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MEDSUDMED - TECHNICAL DOCUMENTS

**Report of the workshop on
octopus stocks and related fisheries
in the MedSudMed area
(Salammbô, Tunisia 18-20 November 2008)**



Cover photograph:

Fishing pots for *Octopus* spp. in Sfax, Tunisia. Courtesy of Petri Suuronen



MedSudMed

GCP/RER/010/ITA

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The conclusions and recommendations given in this and in other documents in the *Assessment and Monitoring of the Fishery Resources and Ecosystems in the Straits of Sicily* Project series are those considered appropriate at the time of preparation. They may be modified in the light of further knowledge gained in subsequent stages of the Project. The designations employed and the presentation of material in this publication do not imply the expression of any opinion on the part of FAO or MiPAAF concerning the legal status of any country, territory, city or area, or concerning the determination of its frontiers or boundaries.

Preface

The Regional Project “Assessment and Monitoring of the Fishery Resources and the Ecosystems in the Straits of Sicily” (MedSudMed) is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Italian Ministry of Agriculture, Food and Forestry Policies (MiPAAF). The Italian Regione Siciliana funded a project aimed at strengthening MedSudMed’s effectiveness on issues related to demersal resources, namely crustaceans, for 18 months, starting from May 2011.

MedSudMed promotes scientific cooperation between research institutions of the four participating countries (Italy, Libya, Malta and Tunisia), for the continuous and dynamic assessment and monitoring of the state of the fisheries resources and the ecosystems in this area of the Mediterranean.

Research and training are supported to increase and use knowledge on fisheries ecology and ecosystems, and to create a regional network of expertise. Particular attention is given to the technical coordination of the research between the countries, which should contribute to the implementation of the Ecosystem Approach to Fisheries. Consideration is also given to the development of an appropriate tool for the management and processing of data related to fisheries and their ecosystems.

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GCP/RER/010/ITA Publications

The MedSudMed Project publications are issued as a series of Technical Documents (GCP/RER/010/ITA/MSM-TD-00) related to meetings, missions and research organized by or conducted within the framework of the Project.

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Preparation of this document

This document is the final version of the report of the Workshop “Octopus stocks and related fisheries in the MedSudMed Project area” organised by the MedSudMed Project (*Assessment and Monitoring of the Fishery Resources and the Ecosystems in the Straits of Sicily*) on 18-20 November 2008 in Salammbô (Tunisia), hosted by the Institut National des Sciences et Technologies de la Mer.

Octopus vulgaris is one of the most important demersal fishery resources in some of the countries surrounding the Straits of Sicily. The fishing areas for this species extends through national and international waters. In some cases fishing grounds for fishing fleets of different countries overlap. In some countries *Octopus vulgaris* stocks is believed to be exploited over the natural limits. A clear definition of the stock boundaries and operational units harvesting this species in the Straits of Sicily is necessary to identify management strategies that ensure fisheries sustainability.

In line with its mandate and coherently with the indications given by the Project Coordination Committee, the issues related to *Octopus vulgaris* fisheries were dealt with by the MedSudMed Project. The MedSudMed Workshop “Octopus stocks and related fisheries” was the follow up to a specific research programme initiated by the Project in Tunisia and in the Project area. The Workshop aimed at reviewing the current knowledge on this species and related fisheries in the Straits of Sicily and establishing the basis for cooperative future work at regional level. In particular, a first identification of Operational Units exploiting the species and a genetic study for the identification of shared stocks were taken into consideration.

This document provides a description of the material presented and the discussions held during the Workshop. A compilation of the technical contributions presented during the workshop is also included. It is addressed primarily to fishery scientists, managers and professionals.

It is hoped that this study will contribute to strengthening international cooperation and to promoting the assessment and monitoring of the fisheries resources in the Straits of Sicily.

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Report of the workshop on octopus stocks and related fisheries in the MedSudMed Project area (Salammbô, Tunisia, 18-20 November 2008).

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ABSTRACT

The Workshop on “Octopus stocks and related fisheries in the MedSudMed Project area” (Salammbô, Tunisia, 18-20 November 2008) was attended by experts from Italy, Libya, Malta and Tunisia. The main objectives of the workshop were: i) to synthesise and compare the existing knowledge on the biology and distribution of *O. vulgaris*; ii) to identify the gaps to be filled for the identification of *O. vulgaris* populations units; iii) to prepare a work plan in order to verify if the population units of *O. vulgaris* belong to shared stock(s); and iv) to contribute to identifying the Operational Units targeting *O. vulgaris* in the Project area. Ten presentations were given on biological aspects, distribution and exploitation (including socio-economic aspects) of *O. vulgaris* in the MedSudMed area. The differences and similarities of biological features, exploitation, market and fisheries regulations among countries in the Project area were also pointed out. A joint exercise to identify the fleet segments targeting *O. vulgaris* in the area was carried out. The gaps to be filled in order to identify population units for *O. vulgaris* and to provide a detailed description of the OUs targeting this species in the MedSudMed area were listed and the outline of a work plan to fill these gaps was agreed upon. Two main thematic areas were identified: i) tentative identification of nursery and spawning areas along the Tunisian coasts by processing available surveys data, ii) genetic studies to compare population units. Moreover, a further field of interest was discussed: the collection of data for the proper identification of OUs targeting *O. vulgaris*.

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**Workshop on octopus stocks and related fisheries in the MedSudMed Project area
Salammbô, Tunisia, 18-20 November 2008**

(i) Opening of the meeting and presentation of the general objectives of the workshop

The Workshop on octopus stocks and related fisheries in the MedSudMed Project area was held in Salammbô, Tunisia, from 18 to 20 November 2008, kindly hosted by the Institut National des Sciences et Technologies de la Mer (INSTM). The Workshop was attended by 13 experts coming from all MedSudMed Project participating countries as well as by the Project staff (see list of participants, Annex A).

According to the Terms of Reference (Annex B), the specific objectives of the workshop were outlined as follows:

- synthesise and compare the existing knowledge on the biology and distribution of *Octopus vulgaris* within the Project area thus highlight possible differences or similarities;
- point out the gaps to be filled for the identification of *O. vulgaris* population sub-units in the MedSudMed region;
- prepare a workplan in order to verify whether the population sub-units of *O. vulgaris* in the MedSudMed sub-region belong to shared stock(s);
- contribute to the identification of the Operational Units (OUs) targeting *O. vulgaris* in the MedSudMed Project area.

The agenda including the list of presentations prepared by the participants are in Annex C. The workshop was organized in three sessions focused on: (i) the presentation of the current knowledge (bio-ecological and fisheries related features) and research activities carried out on common octopus (*Octopus vulgaris* Cuvier, 1797) in the participating countries; (ii) the definition of the main issues to be addressed for the identification of *O. vulgaris* population sub-units and of the fleet segments targeting them in the MedSudMed area; (iii) the discussion of a possible work plan for the identification of population sub-units of *O. vulgaris* in the area.

According to the Agenda items, the presentations by the participants were grouped by theme: (i) Biology and ecology; (ii) Spatial distribution; (iii) Exploitation; (iv) Genetics; and (v) Socio-economy. The abstracts of the presentations are reported below, as well as a summary of the discussions that followed each presentation. Documents related to the presentations given are included at the end of this report.

(ii) Description of the current knowledge on the biology, distribution and abundance of *Octopus vulgaris* and of the fisheries that exploit this species in the MedSudMed subregion

As an introduction, a general presentation was given on the trends of the total capture production of cephalopods and octopuses in the whole Mediterranean and in the MedSudMed participating countries on the basis of the FAO-GFCM capture fisheries statistics. The main biological and ecological characteristics of the common octopus in the Mediterranean region

were also briefly presented as reported in the MedSudMed Technical Document No.15 and in the scientific literature.

Biology and fishery of *Octopus vulgaris* in the Mediterranean Sea (L. Ceriola)

In the MedSudMed sub-region (south-central Mediterranean Sea) cephalopods are valuable fishery target species and among them octopods and *Octopus vulgaris* (Cuvier, 1797) have great importance in terms of economic value and total catch. It is one of the most landed species in Tunisia and Malta and also represents an important resource for fisheries in Libya and Italy (south Sicily). In this presentation the capture production of cephalopods (global and in the Mediterranean Sea) from 1970 to 2005 are described, as well as the total *O. vulgaris* capture production in the Mediterranean Sea and in the MedSudMed sub-region. Moreover, the current knowledge on the biology and ecology of the common octopus in the Mediterranean region and some general features on the fisheries exploiting this species are summarised.

O. vulgaris is distributed from the coastline to the outer edge of the continental shelf (in depths from 0 to 200 m) where it is found in a variety of habitats, such as rocky and grass beds. It is a fast growing species with a life span of less than 24 months. Dorsal mantle length (DML) in the Mediterranean bottom trawl fisheries varies from 50 to 200 mm and the recruitment size ranges from 50 to 60 mm DML. Length-weight relationship is generally isometric for both sexes, even if in western Mediterranean negative allometric values were also found. *O. vulgaris* has an extended spawning period (> 8 months) with a seasonal peak of spawning activity which can vary according to region (generally during spring). Size at maturity ranges from 100 to 120 mm DML with females maturing at larger size compared to males. Juveniles and adults of this species are strictly benthonic but larvae are pelagic and can spread over long distances after hatching and before settling on the bottom. As a result, specimens of *O. vulgaris* belonging to the same stock can spill over and inhabit regions distant from each other. Within the area covered by the MedSudMed Project, *O. vulgaris* is exploited by trawlers or by using different kinds of “traps”, and occasionally by gill or trammel netters. The total capture production of this species in the Sicily Channel generally fluctuates over time with total landings varying according to the country and to the fishing techniques (ranging from 400 to 4000 tons per year). To date, no studies have been carried out and very little information is available on the occurrence of shared stocks for the common octopus in the Mediterranean region.

The gaps that still need to be filled to provide an appropriate background to the identification of appropriate management strategy for *O. vulgaris* were tentatively listed. The importance of the identification of population units and the possible occurrence of shared stocks in order to put into practise management measures in line with an ecosystem approach was also underlined. Finally, the aims of the MedSudMed workshop on “*O. vulgaris* stocks and related fisheries in the MedSudMed Project area” were presented and the available methodological tools for fulfilling the described gaps were illustrated.

After the presentation, the potential role of cephalopods as exploitable fishery resources and the worldwide increase of their total capture production were underlined. It was recalled that the increase of the total biomass of cephalopods is often regarded as a sign of over-exploitation of the stocks of traditional fisheries target species, as long living late maturing fin fishes. An example was given from the Northern Atlantic where cephalopod and squid fisheries developed substantially after the depletion of cod stocks. In addition, it was remarked that only the combined information on landings and fishing effort would provide a more complete description of the overall evolution of the fisheries.

The importance of *O. vulgaris* for the fisheries in the countries participating in MedSudMed was briefly addressed. The possibility that the population units of this species in the area belong to one or more shared stock(s), as well as the importance of addressing shared stocks in the Mediterranean region were also recalled.

Regarding the identification of population units and the main biological features of *O. vulgaris*, the meeting was reminded that the population concept is strictly related to spatial distribution, therefore the description of the distribution and the location of spawning and nursery areas are of utmost importance. It was also mentioned that several features should be taken in consideration for the identification and/or comparison of population units: e.g. the

morphologic and biological characteristics and the homogeneity in specific genetic markers. Participants agreed on the importance of understanding the underlying factors explaining the spatial distribution and the possible migration patterns of the different populations in the region, such as the main environmental parameters (e.g. trophic features, water mass flows) and the occurrence of spawning areas.

As regards growth studies, the applicability of the Von Bertalanffy Growth Function to *O. vulgaris* was questioned and the pros and cons of the most commonly used methods to study growth in this species were discussed (i.e. statoliths/beaks analysis, length frequency distribution analysis).

Current knowledge on the bio-ecology of the common octopus *Octopus vulgaris* from Tunisian Southern coasts. (S. Ezzeddine, B. Chemmam, O. Jarboui).

The common octopus, *Octopus vulgaris*, has been important in the Tunisian economy since the eighties. Subsequently the research interest increased continuously leading to the planning and execution of studies on the bio-ecology and population dynamics of this species and, more recently, on the settlement of the stock. These studies focused in particular on the southern coastal area, due to its utmost importance for fishing: octopus caught in this region represents more than 80% of the national capture production. The current knowledge on the biology of *O. vulgaris* regards reproduction, growth, feeding behaviour and ecology. A summary of this knowledge is provided.

Reproduction:

- The size at sexual maturity, expressed in dorsal mantle length (DML) (i.e. the DML at which the 50% of the population is mature), is 13 cm in females and 9 cm in males;
- The spawning season lasts from February to late November with a peak in spring (from May to July);
- The success of reproduction is significant thanks to the internal mating; the observed fecundity is 100 spermatophores per kg of body weight in males and 4000 mature ovarian eggs per kg of body weight in females. Large females may exceed 5000 mature ovarian eggs per kg of body weight.

Growth:

- An indirect method based on the evolution of size frequencies modes has been adopted to study growth;
- The adjustment of the von Bertalanffy growth equation allowed estimation of the growth parameters and the life span duration: *O. vulgaris* in Tunisian waters is characterised by a relatively short life span, less than 13 months, with an estimated maximum size of 29 cm DML.

Feeding and ecology:

- The feeding behaviour of octopus was also qualitatively and quantitatively studied by analysing the stomach contents: fish and shellfish are the dominant preys in the investigated area, followed by molluscs as accessory preys. Seasonal migrations of genetic and trophic nature were evidenced. *O. vulgari* is a species with a solitary innate affinity, acquiring a gregarious instinct when it undergoes seasonal migration towards shallow waters.

Studies carried out in Tunisia since 1986 on the biology, ecology, biochemistry, stock assessment of *O. vulgaris* show no significant differences in the biological characteristics of coastal (targeted by artisanal fishery down to 10 m depth) and off-shore octopus (caught by trawlers around 50 m depth) populations.

Differences in the recruitment and spawning season between the Tunisian and south-Sicilian coasts were highlighted during the discussion. The bulk of reproduction and recruitment in Tunisia occurs in spring and in autumn respectively. Samples coming from the South Sicilian coast showed an important fraction of recruits during spring. The occurrence of spring recruits was tentatively explained by the presence of females living in deeper and cooler waters. In deeper water, female gonad maturation may require more time compared to females living in shallower and warmer waters, resulting in a delay in egg deposition which occurred during late autumn (at the end of the observed spawning period). In addition, the temperatures of the following winter would slow down the egg incubation process, thus delaying the hatching time and leading to the observed recruitment during spring.

It was also underlined that the results presented were based on fishery dependent data, which hence reflect the trends in fisheries and are strictly related to the national regulations (limited

fishing season and minimum landing size). Therefore, these results may provide an incomplete picture of the size structure of the stock and of the recruitment process in Tunisian waters.

In order to better understand these features, as well as the possible migration and growth process in *O. vulgaris*, participants mentioned tagging experiments. The difficulties of such monitoring activity due to the life cycle and ecological behaviour of this species were also underlined and agreed on. According to the participants, several constraints still prevent the implementation of tagging and recapture experiments on *O. vulgaris* in the MedSudMed area: e.g. given the short life-cycle of the species tagging of common octopus needs to be carried out with a sharp calendar; the semelparous strategy (adults die after reproduction) would greatly affect the re-capture rate. Moreover, tagging experiments require a close and reactive collaboration with fishers, which is not fully achievable in the Project area at present.

Biology and fishery of common octopus, *Octopus vulgaris* Cuvier, 1797, in the northern sector of the Strait of Sicily. (F. Fiorentino, M. Gristina, G. Sinacori, L. Labanchi).

Information on biology and fisheries of *Octopus vulgaris* in the northern sector of the Strait of Sicily is provided on the basis of fishery independent and fishery dependent data. Biological data come from trawl surveys carried out by IAMC_CNR during spring (MEDITS series from 1994 to 2007) and autumn (GRUND series from 1994 to 2006). Investigated areas include the GSAs 15 (with exclusion of Maltese Management Fishing Zone) and 16. Catch and effort information was obtained from data collected by IREPA (Istituto di Ricerche Economiche per la Pesca e l'Acquacoltura) within the European DCF (Data Collection Framework). Monthly data on the sea surface temperature (°C) were downloaded through the POET interactive on-line system (from site <http://podaac.jpl.nasa.gov>).

The biological data were processed using the Seatrim software to produce standard information on abundance indices in terms of both kilograms (biomass index) and number (density index) per square kilometre, length frequency structures, sex ratio, percentage at maturity stage and length-weight relationships. The variation in abundance, demography and catches of the common octopus in the investigated area are discussed and tentatively related to the seasonal variation in SST.

Information on yield of the common octopus in the south of Sicily is provided since 2004; no data on the capture of this species are available before 2003. More than 90 % of the catch comes from trawlers for which *O. vulgaris* is not a main target species. A monthly variation of the catch was observed, with autumn and spring as most productive seasons. 2004 was the year with the highest production (1600 tons) and November was the most productive month.

The results highlight the effect of temperature (SST) on the recruitment process and on the overall abundance of *O. vulgaris* over time. The variations in SST explain 50% of the recruits abundance and total biomass variations. The results were discussed and an explanation was tentatively provided. The different water mass flows occurring in the northern and southern part of the MedSudMed sub region (upwelling along the south Sicilian coast and downwelling along the Tunisian coast), as well as their strong effect on the recruitment process were recalled. Considering the apparent positive effect of higher temperature on recruitment in the south Sicily, it was hypothesised that relatively higher SST would increase the water stratification process, hence reducing the upwelling phenomenon. This reduction would probably reduce the dispersion of pelagic octopus larvae, favour their settlement and enhance the success of recruitment.

During the discussion, the possible effects of environmental parameters on morphometric and biological features of cephalopods (higher water temperature generally accelerates growth and sexual maturation processes, leading e.g. to smaller size at maturity and shorter life span) were recalled. The possibility that the local differences in environmental parameters between south and north of the MedSudMed area may result in differences in octopus populations was also pointed out, even if genetic studies may not detect any distinction between them. Accordingly, it was hypothesised that the population units of *O. vulgaris* in the MedSudMed area could have specific morphometric features and migration patterns even if they belong to the same shared stock(s).

Spatial distribution of recruits and adults of *Octopus vulgaris* in GSAs 15 and 16 (central Mediterranean). (G. Garofalo, F. Fiorentino, M. Camilleri, M. Dimech, M. Gristina).

The focus of this study is the population spatial structure of the common octopus, *Octopus vulgaris* (Cuvier, 1797), in the GSAs 16 and 15 of the Strait of Sicily (central Mediterranean). Specifically, the depth distribution of the species and the geographic position of nursery and spawning grounds were investigated. Abundance indices by life-stage (recruits and spawners) were obtained from two series of scientific trawl surveys carried out in the Strait of Sicily in the period 1994-2007 within the framework of the international programme MEDITS and the Italian national program GRUND. Distribution maps of density indices were generated for each life stage and year by Inverse Distance Weighted (IDW) deterministic interpolation. Specifically, 14 distribution maps of recruit density indices were produced for the autumn season (main recruitment period), whereas 9 density maps of adult females were obtained for the spring season (main spawning period). Moreover, 4 years of data were available to produce distribution maps of recruit density indices in the African platform of GSAs 13 and 14. Nursery and spawning areas were identified by means of GIS techniques, applying criteria based on the persistent presence of high values of recruits and adult females respectively, throughout the period of study.

A stable spawning ground was identified in the north-west sector of GSA16. Several nurseries were found along the south Sicily and north Malta coasts. An important stable nursery was found in the south-east corner of GSA 13. It was pointed out that the highest value of spawners and recruits abundance occurred around 60 m.

The discussion that followed the presentation focused on the importance of identifying nursery and spawning areas of common octopus in the whole MedSudMed sub-region, in order to understand the possible migration patterns and connections between stock units. The relationship between water mass flows and location of nursery areas, as well as the possible occurrence of further spawning and/or nursery grounds in Tunisian and Libyan waters were discussed.

Regarding the results presented, it was highlighted that the common octopus was not a target species during all the surveys carried out in the study period (1994-2007), therefore the information on the abundance of individuals at different maturity stages and on some biological characteristics (e.g. length frequency distribution, percentage of maturity stages, occurrence of recruits) should be considered preliminary and not exhaustive.

