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PhD thesis

"Analysis of the Adriatic macrobenthic assemblages along a spatio-temporal gradient. Habitat mapping as a tool to address restoration and recovery of marine resources"

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SOMMARY

CHAPTER 3. LARGE MARINE PROTECTED AREAS (LMPAS) IN THE MEDITERRANEAN SEA: THE OPPORTUNITY OF THE ADRIATIC SEA

ABSTRACT

The Adriatic Sea is one of the most studied basin worldwide. However, only few ecological studies have been conducted at the whole basin scale and taking into account the deepest, offshore areas of the basin. The most of the available information, moreover, lacks a clear spatial geo-referencing making difficult to determine the boundaries and the localisation of certain biocoenosis of the Adriatic Sea. Even more complicated is the possibility to define and describe spatio- temporal patterns that the communities of the soft bottoms of the Adriatic basin suffered due to increasing natural and human impacts and threats.

Recently, the use of new technologies, e.g. ROV survey, and the plethora of national and international projects underway in the Adriatic basin (Devotes, CoCoNet, Adriplan, Mediseh, Netcet, Shape, Adriamed, Powered) have rapidly increased the available knowledge of the Adriatic marine environment and they are providing fundamental information to define and identify Adriatic ecosystems functioning and services. Understand the role and the value played by the Adriatic marine environments is needed to develop a sustainable management and use of the Adriatic resources.

This PhD thesis focused on the analysis of the macrobenthic community of the Adriatic soft bottoms, with a particular attention on species without any peculiar commercial value, but that are important from a functional point of view such as Essential Fish Habitats (EFHs) or Vulnerable Marine Habitats (VMHs). Time-series data and the use of software that allow to perform spatial analysis gave us the possibility to map and describe past and current changes of macrobenthic populations of the basin.

In particular, this PhD thesis is structured as follow:

- The introduction is an overview of the current international regulations and conservation frameworks of the Adriatic Sea and of the management of marine resources. The existent gaps, opportunities and challenges in the protection and management of the marine environments are highlighted. The main aims of the PhD works will be list and described.

The chapters will be presents in the form of scientific papers.

- Chapter 1 is an analysis and description of the principal changes occurred in the macrobenthonic communities of the North and Central Adriatic Sea. The negative and declining trends of soft bottoms populations resulted by our analysis will be related with the potential sources of impacts, in particular with trawls fishery that is one of the most destructive human practice.
- Chapter 2 is a mapping of the current presence of EFHs and VMHs in the Adriatic Sea. Sea pens have been classified both as EFHs and VMHs. For this reason, they were selected as case study to define the potential and the possibility to the recovery of Adriatic soft bottoms marine habitats. A taxonomical description of some of the sea pens living in the Adriatic Sea has also produced in order to solve misidentifications of the most common species present in the basin.

- Chapter 3 is a proposal for the establishment of a new and efficient management tool for the recovery of Adriatic macrobenthic communities with the consequent replenishment of the Adriatic marine resources.

1. INTRODUCTION

1.1 THE MACROBENTHONIC COMMUNITIES OF THE ADRIATIC SEA

The Adriatic Sea is a semi-enclosed basin with an area of about 138,000 km^2. The northern and central region of the Adriatic Sea constitute the widest continental shelf in the Mediterranean Sea (Pinardi et al. 2006), with depth that never exceed 100 m, exception made for the Jabuka/Pomo pits (around 250 m depth). The southern basin is the only portion with bathyal features, reaching a maximum depth of 1260 m (Artegiani et al. 1997).

The most of Adriatic bottoms, about 70% of the sea floor, are soft bottoms, mainly formed by fine and very-fine sediments (Bramanti et al., 1983; Spagnoli et al. 2014). There have been many reports of scattered rocky outcrops off the Italian northern Adriatic shores (Conti et al., 2002) or carbonate concretions in the offshore central and southern basin (Capozzi et al. 2012; Angeletti et al. 2014; Taviani et al. 2015).

The conspicuous amount of fresh water, mainly discharged by the Po River and others western Apennines rivers, makes the basin among the most productive basin of the Mediterranean (Ott, 1992).

The Adriatic basin is one of the Mediterranean areas with the highest invertebrate species richness (approximately 2300 macrobenthic invertebrates species; Ott 1992), seabirds and marine mammals (Coll et al. 2012). It hosts several important and endemic habitats ranging from *Posidonia* and other seagrasses (the Adriatic Sea hosts about 550 species of benthic algae and seagrasses; Ott 1992), coralligenous habitats and bathyal coral habitats (Guidetti et al. 2002; Casellato & Stefanon 2008; Sanfilippo et al. 2013).

The easy access to the Adriatic Sea bottoms, due to shallow and soft bottoms, and the high productivity have favoured from one hand the development of scientific knowledge of benthic biocoenosis and macrobenthic communities (in particular of the Northern basin), and by the other hand, the intense exploitation of the Adriatic Sea, affecting its marine resources and ecosystems (Coll et al. 2007; Coll et al. 2009; Lotze et al. 2011).

The description and distribution of Adriatic macrobenthic communities have been studied from ancient time. The most of historical studies provide qualitative information, with descriptions of benthic communities mainly related to the production of fishing maps of the Adriatic sea floor (Paolucci 1923; Pasquini 1926). The description of the Adriatic bottoms of the first 20th Century reported marine bottoms full of epibenthic organisms (sponges, sea pens, holothurians, echinoderms, hydroids). The central, offshore portion of the Gulf of Venice was almost completely covered by epifauna species, in particular sponges belonging to the genus *Geodia* sponges. The abundance of those specimens was so high that trawling fishery was allowed only in few, small, clean channels (Mancini, 1927). Very similar condition was described for the bottoms off Marche region (from 12 nm off the coast, to a wide portion of the central basin). These bottoms hosted sea stars, brittle stars, crinoids, sea pens, *Alcyonium* species (Paolucci, 1923) and bottom trawling was avoided or very limited there, since the difficulties to haul the fishing nets on board because full of discard. However, the habitat formed by the epifaunal organisms was ideal for both target and no target species (Paolucci, 1923).

The majority of the studies analysing the biocenosis hosted by the basin were carried out at regional or local level (Scaccini and Piccinetti 1967, 1969; Crema et al., 1991). Only few, described exhaustively the biocoenosis of the Adriatic Sea on a larger scale (Vatova 1949; Gamulin-Brida 1974). All eulittoral and sublittoral biocoenosis recognised by Pérès and Picard (1964) have been recorded in the Adriatic Sea (RAC/SPA, 2014).

Sediment typology is one of the main factors influencing the distribution and composition of the soft benthic communities. The differences in the grain size and mineralogy of different bottoms influence the species assemblages and population that could colonised the different Adriatic bottoms (Van Straaten, 1970; Cerrano et al., 1999). Nutrient availability is another important factor influencing the composition and structure of benthic community (Zuschin & Stachowitsch 2009).

Western assemblages, grade in the form of parallel zones, from sandy beaches over subtidal shallow fine sands to the prevailing muddy off-shores bottoms. They were in most of cases dominated by endobenthos (e.g. bivalves, polychaetes), that in some areas such as near the Po delta, could constitute the 90% of the whole community (McKinney 2007). The eastern side showed higher epibenthic suspension-feeders species, with a mosaic pattern of biocoenosis (McKinney 2007).

The sandy and muddy bottoms of the offshore Adriatic may host sponges, seapens, hydroids, ascidians, supporting, thanks to their structural and functional effects (Cerrano et al. 2015), a rich infauna.

The long-term overexploitation of the basin, in particular of bottom trawling fishery, together with natural changes, have been caused the decline of benthonic habitats and target species living on the Adriatic sea bottom (Kollmann & Stachowitsch 2001; Pranovi & Link 2009; Savini & Occhipinti-Ambrogi 2006). The anoxia/hypoxia events occurred on the last decades in particular in the North Adriatic caused benthic mortalities, sometimes resulting in a changing of the community structure (Danovaro et al. 2009). From 2007 to 2011, two strong disease outbreaks were reported from the Conero Promontory at the end of the summer. These events have cause the shift in the benthic assemblages living on hard substrate of the areas affecting primarily cnidarian and sponges with an estimated reduction of in the filtration efficiency of the local benthic community by over 60% (Di Camillo & Cerrano 2015). The reduction of oyster reefs, that in some areas reached a functional extinction, has been recorded in different areas of the Adriatic basin (Venice lagoon, Grado lagoon, Mali Ston Bay) (Beck et al. 2011). The decline of important habitats such as seagrasses meadows (*P. oceanica*, *Zostera noltii*, *Zostera marina*) or target species, such as *C. gallina,* have been described (Romanelli et al. 2009; Anon n.d.).

Recently the Adriatic Sea has been ranked as one of the priority conservation areas of the Mediterranean Sea (Micheli et al. 2013). The degradation of several marine ecosystems and the cumulative impacts affecting the Adriatic basin require a more pervasive and urgent development of the management of marine resources.

1.2 INTERNATIONAL REGULATIONS AND CONSERVATIONS FRAMEWORKS IN THE ADRIATIC SEA

The Adriatic Sea has peculiar and unique physical and biological features (report RAC/SPA), and it is considered as a specific sub-region of the basin. The Adriatic Sea is bordered by six counties: three EU members States (Italy, Slovenia and Croatia), one candidate country (Montenegro) and two potential candidates (Albania and Bosnia-Herzegovina). Each of these countries may adopt or take advantages from different global or regional legal framework or instruments to the conservation and management of the Adriatic marine resources.

The main global legal instruments to the governance, conservation and protection of living marine resources and environment is the United Nations Convention on the Law of the Sea (UNCLOS), adopted on December 1982 and entered into force on November 1994. The UNCLOS measures include the prevention and control of pollution from any sources and the protection of rare, fragile ecosystems and habitats of endangered species and others threatened marine species.

Others global conservation instruments are the ones related to the Biodiversity. The Convention on Biological Diversity (CBD), adopted in 1992 in Rio de Janeiro established as objectives the conservation of biodiversity, also focus the attention in the definition of tools, such as marine protected areas (MPAs) to promote the sustainable use of the biological diversity. The Ramsar Convention on Wetlands aims to preserve and manage the use of wetlands, while the Convention on Migratory Species of Wild Animals (CMS or Bonn Convention), emphasizing the protection of habitats and corridors. An example of Mediterranean agreement born under the CMS is the Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous (ACCOBAMS). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulate the trades, import and export of species, in order to guarantee the survival of threatened terrestrial and marine species.

In addition to the global conventions, there are several regional instruments and agreements particularly devoted to the conservation and management of Mediterranean Sea, and therefore, they could be apply throughout the Adriatic basin.

The first-ever plan adopted by the European members states as a Regional Seas Programme under United Nations Environmental Program (UNEP) was the Mediterranean Action Plan (MAP) in 1975. One year later, the plan was replaced by the Barcelona Convention, which has given rise to seven Protocols each one addressing different aspects of the marine environment conservation. An example is the Protocol for the Specially Protected Areas and Biological Diversity in the Mediterranean, which resulted in the establishment of a list of Specially Protected Areas of Mediterranean Interest (SPAMI List). The list include sites with habitats, ecosystems or species that are important or endangered for the conservation of the diversity of Mediterranean Sea. In Italy 10 sites have been included in the SPAMI list, and only two, the Miramare MPA and Torre Guaceto MPA and natural reserve from the Adriatic basin (http://www.rac-spa.org/spami).

The Natura 2000 network, developed under the Habitats Directive (92/43/EEC), aims to assure the most threatened species and habitats of the Mediterranean basin. It has been adopted by EU member states, and it includes thousands of areas, most of time terrestrial. Inside the Adriatic Sea the Natura 2000 sites are strictly linked to coastal areas (within the first 10 nautical miles from land). The Natura 2000 sites include the so call Site of Community Importance (SICs). Several SICs have been identified throughout the Adriatic basin, mainly overlapping maerl beds, coralligenous and/or phanerogames distribution. SICs sites have been adopted by the European Community, but not yet formally designed by the government of each countries. The eastern side of the Adriatic Sea, in particular along the coastlines of non EU member states, the Natura 2000 sites are replaced by the EMERALD network. This network works under the Berna Convention and its main aim is the conservation and protection of wild flora and fauna and their habitats.

The Water Framework Directive (WFD, 2000/60/EC) which aims was to classify and monitoring the ecological status of water bodies including transitional and coastal waters, should had promote actions for getting all the European waters into good by 2015 (http://ec.europa.eu/environment/water/water-framework/info/intro_en.htm) .

Finally, the Marine Strategy Framework Directive (MSFD) represents the first legislative instruments that EU members states have to protect marine biodiversity and to reach a Good Environmental Status (GES) of marine waters by 2020.

The majority of the cited global and regional instruments may be applied to the protection and conservation of biological diversity. However, there is no an international governance framework for regulating or coordinating tools, such as MPAs in high sea.

1.3 FSHERY REGUALTION IN THE ADRIATIC SEA

The General Fisheries Commission for the Mediterranean (GFCM) is the regional fishery management organization (RFMO) with competence on the Mediterranean and Black Sea.

The Common Fishery Policy (CFP) is the main regulations adopted by European States with or without Mediterranean coasts regards fishery management (Regulation (EC) n°2371/2002). The CFP has particular attention not only to the recovery and sustainable exploitations of target species, but also to the conservation of habitats, and vulnerable marine ecosystems. The CFP sets up the total allowable catches, technical measures and limitation of fishing efforts to prevent the collapse of marine resources. The prohibition of some fishing gears in particular habitats (such as maerl, coralligenous, seagrasses meadows) is an example of the intent to preserve habitats and not only the species of the Mediterranean Sea.

The GFCM is one of the few RFMOs entitled to adopt spatial management measures, including the reduction or banning of human activities even in high seas (http://www.fao.org/gfcm/en/). Examples are the decision to prohibit trawls activities at depth bigger than 1000 m, or the establishment of Fishery Restricted Areas (FRA) in Mediterranean waters.

In general, the current trawl fisheries management in Mediterranean Sea is based on technical measures (e.g. regulation of mesh size; (Colloca et al. 2013)), spatial closures (trawl banning within 3 nautical miles, or <50 m depth and at depth bigger than 1000 m) or temporal closures varying from countries (Demestre et al. 2008; Martín et al. 2014) and control of fishing capacity (Demestre et al. 2008; Colloca et al. 2013).

In the Adriatic Sea trawling fishery is managed using all the above-mentioned tools. Temporal closures include the prohibition of fisheries activities of 43 consecutive days in a period that goes from 26th of July to 6th of September in the norther basin and between 16th of August and 27th of September for fleets from Pesaro to Bari (DECRET 3rd July 2015). Technical closures contemplate the stop of all trawlers during Saturdays, Sundays and public holidays. The technical measures such as the selectivity of trawl nets (mesh sizes) or minimum length of landed species follow the European regulations.

In the Adriatic Sea there are different, small areas permanently closed to trawl fishery and they are: the Tremiti Islands, Tenue areas, Miramare, the area off Ravenna, around the Barbare platform and a small are off the Apulia region. A bigger area (of about 2000 km^2) where there has been established a one year closure for bottom trawling is the Pomo pits area (DECRET 3rd July 2015) (fig.1).

Despite the listed strategies, the management of the Adriatic fishery, in particular of trawls activities has not produce stable results in terms of sustainability and stock recovery. The reduction of the fishing capacity, together with the fishing effort, the temporary suspension of fishing activities and the reduction of days at sea, should be implemented with more efficient conservation tools (Fouzai et al. 2012).

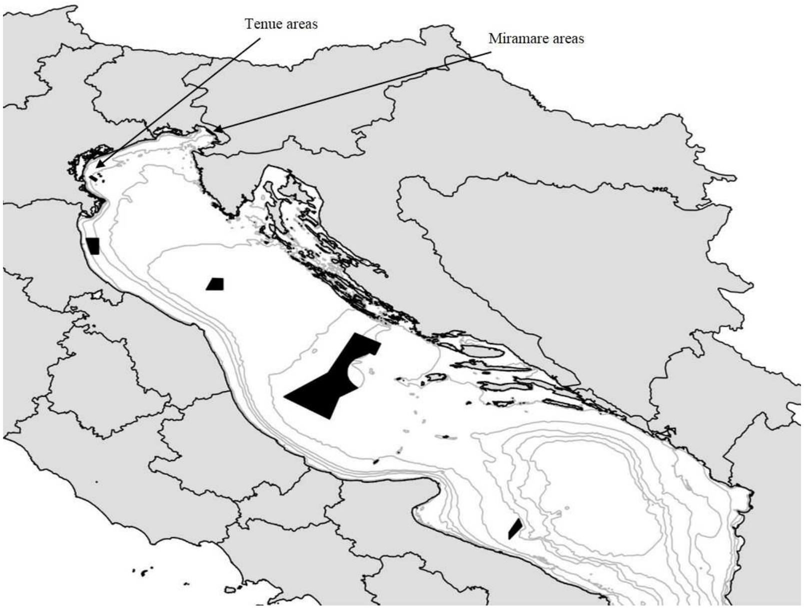

Fig.1: Adriatic no-trawl areas. The black polygons define the boundaries of the different Italian areas permanently closed to trawl fishery.

1.4 MAIN GAPS FOR THE CONSERVATION OF ADRIATIC MARINE BENTHIC HABITATS

The declining trends of the Adriatic marine ecosystems cause a reduction in the quality, in the functioning and in the human uses of the marine resources. The central role played by benthic communities, in particular of soft bottoms communities, in the ecology of the basin is increasingly recognised (Trush & Dayton, 2002.; Lohrer et al. 2004). Epibenthic mega and macro specimens may increase biodiversity, providing refuge for small invertebrate and fishing, or providing substrate to others sessile organisms. *Atrina fragilis* shells, for example, may protrude through sandy bottoms up to 10 cm. Its shells may be used as hard bottoms by others organisms (e.g. pectinids), favouring their settlements (Hall-Spencer et al. 1999). Filter feeding communities are involved in benthic-pelagic coupling, in nutrient regeneration, in the control of phytoplancton community, influencing the primary production (Norkko et al. 2006; Sandwell et al. 2009). Despite the primary role of soft bottoms community of the Adriatic Sea, and the increasing interest and attention in establishing ecosystem-based management plans, a high portion of benthic habitats remain poorly known. In recent years, there has been a great effort in produce geo-referenced information important and sensitive habitats of the Adriatic basin (Casellato and Stefanon, 2008; Biomap project; Mediterranean Sensitive Habitats (MEDISEH), 2013). However, the most of time only shallow environments are considered, with poor description of the deepest areas of the Adriatic Sea.

Moreover, 84% of European stocks are still overexploited (Colloca et al. 2013) highlighting the lack of efficacy in fishery management in European waters. However, no standard procedures to identify and protect important fish habitats have been implemented in European waters yet.

In the USA, at the contrary, where the experience in protection of fish habitats is different, there have been identify fragile habitats based on the sustainability of fishery resources (Report EFH). In particular, criteria to discriminate Vulnerable Marine Ecosystems (VMEs) and Essential Fish Habitats (EFHs) have been produced. VMEs are habitats both easily disturbed and very slow to recover, that may be physically or functionally fragile, such as deep-water corals, sponges, seamounts, chemosynthetic communities (FAO, 2009). EFHs include biological and ecological habitats (both "waters and substrate" habitats) essential to manage the critical life history stages of commercial species (such as nursery and spawning areas, foraging areas) which require protection to guarantee stocks and their long-term sustainability (Benaka, 1999).

The Adriatic basin hosts habitats that fall in the definition of both EFHs and VMEs. Increasing effort to determine the spatial distribution of these habitats and to define shared management plans of the human activities (in particular of trawling fisheries) should be a priority for the Adriatic bordering states.

