		Cyprus Island	25	
		South Levant	26	
		Levant	27	

Trachurus trachurus

Two hypotheses among those considered more likely in WP4 have been selected: the 5 units (Holistic Acceptability Index= 0.8) and the 8 units (HAI=0.95) hypotheses. Both were based upon 5 biological indicators from the trawl survey and 6 thematic descriptors, thus results were considered robust. The 5 units hypothesis had the higher Cohen's Kappa coefficient, though the 8 units hypothesis was in the first rank of acceptability. However the 5 stock units was selected, especially taking into account the results of the thematic descriptor on parasite which is contrasting with a higher fragmentation of stock units and is more in accordance with the species pelagic behaviour. According to the semi-quantitative robustness index (RI=2.2) the 5-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.8.

According to this configuration there are only two border zones in which rectangles from a cluster extend in another one. Few rectangles from GSA18 expand into GSA17, which were associated to GSA17 considering that the fishery of small pelagics is more intense in the north and central Adriatic. In addition, just one rectangle from the GSA9 cluster expands in GSA7, as well as in the border between GSA20 and 22-23. A similar situation was observed also in the eastern Mediterranean for one rectangle that from GSA22 and 23 expands into the GSA 24 and was associated to this GSA. This configuration of 5 stock units is reported in the table 3.7.



Trachurus trachurus - Num of stocks = 5 - HAI = 0.8

Fig. 3.8 – Atlantic horse mackerel. Configuration of five stock units with the overlay of the GFCM GSAs.

Tab. 3.7 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for Atlantic horse mackerel (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Atlantic horse mackerel
		Northern Alboran Sea	1	
		Alboran Island	2	
	Balearic (Division 37.1.1)	Southern Alboran Sea	3	
		Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
	Sardinia (Division 37.1.3)	Ligurian and North Tyrrhenian Sea	9	
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
	Ionian (Division 37.2.2)	Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)		South of Sicily	16	
		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
Eastern Mediterranean (Subarea 37.3)	Aegean (Division	Aegean Sea	22	
	37.3.1)	Crete Island	23	
	Levant (Division 37.3.2)	North Levant	24	
		Cyprus Island	25	
		South Levant	26	
		Levant	27	

Trachurus mediterraneus

For the Mediterranean horse mackerel, results from WP4 highlighted that the configuration with 8 clusters was characterized by both the highest Cohen's Kappa and the highest level of acceptability (HAI=0.96), thus it has been chosen as the best stock structure hypothesis for the Mediterranean horse mackerel. The results, based on 5 biological indicators and 4 thematic layers of information, are considered reliable. According to the semi-quantitative robustness index (RI=2.2) the 8-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.9.

According to this configuration one rectangle from the cluster of GSA9 expanding in the Gulf of Lion was associated to the GSA7. In the case of southern Adriatic, some rectangles from the cluster of GSA19 expand into the southernmost side of GSA18, which is for the major part clustered with GSA17. Following the signal of three thematic descriptors (correlation of biomass trends, EFH and connectivity and Oceanographic systems), these rectangles were associated to GSA17. In this GSA, a small inner cluster could be interpreted as a spurious signal, probably due to the effect of the MEDITS survey indicators. In the eastern Mediterranean, one rectangle belonging to the cluster of Aegean Sea on the border between GSAs 20 and 22 was associated to the GSA23 were assigned to GSA22. Finally the trade-off for the most suitable configuration is based on 7 stock units (tab. 3.8).



Trachurus mediterraneus - Num of stocks = 8 - HAI = 0.96

Fig. 3.9 – Mediterranean horse mackerel. Configuration of eight stock units with the overlay of the GFCM GSAs.