In addition, the participants pointed out that nurseries are located at the edge of circular systems and where thermal fronts exist. Accordingly, it was preliminary hypothesised that the distribution of nurseries is somehow related to the vertical water mass flows (i.e. upwelling) that the circular systems support. It was also highlighted that the nursery area that was found south of Lampedusa coincides with the fishing grounds of the Tunisian trawlers. In this regard, evidence of the possible presence of a nursery area in Kerkennah (South of Lampedusa) was examined and discussed. Moreover, given the general water circulation and the symmetry in the distribution of demersal resources between the south Sicilian and the Tunisian (and conceivably the Libyan) coasts, the occurrence of an additional spawning area in GSA 13 was considered plausible. Finally, it was underlined that as the spawning area found along the south-western Sicilian coast probably “sustains” the octopus population along the GSA 16, likewise the possible spawning area in the western Tunisian coast could have the same role in “sustaining” the octopus population along the southern part of the MedSudMed region. If this was confirmed, it would provide preliminary evidence of the separation of the stocks in the MedSudMed area. Based on this discussion, the meeting agreed that further studies on the distribution of spawning grounds should be planned.

Exploitation of octopus *Octopus vulgaris* in the Tunisian Southern area: current state and future prospects. (S. Ezzeddine, B. Chemmam, O. Jarbouï).

Among the common fisheries target species (cuttlefishes, octopuses, eledonids and squids), *Octopus vulgaris* is the most targeted species in the Tunisian Southern area due to its commercial value. This region provides 83% of the national octopus capture production. Currently cuttlefish represent more than 65% of cephalopod landings, whereas the common octopus was the dominant species in the catch during the eighties.

In spite of the existing fishing regulation (adopted in 1987 and amended twice so far) aiming at preserving the stock of common octopus, the total capture production of this species showed numerous fluctuations between 1975 and 2007, with a tendency to significant reduction beyond 1990. Consequently, a decrease in capture production and revenue of small-scale fishery was observed in the same period, along with an increase in the landings and revenue of trawlers.

The assessment of the southern stock of *O. vulgaris* revealed an optimal exploitation level of the resource tending to the over-exploitation. Several factors caused the modification of cephalopod fishing profile which involved the whole fisheries in the southern area. Among these factors, the over fishing and the lack of respect for regulations were quoted, along with the deterioration of the area reserved for coastal vessel activities. This factor obliged some of the fishermen to replace their artisanal gears with more invasive fishing equipment in order to maintain the same level of capture. Environmental factors also contribute to limiting the expansion of octopus. In order to better manage stock, some recommendations are suggested. A brief description of biocenoses characterising the main fishing grounds for *O. vulgaris* was also provided. In this context it was found that red calcareous algae (*Lithophylum* spp.) is always present in the most productive areas.

The evolution of the fisheries, the level of exploitation of *O. vulgaris* stock along the Tunisian southern coast, as well as the impact of sea water temperature variation on the total biomass of this species were discussed after the presentation. An evolution in the fisheries was observed over time with a relative decrease in common octopus landing in favour of an increasing exploitation/landing of cuttlefishes. The Tunisian off-shore fleet specifically targets octopus, and for some vessels this species represents 80% of the total landing.

The INSTM experts reported that the southern *O. vulgaris* stock seems fully or over exploited and argued that a reduction in the fishing effort in the Tunisian southern area may be envisaged as precautionary measure. It was also noted that there is a concentration of fishing effort, mainly due to trawlers, in Kerkennah (south of Lampedusa) with fishers claiming to be able to fish the “Italian” rather than on the “south Tunisian” stock.

A cyclic variation (about 7 years) in the capture production of *O. vulgaris* was pointed out by Ezzeddine *et al.* and the possible linkage of this periodicity to the natural fluctuation of environmental parameters, especially water temperature, was explored during the discussion. There seemed to be an inverse relationship between common octopus capture production and higher water temperature, with an increase of biomass after a decrease in temperature. This correlation is opposite, but not in contradiction, to what was found in the northern sector of the MedSudMed area. On the contrary, this correlation may support the hypothesis on the effect of upwelling/downwelling on recruitment. Along the southern part of the MedSudMed sub-region the occurrence of downwelling is recorded and this probably favours the settlement of paralarvae and the success of recruitment. In this context, higher water temperature would increase the water mass stratification weakening the down-welling and negatively affecting the recruitment as observed along the Tunisian coast. This hypothesis would explain the opposite effect of temperature on abundance in the two sectors of the MedSudMed area.

Current status of knowledge about the fishery of *O. vulgaris* in the Maltese Islands and data available. (R. Pace, L. Knittweis, M. Dimech).

Octopus vulgaris is of high economic importance for the Maltese fisheries since it is the most caught species by weight after Malta's main targeted species (*Coryphaena hippurus*, *Thunnus thynnus* and *Xiphias gladius*). This was only realised recently, since prior to 2003 when only landings data from the fish market were available, landings by weight of this species ranked at around eleventh. However, when this data was combined with small scale fishery (< 10 m) sampling survey data, catch estimations increased considerably as this species is mostly sold directly by the fishermen and does not go to the fish market.

A series of landings data since 1950 show that in the 1970s a peak was reached which declined drastically in less than ten years. The sudden increase coincides with the period in which several trawlers were introduced to the Maltese fleet, while the decline corresponds to the change of the Maltese fleet from in-shore to off-shore fishing.

Besides landings data, log book data is available from 2006 onwards and applies to vessels longer than 10 m. The small scale fishery survey data, which is aimed at smaller vessels, is available from 2003, while biological parameters of *O. vulgaris* in GSA 15 can be obtained from MEDITS (Mediterranean International Trawl Survey) data since 2002.

Furthermore, data concerning recreational harvesting of *O. vulgaris* especially where shore based and underwater fishing are involved, is missing. In Malta these types of fisheries are important and it is well known that considerable amounts of *O. vulgaris* are caught by spear fishermen and other amateurs using boats. However, to date there is no data to document such catches.

During the discussion the meeting was informed that collecting data (both fishery dependent and independent data) on the common octopus is somewhat difficult in Malta due to the extension of the shelf and to the type of fisheries targeting this species. Moreover, according to the sampling scheme of the trawl surveys carried out in this country and to the ratio between shelf and slope extent, only 15% of the hauls is allocated in the depth strata between 10 and 200 m, significantly reducing the possibility to catch *O. vulgaris*. In addition, the common octopus in Malta is caught mostly by small scale fisheries (especially using pots, with trawlers contributing only by a small fraction to the total capture production); it is sold directly at the landing site and as a consequence it is difficult to collect information on landed quantity and on the length frequency distribution of the catch for this species.

Current status of *Octopus vulgaris* fisheries along the coast of Libya. (H. M. Ghmati).

In recent years the importance of *Octopus vulgaris* for fisheries at local level increased in Libya owing to a significant increase in its economic value. Several surveys were carried out during the last decades and a great abundance of this species along the western coast of Libya was found.

Accurate information on the fisheries targeting *O. vulgaris* is available in Libya, whilst detailed information on the biology and distribution of this species is still missing. Location of landing sites, number of vessels, type of vessels and gears specifically used to target *O. vulgaris* were described. The need to obtain information on the biology and migration pattern of *O. vulgaris* in Libya, so as to be able to design a management strategy for this species, is now a priority.

As trawlers have a limited activity in Libya (the fleet is not active all year round and industrial fishery does not operate during the summer) they have a relatively low impact on common octopus stock. The greatest part of the catch for this species comes from artisanal fishery.

The common octopus in Libya is caught mainly in the Farwa lagoon in shallow waters (2 m depth) using pots from November to March. Trawlers also catch this species even though it is not the main target species. In terms of total landing and commercial value *O. vulgaris* and cephalopods in general are valuable resources in the country and only incomplete information is currently available on their biology and distribution. However, some projects focusing on this group of species are about to start in Libya and specific information should be available in a relatively short time. In particular a biological data collection is planned to take place in Tripoli and Benghazi starting from 2009.

During the discussion that followed the presentation, participants were also informed that *O. vulgaris* in Libya has considerable economically value owing to the amount of exportation and the high value of this species on the international market. In this respect it was also

underlined that a large part of the Libyan *O. vulgaris* capture production is exported, mainly to the Tunisian or European markets.

Genetic analysis of the *Octopus vulgaris* population from the areas of Sfax-kerkennah and Zarzis by means of mitochondrial DNA Sequences. (K. Fadhlaoui-Zid).

The use of genetic methods in fishery population studies is an important facet of both fisheries stock assessment and management. Among the genetic molecular markers used, intra-specific sequence variation within mitochondrial DNA (mtDNA) has proven to be a powerful tool for studying population structure in marine organisms. To date, few population geneticists have made use of DNA sequences of population samples and the corresponding statistical tools for shellfish research.

This study focused on the common octopus, *Octopus vulgaris* that is an important exploited resource. The molecular analyses of octopus phylogeny have focused on allozymes, and mtDNA (gene coding regions: COI, COII, COIII and 16s rRNA). Studies on octopus and *O. vulgaris* in particular, have focused on the COIII gene region of mtDNA. The aims of this study is to investigate the genetic variation within Tunisian *O. vulgaris* in the mitochondrial DNA (mtDNA) cytochrome oxidase subunit III (COIII) region, to determine population structure and, also, to deduce by means of a phylogenetic comparison whether different populations exist in Tunisian waters and other Mediterranean areas.

A total of two sets of samples of *O. vulgaris* were collected from the areas of Sfax-Kerkennah and Zarzis. For each sample, a fragment of COIII gene will be amplified by PCR analysis and subsequently sequenced. Sequences of the COIII region will be aligned using the BioEdit v.7.0.7 program. Haplotype frequencies, standard diversity indices, pairwise genetic distances (Fst) and analysis of molecular variance (AMOVA) will be calculated with Arlequin software and compared to results obtained from other Mediterranean areas.

The discussion following the presentation focused on the problems faced by the Tunisian team which is currently carrying out a pilot study on the population genetics of *O. vulgaris* using samples from 2 regions in Tunisia: Kerkennah and Zarzis (n= 20 per site). The meeting was informed that, at present, the team is working on the optimisation of DNA extraction techniques prior to attempting PCR amplifications. The possible options for sequencing the amplified DNA were examined and two possibilities were considered suitable: (1) the use of sequencing machines available in Tunisian Institutions; (2) sending samples to a sequencing service abroad.

Moreover, the participants underlined that expertise on genetic comparisons/studies is available in the Project participating Institutes and thus advantage from this expertise should be taken if a genetic study is planned in the Project area.

Finally, the utmost importance of a genetic study on all the population sub–units exploited by the national fishing fleets in the MedSudMed region, to define if they belong to shared stock(s) was widely acknowledged and agreed upon. The areas where samples should be collected were also identified: they coincide with the spawning and nursery areas that were identified.

Management measures in the MedSudMed area

Management measures regarding *O. vulgaris* stocks differ among countries and in particular no specific instruments regarding this species exist in Italy and Malta. However, due to the economic importance of this species in the sub-region, there was a general agreement on the need to harmonise management strategies in the different countries. This harmonisation is even more necessary if the population sub–units exploited by each country belong to shared stock(s) or if there is an interconnection between different stocks.

The current fishing regulations regarding *O. vulgaris* in the MedSudMed participating countries were recalled by the participants.

Tunisia has a specific regulation for the management of octopus fisheries: i) fishing is generally authorized from 16 October to 14 May each year; ii) the species is exploited both by artisanal and industrial fleets; iii) a minimum landing weight is set to 1 kg (forbidden to land/commercialize specimen of less than 1 kg); iv) the fishing closure applies to both fleets from 15 May to 15 October.

In **Libya**, fishing is authorized all year round, with a minimum landing size of 500 g.

Italy and Malta apply the Mediterranean regulation of the EU. There is no fishing closure during the year and no particular specification for *O. vulgaris* in the fisheries regulations. The current law on mesh size (40 mm diamond mesh) and the minimum distance from the coastline or depth for trawlers and limitations for scuba divers (no use of spear guns) and spear-fishing (forbidden during the night and with tanks) are not specific to octopus but indirectly regulate the exploitation of this species. It was underlined that in these two countries there is no specific industrial fishery targeting this species and the average annual landings are relatively low (about 500 tons in South Sicily, 30 tons in Malta in 2007), which explains the lack of specific regulations focusing on the management of *O. vulgaris*.

Participants once more underlined the discrepancies between national regulations and the relevance of harmonization where the *O. vulgaris* populations belong to one or more shared stock(s). However, it was also recalled that the introduction of the square mesh size would reduce the capture of smaller and immature individuals, thus enhancing the effectiveness of the national regulations for trawlers and positively affecting the populations of this species

Operational Units targeting *Octopus* spp. in the MedSudMed sub-region (L. Ceriola)

The strategy of the General Fisheries Commission for the Mediterranean (GFCM) to manage fisheries and the general framework that led to the definition of the Operational Unit (OU) concept were presented. The main parameters used to define the OUs were also described, as well as the tools provided by the GFCM for the OUs identification and listing.

The GFCM endorsed the strategy to manage fisheries through effort control by OUs. The conceptual framework for identifying OUs was developed according to three main criteria: geographical (the GFCM Geographical sub-Areas division), characteristics of the fisheries (fleet segments identified by fishing gear and capacity), type of resources targeted (group of target/accessory species). Based on these criteria, the following definition of OU was adopted: “*For the sake of managing fishing effort within a Management Unit, an Operational Unit is the group of fishing vessels practising the same type of fishing operation, targeting the same species or group of species and having a similar economic structure. The grouping of fishing vessels may be subject to change over time and depends on the management objective to be reached*”. As tool for the identification and description of OUs, the GFCM Task 1 Matrix was endorsed by the GFCM, as well as a timetable for gaining information at country level on the first two sub-units of this matrix. The information OUs provided to the GFCM by each country participating in the MedSudMed Project was briefly presented and discussed in the light of the aims of the workshop; the possibility to take advantage of this tool to match these aims was also highlighted.

During the discussion that followed the presentation, the possibility to identify OUs targeting the common octopus in the MedSudMed participating countries was considered and the importance of identifying the main fishing fleet segments which exploit this resource was pointed out. It was also underlined that most of the information needed for the identification of OUs according to the definition and to the conceptual framework proposed (GSA where fishing take place, active fleet segments, type of resource targeted) is already available at country level.

Accordingly, a joint exercise to list the fleet segments that exploit *O. vulgaris* in each country at GSA level was proposed and carried out.

The fleet segmentation proposed by the GFCM, in terms of fishing gear and vessel size, was followed for the joint exercise. In Table 1 almost all the information needed for identification of the OUs by GSA is reported. However, the number of vessels and more accurate information on some fleet segments, as well as socio-economic related information is missing. Accordingly, the table can be considered a significant contribution for the identification of OUs targeting *O. vulgaris* in the MedSudMed sub-region.

Table 1. Summary information on fisheries exploiting *O. vulgaris* in the MedSudMed sub-region. GSA: Geographical sub-Areas; fleet segmentation: the main division proposed by the GFCM (Resolution GFCM/31/2007/1) was adopted; LOA: length overall;

Fisheries		Tunisia		Libya (Farwa lagoon)		Malta		Italy (S. Sicily)	
Fleet segment	Main features	GSA 12	GSA 13	GSA 14	GSA 21	GSA 15	GSA 16		
Minor gear without engine	gear period of the year	Pots 15 October-15 May	Pots 15 October-15 May	Pots 15 October-15 May	Pots November-March	N/A	Traps (minor) + trammel nets + lines		January-December
Minor gear with engine LOA <6m	gear period of the year	Pots 15 October-15 May	Pots 15 October-15 May	Pots 15 October-15 May	Pots November-March	Octopus traps	Traps (minor) + trammel nets + lines		January-December
Engine LOA > 6m	gear period of the year	-----	-----	-----	November-March	-----	-----		-----
Engine LOA 6-12 m	gear period of the year	-----	-----	-----	(not specific octopus)	-----	-----		-----
Trawlers LOA 12-24 m	gear period of the year	Bottom trawl 15 October-15 May	Bottom trawl 15 October-15 May	Bottom trawl 15 October-15 May	Bottom trawl* January-December	Bottom trawl* January-December	Bottom trawl* January-December		Bottom trawl* January-December
Trawlers LOA >24 m	gear period of the year	-----	-----	-----	-----	Bottom trawl*	Bottom trawl*		-----
		-----	-----	-----	-----	January-December	-----		-----

* *O. vulgaris* is not a main target species

Socioeconomic study of the octopus fishery in the southern area of Tunisia (gulf of Gabès) (Scander Ben Salem).

The objective of this study is to describe and analyze the socio-economic profile of the octopus fishery in the southern area of Tunisia (gulf of Gabès).

The first stage of this study consisted in gathering existing institutional data at various levels such as:

- Identification and characterization of the ports and sites of fishing of octopus in the area of the gulf of Gabès;
- Characterization of the fleet which targets the octopus (number of boats, types of boats, technical characteristics, fishing gears used, etc);
- Annual evolution of octopus production by gear and by port;
- Annual evolution of the fishing effort in number of boats and fishing trips and as production/yield per vessel and fishing trip (kg boat^{-1} and kg trip^{-1}).

The second stage of this study relates to the socio-economic surveys which constitute the main source of data regarding the social and economic conditions nearest to the reality of the activity of octopus fishery. This is in progress in four ports (El Kef, Houmet Essouk, and two ports in the Kerkennah island: El Attaya and El Kraten). The specific objectives of this stage are to get information on:

- The economic profitability of the fishing vessels and commercial production chains;
- The adequacy and application of the regulation in force by the community of the fishermen;
- The degree of reliance of the communities of fishermen on the fishing zones to octopus.

During the discussion that followed the presentation, the importance of artisanal fishing “gears” (e.g. pots and/or traps for common octopus in Tunisia and Malta), as well as their profitability and sustainability for the stocks were recalled. The relationship between the profitability of such selective fisheries with the “integrity” of the exploited stock and with the concurrent implementation of other non selective fishing activities was also pointed out. The experience carried out on Spiny lobster (*Palinurus elephas*) in Marettimo (Trapani, Sicily) was recalled highlighting that the traditional and selective fishing methods proved to be economically sustainable as long as the targeted stock was partially exploited and the abundance relatively high. On the contrary, the traditional fishing methods became unsustainable after the depletion of the stock due to the impact of non-selective fisheries.

Participants were also informed that the fishery of *O. vulgaris* in Tunisia mainly uses pots and, while not deeply affecting the stock, it involves and provides income for a great number of stakeholders. Mention was also made of the considerable extent of exportation of *O. vulgaris* landed along the Tunisian coasts towards foreign markets. Finally, the meeting was informed that socio-economic information on the fishery targeting the common octopus in Tunisia country is not complete yet.

The importance of socio-economic studies in Tunisia with the final aim to have complete and updated information on all the aspects related to the fishery of common octopus, and on fisheries in general was pointed out by national experts. Accordingly, the importance of extending such studies to two other landing sites (i.e. Jerba and El Kef) was underlined and agreed on.

In order to have complete knowledge on the fisheries socio-economic data collection and monitoring system currently implemented in the MedSudMed participating countries, the current monitoring activities carried out at national level were recalled by the participants.

In Malta and Italy, according to the EC regulation, a monitoring activity on the entire fishery sector is carried out by national Institutions (Fisheries Directorate and IREPA for Malta and Italy respectively) while no specific activities on *O. vulgaris* are currently implemented. In Italy, IREPA collects economic information and catch and effort data to estimate total catch and total effort and other economic indicators at GSA level for all fishing activities around Italy.

In Libya surveys on fisheries socio-economic aspects (e.g. number of fishermen, general income) were carried out along the entire coast, but they were not specifically on octopus fisheries. The activities implemented with the support of the FAO-CopeMed Project in the country for the collection of socio-economic data were recalled, as well as the need to update this information.

(iii) Definition of the knowledge gaps to be filled for the identification of the stock unit(s) of *O. vulgaris* in the MedSudMed sub-region and of the fleet segments that exploit them in agreement with the OUs concept

The discussion that followed the presentations covered several aspects of the biology and fisheries of the common octopus in the MedSudMed region and highlighted some general gaps that should be filled in order to: i) identify possible population units and define the possible occurrence of shared stock(s); ii) provide a detailed description of the OUs targeting *O. vulgaris*; and iii) better understand and possibly describe the bio-ecology of this species.