Towed gears caused a variety of disturbing and destructive effects on soft bottoms, species and habitats (see Table 1 for a summary). However, few studies have been focused on Adriatic bottoms and there are still very scant information about how and in which time the recovery of soft bottoms community happens (Collie et al. 1997; Kaiser et al. 2001; Collie et al. 2009). Recovery and restoration rates of soft bottoms depend on the growth rate of the community living in the sea floor (Collie et al., 2000; Kiser.et al.,2001). Typical return time of benthic species after trawling disturb may vary from 40 days to 10 years, while the recovery of the community to a pristine or original status may occurred in years or centuries (Watling & Norse 1998).

Increasing effort should be employ in studying the effects that intense impacts, cause on the structure of the Adriatic soft bottoms communities.

Tab.1: Summary of direct and indirect effects caused by bottom trawling

DIRECT EFFECTS	EXAMINED GEARS	LOCATION	REFERENCE
By catch of demersal species and no-target species	Trawls and dredges Rapido	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; Mediterranean (Adriatic Sea)	Dayton.etal.1995; Jennings.Kaiser.1998; Hall-Spencer.etal.1999
Physical damage of benthic species (both epiauna and infauna)	Trawls and dredges Rapido	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; New Zeland; Australia; Georges Bank; Mediterranean (Adriatic Sea)	Dayton.etal.1995; Jennings.Kaiser.1998; Jones.1992; Trush.Dayton.1992; Collie.etal.2000; Hall-Spencer.etal.1999
Physical damage and destruction of habitats	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; Eastern Florida; Mediterranean (Iberic Peninsula)	Dayton.etal.1995; Jennings.Kaiser.1998; Kaiser.etal.2001; Reed.etal.1995; Trush.Dayton.1992; Bordehore.etal.2003;
Reduction in species diversity	Trawls and dredges	Baltic Sea; WaddenSea; North Sea; Irish Sea; Mediterranean (Adriatic Sea)	Jennings.Kaiser.1998; Trush.Dayton.1992; Barausse.etal.2011
Changes in abundance and biomass (both of target and no-target species)	Trawls and dredges	Eastern Florida; Georges Bank; Mediterranean	Reed.etal.1995; Trush.Dayton.1992; Collie.etal.2000; Demestre.etal.2008; Jukic-Peladic.etal.2001
Physical disturbance of substratum (e.g. trawl marks; sediment resuspension)	Trawls and dredges Rapido	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; New Zeland; Australia; Georges Bank; Mediterranean (Adriatic Sea)	Dayton.etal.1995; Jennings.Kaiser.1998; Jones.1992; Kaiser.etal.2001; Collie.etal.2000; Hall-Spencer.etal.1999

INDIRECT EFFECTS	EXAMINED GEARS	LOCATION	REFERENCE
Increase turbity	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea;	Dayton.etal.1995; Jennings.Kaiser.1998
Increase in anoxic conditions	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; New Zeland; Australia	Jones.1992
Alteration of sediment type	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska	Dayton.etal.1995
Alteration in the functional groups (shift from long-lived suspension-feeding organisms to community dominated by detritus feeders)	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; New Zeland; Australia; Mediterranean (Adriatic Sea)	Dayton.etal.1995; Jones.1992; Barausse.etal.2011
Alteration in the comunity structure	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; North Sea	Dayton.etal.1995; Jennings.Kaiser.1998; Trush.Dayton.1992; Frid.etal.2000
Alteration in the food-webs	Trawls and dredges	Southeast Asia; North Sea; Bering Sea; Gulf of Alaska; Baltic Sea; WaddenSea; Mediterranean (Sicily)	Dayton.etal.1995; Jennings.Kaiser.1998; Kaiser.etal.2001; Badalamenti.etal.2002

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2. AIMS OF THE STUDY

The Adriatic basin is a priority area in need of conservation, with its high biodiversity, high productivity and overexploitation that ranked the basin as one of the most exploited sectors of the Mediterranean Sea. Despite the Adriatic Sea is one of the most studied basin in the world and the great amount of scientific literature describing its macrobenthic communities, there are still gaps in the knowledge of deepest areas. Scant are studies describing the historical changes occurred on macrobenthos, non-target specimens living on the soft, offshore bottoms of the Adriatic Sea and almost unexplored are studies considering the ecological effect related to their changes. Because the crucial ecological role played by the soft bottoms macrobenthic communities and because the current management of human activities have not taken into account those fundamental component, we think that there is a urgent need to highlight the importance of benthic soft bottoms assemblages and of their conservation.

In particular, the main aims of this PhD thesis are

1) to describe the composition and the distribution of macrobenthic communities of the Adriatic Sea in the last 70 years;

2) to identify and map some Vulnerable Marine Ecosystems (VMEs) and Essential Fish Habitats (EFHs) still presents in the off shore sandy-muddy bottoms of the overexploited Adriatic basin;

3) to present a rationale for establishing a transboundary Large Marine Protected Area (LMPA) – specifically a no-trawl area – in the Adriatic Sea for the recovery of damaged EFHs and VMEs, as a possible solution to trigger fish stocks increase.

CHAPTER 1

Trends and implications of 60-years of changes of the Adriatic macrobenthic communities.

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Keywords: Adriatic; macrobenthic community; trawling; changes

Abstract

The Adriatic Sea is one of the most depleted regions of the Mediterranean Sea due to a cumulative impact of multiple stressors, such as climate change and a long history of intense exploitation. Severe declines of target and by-catch species call for urgent conservation measures.

In this paper, we highlight changes on macrobenthic communities of the Adriatic Sea. We analysed data from scientific surveys over the period 1934 – 1998 to assess changes in the structure and abundance of benthic communities. Overall change are the decline of epifauna organisms and of the most fragile macrobenthic species. We show that sponges (Porifera) and Echinoderms (Echinodermata) declined by 90 – 70% since the 1900s.

The decrease of the distribution of macrobenthic community can lead to alteration of food-webs, reduction of water quality, or nutrient cycling. An efficient ecosystem-based management focusing on the recovery of key benthic habitats is needed to promote recovery of stocks and to develop a sustainable exploitation of marine resources.

1. Introduction

Historical studies are fundamental to understand long-term changes occurring in marine ecosystems. Spatial and temporal ecosystem dynamics must be considered to define management actions aiming an effective recovery and restoration of marine resources (Boero 2014). Studies of temporal and spatial changes of Adriatic macrobenthic communities are rare (Lotze et al. 2011). Due to a lack of data on the status of pristine macrobenthic communities, define a baseline of macrobenthic species of the Adriatic Sea is complicate. The fisheries literature is rich in cases describing the intergenerational loss of information that afflicts scientists about what is considered natural for an ecosystem under study. This is called "the shifting baseline syndrome" (Pauly 1995). This syndrome is expected to be greater for non-target species and benthic communities because of the lower attention and interest by marine users on those components of the marine environment. Thus, there is the need to increase the knowledge on macrobenthic communities and the urgency to understand the potential to re-establish a pristine and efficient ecosystem-functioning community as recommended by the Marine Strategy EU Directive.

The Adriatic Sea hosts a variety of endemism (Lotze et al. 2011; Coll et al. 2012), of vulnerable marine ecosystems and essential fish habitats, i.e. areas used by fishes as nursery, spawning ground or foraging (de Juan & Lleonart 2010; Colloca et al. 2015). The Adriatic Sea is affected by historical and cumulative impacts of anthropogenic and environmental-mediated stressors (Coll et al. 2009; Lotze et al. 2011; Giani et al. 2012; Zenetos et al. 2011), acting at different spatial-scale. In the late 1980s, for examples, a change in the main circulation patterns, salinity and nutrient budget caused an increase in mucilage events, red tides and qualitative changes of phytoplankton and zooplankton, with important consequences even in the higher trophic levels (such as anchovy stocks decline) (Danovaro et al. 2009; Conversi et al. 2010). Trends occurring in the Mediterranean Sea or a global scale, such as the increase of non-indigenous and tropical species due to temperature rise, species extinction or transition (e.g. cold-waters species overcome by warm-waters affinity species, or jellyfish blooms) have been described through the Adriatic Sea (Boero 2014). Episodic effects such as mass mortalities of benthic macrofauna have been reported in the Adriatic Sea in association with hypoxia/anoxia events in the 1970s – 1980s, and then no more reported in the last decades at basin scale (Giani et al. 2012). However, Adriatic mass mortalities events have continued to occur at local scale such reported for the Conero Promontory (Ancona, Italy) with consequent shift in community composition and alteration of the function of the marine environment (Di Camillo & Cerrano 2015).

The Adriatic Sea is one of the most exploited Italian basin by trawling fishery and more than 90% of its marine resources are depleted (Lotze et al. 2011). The easy access of Adriatic bottoms and its high productivity are the main reasons of the overexploitation of the basin. Overfishing is one of the main human driven impacts that affects Adriatic marine resources (Coll et al. 2010; Lotze et al. 2011; Ferretti et al. 2013) and prevent ecosystem recovery. An example comes from the decline of clam populations occurred in the 1980s in the Gulf of Trieste (where *C. gallina* in the first half of 1970s showed biomasses of 370 g/m^2) whose recovery was hampered by anoxia events and intense dredging (Kollmann & Stachowitsch 2001). In the northern Adriatic, *rapido* trawlers (a specific Adriatic fishing gear, mainly used to catch sole and scallops) can sweep the available fishing grounds up to eight times per year (Raicevich et al., 2004). In practice, soft bottoms communities are continuously subjected to fishing disturbance. Although trawl vessels are the 22% of total number of vessels composing the Adriatic fishing fleet, the trawl fleet is the most important in terms of fishing capacity. It produces 60% of the total tonnage of Adriatic fishing (Piano di Gestione, GSA17). In the North Adriatic Sea, benthic invertebrates contribute for the 67% of the total catch composition, while small pelagic fish represent the 25%. The incidence of discarding of total catch is around 26%. These values are indicative of very high probability of low sustainability of the Adriatic fishing activities (Pranovi & Link 2009).

The increasing effort of trawl fishing contributed to dramatic changes of Adriatic macrobenthic communities. In the Adriatic Sea, there has been a shift from heterogeneous communities characterised by the presence of rich, structuring, filter-feeding epifauna organisms (such as sponges, seapens, ascidians, holoturians, large bryozoa) to a community dominated by infauna and scavengers (Pranovi et al. 2003; Lotze et al. 2011). Experiments on the effect of *rapido* trawl showed that almost 50% of epifaunal organisms and debris were removed after the trawl activity in the northern Adriatic basin (Pranovi et al. 2000). Macrobenthic changes, both in species richness and density, of the Adriatic communities along the shallower waters of the western coasts, have been reported in relation to the intensity of hydraulic dredges fishery (Morello et al. 2005). Changes of the community structure of soft bottoms (that include biodiversity loss, modification in species composition) could result in alteration of food-webs, reduction of water quality, or nutrient cycling (Coll et al. 2009; Sandwell et al. 2009; Lotze et al. 2011). To prevent such alteration in the marine ecosystem functioning the recovery of impacted populations is fundamental. However, recovery rate have been poorly studied, and it may varying between <1 to more than 10 years, depending on the group, bottoms characteristics (hard or soft bottoms) and degree of disturb (number, time and intensity of fishing activities in a same fishing ground) (Kaiser et al. 2001; Lambert et al. 2014).

Currently, the Adriatic has been ranked as one of the most threatened basins of the Mediterranean Sea needing urgent priority conservation actions (Micheli et al. 2013). Several international conventions and directives (Marine Strategy Framework Directive (MSFD), Barcelona Convention) have been implemented in order to develop an ecosystem-base management (EBM) approach and contribute to define a long-term sustainable management of human activities, recovery of stocks and marine services. So far, management of trawl fisheries in the Adriatic (e.g. the reduction of the fishing capacity, together with the fishing effort, temporary suspension of fishing activities, reduction of days at sea) has been ineffective in ensuring sustainability or promote stock recovery (Fouzai et al. 2012; UNEP-MAP-RAC/SPA, 2014.).

Our work analyse and compare the spatio-temporal changes of Adriatic macrobenthic communities from 1940s, the period between the two World Wars when fishing pressure was moderate, to more recent years (end of 1990s), when fishing impacts reach its maximum development. Our aim was to define areas in which changes occurred with higher magnitude and discuss the effects on Adriatic ecosystem functioning of these structural modifications.

2. **Materials and methods**

We assembled a database of surveys targeted on benthic macrofauna over a period of 60 years since the 1934. Data differed in spatial and temporal resolutions, and sampling methods. Below we describe the datasets used.

VATOVA SURVEY:

Data comes from four cruises performed between 1934 and 1936 in the North and Central Adriatic Sea (fig.1). Samples were collected using a Petersen grab of 0.1 m^2 over different sediment type. In hard bottoms, grab penetration was more difficult and thus sometimes more replicas were collected in the same station to have a sufficient number of animals to analyse. During the four cruises, 417 samples were collected, in the North and Central basin, and 592 in the area off Rovinj (fig.1). The total sampled surface was only 180 m^2 of the whole Adriatic Sea surface (132000 km^2). However, the Vatova survey is one of the most extensive and quantitative available dataset for Adriatic macrobenthic communities of the first decades of the 1900. All the sampled specimens were classified at the lower taxonomical level, counted and weighted. In the original publication, species density in each sampling operation was reported as number organisms per m^2. We converted these indices in the total number of organism expected in each sample by multiplying the density by the sample surface, (which were 0.2 – 0.1 m^2). All sampling stations were geo-referenced by using QGISS 2.4.0 and their latitude, longitude and species composition were recorded. Sample depth (in meter) and sediment type were then associated by merging our dataset with the EMODnet Bathymetry dataset (http://www.emodnet.eu/bathymetry) relative to the Adriatic Sea and the ADR_ONE dataset (http://instaar.colorado.edu/~jenkinsc/dbseabed/coverage/adriaticsea/adriatico.htm) with information on sediment composition of the Adriatic Sea. Taxonomic classification of each species was checked in accordance with World Register of Marine Species (WoRMS). Species with old, no more valid names were renamed following the current accepted scientific classification. For each species, relative phylum and class was added.

PIPETA SURVEYS:

The available data from Pipeta trawl surveys were carried out during 1982 along 10 transect with a total number of 58 to 60 fixed stations from the North Adriatic up to the Gargano promontory (fig.1). Sampling were made during day and night, and during spring and winter, from 24/04/1982 to 16/12/1982 over different sediment types. They were carried out by using a commercial vessel ("Pipeta") which was equipped with a standard Italian trawl bottom net (otter trawl net) with 40 mm square mesh size on cod-end (Piccinetti, 1972) (fig.1). The technical features of the otter trawl and sampling methods (e.g. mesh size, door opening and vessel speed) were maintained constant during all sampling activities. Hauls duration was mainly one hour and the swept area (in km^2) was calculated by multiplying the estimated net horizontal opening by the distance trawled. For each station, catch was expressed as number of specimens per hour (number h^{-1}), and as biomass per square kilometre (g km^{-2}). In order to compare the data from the Pipeta surveys to the data of the others surveys, the number of specimens per hour were converted in number per km^2 or number per m^2 (number km^{-2}; number m^{-2}). Sample depth (in meter) and sediment type were then associated by merging our dataset with the previous cited databases (ADR_ONE and EMODnet Bathymetry). A taxonomical check in accordance with current WoRMS classification was performed also for Pipeta species list.

PRISMA2:

PRISMA2 was an Italian Research Project during which macrozoobenthic datasets were collected. Sampling activities were carried out during summer (June to August) in 1996 – 1998, by means of a Van Veen grab (0.1 m^2 opening). Samples were collected along 19 transects (A-R) perpendicular to the coast, in a depth range depth between 5 – 61 m (fig.1). For each stations, latitude, longitude, depth (in meter), species name and number of individuals per m^2 were reported. The class and phylum of each described species were added in the datasets. Sediment type were then associated by merging our dataset with the previous cited database (ADR_ONE). A taxonomical check in accordance with current WoRMS classification was performed also for PRISMA2 species list.

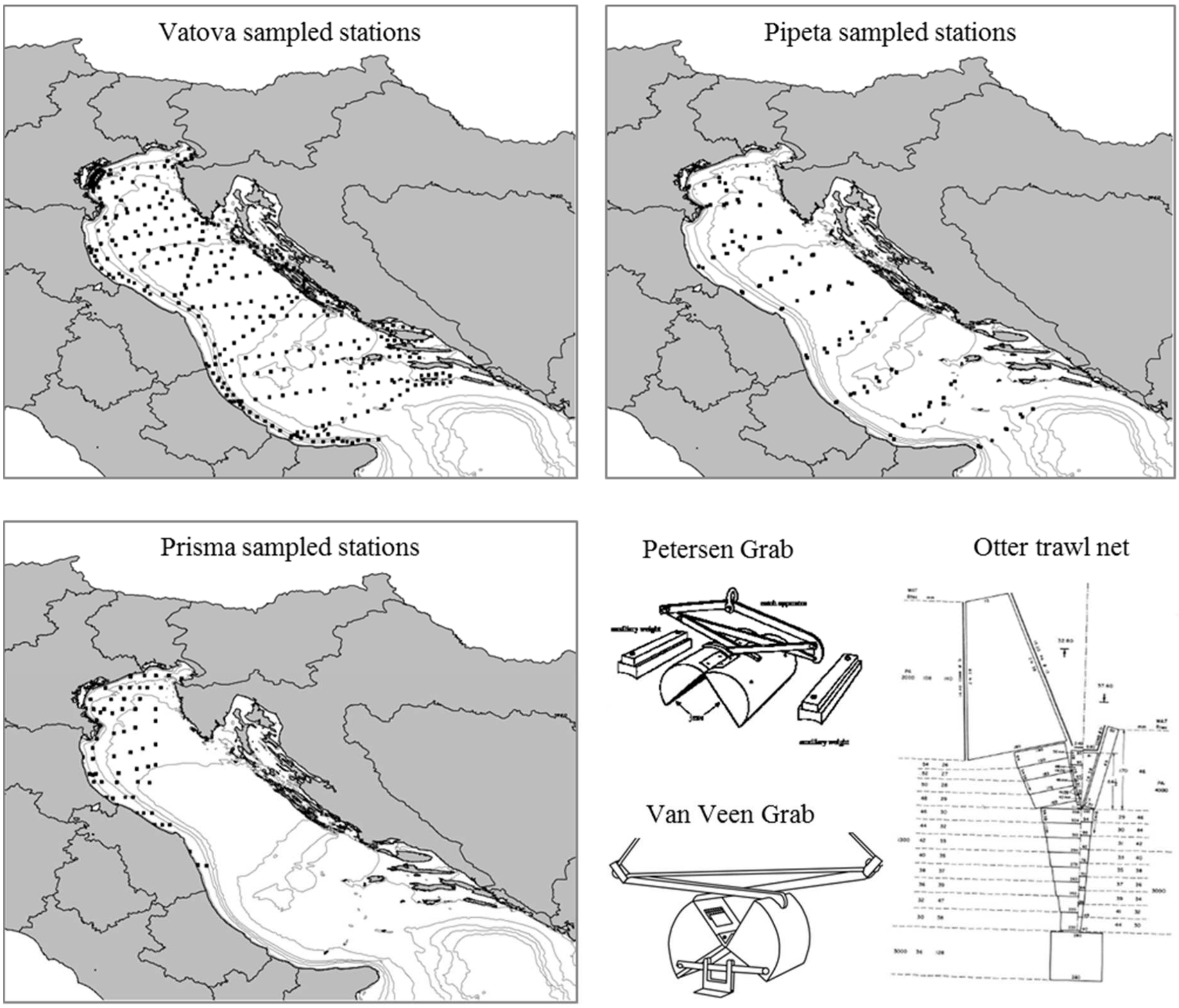

Fig.1: Maps of the sampled stations and of the sampling gears of the three analysed Adriatic surveys. The black squares in the maps shows the position of the sampled stations during the different surveys. In the bottom left, the different sampled gears used during the three studied surveys.