Tab. 3.8 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for Mediterranean horse mackerel (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Mediterranean horse mackerel
Western	tern rranean ea 37.1) Balearic (Division 37.1.1)	Northern Alboran Sea	1	
Mediterranean (Subarea 37.1)		Alboran Island	2	
		Southern Alboran Sea	3	
		Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
		Sardinia (west)	11.1	
	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
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		Corsica Island	8	·
	Sardinia (Division	Ligurian and North Tyrrhenian Sea	9	
	37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)	Ionian (Division 37 2 2)	South of Sicily	16	
(5050100 57.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division	Aegean Sea	22	
Factory	37.3.1)	Crete Island	23	
Eastern		North Levant	24	
(Subarea 37.3)	Levant (Division 37 3 2)	Cyprus Island	25	
(South Levant	26	
		Levant	27	

Engraulis encrasicolous

In the case of anchovy two hypotheses have been selected from the results of WP4, the "5 stock units" and "6 stock units" because gained acceptability index for the first rank of the Holistic Acceptability Index (HAI= 0.87 and HAI= 0.90 respectively). However the mean Cohen's Kappa, evaluated on four layers of information, shows a clear peak at 5 clusters. Thus the five stock units was selected as more suitable option. Results are based on 3 biological indicators from the trawl survey and 4 thematic layers of information (Genetics, L50, EFH and connectivity, Oceanographic systems–surface). According to the semi-quantitative robustness index (RI=2.2) the 5-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.10.

In order to reconcile this configuration with the current GSAs, few rectangles from the cluster of GSA8 expanding in GSA9 have been associated to this GSA and similarly between GSAs 9 and 10 (rectangles belonging to the cluster of GSA9 and expanding in GSA10 should be associated to this GSA). In the GSA 18 the rectangles belonging to the cluster of GSAs 19-20 should instead be associated with the cluster of GSA17 which in part includes also GSA18. Besides consideration on the distribution of fishing effort in this area, this aggregation can be supported by the information from the thematic descriptors of EFH and connectivity and Surface Oceanographic System. It seems that in this case the pattern of the clusters from the constrained clustering was fairly driven by the abundance indices and mean weight that were the only indicators available from the MEDITS trawl survey for this species. Analogous considerations hold for the

GSA22, where, in the northernmost part, a cluster is separated from the rest of GSA. However, information from the thematic descriptor of Genetics and L50 could support the hypothesis of a unique cluster in the Aegean Sea. Finally the trade-off for the most suitable configuration is based on 4 stock units as proposed in table 3.9.



Engraulis encrasicolus - Num of stocks = 5 - HAI = 0.87

Fig. 3.10 – Anchovy. Configuration of five stock units with the overlay of the GFCM GSAs.

Tab. 3.9 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for and	hovy
(shaded areas=no information available).	

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED anchovy
		Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
	Balearic (Division 37.1.1)	Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
		Ligurian and North Tyrrhenian Sea	9	
	Sardinia (Division 37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	

	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
Mediterranean	Ionian (Division 37.2.2)	South of Sicily	16	
(Subarea 37.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division 37.3.1)	Aegean Sea	22	
F actoria		Crete Island	23	
Eastern Mediterranean (Subarea 37.3)		North Levant	24	
	Levant (Division 37 3 2)	Cyprus Island	25	
		South Levant	26	
		Levant	27	

Sardina pilchardus

In the case of sardine two hypotheses have been selected from the results of WP4, the "4 stock units" (fig. 3.11) and "8 stock units" because gained acceptability index for the first rank of Holistic Acceptability Index (HAI= 0.89 and HAI= 0.92 respectively). Both hypotheses also had an equivalent mean Cohen's Kappa coefficient. Results were based on 3 biological indicators (inverse of CV of density, biomass and mean weight) and 4 thematic layers of information (Correlation of Density Index, Genetics, EFH and connectivity, Oceanographic systems–surface). However, the examined stock units should be considered unreliable, as the semi-quantitative robustness index (RI=1.4) was lower than the upper limit of the 1st quantile. Thus the cross-cutting table is not provided.

Sardina pilchardus - Num of stocks = 4 - HAI = 0.89



Fig. 3.11 – Sardine. Configuration of four stock units with the overlay of the GFCM GSAs.

Parapenaeus longirostris

For the deep water rose shrimp, the configuration with 5 clusters was considered the best candidate. This configuration was characterized by both the highest Cohen's Kappa and a higher level of acceptability (HAI=0.83). The results were based on 6 biological indicators and 5 thematic layers of information. According to the semi-quantitative robustness index (RI=2) the 5-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.12.