Identification of shared stock(s)

The GFCM SAC adopted four main criteria for the identification of shared stocks, i.e. population (the population units should be identified by genetic and/or morphometric methods), exploitation (the population should be currently exploited), fleet (the fleets of different GFCM countries are currently exploiting the population) and GSA (the fishing activities are carried out and catches produced in one or more GSA/s) criteria. According to these criteria and to the information provided during the workshop, the main gaps for the identification of shared stock(s) of *O. vulgaris* in the MedSudMed region were identified as follows:

1. Spatial distribution of the critical life stages such as recruits, adults/spawners (namely identification of possible nursery and spawning areas) and of the main migratory pattern of this species. The identification of sensitive areas such as spawning and nursery areas was considered necessary to understand several aspects of the biology and ecology of marine resources and to preliminarily draft the active and passive migration in the distribution area. During the first day of the workshop, the presence of four potential spawning areas (one in GSA 16 and three, less well defined, in GSAs 15 and 13) and two main zones of concentration of juveniles (one in GSA 15 and one in GSA 13) was pointed out according to the persistent occurrence of spawners and juveniles respectively. The identification of several potential spawning areas, along with the information on water mass flows in the region (e.g. currents, up- and down-welling) suggests the presence of more than one population sub-unit. They also support the hypothesis of the possible occurrence of transport of paralarvae by the main water movements in the region. However, participants agreed that the lack of specific data in the Tunisian and Libyan waters (i.e. GSAs 12, 13, 14, and 21)

prevents completion of the information on this aspect, suggesting the areas on which to concentrate further specific studies.

2. Characterisation of population structure using specific genetic markers. During the discussion participants agreed that understanding the level of similitude among populations in terms of genetic markers can be considered the most appropriate means to identify possible population sub-units within a specific geographic area. The importance of such studies has been widely acknowledged especially where fisheries management is concerned. Accordingly, the lack of such information probably represents the main gap that needs to be filled in order to describe the occurrence of shared stocks in the MedSudMed region.

Identification of OUs targeting *O. vulgaris*

Recalling the GFCM definition of OUs and following the identification of fisheries fleet segments targeting *O. vulgaris* in the MedSudMed sub-region carried out during the workshop, the gaps that still need to be filled for the identification and characterisation of OUs were highlighted by country.

In Tunisia, a pilot study on the socio-economic aspects of the fisheries on this species was carried out in 2008. However, complete information on catch (length frequency structure, sexual maturity stage) and effort (number of vessels targeting directly or indirectly *O. vulgaris*), as well as on some socio-economic aspects related to the fisheries is still lacking. This gap was considered as a limiting factor for the proper identification of OUs targeting *O. vulgaris* (e.g. number of people involved in the fishing sector, costs and market prices, quotas of landing exported).

In Italy and Malta, where common EC regulations on fishing monitoring are in place, the main gaps for the identification of OUs targeting *O. vulgaris* are related to the lack of specific data on this species, as it is not a target species for fisheries in general. However, the current implementation of national monitoring system focuses on small (<10 m overall length) vessels in Malta, along with the adoption of an EC regulation on the internal communication of fishery statistic data in both countries, will allow research institutes to gain more accurate information on this species.

A different situation was highlighted for Libya, where the lack of information on the main fishing segments targeting the common octopus is due to the lack of a comprehensive system for the monitoring of the socio-economic aspects of the fisheries in general.

Bio-ecology of *O. vulgaris*

The understanding and description of the bio-ecology of the common octopus in the MedSudMed sub-region was also considered important for the identification of a harmonised management strategy. Accordingly, two general fields of investigation were considered relevant:

1. Factors affecting success of recruitment. Participants recognised that defining the relationships between recruitment and spawning stock biomass, as well as the impact of environmental factors on the first ontogenetic stages of cephalopods in general including *O. vulgaris*, is a matter of discussion at global level. In this regard, it was

observed that in the GSA 15 and 16 the temperature variation can explain almost the 50% of variation in terms of recruitments. Likewise, a strong relationship between temperature and capture production in Tunisian waters was pointed out, but it was not enough to explain all the variation in recruitment and total abundance. The identification of the main environmental factors other than temperature affecting the recruitment of *O. vulgaris* in the MedSudMed region was considered useful information that is still missing in the region to fully understand the dynamics of recruitment.

2. Identification of possible breeding grounds. With the aim of covering all aspects of the biology and ecology of common octopus in the MedSudMed region, the participants highlighted that the parental behaviour of females after spawning and their need for a specific habitat in which to lay eggs (namely the breeding grounds) suggests a potential field of investigation on identification of possible breeding grounds. During the discussion it was suggested that egg deposition could take place in different areas respect to spawning. According to this hypothesis, females, after mating, would migrate to appropriate grounds to lay eggs and take care of them. Therefore, the validation of this hypothesis and the identification of breeding areas were considered gaps that still limit the knowledge of common octopus ecology and a possible direction for future research activities.

(iv) Drafting a work plan for the identification of population units in the area covered by the project and of the OUs that exploit this resource;

In line with the ToRs of the Workshop, a work plan aiming at the identification of possible shared stocks exploited by the countries participating in the MedSudMed Project was agreed on. Two main thematic areas were identified: i) identification of nursery and spawning areas along the Tunisian and Libyan coasts by processing available survey data, ii) genetic study to compare population units. Moreover, interest was expressed for the collection of data for the proper identification of OUs targeting *O. vulgaris*.

1. Tentative identification of nursery and spawning areas

Based on the results obtained on GSAs 15 and 16, participants agreed on the importance of taking advantage of the available fishery-dependent and fishery-independent data, especially in the GSAs 12, 13, 14 and 21, for the complete identification of nursery and spawning areas. The identification of such areas in the GSA 13 following the same procedures used for GSAs 15 and 16 was considered a suitable way to progress in the identification of population units.

2. Genetic study

The execution of a genetic study to understand whether the existing populations units in the MedSudMed sub-region belong to shared stock(s) was identified as a priority and a draft program was discussed (Annex D). Participants agreed to collect samples from all the countries in the sub-region and especially from the areas that were identified as potential spawning and nursery grounds, in order to highlight possible relationships between them. "Laboratory activities", from the identification of suitable DNA markers to the comparison of genetic structure between populations, were discussed with the experts on genetics who attended the workshop.

3. Socio-economic data collection

In the view of an ecosystem approach to fisheries and for the proper identification and depiction of OUs targeting *O. vulgaris*, the further collection of comprehensive socio-economic data in Tunisia and Libya was considered. The importance of taking advantage of existing national monitoring systems was discussed and agreed upon.

4. Other activities

The importance of planning and carrying out a specific trawl survey in the southern part of the MedSudMed area to study the common octopus was also discussed. It was pointed out that, due to the lack of stable national survey programmes, if a trawl survey was planned it should not focus on a single species only but rather on the whole demersal assemblages and should cover the areas not previously/recently investigated, i.e. the central-eastern part of the MedSudMed region. Participants agreed on these statements and acknowledged that the execution of such trawl surveys would be of great importance to partially fill a gap in the knowledge not only on *O. vulgaris* but on the demersal resources in general of the region. As preliminary step a general agreement on the region to be investigated, the possible period of execution and the protocol to be adopted for the survey was achieved. The survey would be tentatively planned to be carried out in Western Tunisia-Eastern Libya, during late spring early summer period following the MEDITS protocol. The possibility that this survey takes advantage of the ongoing national and international programmes was also discussed and agreed on.

Finally, the importance of providing updated information on the status of the stock/s of the common octopus in the MedSudMed sub-region was underlined. The possible application of global models (namely Surplus Production Models) to the time series of catch and effort (either number of vessel or fishing days) data available in the MedSudMed participating countries was discussed. It was underlined that even if these methods have several constraints and strong basic assumptions, their application would provide a preliminary picture of the status of the stocks of *O. vulgaris*. Accordingly, the tentative exploration of data availability in Malta and Tunisia and a preliminary analysis was included in the work plan.

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**Workshop on octopus stocks and related fisheries in the MedSudMed Project area
(Salammbô, Tunisia, 18-20 November 2008)**

Terms of Reference

1. Background and general information

The understanding of the number and boundaries of population units within fishery stocks is relevant for successful stock assessment and fisheries management. In addition, in many Mediterranean regions, where fishery stocks are likely to be transboundary and exploited by more than one country, the knowledge of population biology and the identification of population units assume international relevancy, as highlighted by the Scientific Advisory Committee of the General Fishery Commission for the Mediterranean (GFCM-SAC).

In the area covered by the MedSudMed Project, some cephalopods such as *Octopus vulgaris* have a significant economic importance for both the artisanal and the industrial fleets. *O. vulgaris* is a neritic species distributed from the coastline to the outer edge of the continental shelf (in depths from 0 to 200 m), where it is found in a variety of habitats, such as rocky and grassy beds. Juveniles and adults of this species are strictly benthonic but larvae are pelagic and can spread over long distances after hatching and before settling on the bottom. As a result, specimens of *O. vulgaris* belonging to the same stock can spill over and inhabit regions distant from each other. For these reasons, a single stock of *O. vulgaris* can occur over a wide area and be potentially exploited by the fishing fleet of different regions or countries. Within the area covered by the MedSudMed Project the stock of *O. vulgaris* is likely to be shared by different countries; yet, different management measures are currently applied in the area.

The FAO Regional Project MedSudMed “Assessment and Monitoring of the Fishery Resources and the Ecosystems in the Strait of Sicily”, within the framework of the project component “Spatial distribution of demersal resources and the influence of environmental factors and fishery characteristics”, aims at supporting scientific communities of the participating countries in characterizing the spatial distribution of demersal resources and identifying the underlying factors. One of the activities proposed within this project component is the identification of potentially shared stocks in the Strait of Sicily and the description of the fisheries that are exploiting them.

The 6th Meeting of the MedSudMed Coordination Committee (Salammbô, Tunisia, 4-5 February 2008) highlighted the necessity to collect information on *Octopus vulgaris* and the fisheries that are exploiting this species in agreement with the Operational Unit (OU) concept. A specific activity on this issue was thus launched in Tunisia where *O. vulgaris* has significant economic importance. Biological, ecological and socio-economic data are being collected in this country to describe and analyse the intrinsic parameters of the species’ population, and to assess the characteristics and profitability of the fishery targeting *O. vulgaris*, as well as the appropriateness of the current legislation on octopus fisheries. The overall aim is to gain scientific evidence on the stock unit(s) and, according to the data collected, to identify the fishing segments targeting this species.

The MedSudMed workshop aims at synthesizing the available information on this species in the Project area. The workshop will involve national experts from the participating countries with the aim of providing the basis for a working programme that seeks to identify the

population units of *O. vulgaris* in the Strait of Sicily and to assess which population units are being exploited by the fishing fleets of each participating country.

2. Objectives and themes of the workshop

The main objective of the workshop is to develop a synthesis of the existing knowledge on *O. vulgaris* in terms of spatial distribution, morphometric characteristics, reproduction and growth within the project area, and of the factors that are affecting these features (environmental parameters and fisheries characteristics) in each participating country, so as to highlight possible differences or similarities. In addition, the workshop will represent an occasion to list the gaps that still need to be filled in order to understand whether the populations units being exploited by the national fishing fleets belong to one or more shared stock/s. This should eventually contribute to the identification of the OUs that are targeting on *O. vulgaris* and of possible management options for this species in the MedSudMed region.

The workshop will be organized in two sessions. The first session will include the presentation of national contributions and of the bibliographic synthesis carried out in the MedSudMed area on *O. vulgaris*. The national contributions should consist of the biological description of the *O. vulgaris*, of the fisheries that are exploiting this resource, and of the studies conducted on the species and its environment. The information should be based on all the available fisheries, biological, ecological, physical and, where applicable, the socio-economic data related to this species. During the second session, participants are expected to identify the issues that need to be addressed for the identification of the population units of *O. vulgaris* and the fleet segments that are targeting this species.

The outcome of the workshop would be the possibility to list the gaps in the knowledge and consequently draw up a working programme to be agreed upon by all participating institutes, as well as aiming at identifying the stock units of *O. vulgaris* within the Project area. Proposals for future work to be conducted in the Project area would be discussed on the basis of the information presented during the workshop. The workshop should address the following topics:

- spatial distribution and migration patterns;
- morphometric characteristics: size range (length and weight), length frequency distribution, length-weight relationship and growth;
- reproduction: sex-ratio, size at maturity, spawning season, GSI, cost of reproduction, spawning grounds;
- fisheries exploiting this resource: total catch, seasonality, quantification and spatial distribution of the fishing effort, fishing techniques used in the region, existing management measures.

3. Expected outputs

The main outputs expected are:

- a synthesis of the available information (published and not published) and type of studies carried out in the region;
- a comparison of the results in the different countries in order to highlight possible differences or similarities between the populations inhabiting different areas of the MedSudMed sub-region;
- a list of knowledge gaps that limit the identification of the different population units in the MedSudMed sub-region;
- a work plan for the identification of possible population units in the stocks that are being exploited by the countries participating in the MedSudMed Project;

- a contribution to the identification of fisheries that are targeting *O. vulgaris* in agreement with the OUs concept.

4. Participation

The workshop is open to technicians and researchers from institutes and/or organizations in the countries involved in the MedSudMed Project.

5. Organization

The workshop will be held in Salambô, Tunisia, 18-20 November 2008 and will be kindly hosted by the Institut National des Sciences et Technologies de la Mer (INSTM).

Agenda

- 1. Objectives of the Workshop**
- 2. Current knowledge on *Octopus vulgaris* and related fisheries in the MedSudMed region**
 1. Biology and fisheries of *Octopus vulgaris* (Cuvier, 1797) in the Mediterranean Sea (L. Ceriola)
 2. Current knowledge on biology and ecology of *Octopus vulgaris* along South Tunisian coasts (S. Ezzedine)
 3. Current knowledge on biology and fisheries of *Octopus vulgaris* along the coast of Sicily (F. Fiorentino)
 4. Spatial distribution of adults and recruits of *Octopus vulgaris* in GSAs 15 and 16 (G. Garofalo)
 5. Exploitation of *Octopus vulgaris* in the South Tunisia: Current state and future prospects (S. Ezzedine)
 6. Current status of knowledge about the fishery of *O. vulgaris* in the Maltese Islands and data available (R. Pace and L. Knittweis)
 7. Current status of *Octopus* spp. fisheries along the coast of Libya (H. Ghmati)
 8. Genetics Genetic analysis of the *Octopus vulgaris* population from the areas of Sfax-kerkennah and Zarzis by means of mitochondrial DNA Sequences (K. Fadhlaoui).
 9. Operational Units (L. Ceriola)
 10. Socio-economic study of the octopus fishery in the southern area of Tunisia (S. Ben Salem)
- 3. Definition of gaps for the identification of *O. vulgaris* population units and Operational Units (OUs)**
- 4. Drafting of a work plan for the identification of *O. vulgaris*' population units and OUs**

Other activities related to the GFCM-SAC requests
- 5. Other matters/CONCLUSIONS**

Protocol for population genetic study of *Octopus vulgaris*

Introduction

The use of genetic methods in fishery population studies is an important facet of both fisheries stock assessment and management since the amount of genetic structure is generally thought to be negatively correlated with larval dispersal. A genetic population study of *Octopus vulgaris* may thus provide vital data in understanding both the number and the distribution of population units within the Mediterranean stock of this species. Since such population units may be exploited by the fishing fleets of more than one country, the results of a genetic population study will provide vital information on the necessity for future shared stock assessment exercises as well as their design.

Previous studies of *Octopus vulgaris* genetic population structures have been carried out along the South African coast (Oosthuizen et al. 2004; Teske et al. 2007) as well as around the Iberian Peninsula and Canary Islands (Cabranes et al. 2007). The results have clearly indicated the importance of using more than one marker in the determination of genetic population structuring, with promising results obtained by using mitochondrial cytochrome oxidase markers as well as microsatellites.

Materials and Methods

1. Sample Acquisition

Tissue samples for this investigation would be required from each of the participating project partners, namely the countries Tunisia, Libya, Malta and Italy. For each site 30 replicate samples will be required and at least 3 regions per country should be sampled to acquire sufficient spatial resolution.

2. Laboratory Analysis

The expertise for the laboratory and subsequent data analysis are available in two of the institutes at present involved in the MedSudMed Project: Tunisia (INSTM, National Institute for Marine Science and Technology) and Malta (Malta Center for Fisheries Science, in collaboration with Malta University).

INSTM is at present already involved in the genetic analysis of *Octopus vulgaris* samples from two regions in the South of Tunisia: Kerkennah and Zarsis.

2.1. Sequencing

There are two possible ways of sequencing samples after DNA amplification has been completed: (1) the use of sequencing machines from other institutes (Sfax or Pasteur Institute in Tunisia/University of Malta) or (2) sending samples to a sequencing service (e.g. Macrogen in Korea). The latter option is favourable since costs will almost certainly be less and results will be available more quickly.

2.2. Exchange of Scientists

In order to facilitate the smooth running of the project, it will also be necessary to allow for the exchange of scientists between Malta and Tunisia. Moreover, the visit of institutes with previous experience in the population genetic analysis of *O. vulgaris*, in particular the use of microsatellite markers (University of Oviedo, Spain), will be necessary.

Funds for travel as well as *per diem* for the principal scientists will thus be needed in addition to the above budget estimations for sample analysis.

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Biology and fishery of *Octopus vulgaris* in the Mediterranean Sea

Ceriola L.

FAO MedSudMed and AdriaMed Projects

Introduction

Cephalopod species play an important role in the marine ecosystems, both as predators and prey. They are voracious opportunistic predators of fish and invertebrates and also represent the most important prey category of several fish species and top predators. Cephalopods have gained increasing attention in the past decades as an alternative to more traditional marine resources. They have come to constitute one of the top invertebrate fisheries in the world with catches increasing steadily in the last 30 years from about 1 to more than 4 million tonnes¹ (Figure 1).

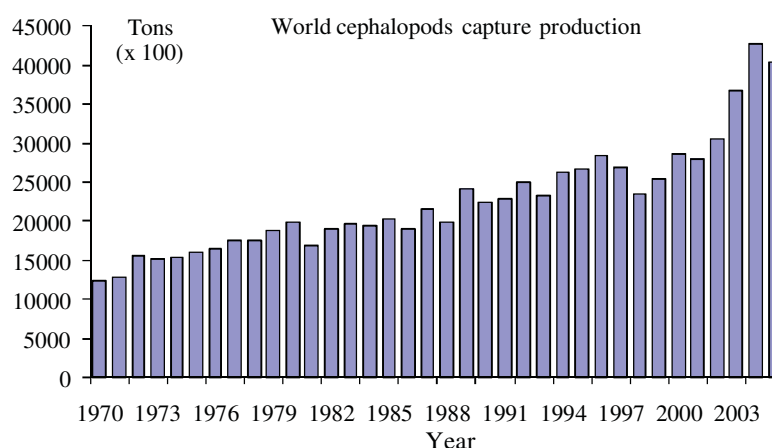


Figure 1. Total capture production of cephalopod species in all the oceanic regions (1970-2005)

In the Mediterranean Sea, cephalopods species are caught by semi-industrial (bottom trawls) and small scale fisheries and in some regions they are regarded as a main fishery target species. Their total capture production in the basin generally fluctuated around 50 000 tons, but increased up to 80 000 tons at the end of 1980s (Figure 2).

In spite of their importance for fisheries, management of cephalopods is still hampered due to uncertainty on biological and ecological characteristics (e.g. growth, spawning-stock recruitment relationship, migratory capability, interaction with environmental factors) and no specific measures are generally adopted in the Mediterranean countries.

In the MedSudMed sub-region (south central Mediterranean) cephalopods are valuable fishery target species and among them octopods and *Octopus vulgaris* (Cuvier, 1797) have great importance in terms of economic value and total catch. It is one of the most landed species in Tunisia and Malta and also represents an important resource for the fisheries in Libya and Italy (south Sicily).

¹ All the data on capture production are from FAO Fisheries Department, Fishery Information, Data Statistics Unit. FISHSTAT Plus: universal software for fishery statistical time series, version 2.3.2. Capture Production 1950–2005.

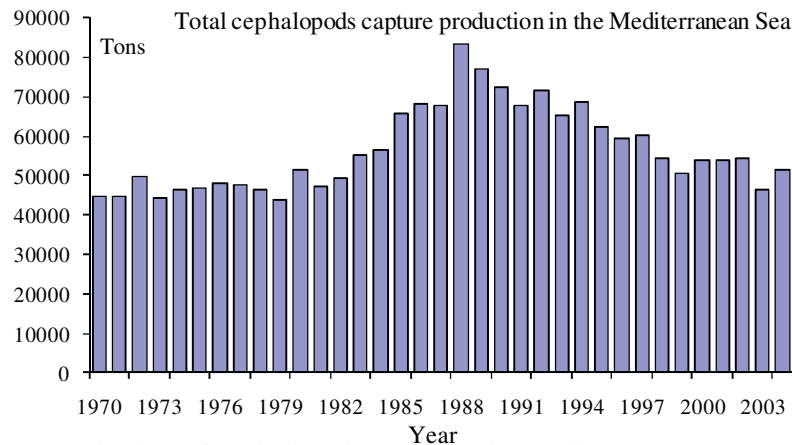


Figure 2. Total capture production of cephalopod species in the Mediterranean Sea (1970-2005)

In this contribution the capture production of cephalopods (global and in the Mediterranean Sea) from 1970 to 2005 are described, as well as the total *O. vulgaris* capture production in the Mediterranean Sea and in the MedSudMed sub-region. Moreover, the current knowledge on the biology and ecology of the common octopus in the Mediterranean region and some general features on the fisheries exploiting this species are summarised.