DATA ANALYSIS:

Different sampling tools can provide different data from the same area. To reduced bias generated by the different sampling gears (trawl vs. grab) Pipeta data were modified with a conversion factor following Jorgensen et al., 2011. In that paper the Authors, comparing samples collected by grab and trawls, showed that “the mean density (individual m^{-2}) of individuals sampled by grab was close to 400 times higher than individuals collected by trawls”. However, the analysis on the class Bivalvia were performed only using the Vatova and Prisma2 datasets. Bivalves are infauna organisms, and samples collected by trawls net can remain greatly underestimated respect those of dredges (not only because trawl net can not sink the soft sediment as dredges did, but also because the net meshes size (40 mm) prevent the collection of smaller bivalves species).

The Adriatic surface covered by the sampled stations was different for each survey (fig.1). To make the results of our data analysis more comparable, we identify each time the minimum convex polygon describing the maximum surface covered by the sampled stations of all the surveys (this means that the analysis were performed only for intersecting areas).

The most of the analysis have been performed at phyla or classes levels, because species were not always available for the oldest dataset. Only two examples at species level have been reported.

In Vatova, Polychaets were excluded from the analysis because it was not possible quantifying their abundances. In the majority of samples, Polychaets were recorded only as fragments. Moreover, the stations sampled in hard bottoms were not included in our database, because the impossibility to know a correct conversion factor to calculate species density.

The Prisma2 dataset that was available for our study was incomplete. Data belongs exclusively to the phyla Mollusca and Echinodermata. Moreover, the sampled station K and N were not available.

The alteration of the species composition of the macrobenthic communities influence the functional role of soft bottoms. Where species level information was available (e.g. for species belongs to the phylum Echinodermata) a description of the changing in species composition were reported. Queirós et al., 2013 provided a functional classification for more than 1000 benthic invertebrate species as tool to estimate the community bioturbation potential (BPc). Followed their classification, we assigned to each species found in the Adriatic Sea surveys a sediment reworking trait, that is a key component of bioturbation trait.

To model the indices of abundance of the three surveys controlling for environmental and sampling covariates we used Generalized linear (GLMs) and generalized additive models (GAMs). We assumed that the number of individual collected in each samples followed a Poisson, quasi-Poisson and negative binomial distribution, and was related linearly to sampling and environmental covariates by a log link function. In GAMs the linear terms of the linear predictors can be not linear functions (smooth functions) of the covariates. Model selection was performed with a backward stepwise selection procedure. We initially specified a saturated model which was used to select the best statistical distribution, and then we retained important covariates according their significance with a likelihood ratio test. Non-significant variables were deleted until it was no longer possible to reduce the Akaike Information Criterion (AIC). All analysis were performed by using the statistical software R (3.2.0) (https://www.r-project.org/).

3. Results

Sponges showed a clear decline. We could compare only the Vatova and Pipeta surveys as the data for sponges of the Prisma2 survey were not accessible to us. Sponge density was much higher in Vatova than in most of the Pipeta stations. Standardized densities reflected the nominal indices of abundance. The best models showed a general declining trends of sponges density throughout the basin from the 1940s to the first years of 1980s. Sponge abundance decreased of 90 % in the majority of the Adriatic Sea. There was a clear west-east gradient in abundance (fig.2).

Sea cucumbers (Holothuroidea) showed a general declining trend (50 – 95% of decrease) throughout the basin. However, in some areas, such as along the western sides of Adriatic along the Emilia-Romagna and Marche regions, they persist with high abundances (fig.2). In Vatova dataset holothuroids were present in the 28% of sampled stations. The species found most frequently during the survey was *Oestergrenia digitata* (Montagu, 1815). It has recorded in the 60% of the stations with holothuroids, with a mean density of about 9 individuals m^{-2} (and a maximum value of 25 individuals m^{-2}). It was followed by *Leptopentacta elongata* (Düben & Koren, 1846) and *Thyone fusus* (O.F. Müller, 1776) both found in the 16% of the sampled station with sea cucumbers (with a maximum of 40 individuals m^{-2} and 5 individuals m^{-2} respectively). *Holothuria* (*Panningothuria*) *forskali* Delle Chiaje, 1823 was recorded in the 9% of the case with mean densities of about 5 individuals m^{-2} and the remnant species were quite rare, observed only in the 3% – 1% of the stations where holothuroids were collected. In Pipeta survey sea cucumbers were collected in the 85% of sampled stations. The most common species was *Ocnus planci* (Brandt, 1835) found about in the 87% of the case, followed by *Parastichopus regalis* (Cuvier, 1817) (recorder in the 45% of station with holothuroids) with density that, for both species, never exceeded 5 individual m^{-2}. *H.* (*P.*) *forskali* was found in the 44% of station where sea cucumbers were collected, with mean abundances of about 5 individuals m^{-2}. Finally, in Prisma2 survey, holothuroids were present in the 41% of sampled stations. *O. digitata* was the most frequent species (found in the 41% of the station with sea cucumbers), that could reach high local density (up to about 500 individuals m^{-2}). It is followed by *L. elongata* (M. Sars, 1857), *Thyone inermis* Heller, 1868 and *Leptopentacta tergestina* (M. Sars, 1857) with mean abundances of about 2 individuals m^{-2} (found in the 37% 33% and 15% of sampled stations with holothuroids, respectively) and then followed by more rare species. Most of the sea cucumbers we analysed are deposit feeders. Only the species belongs to the order Dendrochirotida are suspension feeders (Roberts et al., 2003).

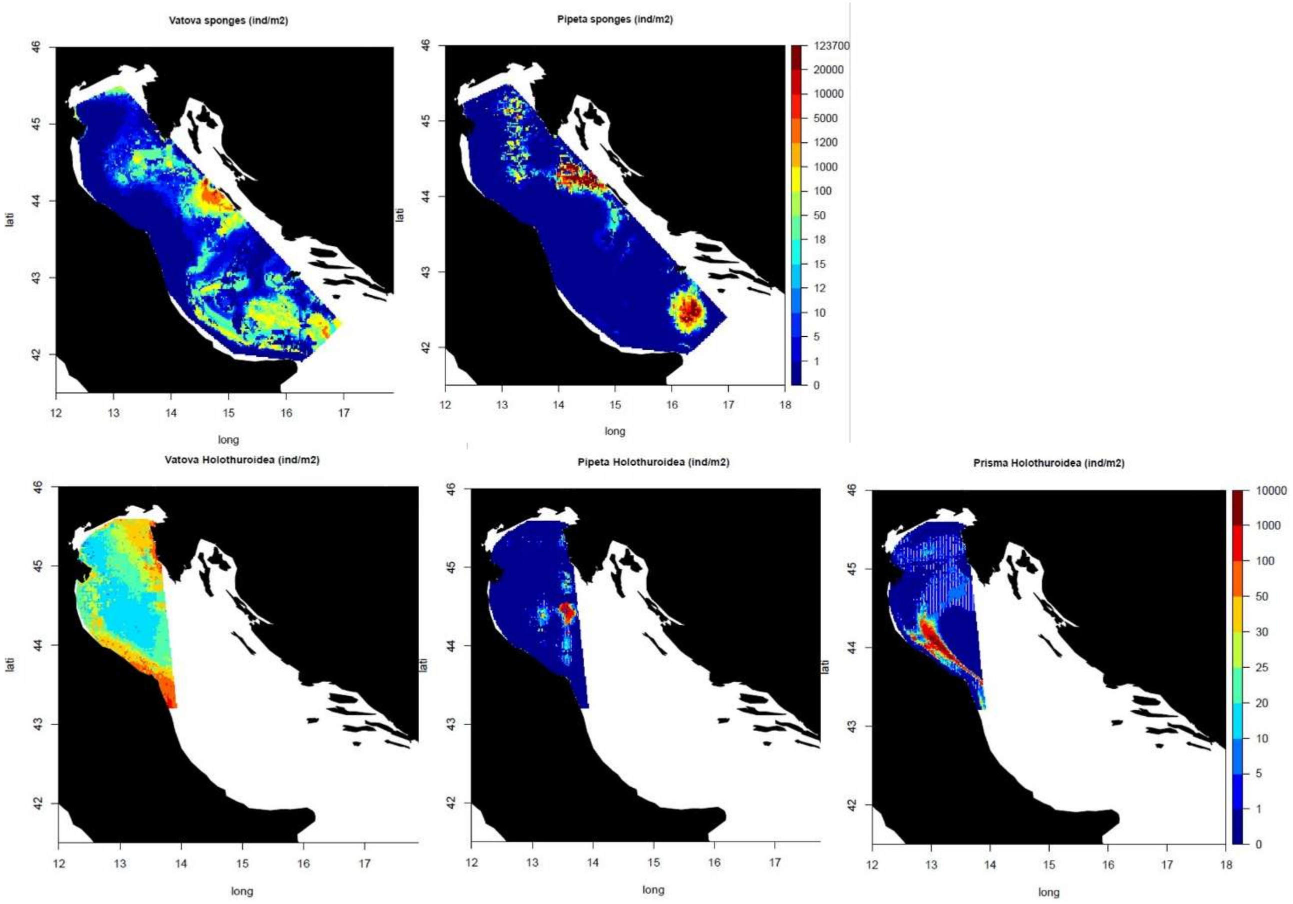

Fig. 2: Maps showing the abundances (individuals m^{-2}) of different phyla. Top: Estimated abundances of sponges in Vatova and Pipeta surveys. Bottom: Estimated abundances of holothuroids in Vatova, Pipeta and Prisma2 surveys.

The class Echinoidea holds genera (*Echinocardium*, *Ova*) which functional importance in soft bottoms is increasingly recognised (Lohrer et al. 2004; Queirós et al. 2013). The frequency and the species composition of echinoids living in Adriatic soft bottoms have changed during the analysed period. In particular, in Vatova samples, the species *Ova canaliferus* (Lamarck, 1816) was the most frequent and abundant sea urchin (with a maximum of around 20 individual m^{-2}), forming in the North Adriatic a specific biocoenosis called by the author the '*Schizaster canaliferus* zoocenosis'. The other echinoids species, in order of decreasing frequency were *Echinocardium mediterraneum* (Forbes, 1844), *Psammechinus microtuberculatus* (Blainville, 1825), and *Echinocyamus pusillus* (O.F. Müller, 1776) with density that reached a maximum of around 15 – 10 individual m^{-2} for each species. The most frequent species found during Pipeta survey were *Gracilechinus acutus* (Lamarck, 1816) followed by *Cidaris cidaris, Echinus melo, P. microtuberculatus* with maximum density of around 5.8, 1, 0.2 and 5 individual m^{-2} respectively. All the other species were found with less frequency. Finally, in Prsima2 the most common echinoids species was *O. canaliferus* followed by *Echinocardium cordatum* (Pennant, 1777), with maximum density of 4 and 36 individual m^{-2}. Fig.3 summarised the frequency of Holothuroidea and Echinoidea species found in the analysed surveys and their respective reworking traits.

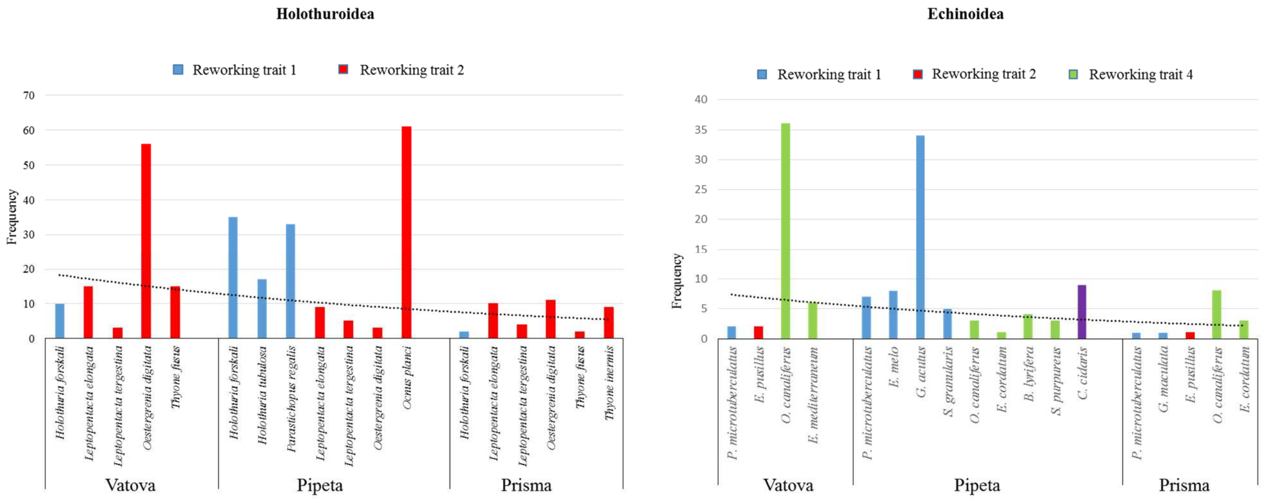

Fig.3: Most common species of Holoturoidea and Echinoidea found in the Adriatic basin in the three different analysed surveys. The frequency indicate the times that each species was found during the sampling activities. The reworking rate give an idea of the ecological importance of the different species (in relation to their bioturbation activities) following the classification suggested by Queiros et al.,2013.

The mean distribution pattern of the Bivalvia class over the analysed 60 years remain similar, with coastal areas hosting the highest bivalve abundances. However, different species followed different trends (fig.4). For example, considering the mean density of the *Chamelea gallina* (Linnaeus, 1758), that is a very important commercial specie, living mainly on Adriatic sandy bottoms from 3 to 12 m depth, it is evident a reduction of its stocks (fig.4). *C. gallina* is a suspension feeders and reaches full maturity when shell length is around 20 – 25 mm and about two years old (Romanelli et al. 2009). Contrariwise, the specie *Corbula gibba* (Olivi, 1792) increased its spatial distribution and abundances in the majority of the basin (fig.4). *C. gibba* inhabits sandy-muddy sediments mixed with gravel and/or shell fragments that it used for byssus attachment. It is a dominant suspension-feeder specie, with rapid growth rate and it is tolerant of a wide range of stress (both natural and anthropogenic disturbances) such as pollution, low oxygen condition and increase of turbidity (Hrs-Brenko, 2006).

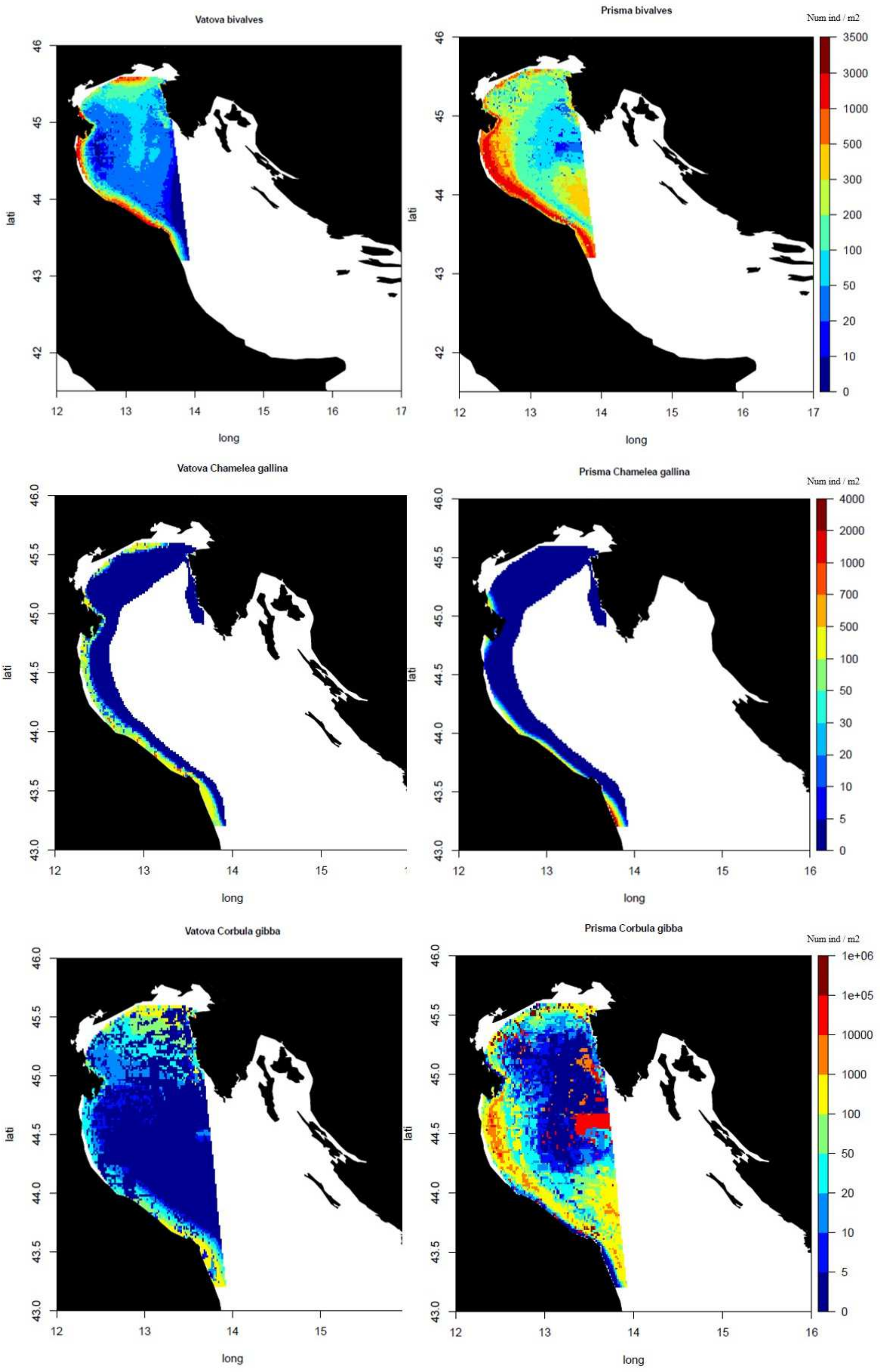

Fig.4: Top: Maps showing the estimated abundances (individuals m^{-2}) of the class Bivalvia; middle and bottom: Estimated abundances of *C. gallina* and *C. gibba* from Vatova and Prisma2 surveys respectively.

4. Discussion

This work highlights the changes occurred on macrobenthic communities in the north and central Adriatic Sea, during 60 years (1934 – 1998). The results obtained by the analyses of different Adriatic surveys suggest that the macrobenthos population of the Adriatic basin follow the same negative trend of stock species and top predators already described by other authors (Froglia 2000; Romanelli et al. 2009; Barausse etal., 2011; Lotze et al. 2011; Ferretti et al. 2013).