In order to compare this configuration with the current GSAs, few rectangles in the GSA9 belonging to the cluster of GSA10 should be instead aggregated to the GSA9 and few rectangles belonging to the cluster of GSA17 and expanding in the GSA18 should instead be associated with GSA18, viceversa for the rectangles belonging to the cluster of GSA18 and espanding in GSA17. Similar considerations hold in the GSA24, where few rectangles belonging to the cluster of GSA22 should instead be associated with GSA24. The most suitable configuration is based on 5 stock units is reported in table 3.10.





Fig. 3.12 – Deep water rose shrimp. Configuration of five stock units with the overlay of the GFCM GSAs

Tab. 3.10 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for deep wate
rose shrimp (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED deep water rose shrimp
		Northern Alboran Sea	1	
		Alboran Island	2	
	Palaaric (Division	Southern Alboran Sea	3	
		Algeria	4	
	071117	Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
	Sardinia (Division	Corsica Island	8	
		Ligurian and North Tyrrhenian Sea	9	
	37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
Central Mediterranean	Adriatic (Division 37.2.1)	Northern Adriatic	17	
(Subarea 37.2)	Ionian (Division	Gulf of Hammamet	13	
	37.2.2)	Gulf of Gabes	14	

		Malta Island	15	
		South of Sicily	16	
		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division	Aegean Sea	22	
- .	37.3.1)	Crete Island	23	
Eastern Mediterranean (Subarea 37.3)		North Levant	24	
	Levant (Division 37.3.2)	Cyprus Island	25	
		South Levant	26	
		Levant	27	

Nephrops norvegicus

As regards *N. norvegicus*, two hypotheses were selected from the results of WP4 and further analysed for a last choice. The 7 stock units and the 8 stock units hypotheses. The former had the higher Cohen's Kappa coefficient, while the latter had the highest HAI (0.79). Both are quite comparable and informative with results based on 6 biological indicators and 4 thematic layers.. Given the higher Cohen's Kappa and a better accordance between Cohen's Kappa and Calinski-Harabasz indices for the 7 units option, this has been selected. According to the semi-quantitative robustness index (RI=2.2) the 7-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.13.



Nephrops norvegicus - Num of stocks = 7 - HAI = 0.66

Fig. 3.13 – Norway lobster. Configuration of seven stock units with the overlay of the GFCM GSAs.

In order to compare this configuration with the current GSAs, some rectangles in the GSA7 belonging to the same cluster as GSA9 should be instead aggregated to the rest of GSA7, similarly few rectangles in the GSA19 belonging to the cluster of GSA15 should be instead aggregated with the same cluster as GSA18. Likewise is for 2 rectangles in GSA23 which are belonging to the cluster of GSA24 and should be instead associated with the same cluster as GSA23. Some rectangles of the cluster of GSA23 expanding into GSA22 should be instead considered part of GSA22. The cross-cutting among GFCM GSAs configuration and proposed stock units from STOCKMED is reported in the table 3.11.

Tab. 3.11 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for Norway lobster (shaded areas=no information available).

		GFCM GSAs		STOCKMED
FAO SUB-AREA	FAO Statistical Division			Norway
				lobster
		Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
	Balearic (Division 37.1.1)	Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
		Ligurian and North Tyrrhenian	٩	
	Sardinia (Division 37.1.3)	Sea		
		South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
Mediterranean	Ionian (Division 27.2.2)	South of Sicily	16	
(Subarea 37.2)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Access (Division 27.2.1)	Aegean Sea	22	
Eastern Mediterranean (Subarea 37.3)	Aegeall (Division 57.5.1)	Crete Island	23	
		North Levant	24	
	Lovant (Division 27.2.2)	Cyprus Island	25	
	Levant (Division 57.5.2)	South Levant	26	
		Levant	27	

Aristeus antennatus

Regarding blue and red shrimp configurations with 4, 5 and 6 and 8 units had comparable holistic acceptability indices (4 clusters, HAI= 0.87; 5 clusters, HAI= 0.88; 6 clusters, HAI=0.85; 8 clusters, HAI=0.82) though the hypothesis of 4 units had also the higher value of mean Cohen's Kappa, coefficient. Results are considered reliable as based on 6 biological indicators and 5 thematic layers. According to the semi-quantitative robustness index (RI=2) the 4-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.14.