Distribution and capture production²

O. vulgaris is distributed from the coastline to the outer edge of the continental shelf (in depths from 0 to 200 m) where it is found in a variety of habitats, such as rocky and grassy beds. The total capture production of this species in the south-central Mediterranean from 1970 to 2005 fluctuated around 5 000 tons in Italy (considering Ionian and Sardinian Sea) and Tunisia, while it was relatively lower in Malta (about 10 tons per year). A decrease in the capture production was observed in Italy (Ionian and Sardinian sea) from 2000 onwards (Figure 3). In Tunisia capture production oscillated considerably over time, with a ‘period’ of about ten years (Figure 4). The peak in landings was recorded in 1998-99 with about 12 000 tons. Great variability in the captures are observed also in Malta, for which the annual values in recent years are considerably lower with respect to the beginning of the time series (1970) (Figure 5). Unfortunately, specific information on the capture production of *O. vulgaris* in South Sicily is not available through the FAO data base. Regrettably this is also the case for capture production data from Libya, which is not available as the statistics refer to octopods in general and do not discern common octopus from the other species.

² All the data on capture production are from FAO Fisheries Department, Fishery Information, Data Statistics Unit. FISHSTAT Plus: universal software for fishery statistical time series, version 2.3.2. Capture Production 1950–2005.

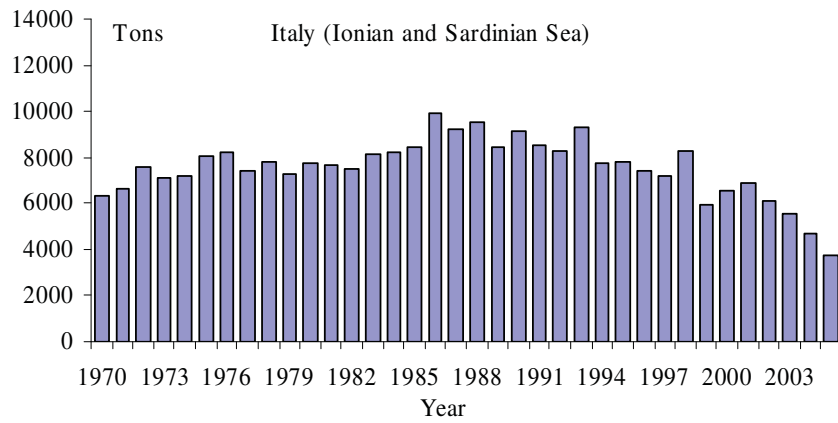


Figure 3. Total capture production of *O. vulgaris* for the Ionian and Sardinian Seas (Italy) from 1970 to 2005

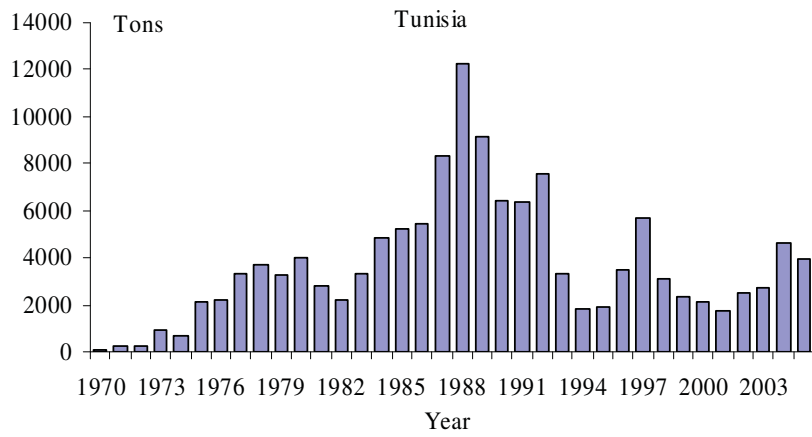


Figure 4. Total capture production of *O. vulgaris* for Tunisia from 1970 to 2005

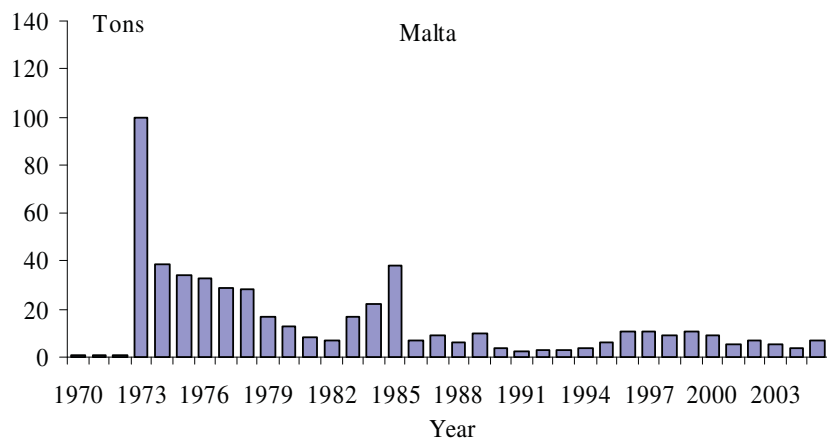


Figure 5. Total capture production of *O. vulgaris* for Malta from 1970 to 2005

Basic biology

O. vulgaris is a fast growing species with a life span of less than 24 months. Dorsal mantle length (DML) in the Mediterranean bottom trawl fisheries varies from 50 to 200 mm and the recruitment size ranges from 50 to 60 mm DML. Length-weight relationship is generally isometric for both sexes, although in the western Mediterranean negative allometric values were also found. *O. vulgaris* has an extended spawning period (> 8 months) with a seasonal peak of spawning activity which can vary according to region (generally during spring). Size at maturity ranges from 100 to 120 mm DML with females maturing at larger size compared to males. Juveniles and adults of this species are strictly benthonic but larvae are pelagic and can spread over long distances after hatching and before settling on the bottom. As a result, specimens of *O. vulgaris* belonging to the same stock can spill over and inhabit regions distant from each other. For these reasons, a single stock of *O. vulgaris* can occur over a wide area and be potentially exploited by the fishing fleets of different regions or countries. Within the area covered by the MedSudMed Project, *O. vulgaris* is exploited by trawlers or by using different kind of “traps”, and occasionally by gill or trammel netters. The total capture production of this species in the Sicily Channel generally fluctuates over time with total landings varying according to the country and to the fishing techniques (generally ranging from 400 to 4000 tons per year). To date, no studies have been carried out and very little information is available on the occurrence of shared stocks for the common octopus in the Mediterranean region.

Management measures and identification of shared stocks

Octopus vulgaris in the MedSudMed Project area is generally caught by trawlers, small scale fisheries (traps, trammel nets) and recreational fisheries. For Italian and Maltese trawlers, however, common octopus is not a main target species, whilst it is an important species for the Tunisian trawlers. Owing to its economic importance, specific regulations for fisheries targeting *O. vulgaris* are in place in Tunisia, where fishing of this species is permitted only during some months (generally from October-November to May) and a minimum landing size is set (1 kg). In Italy and Malta management strategies focus on trawlers generally (minimum distance from the coastline, minimum depth, seasonal fishing closure, gear mesh size regulation), and only indirectly on the protection of common octopus.

The gaps to be filled to provide an appropriate background to the identification of a suitable management strategy for *O. vulgaris* are tentatively listed according to two main groups: the bio-ecology of the species and the fisheries exploiting it. In particular they comprise:

- Bio-ecology: Population structure, growth, maturity, identification of spawning grounds and nursery areas, population units identification (occurrence of shared stocks), state of stock/s;
- Fisheries: Fishing capacity (e.g. number of vessels, total GT/GRT, vessel equipment), social aspects (e.g. number of people directly or indirectly involved in the fisheries, landing per crew, landing per vessel), economic issues (e.g. costs and revenues evaluation), identification of OUs targeting *O. vulgaris* (target or accessory species), current management measures adopted.

The identification of population units and the possible occurrence of shared stocks is a priority in order to put management measures into practice in line with an ecosystem approach.

Biology and fishery of common octopus, *Octopus vulgaris* Cuvier, 1797, in the northern sector of the Straits of Sicily

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Abstract

Updated information on the biology and fisheries of *Octopus vulgaris* in the northern sector of the Strait of Sicily are given. Catches derived from data collected by IREPA within the framework of the European Data Collection Regulation. Biological data derived from trawl surveys carried out by IAMC–CNR in spring (MEDITS series from 1994 to 2007) and Autumn (GRUND series from 1994 to 2006). The area investigated includes the GSA 15 (with the exclusion of the Maltese Management Fishing Zone) and GSA 16.

Biological data were processed by the Seatrim program to produce standard information concerning abundance indices, in terms of biomass and number, length structures, sex ratio, percentage at maturity stages and length-weight relationships.

The observed variation in abundance, demography and catches of the common octopus in the investigated area are discussed.

Introduction

The common octopus, *Octopus vulgaris* Cuvier, 1797, is a benthic cephalopod distributed on rocky, sandy and muddy bottoms from the coastline to the edge of the continental shelf (Belcari and Sartor, 1999). According to Mangold, 1997 the distribution of *O. vulgaris* in a strict sense may be limited to the Mediterranean Sea and eastern Atlantic Ocean. In the various GSAs sited in the Strait of Sicily, the species is fished by various small-scale gears, such as traps, pots, trammel nets, lines and harpoons, although off the southern coasts of Sicily (GSA 16) octopus is mainly caught by trawlers fishing on the continental shelf. This technical note aims to give updated information on the biology and fisheries of *Octopus vulgaris* in the northern sector of the Strait of Sicily, i.e. GSA 15 (with the exclusion of the Maltese Management Fishing Zone) and GSA 16. Since Faure *et al.* (2000), studying *O. vulgaris* off Mauritanian coasts, have outlined the importance of coastal hydrological retention in upwelling areas as a key factor for recruitment success, an attempt to relate recruitment strength to surface seawater temperature was made.

Materials and methods

Catch and effort information derived from data collected by IREPA within the framework of the European Data Collection Regulation. Biological data derived from trawl surveys carried out by IAMC_CNR in spring (MEDITS series from 1994 to 2007) and Autumn (GRUND series from 1994 to 2006). The area investigated mostly includes the GSA 15 (with the exclusion of the Maltese Management Fishing Zone) and GSA 16.

Biological data were processed by the Seatrim program (Garofalo *et al.*, 2006) to produce standard information concerning abundance indices, in terms of biomass and number, sex ratio, percentage at maturity stage and length frequency distributions.

Availability of surface sea water temperatures (SST) from remote satellite monitoring (Source of data: VHRR Oceans Pathfinder SST v5 remote sensing data (4×4 km spatial resolution), PO.DAAC Ocean SIP Tool (POET), NASA Jet Propulsion Laboratory, Pasadena, CA. <http://podaac.jpl.nasa.gov/poet>), allows investigation of the relationship between adults, recruits and SST in terms of anomalies from the mean values, expressed as $(x_i - \bar{x}) / \bar{x}$.

Results

Landing

According to Andreoli *et al.*, (1995), the estimated yield of *Octopus sp.* in GSA 16 between April 1985 and March 1986 was about 94 tons; the year after it amounted to 123 tons. Considering that overall yield was about 9670 tons in the first year and 8050 tons in the second year, *Octopus sp.* landings represented about 1% of total yield of coastal trawling in the area.

Yield of *O. vulgaris* in GSA 16 (all fishing boats combined) was about 650 tons in 2003, of which 370 were due to the artisanal fisheries (about 56%) (source: IREPA). During the last years yield ranges between 631 t (2006) and 1419 t (2004), with the lowest yield occurring in August – September (source: IREPA) (Fig. 1).

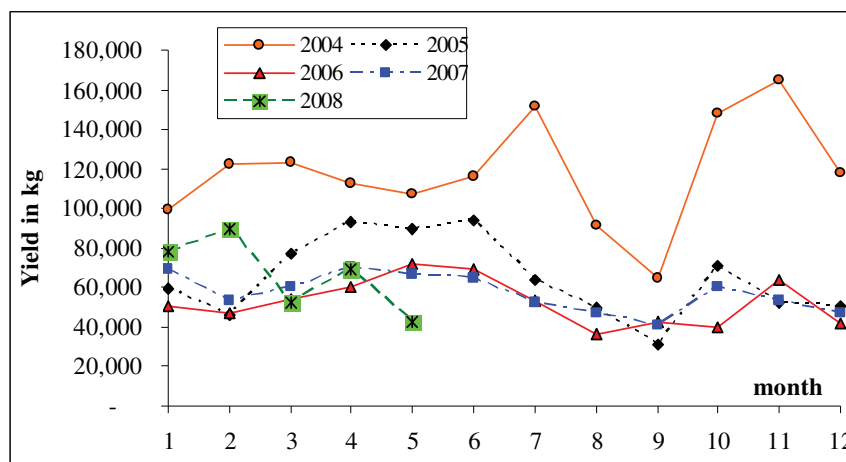


Figure 1. Landing by month of *O. vulgaris* in GSA 16 (source: IREPA).

Abundance indices

Trawl surveys abundance indices from shelf bottoms (10-200m) estimated during the MEDITS programme in GSA 15 and 16 remained stable at around 10 kg/km² from 1994 to 1999. They then fluctuate between 15 kg/km² (2001, 2004 and 2007) and 5 kg/km² (2002-2005).

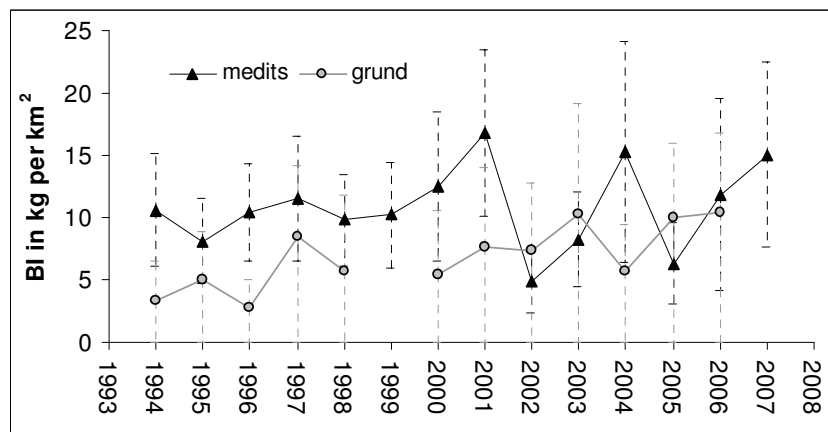


Figure 2. Biomass indices from trawl surveys in Spring (MEDITS series) and Autumn (GRUND series) in GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16. Only the shelf (10-200m) hauls were considered.

Abundance indices estimated from the GRUND (Autumn) programme were in general lower than the MEDITS (Spring) ones. A progressive increase from 1994 (about 3 kg/km²) to 2006 (about 10 kg/km²) was observed (fig.2). Considering the most productive depth strata only (10-100m), the increase of abundance during the Autumn surveys (GRUND) was only observed in the more shallow stratum (10-50m) (Fig.3).

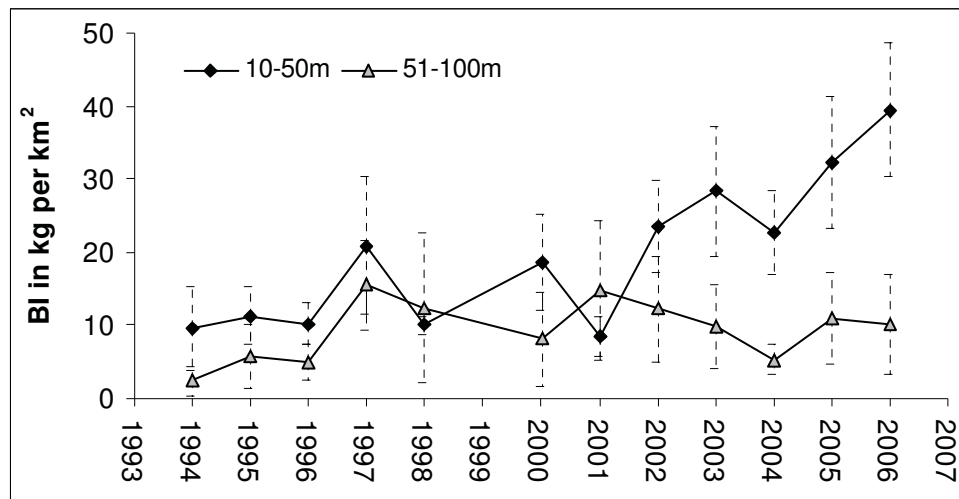


Figure 3. Autumn biomass indices of *O. vulgaris* by depth strata in GSA 16.

Biological information

Length structure

The length structure from spring trawl surveys (all years combined) resulted bimodal regardless sex. The immature juveniles had modal MDL ranging between 40 and 70mm, whereas the modal MDL of the adults ranged between 100 and 110mm (Fig. 4).

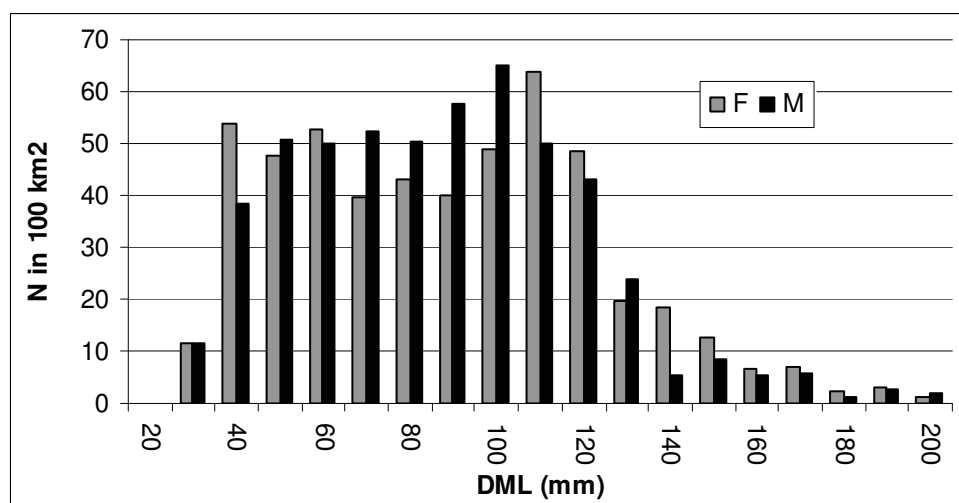


Figure 4. Spring LFD of *O. vulgaris* in GSA 15 (MMFZ only included from 2003 to 2005) and 16. All MEDITS surveys (1994-2007) were combined.

On the contrary, the length frequency distribution in autumn showed that most of the stock was made up of juveniles, with modal DML ranging between 60-70 mm in GSA 15 and 16 (fig.5), and 70-80 mm in international waters of GSA 13 and 14 (Fig. 6)

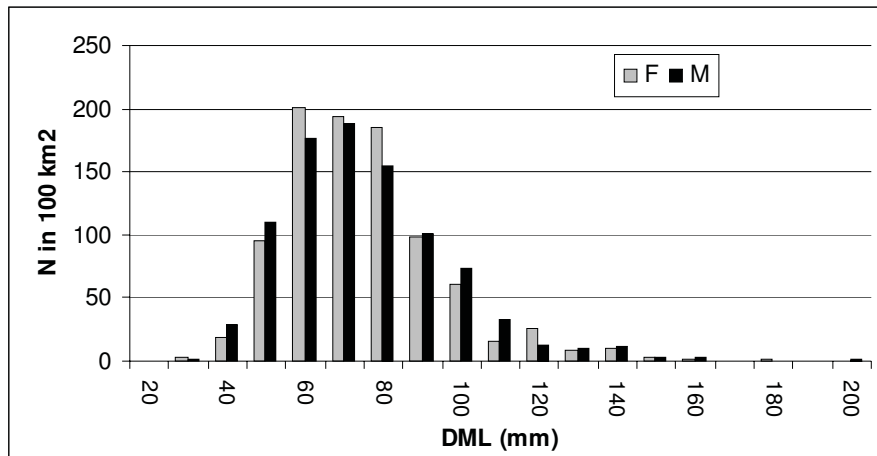


Figure 5. Autumn LFD of *O. vulgaris* in GSA 16. All GRUND surveys (1994-2006) were combined.