According to previous studies did in the northern basin (Raicevich et al., 2004), suspension feeders organisms, such as sponges, are one of the trophic groups that suffered the steepest declines throughout the basin. Our results highlight the higher decrease of sponge abundances in the whole north and central Adriatic basin respect others organisms. The genera *Geodia,* one of the most common, abundant and big sponges of muddy and sandy bottoms of the Adriatic Sea before the onset of industrial fishing, have almost disappeared (personal communication). In the Adriatic Sea, sponges were targeted by a commercial fishery bath-sponges (Pronzato & Manconi 2008), which contributed to deplete the population. This decline likely determined a reduction of ecosystem services they provided. Sponges affect water quality, nutrient cycling (carbon and nitrogen cycles), and modify the primary production They can control diseases by removing organic matter, organic particles and bacteria from water column (Peterson et al. 2006; Bell 2008). The sever depletion of suspension-feeders in Florida Bay caused the persistent and widespread phytoplankton and cyanobacteria blooms recurred since summer 1987, have coincided with the large scale loss of sponges community (Peterson et al. 2006). Increase of phytoplankton may cause reduction of water transparency which in turn reduce the available light for benthic primary producers affecting also their associated communities. Finally, sponges play others important ecosystems functions: they can stabilize the sediment, form big assemblages (sponges gardens) providing refuge for small invertebrates and fishes from predators, and favour larval settlement (Bell 2008).

The negative trends of Holothuroidea and Echinoidea was relevant during the 60 years of analysed data. Sea cucumbers may form high density population (Vafeiadou et al. 2010; Kazanidis et al. 2010). Some species, such as *H. tubulosa*, prefer eutrophic areas, with high food availability (Vafeiadou et al. 2010). Others e.g. *O. digitata* are characteristic of reduced fluid mud and can dig deep down into the sediment (at least 25 cm) (Charles et al., 2014). Both species are deposit feeders. These ecological and behavioural needs may better explain the patterns described in the Adriatic basin. The high nutrient levels of the western, eutrophic areas south of the Po River mouth, may determine suitable areas for sea cucumbers, producing areas where high abundances are still presents. However, in the most of the muddy areas of the Adriatic Sea, with high fishing trawling activities, sea cucumbers declined. A significant factor in the species distribution and persistence is related with sediment composition. The muddy sediments, that are the most part of Adriatic bottoms, are the most affected by trawling impacts and the communities living on muddy bottoms may suffered higher damages and alteration by trawlers (Collie et al. 1997; Collie et al. 2009). Probably only species able to burrow deeper than the used fishing gears persist. This hypothesis has confirmed by the study conducetd by Mangano et al. (2014) where the specie *O. digitata* that could burrow until 25 cm inside the soft sediments, is a characteristic specie of heavy trawled bottoms.

Sea cucumbers together with sea urchin and other burrowing megafauna influence the sedimentary geochemical environments of soft bottoms through their bioturbation activity (Lohrer et al. 2004; Queirós et al. 2013). Bioturbation produced by deposit feeders such as spatangoid urchins, influence nutrient regeneration, mainly with the breakdown of particulate matters that increase the total surface available for bacterial colonisation (Lohrer et al. 2004). Moreover, sea urchin and other burrowing macrofauna specimens determined the mixing of particle and the transport into deeper sediments of organic matter and oxygen, increasing nutrient exchanges with the water column and sediment, enhancing ecosystems functioning contributing to the global element budget (Welsh 2003; Lohrer et al. 2004). *Echinocardium* for example, has resulted important in benthic interactions, favouring complex feedbacks in the NH4-N fluxes and influencing benthic primary production (Lohrer et al. 2004). Thus the decline and the changing in species composition, as resulted in our analysis, may alter the community bioturbation potential (BPc) a parameter that, combining abundance, biomass and life traits of macrobenthic species (such as reworking trait and mobility), provides an estimate of the extent to which benthic community are likely to affect important ecosystems functioning (Queirós et al. 2013). The alteration of specimens belong to the class Echinoidea could mainly be related with the different species behaviour and to theirs different resistance to mechanical and physical disturb. The fragile *E. cordatum*, for example, has a high catchability, meaning a high correlation with intense fishing activity (Morello et al. 2005). However, it can buries between 2 to 20 cm depth in the sediment, and in areas where fishing activities are practices with gears whose penetration do not exceed big depth, it may survive.

Finally, Bivalvia is the showed the least change over time, when all species were considered together. However, the decline of important stocks has been documented. The collapse of *C. gallina* stocks, already reported by other authors (Froglia et al., 2000; Romanelli et al. 2009) has been confirmed by our analysis. The differences observed in the specie *C. gibba*, could be related to the increase of perturbation, such as bottom trawling. The presence of *C. gibba* is an index of unstable environments, e.g. costal and offshore areas suffering occasional or seasonal disturbances (Hrs-Brenko 2006). Its ecology makes it a pioneer species colonising areas with low species richness, as may occur after a catastrophic event (Hrs-Brenko 2006). The increasing abundance of *C. gibba* as showed by our analysis, in the overexploited and eutrophic Adriatic Sea, indicate that the benthic community of the basin is affected by continuous perturbation limiting the development of long-lived species and stable community.

Several factors may determine the sharp decline occurred on macrobenthic species living on soft bottoms of the Adriatic basin. For example, sponges decline have been related both with anoxia events (Fedra et al. 1976), climate change (Di Camillo et al. 2013) and intense direct and indirect human activities (Pranovi et al. 2003; Pronzato & Manconi 2008; Lotze et al. 2011). Despite the natural changes may play important roles in the decline of macrobenthic specimens, our analysis support the hypothesis that the overexploitation and long history of trawlers in the Adriatic Sea may have been one of the main factors determining the alteration of the Adriatic soft bottoms communities. The current composition of Adriatic habitats may be related to the selection of populations more resistant or resilient to the impact of fishing. The increase (around six times higher) in the density of *O. digitata* and of *C. gibba* at the end of 1990s is a clear indicator of the establishment of species characteristic of instable communities. Historical review of Adriatic bottoms showed that past communities, in particular epibenthic organisms (sponges, seapens, hydroids) were abundant in the whole Adriatic basin (Paolucci 1923; Pasquini, 1926). The areas with the highest heterogeneity, and high epibenthic species was unsuitable for trawlers of the first 1900s since the abundance of these discard specimens made difficult to haul the fishing nets. Moreover, the presence of hard bottoms (such as, *Ostrea beds*), once abundant and now almost disappeared in the basin (Beck et al., 2011), increases the possibility of losing or damaging fishing gears. At the same time, those epifauna-rich habitats were the most productive bottoms of the basin, with the presence of the biggest and highest value species such as *Raja* spp., *Lophius* spp., *Squalus acanthias*, *Nephrops norvegicus* (Paolucci 1923; Pasquini, 1926; Scaccini 1967). The low estimated abundances of sponges and holothuroids in the northern and more coastal areas (fig.3), clearly matches with the areas where insist the main Adriatic fisheries (Chioggia and Ancona). The exploitation of the northern basin and occidental coastal areas by Italian fleets and the different fishing effort between the two sides of the Adriatic basin have been well documented (Botter et al. 2006; Ferretti et al. 2013). Thus, the development of professional fishery, with the increase of fishing capacity and of fleet powers, and the inadequate management of the fishing activities have increased the destruction and/or alteration of the rich epifauna bottoms of the offshore soft bottoms, precluding to more eastern and less trawled bottoms, the last long-lived, fragile macrobenthic communities.

5. Conclusion

The historical descriptions and the results of our work highlight the richness and heterogeneity of the past Adriatic benthic communities. The overexploitation of the basin, together with other factors determine abrupt modification of Adriatic bottoms community, with the consequent decline of Adriatic marine ecosystems. The shift from macrobenthic community dominated by structuring sessile organisms, filter-feeding specimens, to a community dominated by opportunistic taxa may determine not only a depletion of stocks, but also a reduction of ecosystem quality and a loss of ecosystem and human services. The importance of the soft-sediment benthos, in fact, is increasingly recognised. Thus, the inability in the recovery of long-living specimens of Adriatic soft bottoms by the frequent disturb cause by trawling activities, determined the reduction of EFHs and VMEs, and a loss or alteration of Adriatic ecosystem functions with negative impacts on the replenishment of the fisheries resources, in the development of no sustainable fishery and human marine uses. However, our analysis about past communities showed that the Adriatic Sea has the potential to promote a recovery of its macrobenthic community. The historical descriptions of Adriatic bottoms highlight the richness in biomass and species diversity of macrofauna species and all the authors admitted the important role played by epifauna organisms (e.g. as nursery, foraging areas). The conservation and management of habitats and sensitive species (e.g. crinoids, seapens, sponges, ophiuroid), such advocated by many national and international directives with the development of an ecosystem-based management approach, should be the priority to reach this objective and to support and promote a sustainable stocks yields. Only through the recovery of the benthic communities of the basin there is the possibility to replenish Adriatic marine resources. Thus, because the failure of the current Adriatic management fisheries practices we suggest that there is the urgency to develop and establish new and efficient conservation tools. The reduction of trawls effort, in particular the creation of no-trawl areas, could be one of the best opportunities to promote benthic community restoration, favouring the recovery of ecosystem functioning and the replenishment of Adriatic marine resources.

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CHAPTER 2

Mediterranean sea pens. From where the recovery of trawled Adriatic sea floor can start

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Abstract

Little information are available on the distribution, ecology and biology of Mediterranean sea pens and the correct identification between different species is difficult because a lack of rigorous morphological studies. Living mainly on mesophotic soft bottoms, sea pens are endangered by trawling activities. Sea pens presence is considered very important from a functional point of view, likely affecting edaphic conditions and representing a possible nursery giving refuge to eggs, invertebrates, and small fishes. As sea pens share the same habitat with some of the most commercial value species (e.g. *Nephrops norvegicus*), they could be very useful indicator of the quality of sand-mud habitats and associated communities.

This study shows a description of the spatial and bathymetric distribution of Mediterranean sea pens, with particular interest to species living in the Adriatic Sea. Models to define the potential suitable habitats of the sea pens *Funiculina quadrangularis*, *Virgularia mirabilis* and *Pennatula phosphorea* have been developed to facilitate the decisions to protect the last habitats structured by their presence.

A detailed morphological description of *P. phosphorea* and *Pteroeides spinosum* living on Adriatic Sea bottoms is also given to clarify the existing taxonomic confusion.

1. Introduction

Pennatulacea (Cnidaria, Class Anthozoa, Subclass Octocorallia) known as sea-pens, are the only octocorals able to live in soft, mainly muddy or sandy, bottoms. They are a specialized and morphologically distinct group of cnidarians with a single, principal axial polyp, the oozoid, from which arise secondary polyps forming the colony. The basal portion anchors the colony into the sediment and an internal axial rod provides the support to the colony in order to maintain the vertical orientation of the sea pens (Williams 1995; Williams 2011; Williams 1998). The ability of Pennatulacea to colonise soft sediment is related to their worldwide distribution that ranges from tropical to polar areas, and from intertidal to abyssal environments reaching depth up to 6000 m (Williams 1998, 2011). Many deeper species are considered cosmopolitan in such habitats (Williams et al. 1992). Despite there are more than 200 species in 34 genera (Williams 1995; Williams and Carins, 2015) the ecology, morphology and reproduction of sea pens remain poorly known.

The ecological role and the fragility of sea pens to human activities have contribute to classified them as essential fishing habitat (EFH) and vulnerable marine ecosystem (VME) (Stecf 2006; Greathead et al. 2007; Rogers & Gianni 2010) and they have started to be considered as target species to develop an ecosystem-based management (EBM) approach of marine environment. Since 2004, the Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic (OSPAR Commission) classified sea pens and burrowing megafauna communities as 'Threatened and/or Declining Habitat'. The NAFO Scientific Council promotes the closure of marine areas to the protection of deep-sea corals, sea pens included (Cogswell et al. 2011).

Studies on the distribution and habitat preferences of sea pens are scant (Greathead et al. 2007; Greathead et al. 2014) and almost absent in the Mediterranean Sea. Only recent surveys started to take into account the presence of *Funiculina quadrangualri*s (Pallas, 1766) as an indicative species of the quality of sandy-muddy habitats (Martinelli et al., 2013). Demersal fishing activities represent the greatest threat to the survival of sea pens colonies and may have had a significant influence on their reduction (Kinnear et al., 1996; Cogswell et al. 2011; Troffe et al. 2005). The large decline of *F. quadrangulairs* and of its associated community, in areas with elevate trawling activities has already been reported in different areas of the Mediterranean Sea (Tunesi & Diviacco 1997; Rogers & Gianni 2010; Stecf 2006) and recently taken in account in the IUCN red list for Anthozoa (IUCN, 2014). The extreme reduction of sea pens and related habitats may determine changes of the ecosystem-functioning of sandy bottoms areas since they add complexity to and otherwise homogeneous habitat and increase biodiversity (Porporato et al. 2012; Porporato et al. 2011). They can act as refuge for eggs or small fishes playing a nursery role (Baillon et al. 2012) or as invertebrate refuge which in turn can be preyed by fishes (Krieger, 1993). They may alter water current flow, thereby retaining nutrients and entraining plankton near the sediment (Tissot et al. 2006). Moreover, species such as *F. quadrangularis* shares the same habitat of commercial value species, in particular with *Nephrops norvegicus* (Linnaeus, 1758) and *Parapenaeus longirostris* (Lucas, 1847) (Greathead et al., 2007; Nouar and Maurin, 2001).

The Mediterranean Sea counts 9 genera with 12 different sea pens species living on soft bottoms ranging from 7-1000 m depth. Six species *Funiculina quadrangularis* (Pallas, 1766), *Pennatula phosphorea* Linnaeus, 1758, *Pennatula rubra* (Ellis, 1761), *Pteroeides spinosum* (Ellis, 1764), *Veretillum cynomorium* (Pallas, 1766) and *Virgularia mirabilis* (Müller, 1776), have been recorded in the Adriatic Sea.

Adriatic sea pens live widespread in the basin and their presence are known from ancient time (Linnaeus 1758; Richiardi 1869; Paolucci, 1923; Pax and Muller 1962; Kruzic, 2007; Sanchez et al., 2007). They occur mostly on muddy and sandy bottoms, some of them forming *facies* of the circalittoral and bathyal biocenosis listed in the priority habitats according to the SPA/BIO Protocol (Barcelona Convention). Until now there are no information about the distribution, the ecology and the abundances (in terms of biomass) of Adriatic sea pens. Moreover, taxonomical classification of the six species is not always clear, with frequent confusion and misidentification of species. The easy access of Adriatic bottoms have allowed the development of intense fishing activities ranking the basin as one of the most exploited basin in the Mediterranean Sea (Micheli et al. 2013a; Coll et al. 2009; Lotze et al. 2011). The decline of demersal and commercial species of the Adriatic Sea is well documented (Jukic-Peladic et al. 2001; Fortibuoni et al. 2010; Lotze et al. 2011; Ferretti et al. 2013). It is possible to suppose that pennatulids and their related habitat have been declined in the Adriatic Sea, following the same negative trend described for other region of the world where trawling is active.

This work aims to increase our spatial knowledge on sea pens living in the sandy-muddy bottoms of the Mediterranean Sea, with a particular focus on the Adriatic Sea. It is the first work with habitat suitability models for sea pens living in the Adriatic Sea and the whole Mediterranean Sea. Habitat mapping of Adriatic soft-bottom communities may contribute to a better identification of priority areas in need of conservation, and support the development of an EBM approach to provide a recovery and replenishment of overexploited resources of the Adriatic Sea. A detailed description of the species *Pennatula phosphorea* Linnaeus, 1758 and *Pteroeides spinosum* Ellis, 1764 is provided in order to clarify taxonomical classification of Adriatic species and limit misidentification.

2. Materials and methods

2.1 Mediterranean sea pens distribution

In order to supply information about the current bathymetric and geographic distribution of the Mediterranean sea pens species, a detailed bibliographic study of the last 30 years, (from 1980 up now) was performed. The records, together with the location and bathymetric range were summarized in a table (Tab.1). The latitude and longitude of each locality were obtained and plotted using the software QGISS 2.4.0 (http://www.qgis.org/it/site/) to map the known distribution of Mediterranean sea pens species. For some literature data, in particular for analysis of the macrofauna of deeper Mediterranean regions surveyed by ROV video, was not possible the identification at species level. Thus, the maps show the distribution of the different sea pens genera, with different symbols and/or colours representing different species, when data were available. Genera which records in the Mediterranean Sea were rare, were mapped together.

2.2 Adriatic sea pens distribution and habitat suitability models

A more detailed analysis was made for sea pens of the Adriatic Sea. Adriatic distribution maps were produced including literature records and samples collected during the "SoleMon" project (Solea Monitoring) conducted from 2005 to 2012 by the National Research Council (CNR-ISMAR,Italy) in cooperation with the National Institute for Environmental Protection and Research (ISPRA, Italy), the Institute of Oceanography and Fisheries (IOF, Croatia), and the Fisheries Research Institute of Slovenia (FRIS, Slovenia). The sampling gear was a modified rapido trawl (width = 3.69 m, weight = 200 kg, cod-end stretched mesh size = 40 mm) (gear description in Hall-Spencer et al., 1999). The approach used during this project allows to have quantitative data (May 2005-Novembre 2012). Finally, occasional records and samples came from Ancona semi-pelagic pair trawl fishery have been taken into account. The gear used by vessels was the so call 'volante'. In this case samples are qualitative, as only presence of entangled Pennatulacea has been recorded. These records were integrate with previous ones to produce the presence/absence habitat suitability models and geographic distribution maps. Models were made only for species with the higher number of records. In particular they have been processed one model for *F. quadrangulairs*, one single model for the species *P. rubra* and *P. phosphorea* and one model for *Virgularia mirabilis* (Müller, 1776). Geographic coordinates of the sampled stations and corresponding bathymetry were identify and used to perform the analysis by the open source statistical language R (3.2.0) (https://www.r-project.org/).Sediments information provided by the ADR_ONE database (http://instaar.colorado.edu/~jenkinsc/dbseabed/coverage/adriaticsea/adriatico.htm) were added to the recorded information. Generalized linear (GLMs) and generalized additive models (GAMs) with binomial distribution were conducted to model the probability of suitable habitats of Adriatic sea pens. The explanatory variable used in the model construction were latitude, longitude, depth and the relation between mud and gravel sediment composition. A backward stepwise selection procedure was performed for the selection of the better model, deleting not significant variables until it was no longer possible to reduce the Akaike Information Criterion (AIC). Predictor was chosen following the maximum likelihood estimation.

2.3 Taxonomical analysis

The collected specimens were preserved in 70° alcohol, or formaldehyde 4%. Preserved materials were used to taxonomical description. Size (total colony length, peduncle and rachis length) of the available colonies were measures with a caliber. The main features of the colonies (organisation of polyp leaves, type and disposition of polyps along the colony, type and disposition of sclerites) were observed by stereo-microscope (Zeiss KL 1500 LCD). Sclerites were prepared by dissolving colony tissue in concentrated sodium hypochlorite, with repeated rising in water and then kept in ethanol 70°, according to Williams (1990). Slides for the microscopical analysis of sclerites were examined under an optical microscope. About 30 sclerites for type (rods and spindles) were measured and mean and standard deviation were calculated.

Moreover, sclerites were mounted onto stubs and sputtered with gold for the ultrastructural analysis with a Scanning Electron Microscope (SEM) (Philips XL 20).

3. Results

3.1 Sea pens in the Mediterranean Sea

Most of the records of pennatulacea are localized on the continental shelves of the northern Mediterranean Sea (fig.1). Information about central and eastern Mediterranean basin are quite limited and comes mainly form the Adriatic Sea and north Aegean Sea. Recent records are available from the Egyptian area (Abdelsalam 2014). There are no scientific reports for the southern regions of the Mediterranean Sea.