Aristeus antennatus - Num of stocks = 4 - HAI = 0.87

Fig. 3.14 – Blue and red shrimp. Configuration of four stock units with the overlay of the GFCM GSAs.

In order to compare this configuration with the current GSAs, few rectangles in the GSA7 belonging to the cluster of GSA6 should be instead attributed to the same cluster as GSA8 (and other adjacent GSAs), one rectangle of GSA20 belonging to the cluster of GSA22 should instead be associated with the same cluster as GSA20. Likewise is for few rectangles in GSA24 belonging to the GSA22 cluster that should be instead associated with the same cluster as GSA25. The cross-cutting among GFCM GSAs configuration and proposed stock units from STOCKMED is reported in the table 3.12.

Tab. 3.12 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for blue and red shrimp (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs	STOCKMED Blue and red shrimp
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		Northern Alboran Sea	1	
		Alboran Island	2	
		Southern Alboran Sea	3	
	Balearic (Division	Algeria	4	
	37.1.1)	Balearic Island	5	
		Northern Spain	6	
Western		Sardinia (west)	11.1	
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	
		Corsica Island	8	
	Sardinia (Division	Ligurian and North Tyrrhenian Sea	9	
	37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
	Ionian (Division 27.2.2)	Gulf of Hammamet	13	
		Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)		South of Sicily	16	
(5454164 5712)		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division	Aegean Sea	22	
Eastern Mediterranean	37.3.1)	Crete Island	23	
		North Levant	24	
(Subarea 37.3)	Levant (Division 37.3.2)	Cyprus Island	25	
		South Levant	26	
		Levant	27	

Aristaeomorpha foliacea

According to this results the configuration with 4 clusters represents the best hypothesis of stock structure as obtained in WP4, as it is based on the highest mean Cohen's Kappa and the higher Holistic Acceptability Index (HAI=0.84). Results are based on 6 biological indicators and 4 thematic layers. According to the semi-quantitative robustness index (RI=2.4) the 4-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.15. The spatial pattern of stock units appears however rather fragmented in the eastern side.

Aristacomorpha foliacea - Num of stocks = 4 - HAI = 0.84



Fig. 3.15 – Giant red shrimp. Configuration of four stock units with the overlay of the GFCM GSAs.

In order to compare this configuration with the current GSAs, some rectangles in the GSA9 belonging to the same cluster as GSA10 should be instead attributed to the cluster of GSA7 (and other adjacent GSAs), some rectangles in the GSA23 belonging to the cluster of GSA20 should instead be associated with the same cluster as GSA23. In the Aegean Sea the situation seems more patchy. Probably GSA22 is more a transition area where the species seems not or poorly present in the northern part. For this reason it is proposed to consider the GSA22 as belonging to the same cluster as GSA23, 24 and 25. The most suitable proposed configuration is thus based on 3 stock units and it is reported in table 3.13.

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs		STOCKMED Giant red shrimp
Western		Northern Alboran Sea	1	
Mediterranean		Alboran Island	2	
(Subarea 37.1)	Balearic (Division 37.1.1)	Southern Alboran Sea	3	
		Algeria	4	
		Balearic Island	5	
		Northern Spain	6	
		Sardinia (west)	11.1	
	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7	

Tab. 3.13 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for giant red shrimp (shaded areas=no information available).

		Corsica Island	8	
	Sardinia (Division	Ligurian and North Tyrrhenian Sea	9	
	37.1.3)	South Tyrrhenian Sea	10	
		Sardinia (east)	11.2	
		Northern Tunisia	12	
	Adriatic (Division 37.2.1)	Northern Adriatic	17	
		Gulf of Hammamet	13	
	Ionian (Division 37.2.2)	Gulf of Gabes	14	
Central		Malta Island	15	
(Subarea 37.2)		South of Sicily	16	
		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
	Aegean (Division	Aegean Sea	22	
Eastern Mediterranean (Subarea 37 3)	37.3.1)	Crete Island	23	
		North Levant	24	
	Levant (Division	Cyprus Island	25	
· · · · · · · · · · · · · · · · · · ·	37.3.2)	South Levant	26	
		Levant	27	