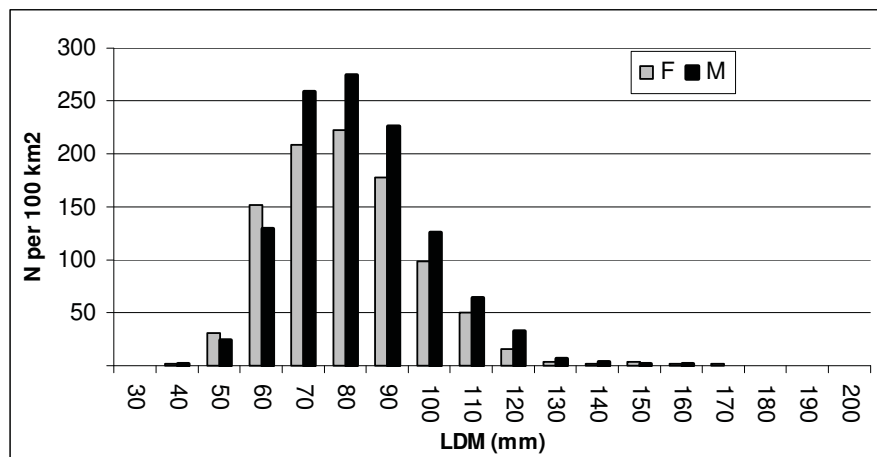


Figure 6. Autumn LFD of *O. vulgaris* in international waters of GSA 13 and 14. Data refers to GRUND surveys 1997, 1998, 2003 and 2004.

Sex ratio and maturity

The temporal trend of sex ratio in the stock showed that females had a significant increase from 1994 to 2007 (Fig. 7).

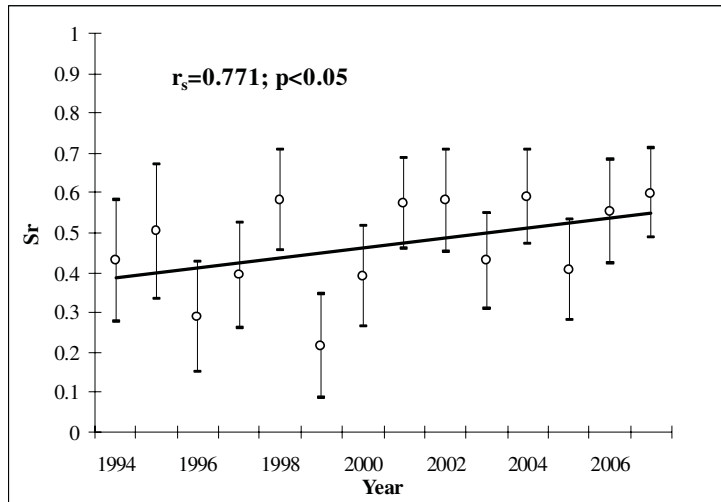


Figure 7. Sex ratio of *O. vulgaris* in GSA 15 (MMFZ included only from 2003 to 2005) and 16.

The sampled stock in Autumn surveys was almost exclusively made up of immature juveniles, whereas the occurrence of specimens in different maturity stages occurred in spring surveys (Fig. 8 and 9).

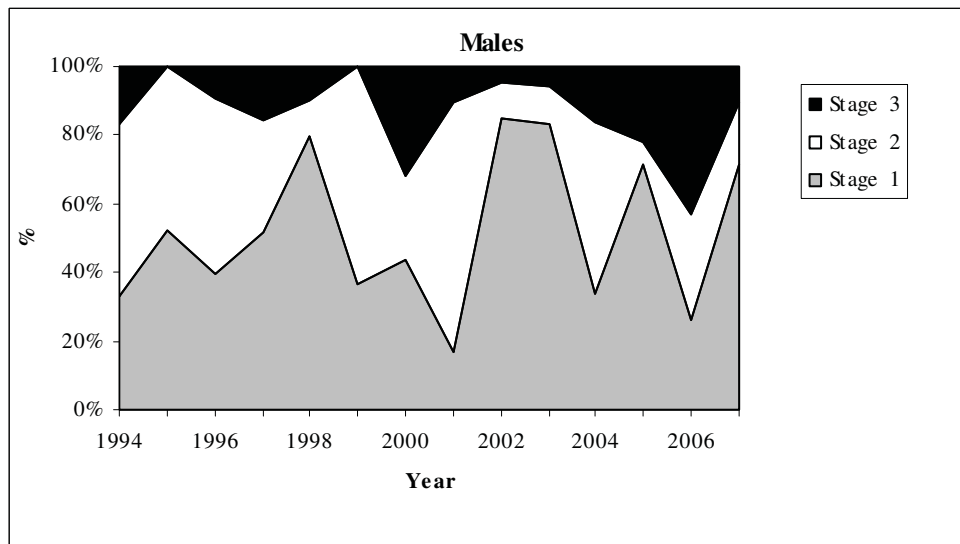


Figure 8. Percentage of males of *O. vulgaris* by maturity stage in Spring (MEDITS surveys). GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16. All MEDITS surveys (1994-2007) were combined.

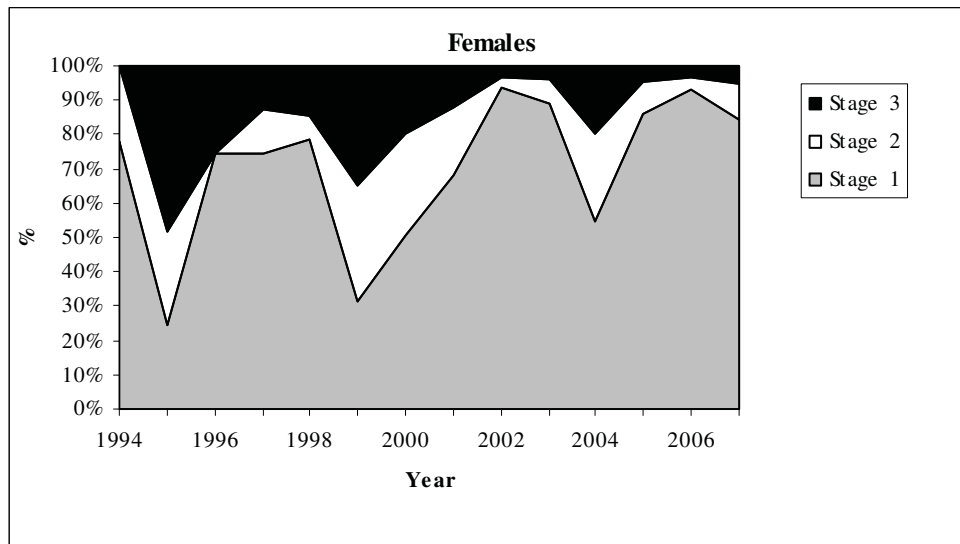


Figure 9. Percentage of females of *O. vulgaris* by maturity stage in Spring (MEDITS surveys). GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16. All MEDITS surveys (1994-2007) were combined.

The length at 50% maturity obtained during the spring surveys was 85 mm DML in males (fig. 10) and 117 mm DML in females (Fig. 11).

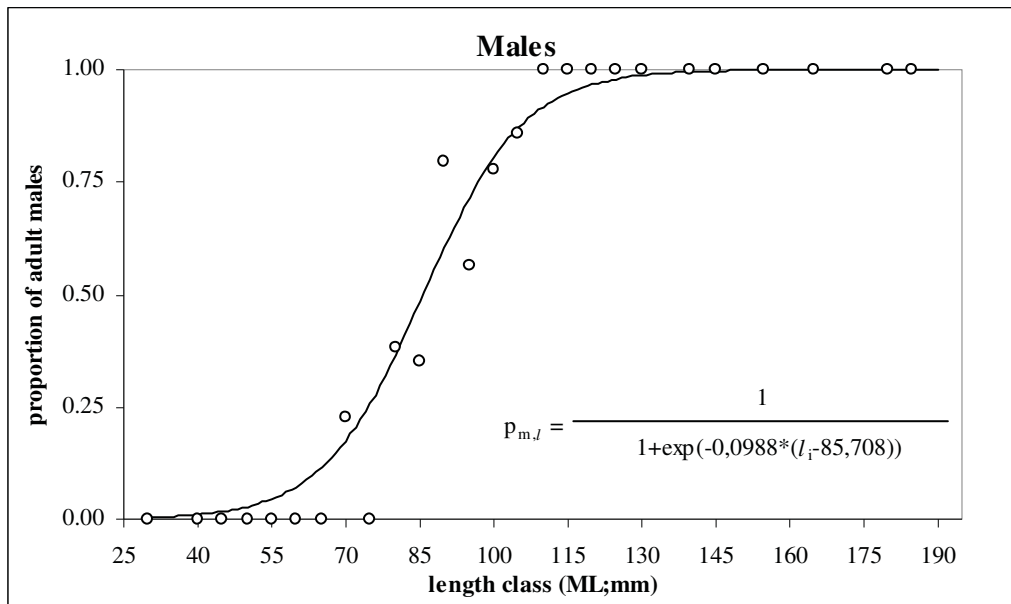


Figure 10. Ogive at maturity of *O. vulgaris* males in Spring (MEDITS surveys). GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16. All MEDITS surveys (1994-2007) were combined.

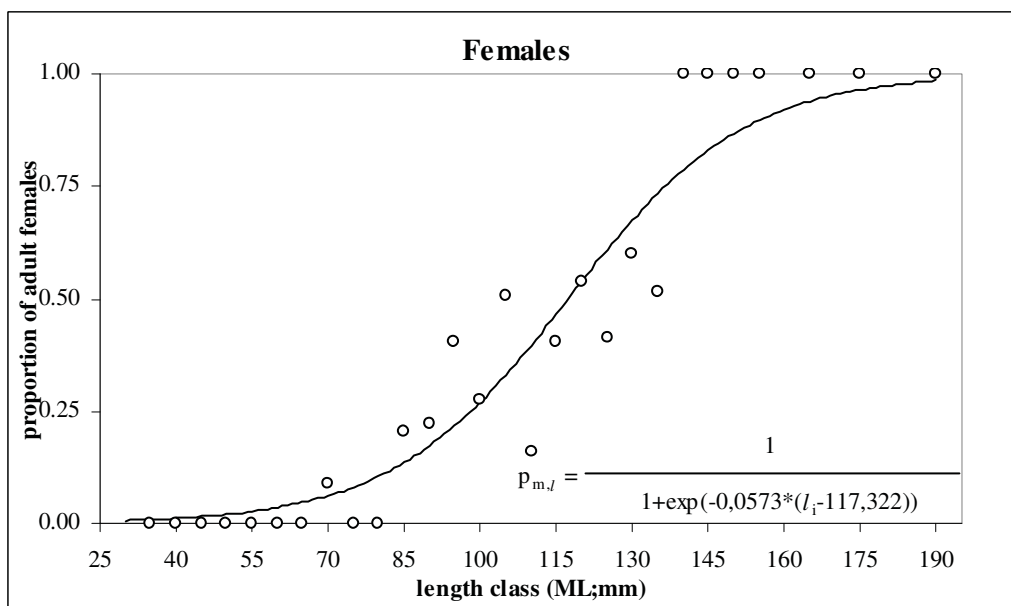


Figure 11. Ogive at maturity of *O. vulgaris* females in Spring (MEDITS surveys). GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16. All MEDITS surveys (1994-2007) were combined.

Adults and recruits

Although no evident trend in adult abundance was detected, recruitment strength, in terms of number of specimens caught in Autumn surveys, showed a significant increase from 1994 to 2006 ($r_s=0.62$; $p<0.05$)(Fig. 12).

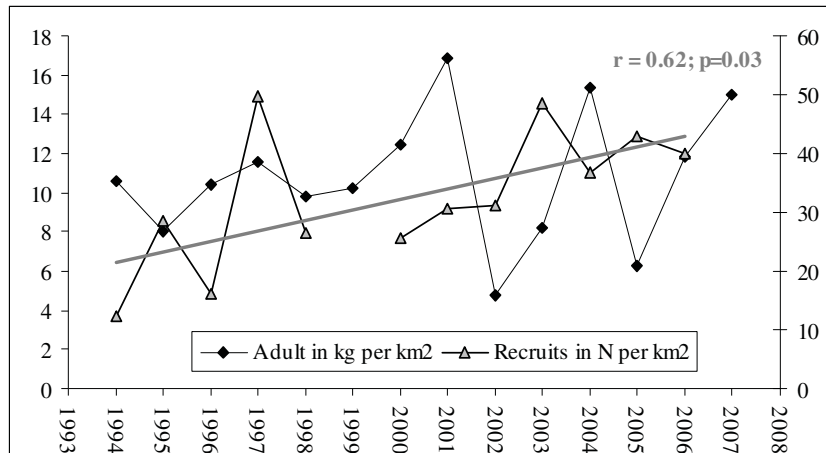


Figure 12. Abundance indices of spawners (kg per km² in Spring on the left Y-axis) and recruits (N per km² in Autumn on the right Y-axis) in GSA 15 (MMFZ only included from 2003 to 2005) and GSA 16.

No relationship between spawning stock biomass (SSB) and recruitment indices was found as reported in Fig. 13.

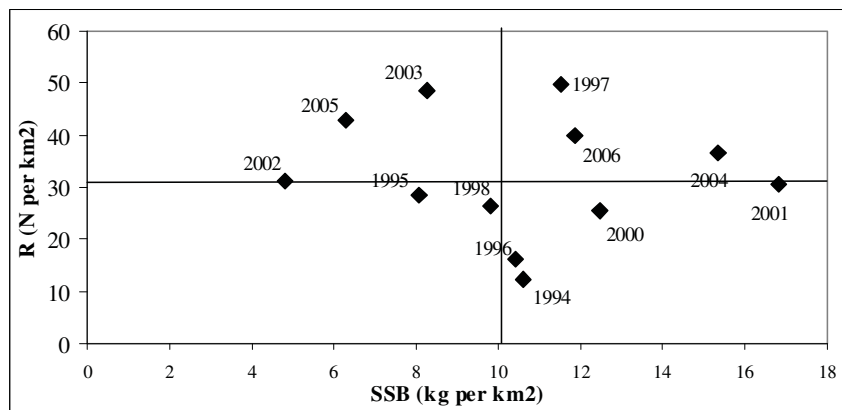


Figure 13. Spawning stock biomass (SSB) and recruitment (R) indices in GSA in GSA 15 (MMFZ included only from 2003 to 2005) and 16. The lines corresponding to median value of indices are also reported.

The behaviour of the mean surface SWT by month in the GSA 15 and 16 from 1994 to 2007 is illustrated in Fig. 14.

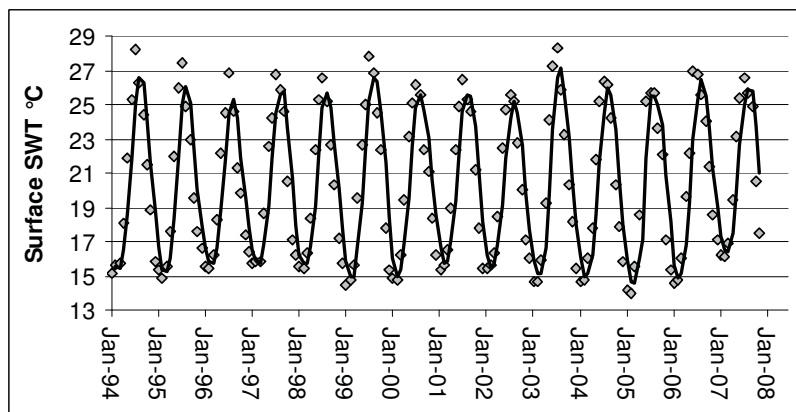
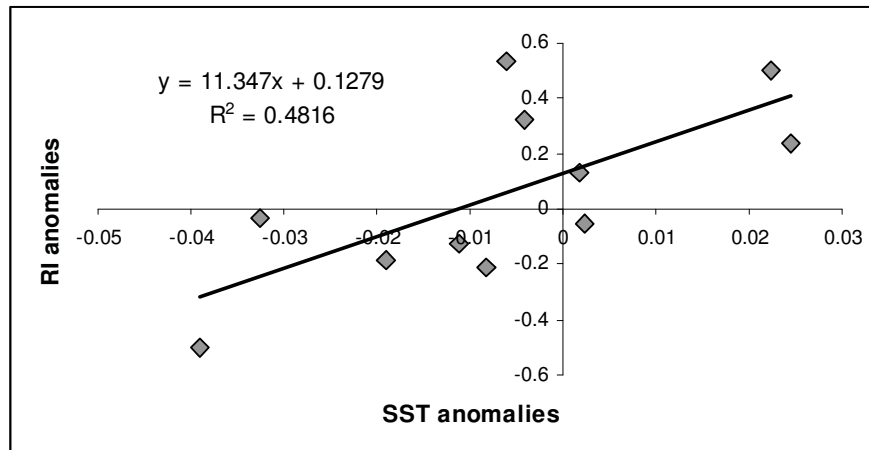


Figure 14. Mean SST from remote satellite in GSA 15 and 16. Monthly estimates are reported.

The anomalies of recruit indices in Autumn showed a significant correlation with the anomalies of SST in July-December of the same year ($r_p=0.693$; $p<0.05$) (Fig.15). This finding suggests that SST warmer than the mean may increase the strength of recruitment. No significant relationships was found for SST and SSB.



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Spatial distribution of recruits and adults of *Octopus vulgaris* in GSAs 15 and 16 (central Mediterranean Sea)

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Abstract

The focus of this study is the population spatial structure of the common octopus, *Octopus vulgaris* (Cuvier, 1797), in the GSAs 16 and 15 of Strait of Sicily (central Mediterranean). Specifically, the depth distribution of the species and the geographic position of nursery and spawning grounds were investigated. Abundance indices by life-phases (recruits and spawners) were obtained from two series of scientific trawl surveys carried out in the Strait of Sicily in the period 1994-2007 within the framework of international and Italian national programmes of experimental trawl surveys. Distribution maps of density indices were generated for each life stage and year by Inverse Distance Weighted (IDW) deterministic interpolation. Specifically, 14 distribution maps of recruit density indices were produced for the Autumn season (main recruitment period), whereas nine density maps of mature individuals were obtained for the spring/early summer season (peak of reproduction). Moreover 4 years of data were available to produce distribution maps of recruit density indices in the African platform of GSAs 13 and 14. Nursery and spawning areas were identified, by means of GIS techniques, applying criteria based on the persistent presence of high values of recruits and adult females respectively, throughout the period of study. A stable spawning ground was identified in the north-west sector of GSA16. Several nurseries were located along the south coast of Sicily and north of Malta. An important stable nursery was found in the south-east area of GSA 13.

Introduction

A central problem in fishery population dynamics is understanding the spatial structure of stocks and the mechanisms that may influence local population size and persistence (Kritzer and Sale, 2004). This issue becomes critical when the natural spatial scale of the population covers areas subjected to different fishery exploitation and management. In this case, the sustainable exploitation of the resource may largely depend on the identification of nursery, spawning and feeding areas, as well as movement patterns and interpopulation connectivity (Jennings et al., 2001; Berkeley et al., 2004).

Similarly to many demersal stocks in the Strait of Sicily, *Octopus vulgaris* is likely to be a shared resources among the countries of the region. Though it is a benthonic species inhabiting a variety of bottoms, mostly up to 250 m depth, its life cycle begins with a planktonic phase (paralarvae) lasting one or two months. Hence, patterns of dispersion may be responsible for wide geographical distribution of the stock.

The current study focuses on spatial and temporal patterns in distribution and abundance of recruits and spawners of *Octopus vulgaris* to preliminarily assess the stock structure in the Strait of Sicily.

Material And Methods

Study Area

The south-central Mediterranean region is characterized by complex bottom morphology (Fig. 1). Along the southern coast of Sicily, the shelf is characterized by two wide and shallow (100 m) banks on the western (Adventure Bank) and eastern sectors (Malta Bank) separated by a narrow shelf in the middle part. The North African shelf is very wide, especially along the Tunisian coasts. In the Gulf of Gabès, the continental shelf with depth shallower than 30 m extends far away from the coast.