The genera *Funiculina*, *Pteroeides*, *Pennatula* and *Veretillum* shows similar spatial distribution (fig.1). Specimens belonging to them have been found in the whole Mediterranean Sea, with some small differences. *F. quadrangularis* (Pallas, 1766) is considered a cosmopolitan species. It is found in Scotland, Sweden, in the Mid Atlantic Ridge, in the Gulf of Mexico, Grand Banks Newfoundland, in South Africa, and in the Marmaran Sea (Williams 1995; Williams 2011). In the Mediterranean Sea it has been recorded in the northern portion of the whole basin, with a depth range from 40 – 1000 m mainly on muddy areas (Tab.1 and fig.1). *Pteroeides spinosum* (Ellis, 1764) is typical of the European Atlantic (from Gibraltar up to Faroe island) and Mediterranean Sea mainly on sandy and muddy bottoms (Williams 1995). It has been found in the northern region of the Mediterranean, with few records in the Tyrrhenian Sea, in the Adriatic Sea and it has been described even along Tunisia and Egyptian coasts (Tab.1 and fig.1). The bathymetric distribution of *P. spinosum* in Mediterranean region goes from 14.5 – 516 m, shallower compere with that of *F. quadrangularis* (Tab.1). Three species belonging to the genus *Pennatula* have been described in the Mediterranean Sea. *Pennatula rubra* (Ellis, 1761) and *Pennatula phosphorea* Linnaeus, 1758 are widespread along the whole basin, with a depth range varying between 20 – 280 m, 16 – 700 m depth respectively. *P. aculeata* Danielssen, 1860 has been recorded only in the most western part of the Mediterranean Sea, from Granada coast and Gibraltar Strait, in a depth range from 100 – 300 m (Tab.1 and fig.1). *Pennatula* species live mainly on sandy, sandy-muddy bottoms. The genus *Veretillum* is known in the Mediterranean region with a single species: *Veretillum cynomorium* (Pallas, 1766). It has been recorded in the norther part of the west and east Mediterranean basin, in a depth range from shallow to deeper waters (17 – 250 m depth) (Tab.1 and fig.1). The genera *Cavernularia* and *Kophobelemnon* have been reported with lower frequency respect than of the previous genera, even if their presence have been recorded both in the western and eastern Mediterranean basin (Tab.1 and fig.1). The species *Cavernularia pusilla* have been recently described even in the Egyptian coast (Abdelsalam, 2014) but it has not been recorded in the Tyrrhenian and Adriatic Sea. Its bathymetric range goes from 20 – 350 m depth. Two species of *Kophobelemnon* have been described in the Mediterranean: *Kophobelemnon leucharti* Cecchini, 1917 and *Kophobelemnon stelliferum* (Müller, 1776). *K. leucharti* is present only in the Tyrrhenian Sea in a depth range form 70 – 200 m, while *K. stelliferum* has a wider distribution and have been recorded at greater depth, from 200 – 500 m. Both species have not been recorded in the Adriatic Sea. *Virgularia mirabilis* (Müller, 1776) has been recorded only for the western Mediterranean and in the Adriatic Sea. It is one of the shallower sea pens species of the basin with a depth range that can vary a lot from 7 to 650 m depth (Tab.1 and fig.1). The genus *Crassophyllum* is present with a single species *Crassophyllum thessalonicae* (Vafidis and Koukouras 1991) and it has been recorded only in the Aegean Sea, while the genus *Protoptilum* has been reported for the first time in the Mediterranean Sea in Santa Maria di Leuca area in 2014 (Mastrototaro et al. 2014) (Tab.1 and fig.1).

Table 1. Summary of the distribution and bathymetric sea pens species recorded in the Mediterranean Sea.

Specie	**Studied area**	**Depth range (m)**	**Reference**
***Funiculina quadrangularis* (Pallas, 1766)**			
Funiculina quadrangularis	Strair of Sicily	163	Pipitone & Tumbiolo, 1992
F. quadrangularis	Cretan sea	40-1000	Smith et al., 1997
F. quadrangularis	Greek Ionian Sea	300-1000	Smith et al., 2010
F. quadrangularis (Pallas, 1766)	North Aegean Sea	49-91	Vafidis et al., 1994
F. quadrangularis (Pallas, 1766)	Catalan continental shelf	40-700	Gili and Pages, 1987
F. quadrangularis	Alboran Sea	200-360	Pardo et al., 2011
F. quadrangularis (Pallas, 1766)	Granada coasts	150-300	Ocaña et al., 2000
F. quadrangularis	Marmaran Sea		Topcu and Ozturk, 2013
F. quadrangularis (Pallas)	Ligurian Sea	180-240	Tunesi and Divaccio, 1997
F. quadrangularis (Pallas)	between La Spezia to the North, and the islands of Montecristo and Giglio (Tuscan Archipelago)	275-280	Morri et al., 1991
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea		SIBM, 2009
F. quadrangularis	Adriatic Sea	128-280	PIPETA, 1982
F. quadrangularis	Adriatic sea (Pomo pits)	200-270	Martinelli et al., 2013
F. quadrangularis	Adriatic Sea	47-80.6	SoleMon project (2005-2012)
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Sant'Eufemia Gulf)	70-140	Bo et al., 2012
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Capo Vaticano Calabria)	105-210	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Ionian Sea (Capo Rizzuto)	60-170	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Carloforte, Sardinia)	115-160	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Secca di Nisida, Naples)	100	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Li Galli, Naples)	100	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Ischia)	90	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Capri)	100	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Canyon tavolara	160-250	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Canyon Caprera	140-150	Bo personal obs.
F. quadrangularis (Pallas, 1766)	Tyrrhenian Sea (Isola del Toro, Sardinia)	100	Bo personal obs.
Funiculina spp.	Gulf of Lion	80-150	Gaertner et al., 1999

***Pteroeides spinosum* (Ellis, 1764) name inquirendum**			
Pteroeides spinosum (Ellis, 1764)	Greek Ionian Sea		Koukouras, 2010
Pteroeides griseum (Linnaeus, 1767)	North Aegean Sea	20-90	Vafidis et al., 1994
P. spinosum Herklots, 1858	Catalan continental shelf	30-280	Gili and Pages, 1987
P. spinosum(Ellis, 1764)	Spain	30-60	Brito and Ocaña, 2004
P. spinosum(Ellis, 1764)	Adriatic sea		Brito and Ocaña, 2004
Pteroides spinosum (Ellis, 1764)	Tyrrhenian Sea (Sant'Eufemia Gulf)	70-130	Bo et al., 2012
P. spinosum Herklots	between La Spezia to the North, and the islands of Montecristo and Giglio (Tuscan Archipelago)	47-68	Morri et al., 1991
P. spinosum (Ellis, 1764)	Strait of Gibraltar		Brito and Ocaña, 2004
P. spinosum (Ellis, 1764)	Tyrrhenian Sea (Sicily)	57-59	Porporato et al., 2011
P. spinosum (Ellis, 1764)	Ionian Sea (Sicily)	10-180	Porporato et al., 2011; 2014
P. spinosum (Ellis, 1764)	Tyrrhenian Sea (Sicily)	108-516	Porporato et al., 2012
P. spinosum (Ellis, 1764)	Adriatic Sea (Croatia)	18-27	Kruzic, 2007
Pteroides spinosum	Girona	70-96	Domenech et al., 2006
P. spinosum	Adriatic Sea	14.5-108	SOLEMON project (2005-2012)
P. spinosum (Ellis, 1764)	Egypt	20-25	Abdelsalam, 2014
P. spinosum	Turkish Straits System (Dardanelles)	45	Barış Özalp and Suat Ateş 2015
Pteroides griseum	Gulf of Lion		Gaertner et al., 1999
P. griseum (Pallas 1776)	Gabes gulf (Tunisia)	40-55	Lakhrach et al., 2011
***Pennatula phosphorea* Linnaeus, 1758**			
Pennatula phosphorea	Tyrrhenian Sea	90-700	Mori et al.,2004
P. phosphorea Linnaeus, 1758	North Aegean Sea	25-91	Vafidis et al., 1994
P. phosphorea Linné, 1758	Catalan continental shelf	30-280	Gili and Pages, 1987
P. phosphorea	Alboran Sea	200-350	Pardo et al., 2011
P. phosphorea Linnaeus, 1758	Adriatic Sea (Croatia)	16	Kruzic, 2007
P. phosphorea Linnaeus, 1758	Adiatic Sea	60-280	PIPETA
P. phosphorea	Adriatic Sea	19.9-94.1	SOLEMON project (2005-2012)
P. phosphorea Linnaeus, 1758	Tyrrhenian Sea (Sicily)	25-35	Porporato et al., 2011
P. phosphorea Linnaeus, 1758	Ionian Sea (Sicily)	57	Porporato et al., 2011
P. phosphorea Linnaeus, 1758	Sicily Channel		IUCN list
P. phosphorea Linnaeus, 1758	Strait of Messina	35	Porporato et al., 2012
***Pennatula rubra* (Ellis, 1764)**			
Pennatula rubra Pallas, 1766	North Aegean Sea	25-40	Vafidis et al., 1994
P. rubra Ellis, 1764	Catalan continental shelf	30-100	Gili and Pages, 1987
P. rubra (Ellis, 1761)	Mediterranean sea		Williams, 1995
P. rubra (Ellis, 1764)	Adriatic Sea		IUCN list
P. rubra (Ellis, 1764)	Granada coasts	30-150	Ocaña et al., 2000
P. rubra (Ellis, 1761)	Marmaran Sea		Topcu and Ozturk, 2013
P. rubra Ellis	between La Spezia to the North, and the islands of Montecristo and Giglio (Tuscan Archipelago)	47-280	Morri et al., 1991
P. rubra	Gulf of Lion		Gaertner et al., 1999
P. rubra	Girona	70-96	Domenech et al., 2006
P. rubra (Ellis, 1764)	Sicily Channel		IUCN list
P. rubra Ellis, 1764	Egypt	20-25	Abdelsalam, 2014

***Pennatula aculeata* Danielssen, 1860**			
Pennatula aculeata Koren and Danielssen, 1859	Granada coasts	150-300	Ocaña et al., 2000
P. aculeata Danielssen, 1860	Strait of Gibraltar		Brito and Ocaña, 2004
***Pennatula* spp.**			
Pennatula spp.	Tyrrhenian Sea (Sant'Eufemia Gulf)	80-140	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Capo Vaticano Calabria)	80-110	Bo personal obs.
Pennatula spp.	Ionian Sea (Capo dell'Armi, Calabria)	120	Bo personal obs.
Pennatula spp.	Banco Amendolara	120	Bo personal obs.
Pennatula spp.	Palinuro Seamount	50-60	Bo personal obs.
Pennatula spp.	Lampedusa	100	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Sardinia)	95	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Isola del Toro, Sardinia)	80-100	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Carloforte, Sardinia)	115	Bo personal obs.
Pennatula spp.	Ligurian Sea (Portofino)	95-100	Bo personal obs.
Pennatula spp.	Ligurian Sea (Secca del Mantice, Savona)	90-100	Bo personal obs.
Pennatula spp.	Maledetti	80-90	Bo personal obs.
Pennatula spp.	Canyon della Luna	90-100	Bo personal obs.
Pennatula spp.	Ligurian Sea (Albenga)	50	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Li Galli, Naples)	85	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Nisida)	100	Bo personal obs.
Pennatula spp.	Tyrrhenian Sea (Pianosa)	50-70	Bo personal obs.
Pennatula spp.	Plemmirio	110-120	Bo personal obs.
Pennatula spp.	Canyon tavolara	110-160	Bo personal obs.
Pennatula spp.	Banco di Graham	80-160	Bo personal obs.
***Veretillum cynomorium* (Pallas, 1766)**			
Veretillum cynomorium (Pallas, 1766)	Agean Sea; (also known by authors in Sea of Marmara; W-Mediterranean; Adriatic Sea)	25-42	Vafidis et al., 1994
V. cynomorium (Pallas, 1766)	Alboran Sea	20-100	Brito and Ocaña, 2004
V. cynomorium (Pallas, 1766)	Catalan continental shelf	30-260	Gili and Pages, 1987
V. cynomorium	Gulf of Lion		Gaertner et al., 1999
V. cynomorium (Pallas, 1766)	Adriatic Sea (Croatia)	17-24	Kruzic, 2007
V. cynomorium (Pallas, 1766)	Tyrrhenian Sea (Sant'Eufemia Gulf)	70-120	Bo et al., 2012
V. cynomorium (Pallas, 1766)	Ligurian Sea		IUCN list
V. cynomorium (Pallas, 1766)	Sicily Channel		IUCN list
V. cynomorium (Pallas, 1766)	Marmaran Sea	25-32	Topcu and Ozturk, 2013
V. cynomorium	Turkish Straits System (Dardanelles)	45	Barış Özalp and Suat Ateş 2015
Veretillum sp.	Girona	50-60	Domenech et al., 2006
***Cavernularia pusilla* (Philippi, 1835)**			
Cavernularia pusilla (Philippi, 1835)	whole Mediterranean Sea		Williams, 1995
C. pusilla (Philippi, 1835)	Catalan continental shelf	200	Gili and Pages, 1987
C. pusilla	Alboran Sea	200-350	Pardo et al., 2011
C. pusilla (Philippi, 1835)	North Aegean Sea	30-80	Vafidis et al., 1994
C. pusilla (Philippi, 1835)	Egypt	20-25	Abdelsalam, 2014

***Kophobelemnon leucharti* Cecchini, 1917**			
Kophobelemnon leucharti Cecchini, 1917	Mediterranean Sea		Williams, 1995
K. leucharti Kölliker, 1872	Tyrrhenian Sea (Sant'Eufemia Gulf)	70-130	Bo et al., 2012
K. leucharti Kölliker	between La Spezia to the North, and the islands of Montecristo and Giglio (Tuscan Archipelago)	97-200	Morri et al., 1991
***Kophobelemnon stelliferum* (Müller, 1776)**			
Kophobelemnon stelliferum (Müller, 1776)	Greek Exclusive Economic Zone		Koukouras, 2010
K. stelliferum (Müller, 1776)	Catalan continental shelf	400-500	Gili and Pages, 1987
*K. stelliferum (*Müller, 1776)	Ionian Sea (Santa Maria di Leuca, Apulia)	404-467	Mastrototaro et al., 2012
K. stelliferum Kölliker, 1872	Sicily Channel		IUCN list
K. stelliferum	Alboran Sea	200-300	Pardo et al., 2011
***Virgularia mirabilis* (Müller, 1776)**			
Virgularia mirabilis (Müller, 1775)	Catalan continental shelf	30-200	Gili and Pages, 1987
V. mirabilis (Linnaeus, 1758)	Granada coasts	7-15	Ocaña et al., 2000
V. mirabilis (Müller, 1776)	Adriatic Sea (Croatia)	21	Kruzic, 2007
V. mirabilis (Müller, 1776)	Adriatic sea (Fano harbour)	50-55	Sanchez et al.,2007
V. mirabilis (Müller, 1776)	Canyon Cavoli	120	Bo et al., 2012
V. mirabilis (Müller, 1776)	Ligurian Sea		IUCN list
V. mirabilis (Müller, 1776)	Tyrrhenian Sea (Sant'Eufemia Gulf)	70-130	Bo et al., 2012
V. mirabilis (Müller, 1776)	Tyrrhenian Sea (Capo Palmeri, Sardinia)	120	Bo et al., 2012
V. mirabilis (Müller, 1776)	Tyrrhenian Sea (Secca di Penna Palummo, Naples)	80-90	Bo et al., 2012
V. mirabilis (Müller, 1776)	South Tyrrhenian Sea	560-620	Porporato et al., 2009
V. mirabilis (Müller, 1776)	Adriatic Sea	10.2-89.9	SOLEMON project (2005-2012)
***Crassophyllum thessalonicae* Vafidis and Koukouras, 1991**			
Crassophyllum thessalonicae Vafidis and Koukouras, 1991	Thermaikos Gulf	37-40	Vafidis and Koukouras, 1991
C. thessalonicae Vafidis and Koukouras, 1991	Thermaikos Gulf	30-60	Fryganiotis et al., 2010
***Protoptilum carpenteri* Kölliker, 1872**			
Protoptilum carpenteri Kölliker, 1872	Santa Maria di Leuca	240-450	Mastrototaro et al., 2014

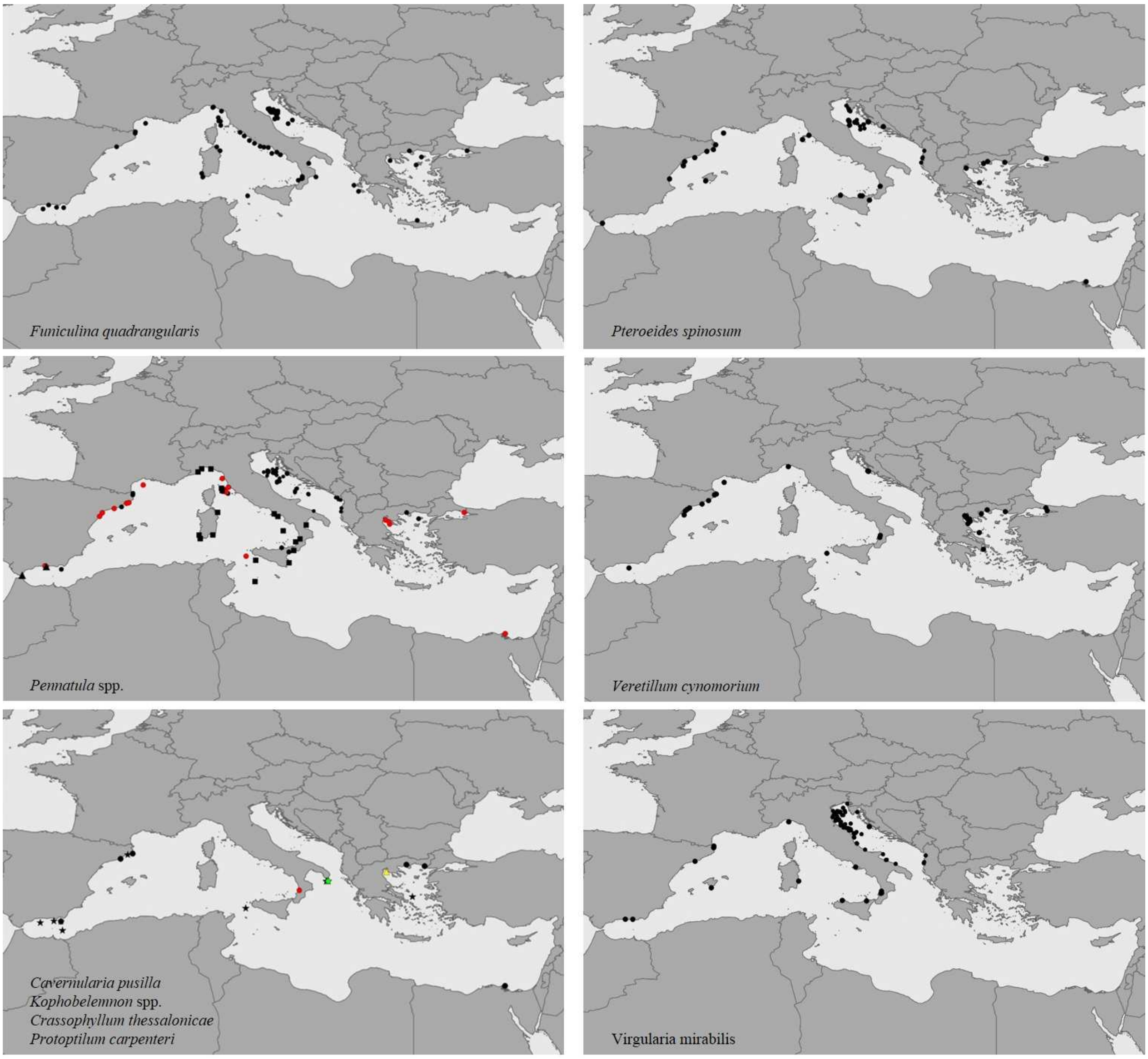

Fig.1. Mediterranean Sea distribution maps of the sea pens species divided by genus. From the top-left to the right: *F. quadrangularis* records; *P. spinosum* records; *Pennatula* spp. records with *P. rubra* in red, *P. phosphorea* blacks circles, *P. aculeata* blacks triangles and not identify species blacks squares; *V. cynomorium* records; map showing *C. pusilla* records (blacks circles); *Kophobelemnon* spp. records with circles *K. leucharti* (red circle), *K.stelliferum* (blacks stars); ; *C. thessalonicae* yellow triangle, and *P. carpenter* green circle*;* finally *V. mirabilis* records.