Illex coindetii

In the case of Broadtail shortfin squid two hypotheses were selected from WP4: 8 stock units with the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.94) and 4 stock units with an HAI=0.85 and the higher mean Cohen's Kappa. This hypothesis was selected. The results were based on 6 biological indicators and 4 thematic layers. According to the semi-quantitative robustness index (RI=20) the 4-units configuration is ranked as reliable, thus it is selected and reported in the figure 3.16.

In order to reconcile this configuration with the current GSAs, two rectangles in the GSA19 belonging to the cluster of GSA18 should be instead aggregated to the cluster of GSA19 (and of other adjacent GSAs), some rectangles in the GSA20 belonging to the cluster of GSA23 should instead be associated with cluster of GSA20, while few rectangles in GSA24 belonging to the cluster of GSA22 should be associated to GSA24. The cross-cutting among GFCM GSAs configuration and proposed stock units from STOCKMED is reported in the table 3.14.

Illex coindetii - Num of stocks = 4 - HAI = 0.85



Fig. 3.16 – Broadtail shortfin squid. Configuration of four stock units with the overlay of the GFCM GSAs.

Tab. 3.14 – Cross-cutting table between GFCM GSAs and stock units from STOCKMED results for Broadtail
shortfin squid (shaded areas=no information available).

FAO SUB-AREA	FAO Statistical Division	GFCM GSAs	STOCKMED Broadtail shortfin squid			
		Northern Alboran Sea	1			
		Alboran Island	2			
		Southern Alboran Sea	3			
	Balearic (Division 37.1.1)	Algeria				
		Balearic Island	5			
		Northern Spain	6			
Western		Sardinia (west)	11.1			
Mediterranean (Subarea 37.1)	Gulf of Lions (Division 37.1.2)	Gulf of Lions	7			
		Corsica Island	8			
	Sardinia (Division 37.1.3)	Ligurian and North Tyrrhenian Sea	9			
		South Tyrrhenian Sea	10			
		Sardinia (east)	11.2			
		Northern Tunisia 12				
Central	Adriatic (Division 37.2.1)	Northern Adriatic	17			
Mediterranean	Ionian (Division 37.2.2)	Gulf of Hammamet	of Hammamet 13			
(Subarea 37.2)		Gulf of Gabes	14			

		Malta Island	15	
		South of Sicily	16	
		Southern Adriatic Sea	18	
		Western Ionian Sea	19	
		Eastern Ionian Sea	20	
		Southern Ionian Sea	21	
Eastern Mediterranean (Subarea 37.3)	Aegean (Division 37.3.1)	Aegean Sea	22	
		Crete Island	23	
	Lovent (Division 27.2.2)	North Levant	24	
		Cyprus Island	25	
	Levalit (Division 57.5.2)	South Levant	26	
		Levant	27	

Octopus vulgaris

Concerning common octopus two hypotheses were formulated in WP4, the 4 clusters that had a high HAI (0.90) and the "3 stock units" configuration (fig. 3.17) characterized by the higher mean Cohen's Kappa and ranked first in terms of both acceptability index for the first rank and HAI (0.93). The analysis was based on two biological indicators (Biomass index, CV % of density) and three thematic descriptors.. However, the examined stock units should be considered unreliable, as the semi-quantitative robustness index (RI=1.6) was lower than the upper limit of the 1st quantile. Thus the cross-cutting table is not provided.

Octopus vulgaris - Num of stocks = 3 - HAI = 0.93



Fig. 3.17 – Common octopus. Configuration of three stock units with the overlay of the GFCM GSAs.