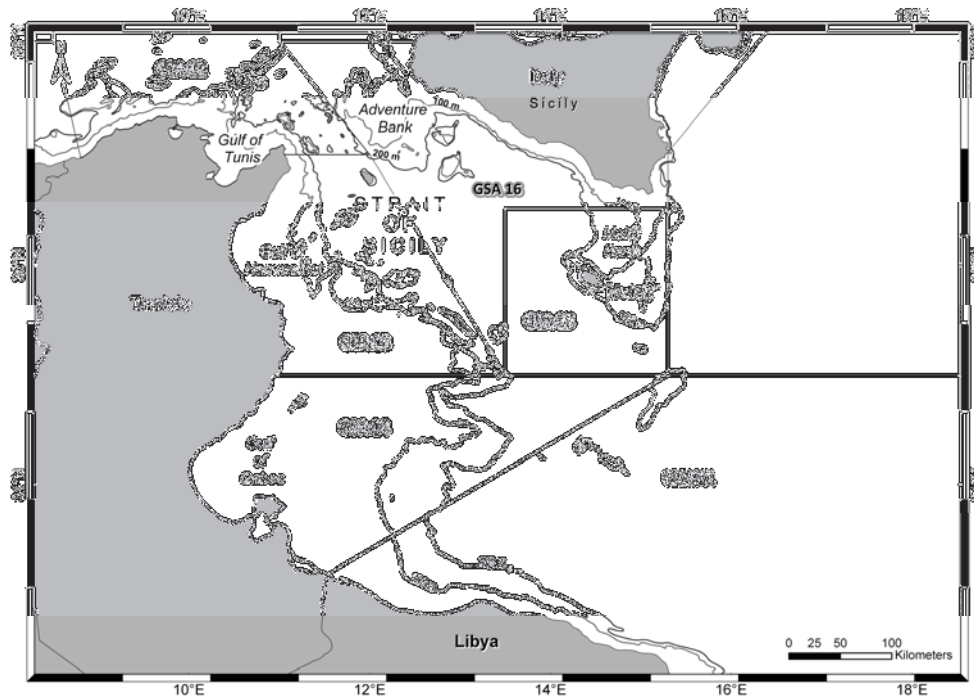


Figure 1. The Strait of Sicily and the boundaries of GSAs. The Italian-Maltese and the North African platforms (100 and 200 m depth contours) are shown.

Data sources

The data were collected within the framework of national and international programmes of experimental trawl surveys planned for the evaluation of demersal resources: Italian national Grund (GRUppe Nazionale risorse Demersali) program (Relini, 2000) carried out in the Autumn season and international Medits (MEDiterranean International Bottom Trawl Surveys) programme (Bertand et al., 2002) carried out in Spring/early Summer season.

Both programmes applied a random sampling with a stratification protocol. The prospected depth strata were: 10-50 m, 51-100 m, 101-200 m, 201-500 m, 501-800 m. Figure 2 gives a picture of the spatial coverage of the surveys carried out in the two seasons from 1994 to 2007. The Autumn surveys differed in having, in some years, a wider geographical coverage than the Spring survey.

For each haul, commercial species were sorted out, weighed and analyzed separately. For cephalopods, mantle length of individuals (ML) was measured to the nearest mm and sex and sexual maturity (three-stage maturity scale) were recorded.

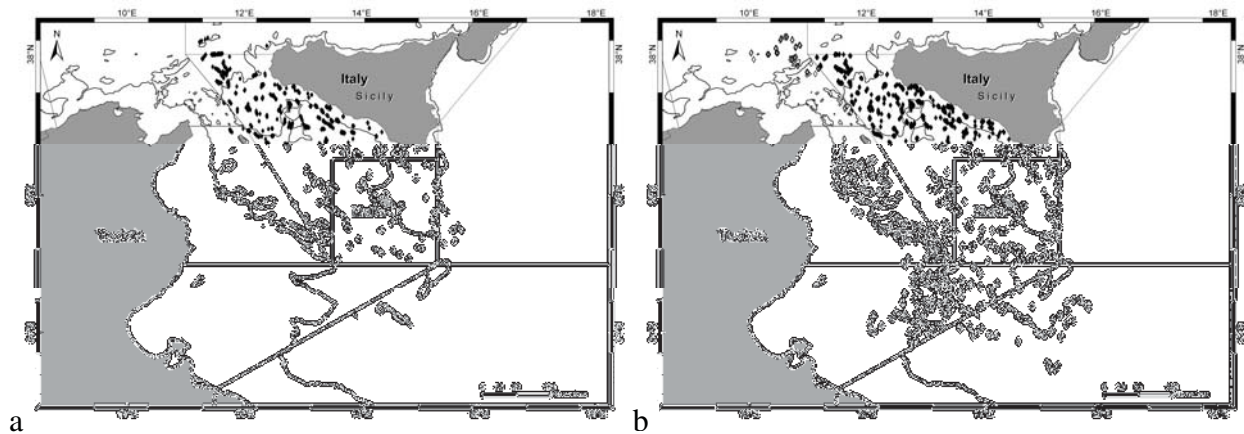


Figure 2. Spatial coverage of trawl surveys carried out during a) Spring/early Summer and b) Autumn.

The time series of data available on *O.vulgaris* spans from 1994 to 2007 for the spring survey. Autumn temporal series includes periods 1994-1998 and 2003-2006 for GSAs 16 and 15, and years 1997, 1998, 2003, 2004 for the enlarged sampling area which includes portions of GSAs 13, 14 and 21 (Fig. 2b). Furthermore, samples inside the Maltese Fisheries Management Zone (MFMZ, i.e. the area surrounding the Maltese Islands within 25 nautical miles from the coast) have only been collected since 2000 in Spring and 2003 in Autumn.

Estimation of demographic indices

Analysis of spawning spatial distribution was based on data from the Spring (main spawning season) surveys. Recruitment indices were derived from the Autumn (main recruitment season) series of data. Density indices were expressed as number of individuals standardized to 1 km² (N/km²) assuming a catchability coefficient equal to 1. Recruits, corresponding to the young of the year, were identified by splitting the overall standardized Length Frequency Distributions (LFDs) into components (combined sexes) and isolating the first modal component. Hence, density index of recruits by haul was calculated as the standardized number of specimens whose length is below $l^- + 1$ sd of the first component of LFD.

Density index of spawners by haul was expressed as the number of individuals (males and females) whose maturity stage was > 2 . Depth range and space occupancy of the two life stages were investigated, respectively, by plotting the mean density index by depth (pooled years) and the percentage number of positive hauls versus population size (over time).

Spatial analysis

Distribution maps of density indices were generated for each life stage and year by Inverse Distance Weighted (IDW) deterministic interpolation. Nursery and spawning areas were identified, by means of GIS techniques, applying criteria based on the persistent presence of high values of recruits and spawners respectively, throughout the period of study. Specifically, once-yearly maps were produced, the area encompassing 75% of the total abundance was outlined over each map to identify the annual nursery and spawning areas. To verify if they were located in the same position consistently through time, an index of persistence (PI) was calculated. It was obtained by overlapping the maps of the entire seasonal series and counting, on a cell-by-cell basis, the number of times a given area was classified as annual nursery or spawning area. The index was expressed in percentage terms and the level of 60% was taken into consideration to define persistent nursery and spawning areas.

Results

Density indices of spawners were generally low throughout the time series; mean values over the shelf ranged from about 3 to 11 N/km². Mean recruitment indices varied between 9 and 47 N/km² in GSAs 15 and 16 whereas they were much higher over the African platform, being comprised between 44 and 81 N/km². Figure 3 shows that although individuals were found up to 200 m, the majority of the population was found at a depth shallower than 100 meters. Specifically recruits were found at a mean depth of 64 m (± 30 *sd*) whereas spawner mean depth was 68 m (± 36 *sd*).

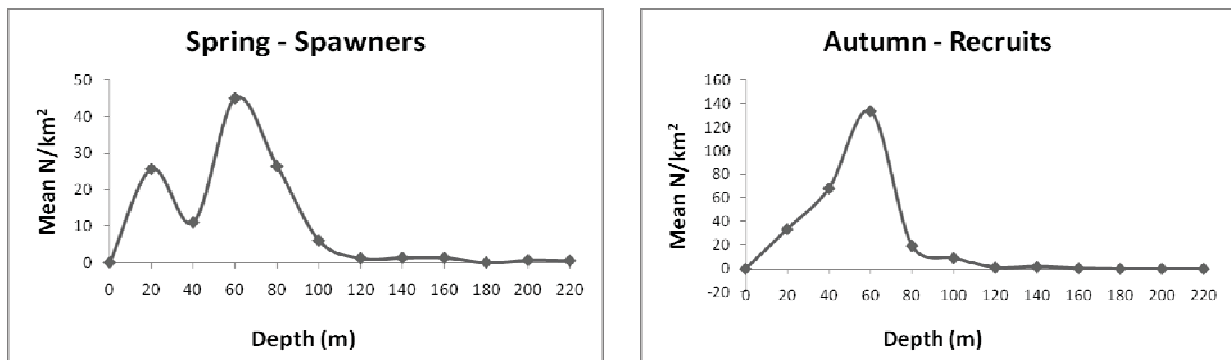


Figure 3. Distribution by depth range of the mean density index of spawners and recruits in the Spring and Autumn season, respectively.

An increasing trend in the relationship between the percentage number of positive hauls and density index (Figure 4) is indicative of a density-dependent effect in the spatial distribution of both spawners and recruits. Though an advanced spatial analysis is necessary to ascertain this aspect, the significance of the trend ($p < 0,05$) for recruits suggests that they occupy a wider area when their abundance increases.

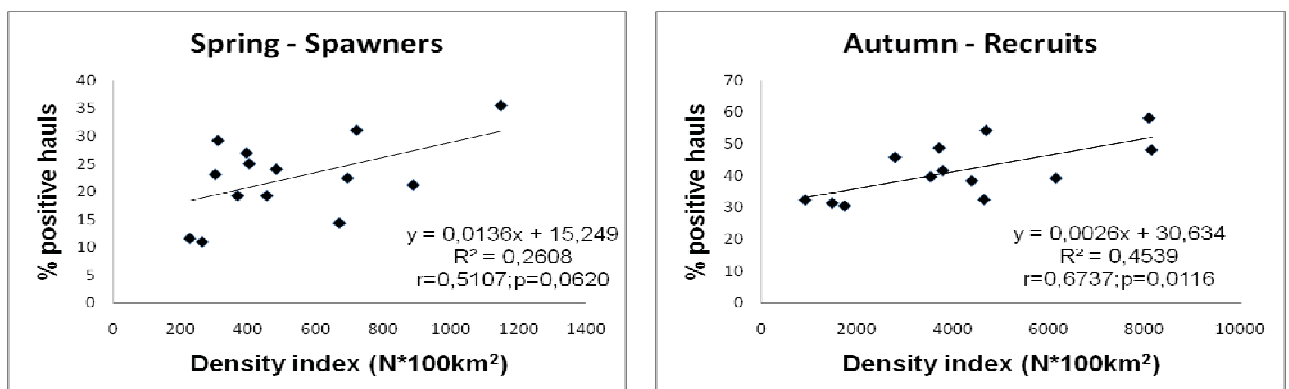


Figure 4. Trend of the percentage number of positive hauls versus density index of spawners and recruits in Spring and Autumn season respectively.

The distribution of spawners (Fig. 5) presents a spatial pattern characterized by a few patches reflecting a highly aggregated structure. The main patch is located over the Adventure Bank off the south-western Sicilian coast (GSA 16). It is variable in size and intensity throughout the years. Minor patches are observed over the Adventure bank (100 m depth), in the central part of the Sicilian coast (GSA 16) and north of Malta (GSA 15). The spawner aggregation north of Malta has only been observed in two years, but it is characterized by high densities.

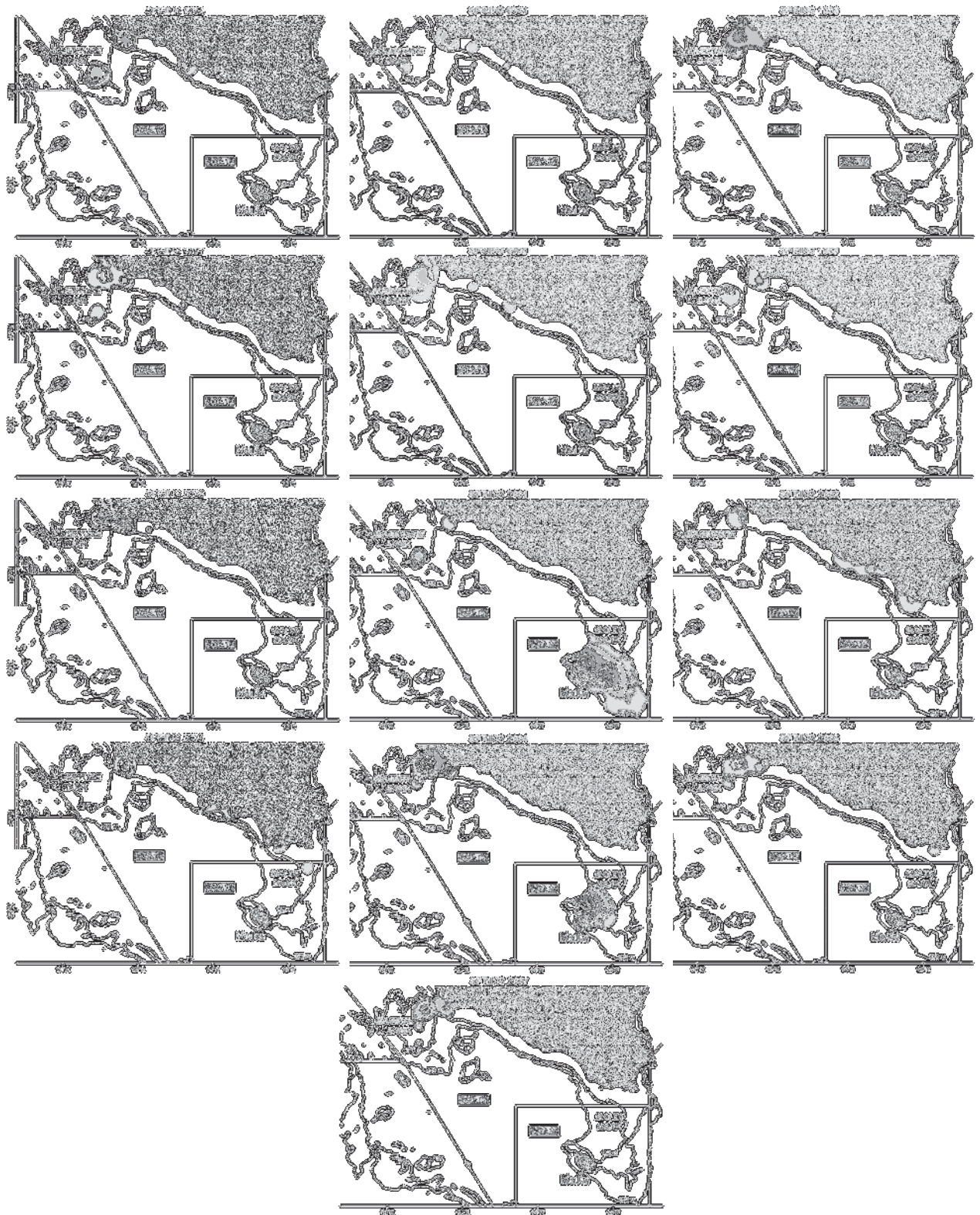


Figure 5. Spatial distribution of spawner density index in the GSAs 15 and 16 during Spring season (1994-2007)

Yearly maps of recruit distribution (Fig. 6) show the occurrence of several areas of high concentration. In some years, recruits spread across inshore areas of most of the Sicilian shelf (GSA 16), whereas in other years the distribution is patchy. Considering that MFMZ (GSA

15) was sampled starting in 2003, the recruitment area observed north of Malta is quite stable. Finally, a large and high aggregation of recruits is found in offshore waters of GSA 13, along the shelf edge of the African platform (Fig. 7).

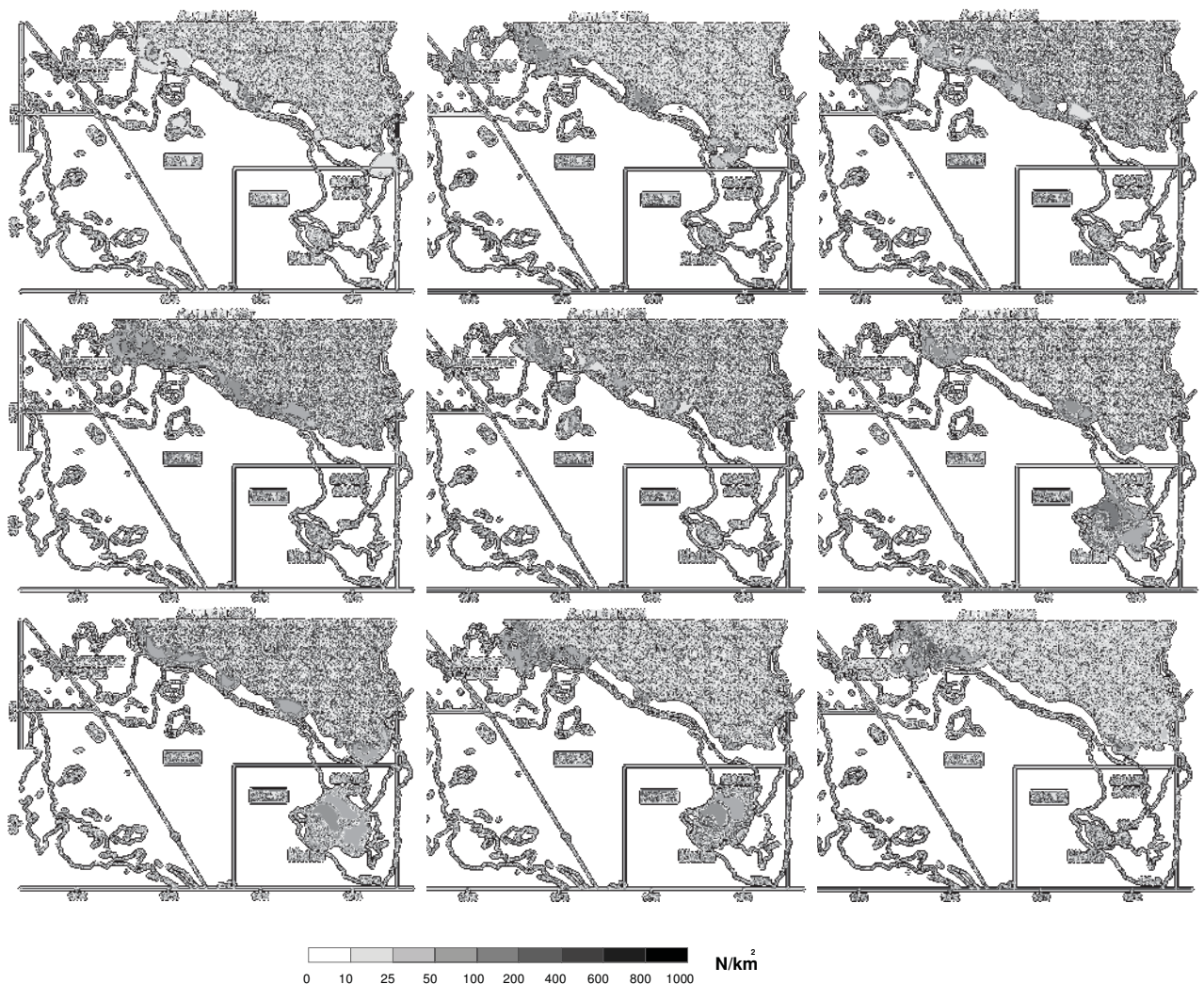


Figure 6. Spatial distribution of recruit density index in GSAs 15 and 16 during the Autumn season (1994-1998, 2003-2006).

Results of persistence analysis are shown in Figure 8. Two stable ($PI \geq 60\%$) nursery areas are identified, during the Autumn season in the inshore waters of Adventure Bank (south-western Sicilian coast; GSA 16) and Malta Bank (north of Malta; GSA 15) respectively, whereas a less stable ($PI < 60\%$) area of recruit aggregation is observed in the central sector of the Sicilian coast. A wide and persistent ($PI \geq 60\%$) Autumn nursery is located offshore on the African platform (GSA 13) within the 100 m bathymetric contour.

During the Spring season an important spawning ground is located close to the south-western Sicilian coast (GSA 16). It is highly stable ($PI \geq 60\%$) and partly overlaps with the nursery area. In some years, a spawning ground is observed north of Malta in Spring at the same location as the Autumn nursery ground.