3.2 Sea pens in the Adriatic Sea: known distribution and habitat suitability models

Of the 12 species of the Mediterranean Sea, 6 are present in the Adriatic belonging to 5 different genera (*Funiculina*, *Pennatula*, *Pteroeides*, *Veretillum* and *Virgularia*). The majority of records were in the central – northern basin and proceeding southward available information decreased (Fig.2 A).

F. quadrangularis is the species with the deepest distribution. It has been recorded from 40 m depth in the offshore of the central Adriatic to 200 m in the Pomo (Jabuka) pits (fig.2 B). Literature data reported two species of *Pennatula* in the Adriatic Sea: *P. rubra* and *P. phosphorea*. They show an overlapping distribution in the offshore central Adriatic, while the presence of the two species seems to be different in coastal areas. *P. rubra* has been found along Apulian and Albania coasts and *P. phosphorea* in Croatian shallow waters (fig.2 C). The bathymetric range of the two species in the Adriatic Sea vary from 20 up to 100 m depth. All the samples collected during our researches belong to the species *P. phosphorea*. The species *P. spinosum* has been recorded in the offshore central portion of the basin and along the northern coast of Istria, Croatia and Albania (fig.2 D). *P. spinosum* is present from very shallow waters (14 m depth) to deeper region of the Adriatic Sea, reaching more than 100 m depth. *V. cynomorium* has been reported only in Telašćica Nature Parck of Croatia. It has been found in shallow waters (17 – 24 m depth) (fig.2 E). Finally, the species *V. mirabilis* is mainly distributed in the north and throughout the Italian coasts, even if records have been also found in Albania, and Croatia shallow waters (fig. 2 F). The bathymetric depth range of the specie goes from 10 to 90 m depth.

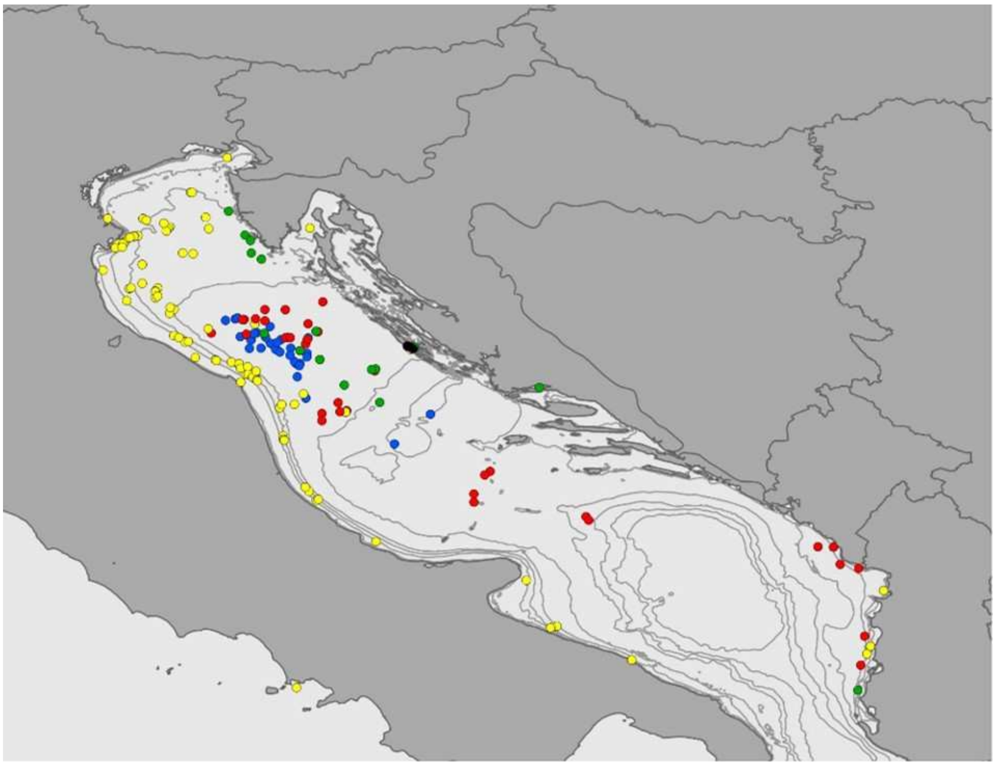

Fig.2: Adriatic distribution of sea pens species. Blue circles *F. quadrangularis*; red circles *Pennatula* species; green circles *P. spinosum*; black circles *V. cynomorium* and yellow circles *V. mirabilis* records.

The presence-absence models, defining the most probable suitable habitat for different sea pens species, highlight different preference depending on each species (fig.3). Of the five variable used to run the models, depth, longitude, latitude and mud content were of primary importance to predict the most probable presence of *F. quadrangularis*. The presence of *Pennatula* spp. was mainly related with depth and latitude.

Finally, the variables influencing the presence/absence model of *V. mirabilis* were depth, mud abundance and latitude. *F. quadrangularis* has the highest probability to be found in areas deeper than 50 m, in particular in the southern and in the central offshore areas of the basin. The probability to find *Pennatula* spp. is high through the central Adriatic Sea. Thus, the mid Adriatic resulted as the area where *F. quadrangularis* and *Pennatula* spp. can be easier find together (fig.3). The model for the predicted distribution of *V. mirabilis* confirmed that it is the sea pens with the shallower distribution along the Adriatic basin. The more suitable habitats of the Adriatic Sea for *V. mirabilis* has resulted to be the norther basin and the sandy-muddy occidental coastlines (fig.3).

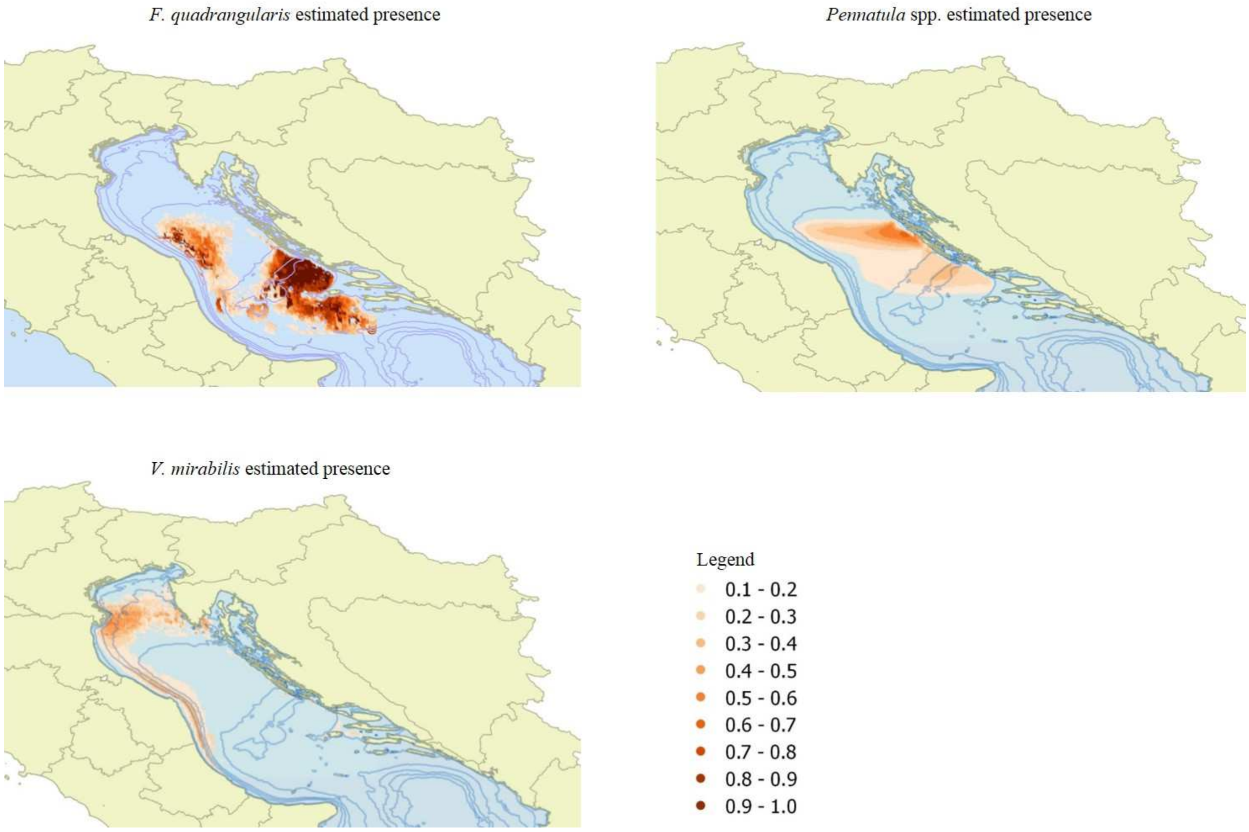

Fig.3: Predicted habitat suitability in the Adriatic Sea for the presence of *F. quadrangularis* (top-left); *Pennatula* spp. (top-right) and *V. mirabilis* (bottom-left). Value range from 0 (lower possibility to find sea pens in the area) to 1 (higher probability to find sea pens in the area).

Data recorded from *rapido* trawling survey (SoleMon) let the possibility to produce quantitative results. The sea pens abundances, reported as fresh weights (kg/km2), show higher value in the north east Adriatic, in front of Istria coast (Fig.4 A). The species have different weights, depending mainly on colonies morphologies and not by the numbers of hauled colonies. *P. spinosum* has the greater biomass because its robust colonies, in particular along Istria coasts (Fig.4 B). The slender *F. quadrangulairs* has the lowest biomasses, with higher values found in the central basin, off Ancona promontory (Fig. 4 C). The slender *V.*

mirabilis and the small *P. phosphorea* show very similar values, even if the stations with the bigger biomasses occurred in different areas. In particular *V. mirabilis* showed higher biomasses in front of the Po delta, while *P. phosphorea* in offshore central Adriatic and along Montenegro coast (Fig.4 D-E).

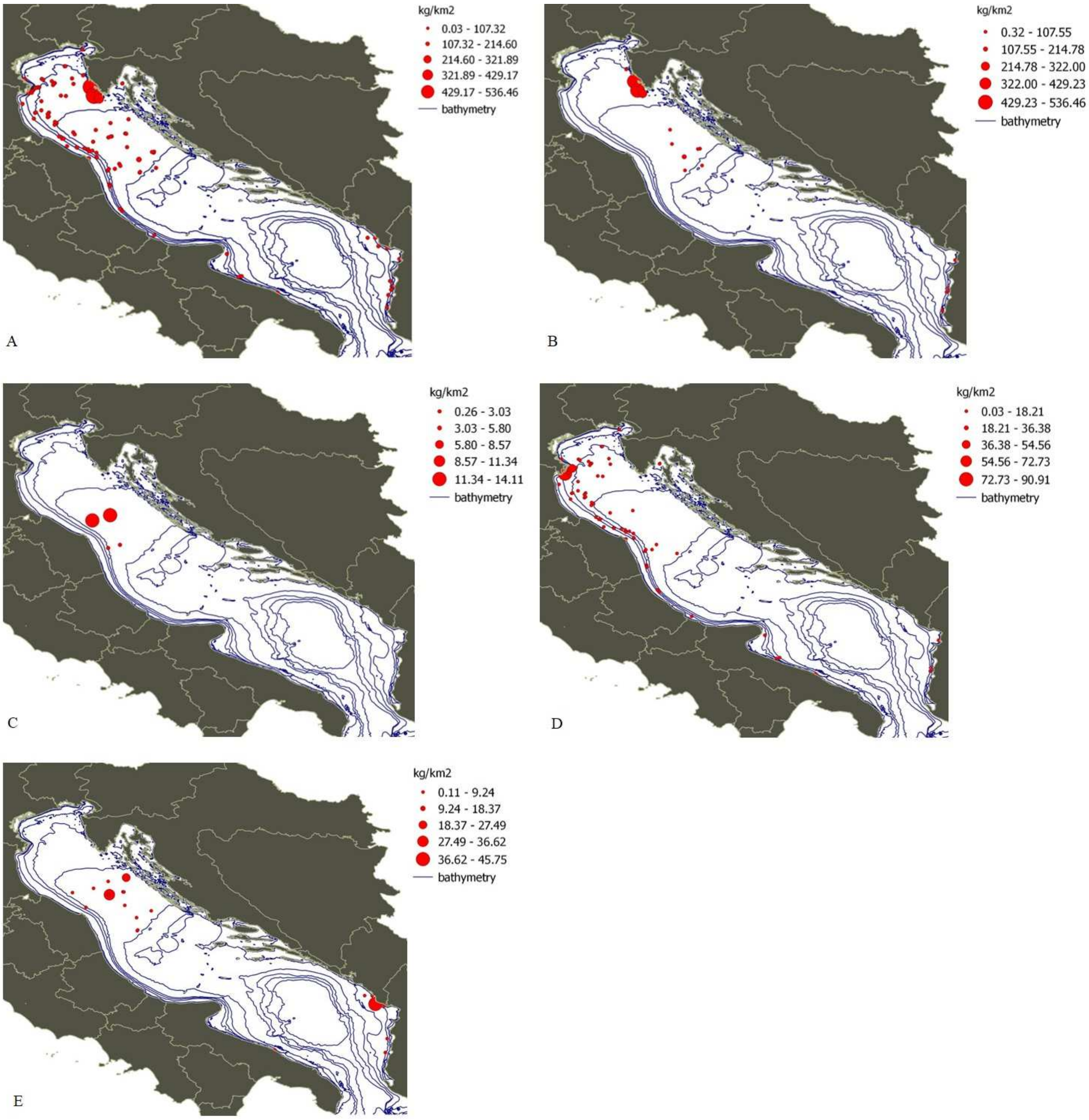

Fig.4. Biomasses distribution of sea pens sampled during SoleMon survey. A) Total sea pens biomasses (kg/km2). B) *P. spinosum* biomasses. C) *F. quadrangularis* biomasses. D) *V. mirabilis* biomasses. E) *P. phosphorea* biomasses.

3.3 Taxonomical descriptions

Pteroeides spinosum (Ellis, 1764) name inquirendum

Materials

8 colonies form the Adriatic Sea.

Description

The colony has a feather-like shape, is fleshy and yellow-brownish in colour colonies, up to 20 cm high (length measured on colonies fixed in alcohol). The internal axis is present throughout the whole colony, excluding only the apical part. It is white-yellow in colour and it has a round section. The peduncle is about 1/3 of the length of the whole colony. Its colour range from yellow-orange to brown-violet and it has a widening in proximity of the rachis. The rachis is well developed (around 2/3 of the colony length), bringing polyps supported by leaves. A primary polyp can be distinguished at the upper extremity of the rachis. There are 19-24 pairs of polyp leaves per colony. Smaller leaves are near the basal portion and in the apical part of the rachis, while the leaves of maximum size are in the middle of the rachis (fig.5 A). Leaves are rigid, due to long smooth needle-like spicules, grouped in bundles and forming the rays (from 10-14 rays per leaves). The sclerites of the ray reaching the surface protrude from it forming spines up to 6 mm long (fig.5 A). Other spicules (spindle and rods) are found between the rays supporting the anthocodiae or lying between them. Autozooids are numerous, brownish in colour and arranged in lines along the edge of the leaves, between the spines. Each polyp has pinnate tentacles (about 15 in each side of the tentacle) and they are free of sclerites. Autozooids can retract inside anthocodiae that appeared as star- shaping. Siphonozooids are smaller than authozooids, like minute protuberances on the surface's leaf. They do not have tentacles and they are organised in a basal plate to the under-side of the leaf. Sifonozooids have not sclerites, and they are partially pigmented, resulting white with small brown shades. Mesozooids are intermediate in size between autozooids and siphonozooids. They are present only in the ventral face of the colony, in the distal portion of the rachis and along the central axis (fig.5 A). They are brown in colour, with no tentacles and they have sclerites surrounding and supporting them. The sclerites of *P. spinosum* are smooth, rods and spindles. The peduncle has rods, more or less flattened of 65 – 144.42 (±49.86) – 250 µm x 15 – 20.38 (±3.29) – 25 µm and spindle of similar size (130 – 160.71 (±24.40) – 205 µm x 15 – 19.64 (±2.92) – 25 µm). Some cross shaping sclerites were observed in the peduncle of about the same length of the smallest rods (fig.5 B). The central axis has rare and small rods of 115 – 199.55 (±43.3) – 275 µm x 10 – 19.44 (±4.82) – 25 µm (fig.5 C). In both the peduncle and central axis, sclerites are widespread through the flesh. Mesozooids have thin spindles of about 90 – 163.00 – 340 µm x 10 – 13.5 – 20 µm (fig.5 D). Polyps leaves have long, slender spindles and rods of different size ranging from 145 – 313.64 (±117.96) – 500 µm x 10 – 12.95 (±4.00) – 20 µm. The longest sclerites, forming the rays and the spines protruding the surface, are smooth, with irregular extremities and can reach up to 8 mm long (fig.5 E).

Remarks

The reconstruction of the story of *P. spinosum* has been really complex. After a deep bibliographic research we can confirm that there is only one specie in the Mediterranean Sea belong to the genera *Pteroeides* (Herklots, 1858). The specimens have a great variability in some morphological features, such as the more or less marked presence of spines protruding polyp leaves. The extent to which these spines project from polyp leaves may vary to some extent according to the mode of preservation (well preserved specimen have tissues less contracted and shortest spines). Over the years there have been a great number of authors describing the same Mediterranean specie, using different specie name causing great confusion in the taxonomical assignation of the specie. A more detailed work should be done to confirm the correct specie name and the correct authorship to the Mediterranean specie.

Pennatula phosphorea Linnaeus, 1758

Materials

50 colonies form the Adriatic Sea.