Eledone cirrhosa

Two hypotheses were considered more probable from the outcomes of WP4, the "7 stock units" which had the maximum of mean Cohen's Kappa coefficient though the accordance between constrained cluster outputs and thematic descriptor was rather flat from 3 to 7 clusters. The "6 stock units" (fig. 3.18) received the highest acceptability index for the first rank as well as the highest Holistic Acceptability Index (HAI=0.93). The results were based on 6 biological indicators and on two 2 thematic layers (correlation of density trends and EFH and connectivity) of information only. However, the examined stock units should be considered unreliable, as the semi-quantitative robustness index (RI=1.4) was lower than the upper limit of the 1st quantile. Thus the cross-cutting table is not provided.



Fig. 3.18 – Horned octopus. Configuration of six stock units with the overlay of the GFCM GSAs.

Eledone moschata

For this species information were insufficient to estimate any pattern of stock units.

4. Identifying knowledge gap and suggest candidate field for further investigations

In our analyses, the situations in which the information was too scant to progress with the identification of stock units, the elaboration process was not continued to the final step. This was the case of musky octopus. In other cases all the available information was used to define stock units and compare these with the current GFCM GSAs. However, not for all the species the same number of descriptors was available and thematic layers differed in terms of information by species. In addition, in some cases, although the thematic layer was available, the spatial coverage in terms of GSAs was poor and this could have affected, to a certain extent, the analyses. Thus a rank of robustness of the results was adopted and for species as *L. budegassa*, *S. pilchardus*, *O. vulgaris* and *E. cirrhosa* results were considered unreliable.

Regarding a quantitative evaluation of quality and quantity of information analysed and used in the project as well as spatial and temporal coverage of this information, a synthesis of the results obtained from the survey among the experts of the STOCKMED project is reported in the tables 4.1 and 4.2 (gap analysis tables).

If we consider the standardized vector of the species over the thematic layers, more information was available for European hake, red mullet, giant red shrimp and anchovy, whilst the information was more scant for cephalopods, black-mouthed dogfish and blackbellied angler. If we consider the standardized vector of the thematic layers over the species, genetics, oceanographic systems and parasites had a lower availability of information, whilst the thematic layers linked to the MEDITS trawl survey data were better represented. The outputs of the MEDISEH project were very useful for the Essential fish habitat and connectivity layer.

	Abundance-	Critical area	Oceano	Genetics	Parasites	Spawning_	Growth	Spawning	Final	Standardized
	trends	connectivity	systems			LSU		_season	score	
M. merluccius	0.057	0.055		0.087	0.048	0.092	0.101	0.055	0.493	0.084
M. barbatus	0.055	0.043	0.053	0.059		0.072	0.108	0.085	0.476	0.081
A. foliacea	0.050	0.072	0.053	0.058		0.071	0.065	0.086	0.455	0.077
E. encrasicolus	0.051	0.063	0.053	0.038		0.062	0.066	0.076	0.409	0.069
S. solea	0.053	0.073	0.053		0.058	0.078	0.038	0.042	0.396	0.067
T. trachurus	0.055	0.067		0.044		0.062	0.086	0.081	0.395	0.067
M. surmuletus	0.053	0.054	0.053			0.064	0.075	0.073	0.372	0.063
T. mediterraneus	0.053	0.025		0.062		0.073	0.077	0.071	0.361	0.061
A. antennatus	0.051	0.017	0.053	0.041		0.045	0.069	0.078	0.355	0.060
N. norvegicus	0.053	0.055		0.041		0.053	0.068	0.046	0.317	0.054
S. pilchardus	0.052	0.075	0.053			0.068	0.031	0.025	0.303	0.051
P. erythrinus	0.049	0.049		0.041		0.044	0.055	0.045	0.283	0.048
P. longirostris	0.048	0.025	0.053	0.063		0.013	0.030	0.020	0.251	0.043
O. vulgaris	0.052	0.059		0.045		0.019	0.019	0.019	0.213	0.036
I. coindetii	0.054	0.065				0.043	0.011	0.029	0.201	0.034
E. cirrhosa	0.054	0.055				0.028	0.028	0.035	0.199	0.034
E. moschata	0.052	0.050				0.022	0.007	0.028	0.160	0.027
G. melastomus	0.054	0.057				0.039			0.150	0.025
L. budegassa	0.052	0.040					0.013		0.105	0.018

Table 4.1. Synoptic table of the gap of information by thematic descriptor and species highlighted by the experts in the survey on the quality, quantity and coverage of information source (standardized results).