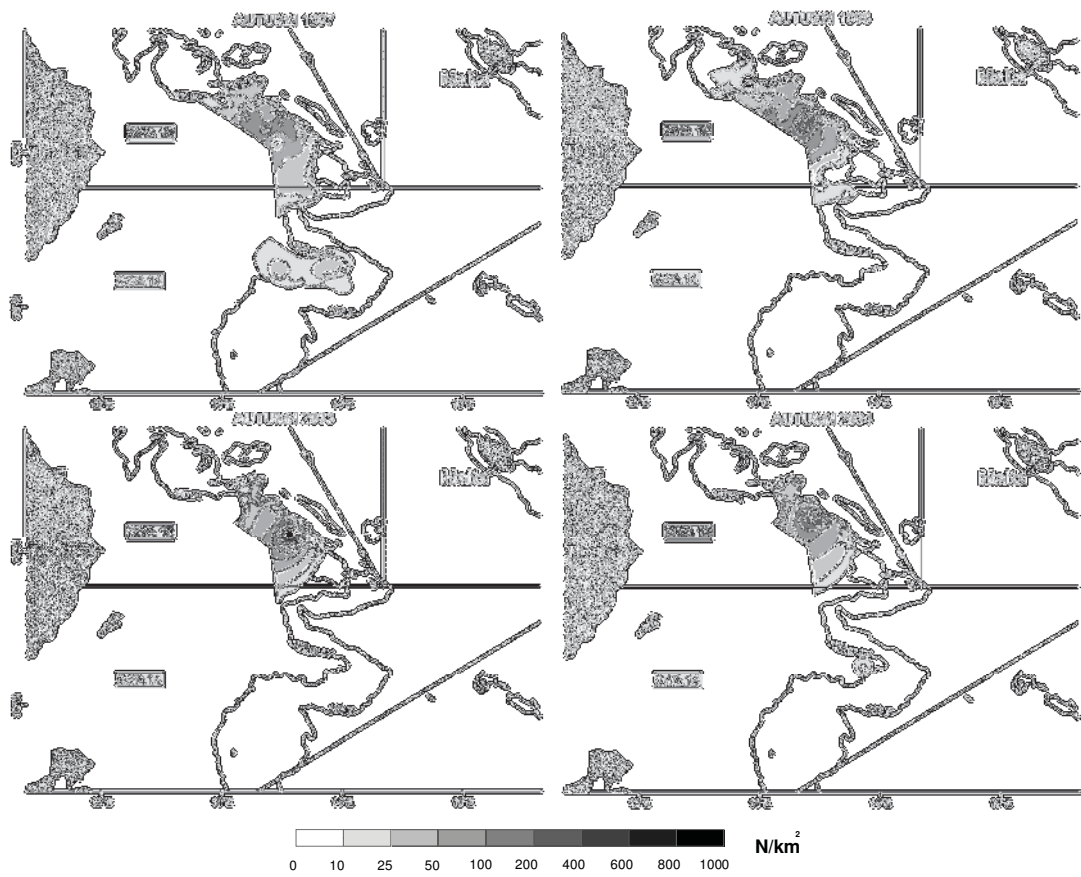


Figure 7. Spatial distribution of recruit density index in the GSAs 13 and 14 during the Autumn season (1997-1998, 2003-2004).

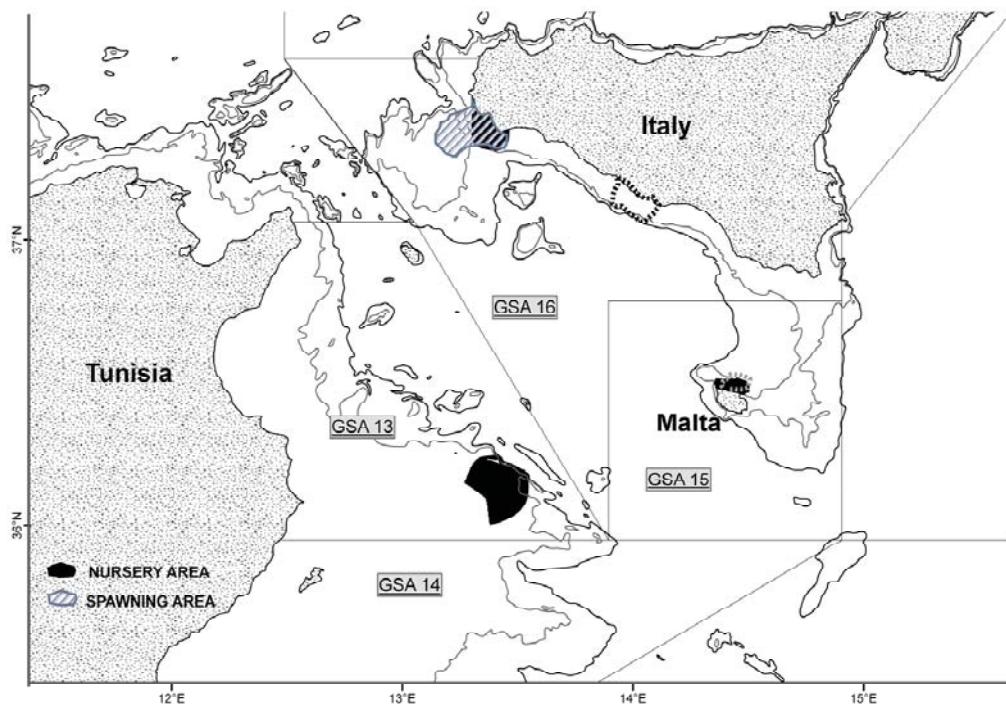


Figure 8. Persistent ($PI \geq 60\%$) nursery and spawning areas of *O. vulgaris* in the Strait of Sicily. The dashed black and grey lines outline intermittent ($PI < 60\%$) nursery and spawning areas respectively.

Conclusions

In this paper the spatial distribution of *Octopus vulgaris* in the Strait of Sicily has been studied considering the two key life phases, Spring spawning and Autumn recruitment. The resource is distributed in moderate quantities over neritic shelf areas of Sicily and Malta while it is more abundant on the African platform.

Spawning is spatially well localised and shows quite a stable pattern throughout years. Particularly, a persistent and wide spawning ground is identified in the north-west sector of GSA16 over the Adventure Bank. A spawning area is just occasionally observed north of Malta (GSA 15) but this result might depend on the low uniformity and size of the sample during the first years of the sampling program in the area surrounding Malta (MFMZ).

Recruitment is spatially more widespread and more variable in time. An extensive and variable distribution of recruits is observed along the southern coast of Sicily, with two main aggregation areas. One area is characterized by high stability and is located over the Adventure Bank off the south-western Sicilian coast (GSA 16), while the other one is sited in the south-central sicilian coast and presents minor stability. A nursery is identified in inshore waters north of Malta (GSA 15). Finally, an important stable nursery is found on the African platform south of Lampedusa (south-east area of GSA 13).

It is worth noting that there is considerable overlap between nursery and spawning grounds both on Adventure Bank and Malta Bank. The lack of data on spring spawners on the African platform (GSA 13 and 14) prevents location of the natal source of the recruits observed in this area. However, aggregation of mature females in inshore areas of the Gulf of Gabès (GSA 14) are reported by Ezzedine and El Abed (2004).

Results of this study provide first evidence about the stock structure of *Octopus vulgaris* in the Strait of Sicily. The distribution of identified nursery and spawning grounds in the area suggests that at least three population units exist. Each potential population unit is located in a different GSA and this would have important implications for stock assessment and management.

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Current status of knowledge about the fishery of *Octopus vulgaris* in the Maltese Islands

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Octopus vulgaris is of high economic importance for the Maltese fisheries since it is the most caught species by weight after Malta's main targeted species (*Coryphaena hippurus*, *Thunnus thynnus* and *Xiphias gladius*). This was only realised recently, since prior to 2003, when only landings data from the fish market were available, landings by weight of this species ranked at around eleventh. However, when this data was complimented with small scale fishery (< 10 m) sampling survey data, catch estimations increased considerably as this species is mostly sold directly by the fishermen and does not go to the fish market. A series of landings data since 1950 show that in the 1970s a peak was reached which declined drastically in less than ten years. The sudden increase coincides with the period in which several trawlers were introduced into the Maltese fleet, while the decline corresponds to the change of the Maltese fleet from in-shore to off-shore fishing. Besides landings data, log book data is available from 2006 onwards and applies to vessels longer than 10 m. The small scale fishery survey data, which is aimed at smaller vessels, is available from 2003, while biological parameters of *O. vulgaris* in GSA 15 can be obtained from MEDITS (Mediterranean International Trawl Survey) data since 2002. Furthermore, data concerning recreational harvesting of *O. vulgaris* especially where shore-based and underwater fishing are involved, is lacking. In Malta these types of fisheries are important and it is well known that considerable amounts of *O. vulgaris* are caught by spear fishermen and other amateurs using boats. However, to date there is no data to document such catches.

Introduction

Maltese fisheries mainly depend on dolphin fish (*Coryphaena hippurus*), blue fin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) catches of which in 2005 amounted to 80% by weight (Darmanin *et al.*, 2007). However, other species also play an important role in the local artisanal fisheries. The common octopus (*Octopus vulgaris*) is one such species which is mainly caught using the species-specific pots and traps as part of the artisanal multi-species fishery (MedSudMed, 2007). Octopus is also specifically fished using the *hiel* or *kulpara*. The former consists of a rosette of three hooks above which a white cloth or an artificial crab is tied while the *kulpara* is made of a weight or a jig to which hooks are attached in a dispersed manner. Such fishing practices generally take place during the day from an onshore area or in coastal zones by means of a small boat, where the hook device is lowered and suddenly pulled up as soon as the octopus crawls onto it (Farrugia Randon, 1995).

On the Maltese islands there is also a huge recreational activity targeting *O. vulgaris*, where skin and SCUBA divers catch octopus using either a large hook attached to a pole or a spear-gun. Even though it is a well known fact that catches by this means can be quite significant, there is no data documenting this activity. In the past, this species was also caught by means of a trident and a lamp at night, where fishers from onshore or a small boat used a bucket with a glass bottom in order to enable them to see clearly underwater. Nowadays these two

methods are being regulated as spear-guns are prohibited if used in conjunction with underwater breathing apparatus (aqualung) or at night from sunset to dawn (Regulation EC No 1967/2006). However, divers can still use the traditional large hook attached to a pole and presumably fishing activity for octopus has not been affected by this regulation. *O. vulgaris* is also caught as by-catch by means of bottom otter trawls, trammel nets and other types of nets such as set gillnets (both anchored and not) and combined gill/trammel nets. There were also times where trawlers fished certain grounds targeting the common octopus as their main species.

The importance of *O. vulgaris* was disregarded in the past since it constituted less than 1% of the total annual landings from the central fish market. However, since 2005 catch assessment surveys have been carried out at various ports around Malta. The results from these surveys have shown that octopus catches are relatively high by the < 10 m vessels and most of the catch is not landed at the central fish market, but sold directly. When considering these results, octopus ranks as the fourth or fifth most species caught (by weight) after the dolphin fish, blue fin tuna and swordfish.

Till now in the Maltese Islands, there are no management regimes regarding *O. vulgaris*. Apart from Regulation EC No 1967/2006 as already described above, and the use of netting smaller than 40 mm mesh size opening for bottom trawlers, there are no other regulations or regimes, such as closed seasons, area closures, and minimum landing sizes specifically protecting this species for a long term sustainable fishery, with maximum harvest yields.

Materials and methods

The data used in this work includes fisheries statistics collected by several means including; the fish market landing statistics (herein referred to as landings) since 1954, logbooks since 2006, the small scale fishery sampling survey which is referred to as the Catch Assessment Scheme (CAS) since 2003 and MEDITS (Mediterranean International Trawl Survey) data since 2002.

The series of landings data was not obtained from a common, single source. Even though the main source was the 'Abstract of Statistics'; a yearly report published by the Maltese National Statistics Office (NSO), from which data was obtained for the years 1954 till 1984, the 1985 till 1993 data series was obtained from monthly reports of the Fisheries Department, while landings data of later years was extracted from the Fish market Scheme of the Fisheries Department. However, these sources are comparable as the original source of the landings data in the 'Abstracts of Statistics' is the Fisheries Department which forwards a monthly report to the NSO. Here it is also important to note, that on the Maltese Islands there is only one fish market in which most of the fish is landed, except when sold directly or exported.

For this study the landings of 'Octopus' from the fish market were assumed to be made entirely of *Octopus vulgaris*. This assumption has to be made in view that all octopoids landed at the fish market are aggregated together in the landing statistics as *Octopus* spp., which is made up of a mixture of Octopoid species, including *Octopus vulgaris*, *Octopus macropus*, *Eledone cirrhosa*, *Eledone moschata*. However, *O. vulgaris* is absolutely the most abundant octopod species in catches from the small scale fishery (CAS survey) especially when pots and traps are used.

Since 2006 owners of vessels larger than 10 metres in length are obliged to keep record of their catch by filling a logbook and report their catches at the Fisheries Department within 24 hours of the end of their fishing trip. Since 2006 logbook data was used in conjunction with the landings. When data was available for the same fishing trip the highest value for either the logbook or landings data was used. This was done to order to avoid duplication of data.

The Catch Assessment Scheme has been running since 2003, where field recorders sample catches in Malta's ports and the data obtained is then extrapolated to all the small scale fleet (< 10m vessels).

MEDITS data has been collected since 2002 following the MEDITS protocol (Anon. 2007). This is the only source of length, weight, sex and maturity stage data of *O. vulgaris* available to date.

The temporal variation of *Octopus vulgaris* catches was examined by gear type, vessel size class, percentage landed at the fish market and percentage sold in ports from 2005-2007. Catch was estimated by summing landings/logbook data, whichever was the higher value for the same trip, and CAS data.

The temporal trend in landings during the last fifty years was examined by studying the historical time series from 1954 – 2007 (only data from the fish market was used). In order to compensate for the increased in fishing effort during this time series, landings per unit effort (LPUE) was calculated as tons per fishing vessel. However LPUE was only calculated since 1963 as data on the number of fishing vessels was available from this year onwards.

The MEDITS data available was used to obtain information about the distribution of *O. vulgaris*. The length frequency distribution, smallest length at maturity and other biological parameters could not be obtained since in the whole data series only 90 specimens of *O. vulgaris* were present. The low number of specimens is due to the fact that, out of the 45 stations sampled each year during MEDITS, only 7 (15%) and 13 (29%) of the stations were in the 0-100 m and 100-200 m depth stratum, respectively and *O. vulgaris* is usually found in depths up to 150 m (Ragonese *et al.*, 1990).

Results

Catch estimation of octopus increased drastically since 2005 when compared to previous years. Whereas before octopus did not rank among the first ten most caught species, between 2005 and 2007 it resulted fourth or fifth due to catches recorded from CAS being included with the landings. In these three years the respective recorded landings of octopus were 6.80, 4.58 and 6.65 tons while the total catches amounted to 39.02, 33.00 and 28.41 tons respectively. The estimated value of these catches was €210,487 in 2005, €198,603 in 2006 and €167,409 in 2007.

Mainly octopods are targeted by the small scale fishery (boats smaller than 10m) (96.3%, 89.4% and 81.6% in 2005, 2006 and 2007 respectively) with an overall mean of 89.1 % (Fig. 1).

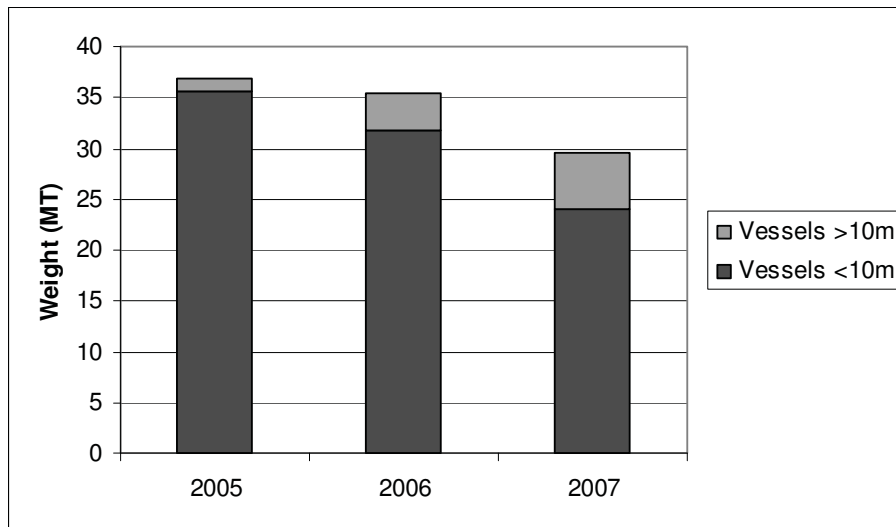


Figure 1. The catch in weight of octopods by vessels smaller and larger than 10 m between 2005 and 2007.

On average, between 2005 and 2007 90.7% of the octopus was caught by means of the species-specific pots and traps, 4.9% as a by-catch of trammel nets and other nets (including set gillnets (both anchored and not) and combined gill/trammel nets), and 4.4% by otter bottom trawling (Fig. 2). The percentage of octopods caught by means of the species-specific pots and traps decreased from 93.3% in 2005 and 90.9% in 2006 to 87.9% in 2007.

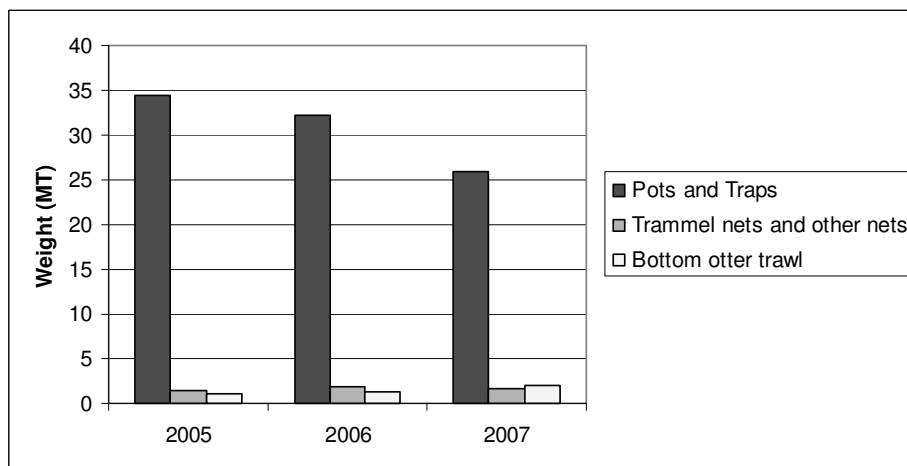


Figure 2. The catch of octopods in weight by gear between 2005 and 2007.

Results shown in figure 3 further indicate that most of the octopus caught is not landed at the fish market but is sold directly. Over the time period analysed it seems that the percentage of catch landed is increasing (from 3.73 % in 2005 to 18.37 % in 2007).

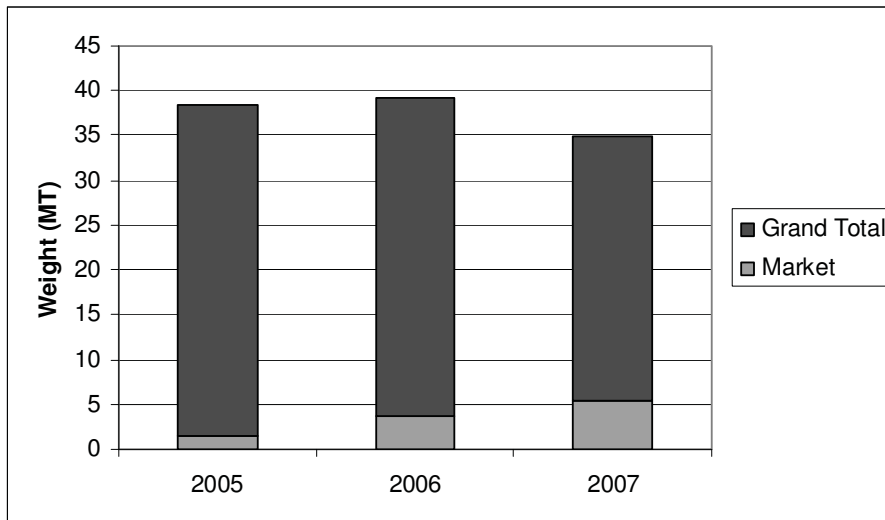


Figure 3. The catch in weight of octopods landed at the fish market and that which was recorded by CAS. Landed values also include catches recorded from logbooks.

Overall landings of octopods decreased drastically from about 30 MT in the 1950s to a mere 5 MT in the late 2000s. Landings and LPUE of the last forty-five years follow a very similar pattern (Fig. 4), implying that the resultant fluctuations represent fluctuations in the octopus population rather than variations in effort. However, a slight increase in LPUE when compared to landings between the years 1996 and 2000 might have resulted due to an increase in effort, although this is not the case when considering effort as the number of registered vessels (Fig. 5).