Description

Feather-like colonies, purple in colour up to 9.3 cm long (fig.6 A). The colour is due to the red sclerites that cover all the portion of the specimens. The internal calcified axis is present throughout the colony. It has round section, tapering at the extremities and ending with a small bent. The peduncle is quite cavernous and it is 1/3 of the length of the colony. It ends with a white small protuberance and it divides form the rachis through an annular restriction. The rachis is about 2/3 of the colony and it comprises many polyps leaves (about 21-22 per side). There is no a primary polyp at the summit of the rachis, even if it is visible a palisade formed by sclerites. The leaves are disposed symmetrically around the axial portion and sustained the autozooids that range from 6 to 16 per leaves (the leaves near the peduncle and the apex are smaller than the central ones). *P. phosphorea* has only two different types of polyps: autozooids and siphonozooids. Autozooids are arranged in one line along the margin of the leaves. They are surrounded by a thick palisade of sclerites, well organised in lines that end forming calyces with 8 teeth where autozooids can retract. Autozooids are white in colour, they have pinnate tentacle and each tentacle and pinnulae contain small sclerites inside of them. Siphonozooids are exclusive of the rachis. In the dorsal face of the colony they are organised in two lines and they are so numerous that the central groove present in this side of the rachis was sometimes poorly visible. Sifonozooids are present also in the ventral face of the colony, grouped in small number at the base of the polyp leaves. They have not tentacles and they are sustained by a palisade of sclerites. *P. phosphorea* have three-flanged rods and spindles of very different size, in relation to their disposition along the portion of the colony. In particular, the peduncle has three-flanged rods of 95 – 213.03 (±70.45) – 380 μm x 10 – 20.07 (±4.48) – 30 μm and spindle of 75 – 137.81 (±60.22) – 290 μm x 7.5 –

14.06 (±4.82) – 25 µm usually smoother than the rods (fig.6 B). Siphonozooids of the dorsal face have three-flanged rods and spindles of different size that range from 77.5 – 273.95 (±153.13) – 620 µm x 10 – 20.83 (±7.02) – 35 µm (fig.6 C). Siphonozooids of the ventral face have three-flanged rods of 125 – 197.35 (±51.72) – 310 µm x 15 – 19.41 (±2.87) – 25 µm and three-flanged spindles of 90 – 164.00 (±39.74) – 220 µm x 15 – 17.67 (±3.07) – 25 µm (fig.6 D). Polyp leaves have three-flanged rods of different size. The biggest sclerites (830 – 1268.18 (±280.17) – 1860 µm x 30 – 38.64 (±6.36) – 60 µm) usually have a smooth cental axis and only the three flanged (fig.6 E). The medium (450 – 591.33 (±110.74) – 750 µm x 20 – 28.33 (±4.88) – 40 µm) and smaller rods (260 – 322 (±40.86) – 370 µm x 15 – 18.5 (±2.23) – 20 µm) are three-flanged for all the length of the sclerite (fig.6 E). Finally, polyps have three-flanged rods of 107.5 – 142.5 (±17.63) – 177.5 µm x 10 – 14.1 (±2.15) – 17.5 µm along the main tentacles, and smaller rods inside the pinnulea of 37.5 – 65.57 (±17.04) – 105 µm x 5 – 7.13 (±1.60) – 10 µm (fig.6 F).

Remarks

The great abundance of sclerites in the whole colony, the distribution of slcerites, in particular around the autozooids where they form calyces with 8 teeth and the presence of three-flanged rods inside the tentacles and pinnulae of the polyps are distinctive feature of *P. phosphorea*. Two different *Pennatula* species are reported in the Adriatic Sea in literature: *Pennatula rubra* (Ellis, 1764) and *P. phosphorea* Linnaeus, 1758. However, all the collected specimens and then taken to us belongs only to the specie *P. phosphorea*. Thus, it was not possible compare both species and it was not possible identify, to better describe, the main characters that differentiate the two species.

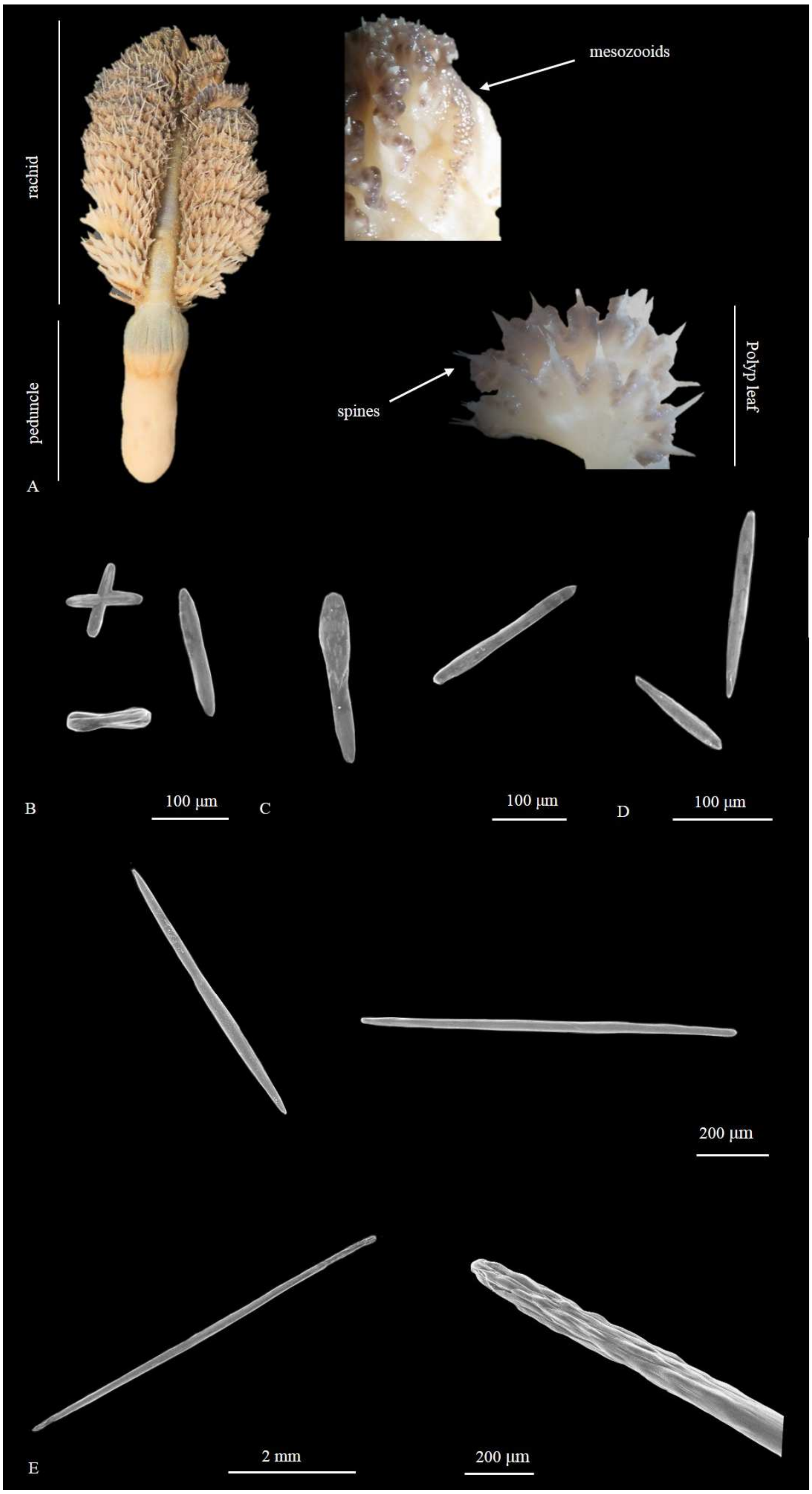

Fig. 5: *P. spinosum* main features. A) Colony and a detailed of mesozooids and polyp leaf. B) Sclerites (rod, spindle and a cross) of the peduncle; C) Rods of the central axis; D) Spindles of the mesozooids; E) In the upper part one spindle and rod of medium size; down one of the longest sclerites forming the spines with a particular of the trabecolate extremity.

Fig. 6: *P. phosphorea* features. A) Colony of *P. phosphorea*; B) Three-flanged rods of the peduncle; C) Three-flanged rods and spindles of the siphonozooids of the dorsal face; D) Three-flanged rods and spindles of the siphonozooids of the ventral face; E) Three-flanged rods of polyps leaves; F) Three-flanged rods of the polyps tentacles and pinnulae.

4. Discussion

According to Williams 2011, sea pens can be divided into three groups depending on their bathymetric distribution: shallow, mid-depth and deep-water species (0-400 m, 400-2000 m, 2000-6100 m respectively). The genera *Veretillum*, *Cavernularia* and *Pteroeides* were considered by Williams in the shallow-waters group. This is confirmed in our analysis of the Mediterranean and Adriatic Seas with the exception of *P. spinosum* that was found even at depth greater than 500 m in the Tyrrhenian Sea. All the reviewed species' records are limited to continental shelves, leaving deep waters habitats of the Mediterranean basin poorly known. Species belong to deep-water genera, such as *Funiculina*, *Pennatula*, *Protoptilum* and *Kophobelemnon* have never been found deepest than 700-1000 m in the Mediterranean basin. Furthermore, the Adriatic Sea never reaches depth bigger than 1200 m. However, the basin is mainly characterised by sandy and muddy bottoms that are suitable habitats for sea pens. The presence of favourable sea pens bottoms in the Adriatic Sea is supported by our distribution maps that shows the widespread distribution of the different species throughout the basin.

The produced suitability habitat maps is also related with the different behaviours of the Adriatic sea pens species. *V. mirabilis* has a large muscular peduncle that gives to the specimens the capacity to entirely retract inside sediment (Greathead et al., 2014). This allow to the specie to colonise more unstable habitats, with a higher content of gravel sediments, usually typical of shallow waters. Moreover, the withdraw capacity of *V. mirabilis* yield a bigger resilience to trawl disturb respect that of other sea pens species. The most suitable habitat for *F. quadrangularis* is the deepest, muddy areas of the Adriatic Sea. *F. quadrangualris* has a really low withdraw capacity, making it highly endangered by bottom trawling activities (Huges, 1998; Greathead et al., 2007).

Sea pens habitats are particularly sensible to the action of bottom trawling that may completely destroy them. Sea pens abundances could be dramatically less, up to six times lower, in areas with intense disturb and the sessile, slow-growing and long-life cycles of sea pens let their recovery difficult and slow (Hixon & Tissot, 2007). In other world region, the best conservation conditions of sea pens and related habitats have been observed only in areas with low fishing effort or near rocky outcrops that offer a natural protection to soft bottoms (Hixon & Tissot 2007; Cogswell et al. 2011).

Currently there are no legislation that protect sea pens and associated communities in the Adriatic Sea, even if they could be one of the most widespread and important habitat of the Adriatic soft bottoms. The cumulative impacts affecting the Adriatic basin, in particular fishing activities that in the Adriatic Sea are among the most intense in the whole Mediterranean regions (Lotze et al., 2011; Micheli et al., 2013a; Micheli et al., 2013b), have definitely contributed to alter sea pens communities. Despite historical information about abundances and distribution of Adriatic pennatulacea are scant and identify a pristine, no disturbed condition is rather complex, we believe that the decline of Adriatic sea pens may be comparable to that occurred in other world regions (such as off of Oregon (Hixon & Tissot, 2007)). The huge decrease of sea pens was reported from Ligurian Sea where in the ‘70s soft coral habitats was very common but currently totally disappeared in trawled bottoms (Tunesi & Divaccio, 1997). The same negative trend has been confirmed in Algeria, where otter trawling fisheries on muddy bottom targeting shrimp *Parapenaeus longisrostris* destroy the benthic habitat associated with *Funiculina* (http://www.fao.org/docrep/007/y5594e/y5594e04.htm#bm4.7). In the Adriatic Sea, *F. quadrangularis* have been described in Pomo pits Norway lobster surveys (Martinelli et al., 2013) with a rare occurrence. Personal communications of local fishermen involved in *Nephros norvegicus* fishery, working on Adriatic grounds, confirm a progressive reduction of *F. quandrangularis* higher than the 50% in these habitats.

Evaluate the fishing impact on soft corals is not easy. An approach adopted by research institutes used fishing surveys to analysed soft corals presence, distribution and threats (Troffe et al., 2005; Greathead et al., 2007). However, surveys on sea pens discard in the Adriatic Sea are scant. The density maps produced by our work are very punctiforms, highlighting the gap in knowledge of these important soft bottoms Adriatic habitats. Moreover, the traditional sampling (e.g. grab, trawl or dredge) can introduced sampling bias, or the species may not be retained by the gear and prevent their collection, with consequently underestimate of sea pens. Finally, the level of the damage depend on species behaviour size and shape of sea pens. Bigger and more fragile species suffered major stress by trawling activities because they may be entangled in nets and their recovery may be prevented by continuous disturbs.

The ecological role of sea pens is not completely understand, even if we know that these octocorals may play a primary role in the ecosystems functioning of soft bottoms areas. They can live in aggregation forming real sea pens fields, contributing to add tridimensionality to an otherwise poorly complex habitat, conferring structural relief on sandy muddy habitats (Tissot et al., 2006). Sea pens have been considered both as vulnerable marine ecosystems (VMEs) and essential fish habitats (EFHs). However, international criteria to define VMEs don’t accept the use of bycatch data as sufficient criteria to map them (Murillo et al, 2011). This means that more accurate research should be done to produce an accurate habitat mapping of VMEs of the Adriatic Sea. However, the identification of presence/absence areas with sea pens may provide a sufficient tool to the selection and proposal of fishery closed areas or of other conservation measure, as already suggest by other authors (Murillo et al., 2011).

Finally, the confused taxonomy of Adriatic sea pens may led to misinterpretation or underestimation of the species present inside the basin.

5. Conclusion

Mediterranean and in particular Adriatic sea pens are poorly studied. Our work gives a base to set the status of sea pens in the whole basin and it sets the base for future analysis on the identification and mapping of the soft bottoms EFHs and VMEs of the Adriatic Sea. The habitat suitability maps, the abundances maps and the taxonomical description of Adriatic species provide the first extended study on these soft bottoms habitat forming species of the Adriatic Sea. Sea pens habitats are one of the few soft bottoms habitat to be classified as EFHs and as VMEs, with increasing attentions by international organisations on these sand-mud seafloor ecosystems. These habitats should be taken into account in the selection of priority areas in need of protection. The occurrence of sea pens habitats could be fundamental to define an EBM approach urgently required to reach a sustainable exploitation of Adriatic marine resources and for stop the biodiversity loss and environmental decline of the basin. However, to define a good management strategy for sea pens and related habitats, more detailed studies should be done. The unregulated fishing practices, and the lack of deep knowledge of the ecological role played by soft bottoms habitats makes difficult define clear management and protection action for the sustainability of Adriatic marine resources.

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CHAPTER 3

Large marine protected areas (LMPAs) in the Mediterranean Sea: the opportunity of the Adriatic Sea

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Abstract

The Strategic Plan for Biodiversity 2011 – 2020 of the Convention on Biological Diversity (CBD) establishes that at least 10% of each of the world's marine and coastal ecological regions should be effectively protected by 2020. Progress towards this target has been slow, though it has recently accelerated through the establishment of several oceanic large marine protected areas (LMPAs). Intensely used and heavily impacted marine regions, including the Mediterranean Sea, are particularly challenging for expanding protection. Current protection of Mediterranean marine ecosystems is limited, and mostly limited to shallow coastal habitats. Hence, there is a strong need of scaling up conservation efforts in the Mediterranean Sea to ensure effective protection of a representative set of marine ecosystems and species from current and escalating threats and to meet the CBD target.

Aim of this paper is highlights current opportunities and expected benefits of establishing a transboundary large marine protected area (LMPA) – specifically a no-trawl area – in one of the most exploited sectors of the Mediterranean, the Adriatic Sea, as a strategy to foster recovery of the local marine ecosystems and economies, and to meet international conservation targets and EU legal mandates. Based on a review of published studies documenting the positive outcomes of previous trawling bans in other regions, and of current initiatives and opportunities within the Mediterranean region, we conclude that large-scale protection of the Adriatic with a no-trawl zone is a promising and feasible approach for reversing ecological and socioeconomic losses in this basin. In particular, ecosystem protection can be established in the Mediterranean through a proposal for a Fisheries Restricted Area (FRA) to the general Fisheries

Commission for the Mediterranean (GFCM). The successful establishment and function of a FRA or LMPA will depend on its support by the governments of the surrounding countries, as well as involvement and participation of key user groups.

1. Introduction

In recent years numerous international conventions have recognised the need to increase protection of marine resources and to reform ocean management to better account for and balance the multitude of human marine uses. Significant efforts are taking place worldwide to reach the objective of protecting 10% of coastal and marine areas by 2020 (Convention for Biological Diversity (CBD) (https://www.cbd.int/)) ratified by all EU countries and non-EU Mediterranean Countries, including comprehensive national and international ocean policies (e.g., the US Ocean Policy, the European Union Marine Strategy Framework Directive (MSFD)), and planning initiatives to expand ocean protection to deep and offshore areas [1,2].

Progress towards the 10% target has accelerated in recent years through the establishment of several Large Marine Protected Areas (LMPAs, 1000s-10,000s Km^2 in surface area) and very LMPAs (VLMPAs, > 100,000 Km^2) ([3]; Table S1 in Suppl. materials). LMPAs and VLMPAs provide unique benefits, but also potential drawbacks and challenges, including the difficulty of excluding or limiting uses and of enforcing regulations over large areas (Table 1).

The Mediterranean Sea is a prime example of the difficulty of establishing comprehensive, coordinated marine conservation and management. The Mediterranean has been exploited for centuries and currently is one of the most intensely-used and most impacted seas in the world [4,5]. Marine resource overexploitation poses major threats for biodiversity, resulting in the decline and loss of marine populations and habitats [6,7]. In turn, consequences of biodiversity loss include changes in ecosystem function and reduction in ecosystem services [8], a scenario that is complicated by climate change [9,10].

Mediterranean marine ecosystems are composed by diverse and ecologically valuable habitats such as seamounts, canyons, hydrothermal vents, cold seeps, mud volcanoes and unique and sensitive habitats (e.g. meadows of the endemic seagrass *Posidonia oceanica* and biogenic reefs) [11]. These habitats make the Mediterranean Sea one of world seas with the highest biodiversity [12,13]. Although the basin covers only the 0.82% of the global ocean's surface, it hosts more than 17,000 described marine species contributing to an estimated 4% – 18% of the world's marine biodiversity [14,15] values that can be much higher considering the hidden deep-sea biodiversity [12]. More than 20% of known Mediterranean marine species are endemic [16], and therefore at risk of global extinction from local extirpation.

As of 2012, 161 marine protected areas have been established in the Mediterranean, covering 4.6% of its surface [17]. Most MPAs are small (66% of Mediterranean MPAs are smaller than 50 Km^2; [17,18]) and concentrated along its northern and western coasts. The only Mediterranean LMPA is the Pelagos Sanctuary for Marine Mammals, which encompasses 87,500 Km^2 [5,19] (Fig. 1) and accounts for 76% of the Mediterranean total protected area (3.5% of the total Mediterranean MPAs surface; [4]). If Pelagos is excluded, only 1% of the Mediterranean Sea surface is actually in MPAs, and less than 0.1% is in fully protected areas that exclude all extractive uses [1,17].

Thus, it is necessary to increase conservation efforts throughout the Mediterranean basin to reach the CBD protection target of 10% in MPAs by 2020 and achieve more effective protection of marine biodiversity and management of multiple marine uses. This goal could be achieved through the establishment of LMPAs.

Among the Mediterranean ecoregions, the Adriatic Sea represents a top priority and opportunity for expanding spatial protection through MPAs [5]. This region has undergone major fisheries overexploitation, causing the widespread degradation of marine habitats, decline of target and non-target species, food-web alterations [6,20–23], and major losses of ecosystem services [24]. The yields of several important commercial fisheries have sharply declined in the last 6-7 decades [25–27]. The basin-scale management of the Adriatic Sea and its resources is challenging because of the presence of a large array of multiple interacting pressures, in addition to fishing [56]. Moreover, marine resource management and ecosystem restoration are also complicated by the exceptional proximity of the various countries bordering the Adriatic Sea, each with their own economic interests and cultural and legal approaches to marine management.