Tab. 4.2. Synoptic table of the gaps of information by thematic descriptor over all the species as highlighted by the experts in the survey on the quality, quantity and coverage of source of information (standardized results).

	Abundance- biomass trends	Critical area and connectivity	Spawning_L50	Growth	Spawning_se ason	Genetics	Oceanographi c systems	Parasites
Final score	1.000	1.000	0.947	0.947	0.895	0.579	0.421	0.105
Standardized	0.170	0.170	0.161	0.161	0.152	0.098	0.071	0.018

However, besides the remarkable gaps of information emerged for genetics (especially study with significant spatial coverage and fine spatial scale representation) and parasite, the lack of knowledge in different key disciplines was experienced throughout the project (see table 4.1). In particular, for the following topics the information available useful for stock identification and definition of stock boundaries was very scant or absent:

- otolith shape and chemical composition;
- analyses of hard structures like vertebrae and spines;
- larval drift associated with the pattern of the currents;
- spatial movements and habitat use at different life stages and for different species;
- spatial and temporal pattern of oceanographic fronts.

5. Concluding remarks and recommendations

The achieved results in terms of stock units identification for 14 species are provided in the synoptic table 5.1, where the proposed stock units are associated to the current GFCM GSAs.

Only for one species (*Eledone moschata*) results were considered not enough for the continuation of the analysis, while for other 4 species (*L. budegassa, S. pilchardus, O. vulgaris* and *E. cirrhosa*) results were considered unreliable for proposing new stock units. However, not for all the species the same number of descriptors was available and thematic layers differed in terms of information by species. In addition, in some cases, although the thematic layer was available, the spatial coverage in terms of GSAs was poor and this could have affected, to a certain extent, the analyses.

The collection of information during the STOCKMED project, the carried out analyses and finally the achieved outcomes highlighted several knowledge gaps regarding the species biological features and life history, genetic characterization, parasites. In some cases the available information was insufficient for stock unit identification. In other ones the available information was not geo-referenced at a fine spatial scale that resulted in an oversimplification of the spatial structure. In addition, some fields of knowledge are quite completely not covered, like for example animal movement and habitat use, that are of crucial importance for stock identification and definition of stock boundaries. Furthermore, information on the spatial localization of fishing effort is in general poorly informative on the true composition by species of the catches or of the main target resources.

Another gap is represented by vast areas of the Mediterranean, in particular on the southern borders, where the information is too scant or sparse for a comprehensive analysis. This implies that the view gathered by the project is mainly localized in the European region of the Mediterranean.

All these considerations entail that the view we gathered should be regarded as a "working in progress" linked to the current level of knowledge, but that can be easily updated along with the improvements of the level of information. Indeed, a methodological framework has been developed that can be adapted to new gathered data.

Given the background knowledge used in the identification of stock units, these should be mainly regarded as homogeneous biological entities. In general, the process of stock units identification resulted in a smaller number of entities compared to the current GFCM GSAs frame and thus with the aggregation of units in bigger areas for all the stocks. In many situations it was necessary to incorporate some GFCM statistical rectangle in one GSA or another, in order to reconcile the current spatial segmentation of the Mediterranean with the stock units identified. This adaptation process was in general limited and supported by considerations related to the information conveyed in the thematic descriptors as well as from the fishing effort spatial intensity as derived from the Deliverable 11.

The aggregation of the current GFCM GSAs in larger areas for stock assessment purposes recalls for a better harmonization of the Data Collection also between different Member States, with the task of sampling allocation reflecting the stock distribution and the relative catch among the relevant GSAs. In addition, this should be realized in terms, for example, of sampling certain métier and period, or gathering and make available a better knowledge of the spatial distribution of the fleet and, in turn, of the intensity and type of fishing effort deployed in certain areas. Data like VMS and AIS would be very important to these purposes.

If the new stock units will be adopted It is also suggested to apply a gradual approach during the stock assessment process, for example conducting the assessment at the spatial scale used so far and then expanding the evaluation to the new stock units in order to make comparison and highlight possible improvements.