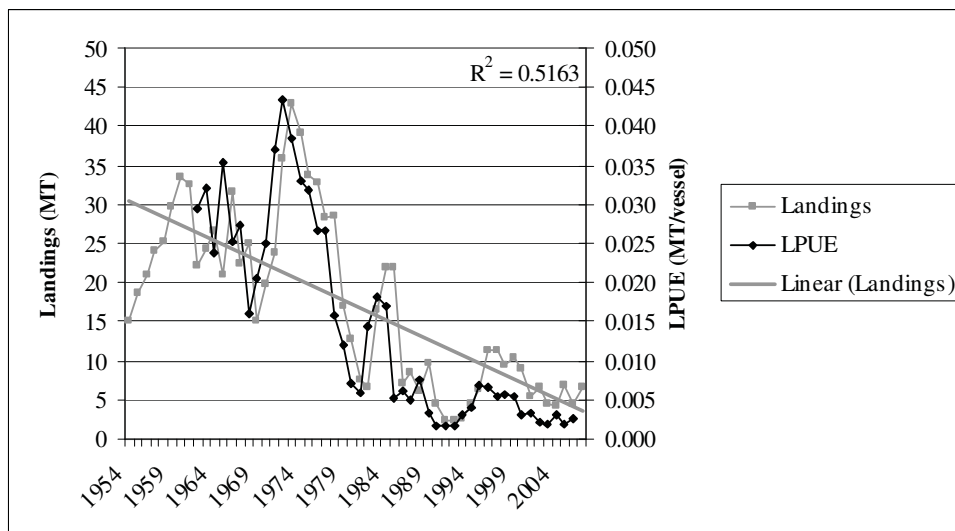


Figure 4. Landings in MT and LPUE in MT/vessel of octopus since 1954 and 1963 respectively.

Since 1954 there have been periods of high catches followed by relatively low ones, which were mainly observed between 1954 and 1969 (fifteen years), 1969 and 1982 (13 years), 1982 and 1993-1995 (about twelve years) and the last cycle from the 1990s till the present day (approximately 13 years). However, as time went by the difference between cycle highs and lows decreased drastically to the extent that this trend almost does not exist anymore in

the last cycle. Here one has to keep in mind that this data does not include *O. vulgaris* only but consists of all the octopods caught on the Maltese Islands.

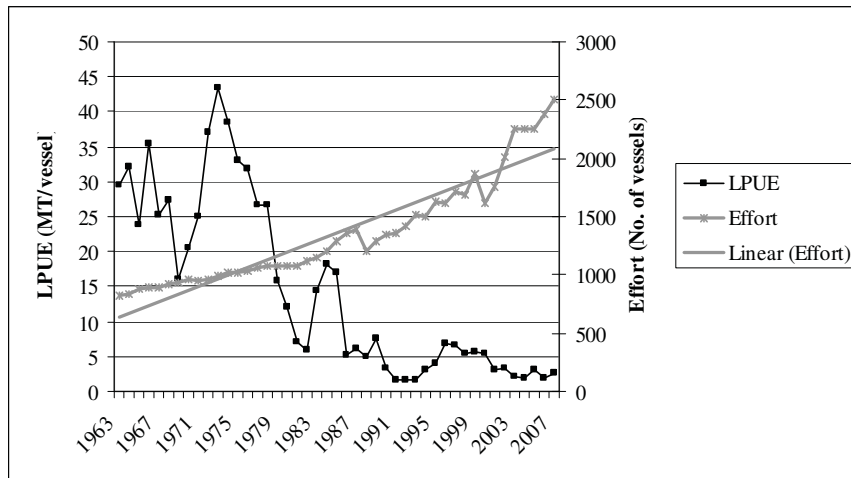


Figure 5. LPUE of octopods and effort since 1963. Effort was considered as the total number of registered vessels, irrespective of whether they contributed to octopod catches or not.

Using the MEDITS data it was noted that *O. vulgaris* in the Maltese Islands is mainly restricted to coastal zones due to the continental shelf being limited to such areas (Fig. 6). Although from this map the common octopus seems to occur north east of the islands in large biomass, it was recorded in that area only during the MEDITS leg in 2007.

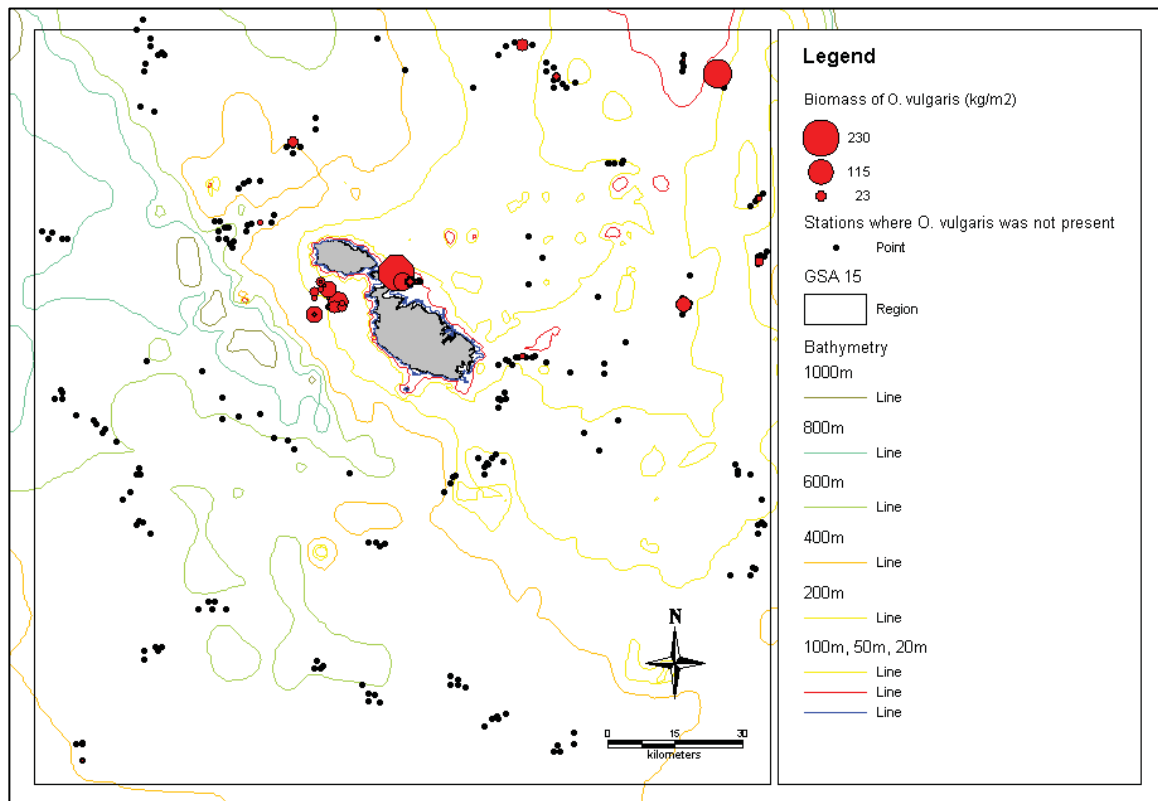


Figure 6. Map showing the biomass distribution of *O. vulgaris* in stations sampled during MEDITS 2002-2007.

Discussion

Octopods are amongst the most important species in the Maltese fishery both from an economical as well as from a social and cultural point of view. They are amongst the first five most-caught species on the Islands and among the first ten species which have had the highest monetary value in the past three years (2005-2007). The importance of octopods would be further amplified if yearly catch estimations had to include catches from recreational activities. Although a proportion of such catches is used for personal consumption, a good percentage is sold directly (catches from recreational activities cannot be landed at the fish market. From a social and cultural perspective octopods are important as they are mainly caught by vessels smaller than 10 m, (> 90% of the fleet), using species-specific artisanal gear such as pots, traps, the *hiel* and the *kulpara*.

In Malta, most of the octopus caught is sold directly and is not channelled through the fish market. This was clearly shown when estimated catch values drastically increased when considering CAS data, and also from results presented in Fig. 3. Thus, when estimating catch dependent parameters, it is very important not to consider landings data only, but also CAS data.

Landings and LPUE values of octopods during the last fifty years, apart from showing a significant decrease, also demonstrated that, probably, local biomass of octopod populations undergo cycles of low and high peaks averaging about thirteen years. Even though low and high peaks arise due to density-dependent factors such as food availability, the reason behind a specific time period between each cycle is still unknown. However, the cycles recorded in the Maltese Islands (1954 till 1969, 1969 till 1982, 1982 till 1993-1995 and 1990s till the present date) coincide with cycles resulting from landings of *O. vulgaris* in the Gulf of Gabes (Tunisia). The landings series from 1975 till 2000 reported by Ezzeddine *et al.* (2004) show cycles between 1975 and 1982 (12 years), followed by a second one lasting till 1994 and a third uncomplete cycle extending until 2000. However, differences were observed between the relative abundances of the peaks; the cycles with the highest peaks do not coincide. Whereas in the Maltese Islands the cycle with the highest peak occurred between 1969 and 1982, with the previous peak being slightly lower and latter ones being even smaller, in the Gulf of Gabes, the highest peak was observed between 1983 and 1994, with both the previous and the latter peaks being lower. These changes in peak heights may have resulted due to differences in fishing effort between the two different landing areas.

The historical analysis in landings, LPUE and effort shows a clear picture of overfishing for this species. The effort has increased since 1963 by ca. 100% from about 1000 to 2000 vessels (Fig 5). For the same period the landings have decreased by 10 fold from about 30 to 3 tons (Fig 4). At present the stock seems to be in an equilibrium state with low catches, with the trend in landings clearly showing that catches were considerably higher in the 60s and 70s.

Although the unit of effort is not ideal as the number of pots and traps used and/or trawling hours spent fishing during would be a better choice the number of registered vessels is the only measure of effort for which a long time series is available. However one also needs to note that more than 90% of the Maltese fleet is artisanal small scale and the increase in the number of vessels since 1963 has also resulted in the increase in fishing effort for octopus using pots and traps.

MEDITS data showed that in summer *O. vulgaris* is mainly limited to coastal areas due to this species inhabiting waters shallower than 200 m (Ragonese *et al.*, 1990). This is also one of the reasons why catches of this species are mainly attributed to small vessels; trawling is prohibited inside the 3 nautical mile zone around the Maltese Islands. This can also help to conclude that octopods caught by means of trawling are mainly *Eledone spp.* which can be found at greater depths.

Where biological parameters such as length, weight, sex and maturity of *O. vulgaris* are concerned, there is still a lack of knowledge which is needed for a better understanding of the population biology in the local environment as well as for improved management of the stock. Furthermore, studies on the percentage composition of *O. vulgaris* in the landed catch of 'Octopus' especially at the fish market need to be conducted. Such studies can be used to improve the historical and future landing statistics of *O. vulgaris*. Further studies may also include efforts to quantify catches of *O. vulgaris* resulting from recreational activities. All these commitments to monitoring and research should eventually lead to a full stock assessment of *O. vulgaris* with proposals for management so that the harvesting yield of this fishery can be increased and sustained on the medium-long term.

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Genetic analysis of the *Octopus vulgaris* population from the areas of Sfax-Kerkennah and Zarzis by means of mitochondrial DNA Sequences

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Abstract

The use of genetic methods in fishery population studies is an important facet of both fisheries stock assessment and management. Among the genetic molecular markers, intra-specific sequence variation within mitochondrial DNA (mtDNA) has proven to be a powerful tool for studying population structure in marine organisms. To date, few population geneticists have made use of DNA sequences of population samples and the corresponding statistical tools for shellfish research.

This study focused on the common octopus, *Octopus vulgaris*, which is of an significant interest as an exploited resource. The molecular analyses of octopus phylogeny have focused on allozymes, and mtDNA (gene coding regions: COI, COII, COIII and 16s rRNA). Studies on octopus and *O. vulgaris* in particular, have focused on the COIII gene region of mtDNA. The aims of this study are to investigate the genetic variation within Tunisian *O. vulgaris* in the mitochondrial DNA (mtDNA) cytochrome oxidase subunit III (COIII) region, to determine population structure and also to deduce whether different population exist in Tunisian waters and others Mediterranean areas by means of a phylogenetic comparison.

A total of two samples of *Octopus vulgaris* were collected from the areas of Sfax-Kerkennah and Zarzis. For each sample, a fragment of COIII gene was amplified by PCR analysis and subsequently sequenced. Sequences of the COIII region were aligned using the BioEdit v.7.0.7 program. Haplotype frequencies, standard diversity indices, pairwise genetic distances (Fst) and analysis of molecular variance (AMOVA) were calculated with ARLEQUIN software and compared to results obtained from other Mediterranean areas.

Introduction

The genetic structure of natural populations has played an increasingly important role to formulate effective management programs on exploited resources. The knowledge of genetic diversity of a species allows the identification the possible existence of genetically distinct sub-groups, joined by a comparatively low level of gene flow. In this context one of the main purposes of conservation biology is to preserve the genetic diversity of species, together with the biological and evolutionary process determining it. The common octopus (*Octopus vulgaris*) is of great interest as an exploitable resource in the whole Mediterranean Sea. In Tunisia, among the four commonly exploited species (cuttlefish, octopus, eledone and squid), *Octopus vulgaris* is the most sought after species in the Southern Tunisian area thanks to its commercial value. This region provides 88% of the national octopus production.

The aims of this study were to investigate the genetic variation within the South Tunisia coast of *O. vulgaris* in the mitochondrial DNA (mtDNA) COIII region, to determine population structure and also to derive the phylogenetic relatedness of South African and Mediterranean *O. vulgaris*.

Recently, significant advances have been made in the field of genetic stock identification (Begg et al. 1999). Genetic markers have increasingly been used in the identification of finfish stocks (Bartley et al. 1992 and Smith et al. 2002).

By comparison, the use of genetic methods in cephalopod population studies has been modest (Carvalho et al. 1992 and Shaw et al. 1999) and genetic studies on octopus specifically have focused mainly on phylogeny (De Los Angeles et al. 1995 and Warnke et al. 2000).

The molecular analyses of octopus phylogeny have focused on allozymes, and mtDNA (gene coding regions: COI, COII, COIII, 16s rRNA, etc.). Studies on octopus and *O. vulgaris* in particular, have focused on the COIII gene region of mtDNA (De Los Angeles et al. 1995, Bonnaud et al. 1997, Söller et al. 2000 and Warnke 1999) with DNA sequences available from individuals from several regions.

Materials and methods

Sample collection

Samples of *Octopus vulgaris* were collected at two sites in the Zarzis and Kerkennah (Fig. 1). From each animal, a small piece of muscular tissue from the tip of the arm was removed and preserved in absolute ethanol.

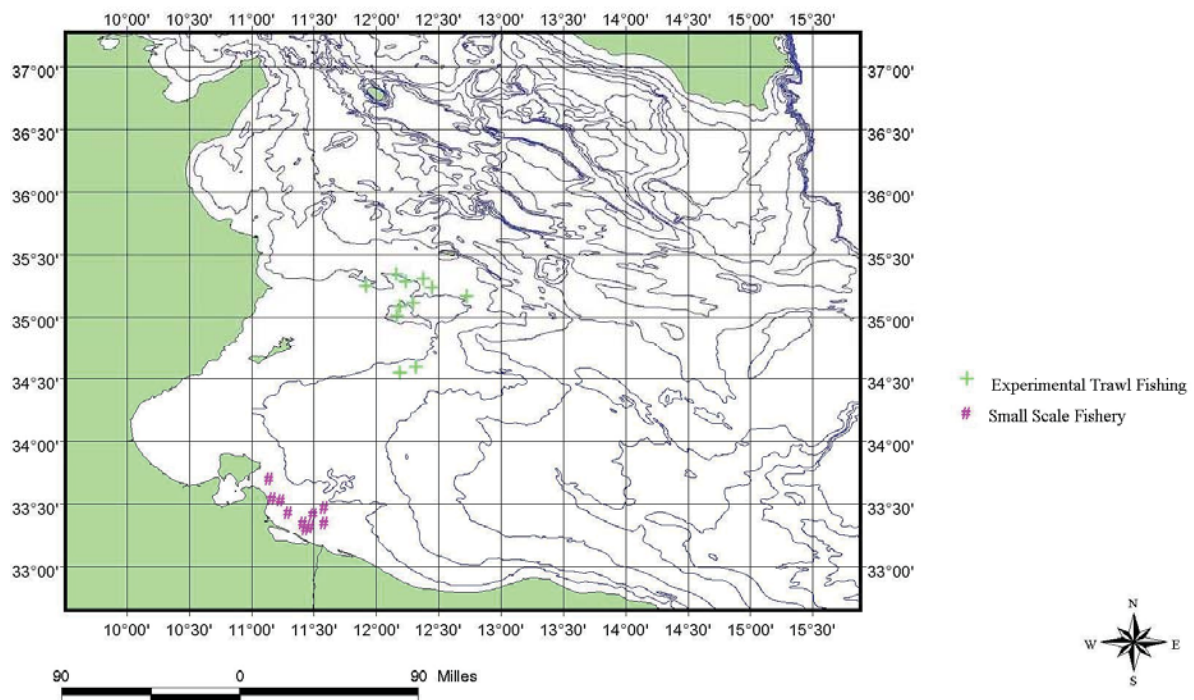


Figure 1. Geographic locations of *Octopus vulgaris* samples

DNA extraction, polymerase chain reaction (PCR), and sequencing

The total genomic DNA was extracted using a QIAGEN DNeasy® tissue kit; after addition of the ATL buffer and proteinase K, the tissue was incubated overnight at 55°C before the protocol was completed. The elute was tested on agarose gel to determine DNA yield.

The COIII region of mitochondrial DNA (380-bp) was amplified by polymerase chain reaction (PCR) using the primers: mtDNA forward 5'-ACCATAATTCAATGTGACGTGATATT-5' and mtDNA reverse 5'-AAATAGAAAATGATGCTTCTATATATTCTAA-3' as described by Ané *et al.* (2004).

The PCR amplification reactions were performed in a total volume of 50 µL containing: 2.5 µl 10mM NH₄ buffer, 2.5 µl 50mM MgCl₂, 2.5 µl BSA, 2.0 µl 100mM dNTPs, 1 µl primer (3.2 pmol), 0.4 µl *Taq* (Go *Taq*® Flexi DNA polymerase) and 1–5 µl DNA (50ng /µL). Thermal cycling was hot-started (96°C for 5 minutes, held at 85°C while *Taq* was added), followed by five cycles of 93°C (50 s), 45°C (50 s) and 72°C (1 min), followed by 28 cycles of 93°C (50 s), 50°C (30 s) and 72°C (1 min). The last step of 72°C was prolonged for 5 min (Söller *et al.* 2000).

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The sequencing reaction of both strands was performed using the Big Dye Terminator (version 3.0) Cycle Sequencing Kit, with AmpliTaq® DNA Polymerase (Applied Biosystems). Sequences were run in an automatic Sequencer ABI377 (Applied Biosystems).

Genetic diversity and phylogenetic analysis

Both strands of a sequence were aligned with the programme BioEdit version 7.0.4.1 (Hall, 1999) and confirmed visually. Multiple alignments of sequences were done using Clustal V implemented by BioEdit (version 7.0.4.1).

Estimates of genetic variation were obtained in the form of haplotype diversity *h* (Nei, 1987), nucleotide diversity *p* (Nei and Jin, 1989), and mean number of nucleotide differences among all haplotypes in a putative population with the software ARLEQUIN (version 3.0; Excoffier *et al.*, 2005).

Analysis of population structure

The significance of population structure was tested by the analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) as implemented in ARELQUIN, which takes into account distances between haplotypes, gamma shape parameters and geographic distribution. U statistics of AMOVA quantify population structure at each level in a given hierarchy. For this hierarchical analysis, the samples were grouped into two putative populations: Zarzis and Kerkennah (Fig. 1). The significance of U statistics was tested by comparisons to null distributions constructed from 10,000 random permutations of the original data matrix.

Conclusions

This work reports the development and application of genetic tools for the identification of *octopus vulgaris* genetic structure. *Octopus vulgaris* is one of the most economically important Tunisian fisheries. A good understanding of dispersal and stock structure is clearly essential for fisheries management. In this study, we are investigating the stock structure of the common octopus, *O. vulgaris*, from South Tunisia using the experimental approach described above. Having already collected samples, we worked with mitochondrial COIII region data to discriminate between different octopus stocks.

Previous studies of the genetic structure of *O. vulgaris* from the Mediterranean Sea using allozymes (Maltagliati et al., 2002) and microsatellite loci (Casu et al., 2002) excluded isolation by distance in *O. vulgaris* Mediterranean populations. Maltagliati et al. (2002) suggested that *O. vulgaris* in the Mediterranean followed a basic island model in a background of high gene flow. Data that obtained from this study should clarify if the South Tunisian *O. vulgaris* can be treated as a single population or two genetically different lineages.

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