A further aspect is related to the necessity of using, developing or adapting stock assessment and forecast tools that allow to take into account the different components of the pressure in terms of fishing mortality and effort by fleet and gear. If the identified stock units will be adopted the partitioning of fishing mortality among fleets and gears is crucial, especially for designing management measures calibrated for the different fleets and gears that are affecting the sustainable harvest of target stocks.

AREA Division OPC/M GARS Image: Constraint of the constraint o	adtail ortfin
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Scale	
Stock units 1 2 3 4 5 6 7 no info absence	

Tab. 5.1 – Synoptic table of the association of the stock units identified in STOCKMED with GFCM GSAs.

ANNEX IV - LIST OF ACRONYMS

- AVISO Archiving, Validation and Interpretation of Satellite Oceanographic Data
- AVHRR Advanced Very High Resolution Radiometer
- AFLP Amplified Fragment Length Polymorphism
- AHP Analytical Hierarchy Process
- CC Constrained Clustering
- CHL Chlorophyll
- CIBM Centro Interuniversitario di Biologia Marina ed Ecologia Applicata "G. Bacci"
- CNR Italian National Research Council
- COISPA COISPA Tecnologia e Ricerca Stazione Sperimentale per lo studio delle Risorse del Mare
- CoNISMa Consorzio Nazionale Interuniversitario per le Scienze del Mare
- DCR Data Collection Regulation
- DCF Data Collection Framework
- DGMARE Directorate-General for Maritime Affairs and Fisheries (European Commission)
- DNA Deoxyribonucleic acid
- DRIFTMED Identification and characterization of the small-scale driftnets fisheries in Mediterranean (Marea Specific Contract N° 8)
- EFH Essential Fish Habitat
- EU European Union
- EUSeaMap European Sea bed Mapping
- FAO Food and Agriculture Organization (United Nations)
- FishPopTrace "the Structure of Fish Populations and Traceability of Fish and Fish Product"
- GBS Genotype by Sequencing
- GFCM General Fisheries Commission for the Mediterranean (FAO).
- GIS Geographic Information System
- GIS-MCDA Geographic Information System Multi Criteria Decision Analysis.
- GRUND GRUppo Nazionale valutazione risorse Demersali.

GSA – Geographical Sub Areas GSSA – Genetic Stock Structure Analysis HCMR – Hellenic Centre for Marine Research ICES - International Council for the Exploration of the Sea IEO – Instituto Español de Oceanografía **INSPIRE - Infrastructure for Spatial Information in the European Community** JRC - Joint Research Centre LFD – Length Frequency Distribution LOA – Length Overall MAREA - Mediterranean hAlieutic Resources Evaluation and Advice MCDA – Multi Criteria Decision Analysis MCFS – Maltese Centre of Fisheries Sciences MEDIAS - Mediterranean International Acoustic Survey MEDISEH – Mediterranean Sensitive Habitats (Marea Specific Project N.2) MEDITS – Mediterranean International bottom trawl survey mtDNA - Mitochondrial Deoxyribonucleic Acid MVA – Multivariate data Analysis NGS – Next Generation Sequencing NSFDSS – Non-Structural Fuzzy Decision Support System nuDNA – Nuclear Deoxyribonucleic Acid PDF - Probability Density Function. POPGENE - The User-friendly Freeware For Population Genetic Analysis RoME - R code to perform multiple and cross checks on MEDITS survey data **RTD** - Research and Technology Development SGMED – Sub Group for the Mediterranean (STECF group) SMAA - Stochastic Multicriteria Acceptability Analysis SNP - Single Nucleotide Polymorphism SOLEMON – Solea Monitoring modified beam trawl survey

- SSR Simple Sequences Repeated
- STECF Scientific, Technical and Economic Committee for Fisheries
- UPGMA Unweighted Pair Group Method with Arithmetic Mean
- UPGMC Unweighted Pair-Group Method using Centroids
- VMS Vessel Monitoring System
- WGAGFM Working Group on the Application of Genetics in Fisheries and Mariculture (ICES)
- WPGMA Weighted Pair Group Method with Averaging
- WPGMC Weighted Pair-Group Method using Centroids