Recognition of these peculiar environmental and geo-political constraints has motivated the development of the European Strategy for the Adriatic Ionian Macroregion (EUSAIR) (http://www.ai-macroregion.eu/), whose objective is to increase cooperation among the countries bordering the Adriatic Sea. The initiative is built upon four main pillars (Table 2), including the quality of marine environment, in line with the ecosystem approach of the CBD. The Adriatic Ionian Macroregion initiative could represent a political opportunity for the establishment of a transboundary LMPA, in particular a no-trawl area, aimed at recovering biodiversity and reversing fisheries losses in the Adriatic Sea. An Adriatic LMPA could promote biological and socioeconomic benefits and effectively address the political and management challenges of large-scale protection of the basin, as reported from other areas of the world’s oceans (Table 1).

This work presents the rationale for, as well as the risks and uncertainties of, establishing a transboundary LMPA in the Adriatic Sea as an option to meet international conservation targets and promote recovery of depleted fish stocks and habitats. First, it is analysed the ecological basis for such an initiative, by assessing key ecosystem services (with a focus on fisheries) that are expected to benefit from the establishment of an Adriatic LMPA. Then it is explored the political opportunities and the national and international legal frameworks that may enable the establishment of a LMPA in the Adriatic Sea. Finally, it is delineated a possible process by which the LMPA may be implemented and identify the remaining challenges, information gaps and uncertainties of this proposed process.

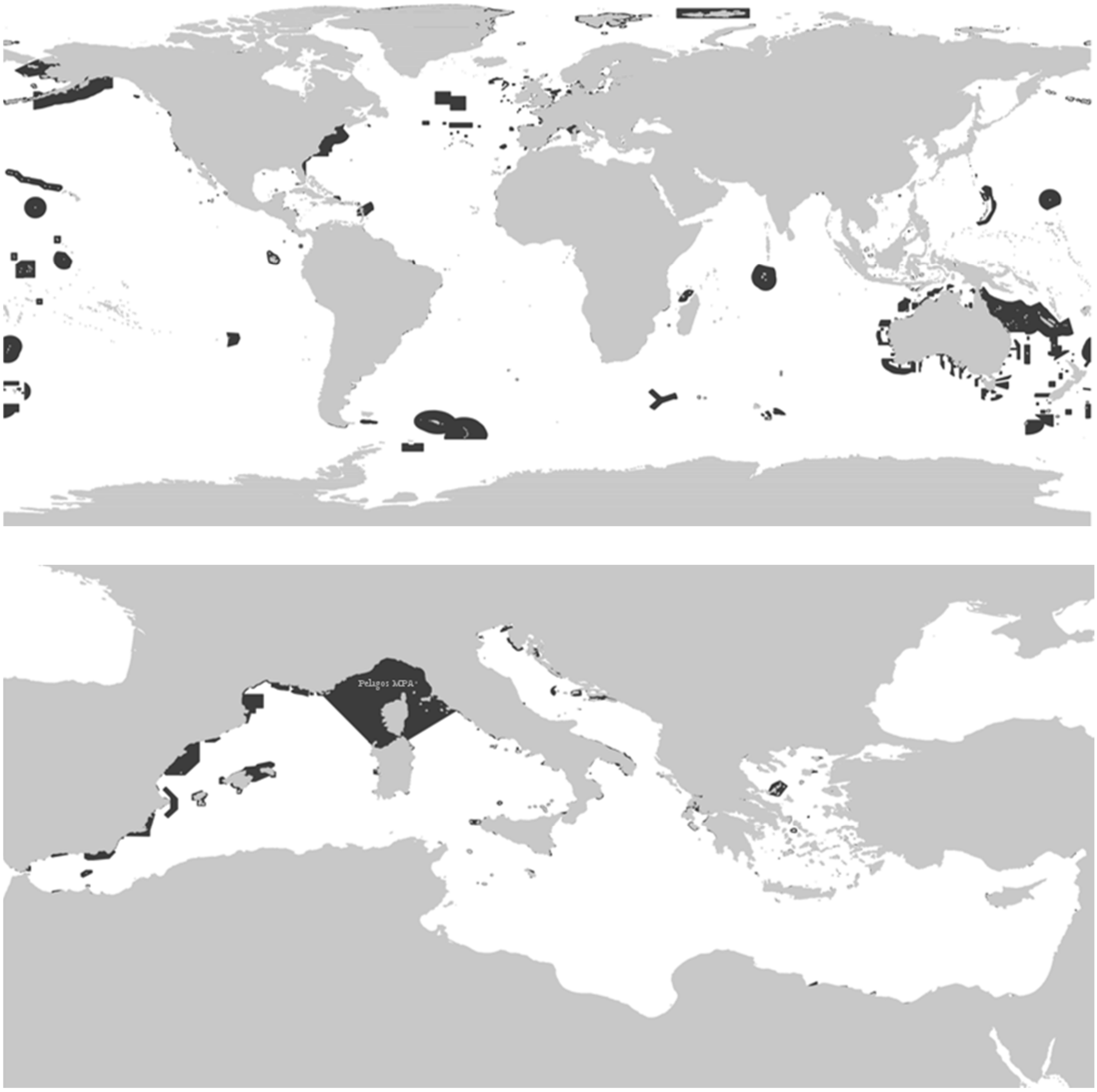

Fig. 1. Existing MPAs and LMPAs. Top: the world LMPAs (black polygons) generated by UNEP World Conservation Monitoring Centre (UNEP WCMC) using data from the World Database on Protected Areas (WDPA). Bottom: Mediterranean MPAs (black polygons), including Pelagos, in the northern Tyrrhenian Sea.

Table 1. Benefits and limitations of large and very large MPAs (LMPAs and VLMPAs).

Benefits
LMPAs may comprise the entire home range of threatened species or overexploited commercial stocks, thereby effectively protecting or recovering these populations [28,29]
By protecting larger portions of the ocean than MPAs, LMPAs ensure connectivity through the dispersal of larvae and early life stages of marine species [30]
LMPAs are effective in protection of migratory species, in addition to sedentary organisms [30–32]
LMPAs have potential economic benefits including enhancement of local fisheries [33], increased sustainable tourism [33,34], and maintenance of ecosystem services [24,33]; LMPAs are expected to provide these benefits and are less expensive per unit area than smaller MPAs [35,36]
LMPAs constitute a mechanism for preventing future overexploitation and degradation of currently remote and near-pristine ecosystems (e.g., Global Ocean Legacy, http://www.pewtrusts.org/en/projects/global-ocean-legacy)
LMPAs allow for expansion of globally protected areas, thereby achieving the conservation targets of international agreements (e.g., CBD) [37–39]
Transboundary LMPAs provide opportunities for international cooperation among States [2,37,40]
Limitations and uncertainties
It is difficult to ensure adequate surveillance and enforcement, and therefore effective protection of LMPAs [30]. New control technologies are needed before LMPAs can become an effective conservation and management tool [41-43].
Reaching agreements between multiple states adds a further layer of complexity in the establishment of LMPAs, if they are transboundary,[44]
Empirical evidence that LMPAs effectively protect exploited populations within their boundaries is still limited [45,46].
Creation of LMPAs redirects fishing effort in other areas that are perhaps less effectively managed than where closure is planned [46]
Because of the large extent of protection, larvae and early life stages will benefit only the area under protection without any benefit for adjacent areas because larval and juvenile export across the MPA boundary is limited [45]
LMPAs are typically established in remote areas and may take resources and political support away from areas where protection is most urgently needed (e.g., densely populated coastal areas) and may provide economic benefits [47,48]
LMPAs can be established only in remote and unpopulated areas where marine ecosystems are in the least need of protection [43]

Table 2. Pillars and topics of the European Strategy for the Adriatic Ionian Macroregion (EUSAIR) initiative (http://www.adriatic-ionian.eu/about/pillars)

	TOPICS
PILLAR 1: BLUE GROWTH	1) Promote research and development of blue technologies 2) Promote sustainable seafood production and consumption (fishery and aquaculture) 3) Improve maritime and marine governance and services
PILLAR 2: CONNECTING THE REGION	1) Strengthen maritime safety and security (maritime transport) 2) Intermodal connections to the hinterland 3) Energy networks
PILLAR 3: ENVIRONMENTAL QUALITY	1) Reach Good Environmental Status (GES) by 2020, halt the loss of biodiversity and degradation of the ecosystem services and restore them (marine environment) 2) Transnational terrestrial habitats and biodiversity
PILLAR 4: SUSTAINABLE TOURISM	1) Diversified tourism offer (products and services) 2) Sustainable and responsible tourism management (innovation and quality)

2. The Adriatic Sea: needs and opportunities for large-scale protection

2.1 *Marine biodiversity and economies under threat*

The Adriatic Sea (Fig. 2) covers 5% (138,600 Km^2) of the total area of the Mediterranean and 1% (35,000 km^3) of its total volume. It is one of the most productive areas of the Mediterranean Sea, supporting a wide diversity of habitats, including rocky and extensive soft bottoms, large estuaries and lagoons, seagrass meadows, and deep water environments [49–51]. This richness of habitats is mirrored by a high level of biodiversity, with high species richness of marine invertebrates, seabirds, marine mammals [4,6], and 18% of the endemic fish species of the Mediterranean [15,23].

High ecosystem productivity and extensive shallow and sandy Adriatic bottoms in its northern and central areas, which provide easy access to fishing grounds, has historically created favourable conditions for intense exploitation. The Adriatic Sea has been exploited for centuries by a variety of fishing activities, ranging from small-scale artisanal fisheries and recreational fishing, to industrial fisheries using hydraulic and trawled dredges for clams and scallops, otter and mid-water trawling for exploiting ground and small pelagic fishes, and pelagic long-lines for tuna [6,25,52–54]. In 2013, there were 3,590 trawlers (dredges, demersal and beam trawlers) fishing in the Adriatic. Of these, 3,105 were Italian, while 485 (mainly smaller than 12 m) where from Slovenia and Croatia (http://stecf.jrc.ec.europa.eu/data-reports). Adriatic fisheries account for 51% of the total capture fish production (landings) in Italy, and 40% of its total value [55]. The main exploited stocks by the Italian fleet are small pelagics such as anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) respectively contributing to 22% and 10% of Italian total landings. European hake (*Merluccius merluccius*) and red mullet (*Mullus barbatus*) are the most important demersal species fished, and together with Norway lobster (*Nephrops norvegicus*), are the next most landed species (together they represent around 4-5% of total landings) [56].

The Adriatic marine ecosystems and the services they provide are affected by a suite of natural and anthropogenic threats [4,57], which have resulted in historical species declines, food web changes, extensive ecosystem degradation, and, more recently, severe regime shifts [6,20,22,24,58–60]. Among all human activities and pressures on Mediterranean marine ecosystems, fisheries exploitation, in particularly bottom trawling and dredging, has been identified as the major threat [7,57,61]. Impacts of fishing are compounded and exacerbated by other stressors and pressures. Eutrophication, for example, is an important stressor in the north and western Adriatic sectors, which are influenced by high nutrient discharge from the Po River (mean flow rates of 1496 m^3 s^{-1} in the period 1917-2008 [62]). Nutrient input combined with alteration in water circulation have caused hypoxia and anoxia events, resulting in episodic mortalities of the Adriatic benthos [60,63,64]. Maritime traffic is also very intense inside the Adriatic basin, causing a significant risk of accidents and spills of oil and other contaminants [65,66]. Finally, climate change is also expected to significantly affect these ecosystems [6,67]. While LMPAs regulating fishing cannot directly address these additional pressures, they are expected to increase population and ecosystem resilience by decreasing cumulative impact and recovering diversity and functional redundancy [68–70].

2.2 *Existing MPAs and other forms of spatial marine management*

Currently, there are 15 coastal MPAs (including all coastal protected areas with a marine component, Table S2 in Suppl. materials) in the Adriatic Sea, altogether covering less than 1% of its surface. Four additional MPAs are planned: two in Albania (Kepi i Rodonit and Porto Palermo) [65], and two in central Italy (Costa del Monte Conero and Costa del Piceno) (http://www.minambiente.it/pagina/aree-marine-di-prossima-istituzione).

The current siting of Adriatic MPAs is not homogeneously distributed: 12 of the 15 existing MPAs are along the eastern coast of the basin, 7 of these in Croatia (Fig.2). Adriatic MPAs are also widely heterogeneous in their regime of legal protection. There are national parks (Briuni, Croatia), nature reserves (Miramare, Italy) and natural monuments (Debeli rtič, Slovenia) (see the glossary for their definitions).

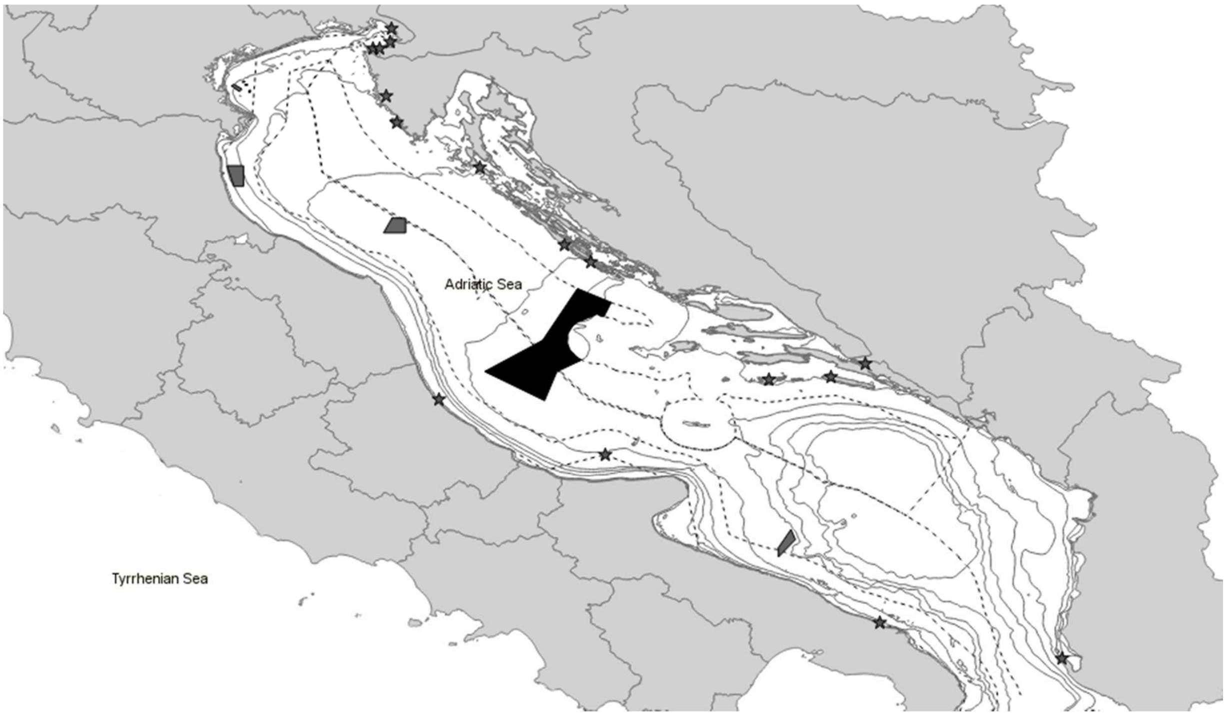

Fig.2. Protection of the marine environment in the Adriatic Sea. The picture shows the current distribution of Adriatic MPAs (black stars), of the temporal closure areas (grey polygons) and the temporary no-trawl areas of Jabuka-Pomo pits (the big black polygon). The dashed lines show the territorial sea boundaries of Italy and Croatia and the limit of the continental shelf.

Outside of the 12 mile national limits of national territorial waters, there is no permanent spatial protection and management of human activities. Current, management of Adriatic trawl fisheries calls for temporal closures (i.e. closing sectors to fishing for a few months, seasons or years; Tremiti Island, Tenue areas, Miramare, area off Ravenna, the area around the Barbara gas platform, a no-trawl area off Apulia region, and the Jabuka-Pomo pits [79], see below). Seasonal closures (e.g. for 30-45 days, as commonly done in Italy) may be too short to influence and recover demersal and benthic species with long-term life cycles [71]. Fouzai et al. [23] modelled the efficacy of different fisheries' management strategies applied in the Adriatic from 1975 to 2020, including the limitation of the number of fishing licenses, the introduction of closed fishing seasons, the establishment of spatial and temporal closures for all or a subset of fishing gears (e.g. seasonal closure of mid-water and bottom trawl fisheries). Their results demonstrate that past fisheries management has not been effective in ensuring a sustainable use of marine resources, and that the current management regime is not expected to promote the recovery of depleted fish stocks unless is augmented with new options, including the establishment of MPAs and an overall reduction of fishing effort [23].

Areas outside territorial waters include important and sensitive fishery areas [72,73]. The Jabuka-Pomo pits (240 m of maximum depth), for example, are nursery areas for some of the most economically important fished Adriatic species, such as the European hake, *Merluccius merluccius*, and the Norway lobster *Nephrops norvegicus* (L.) [74–76], and, as such, their bottoms are regularly and intensively trawled [77].

Due to the importance of this area, there is an ongoing debate on the need to establish here a permanent no-trawl area [73,78]. In July 26th 2015, a temporary no-trawl area covering approx. 2,700 Km^2 was established in the international waters of the Jabuka-Pomo pits to promote the recovery of *M. merluccius* and *N. norvegicus* [79] (Fig. 2). The closure is planned for only one year and it includes a fraction of the nursery and spawning areas identified for these and other commercially important species [76].

3. Ecological, economic and political benefits of no-trawl areas

3.1 *Benefits for target stocks and fisheries*

Empirical assessments of the effects of large no-trawl zones from other oceanic sectors, particularly the few established in intensely exploited regions, are valuable references for anticipating the benefits of establishing no-trawl zone in the Adriatic (Table 3). Several studies report evidence that limiting or banning bottom trawling from large areas can provide some of the positive economic and biological effects expected for LMPAs (Table 1), as well as additional benefits specific to benthic and demersal communities. In particular, no-trawl zones are expected to promote recovery of depleted target populations and benefit adjacent fishing grounds and fisheries through larval, juvenile and adult spillover. In addition, no-trawl zones are expected to increase diversity of benthic and demersal assemblages, and increase complexity of benthic habitats, thereby restoring processes and interactions lost in intensely fished marine ecosystems [80–82].

In the Gulf of Castellammare, a previously intensely-trawled area off Sicily [83], a significant increase in catch of eleven target species (9 finfish and 2 cephalopods) has been reported after only a 4-years trawl ban covering approx. 200 Km^2. The trawl ban promoted recovery of benthic habitats and increased habitat complexity. Areas with no trawling show structured three-dimensional benthic communities [84] with higher diversity and density of invertebrates (e.g. sponges and seapens) than heavily trawled grounds [85,86]. The recovery of large epifauna, such as sponges, hydroids, bryozoan, and tube-dwelling polychaetes in undisturbed areas results in complex biogenic habitats that provide refuges and food to benthic and neritic species, including larval and juvenile stages that are key to the recovery of over-exploited species [87]. Similar positive effects on benthic diversity and habitat complexity were observed at lightly or no-trawling in, e.g., eastern Florida, USA (Table 3). Some of these benthic species are prey of demersal and pelagic species [88], highlighting the importance of an ecosystem approach to the management of fisheries, including the protection of habitat and food-web interactions.

The fishing closures on the Georges Bank, USA, are other examples of the beneficial effects of large no-trawl areas. The Georges Bank is a shallow, mainly sandy submarine plateau with high level of primary productivity that has been one of the most important fishing grounds of the North West Atlantic. The increase of fishing effort since 1960 caused the decline of over 50% of total fish biomass [89]. After the collapse of cod stocks in the 1990s, five large areas, together covering 22,000 Km^2, were year-round closed to all gears targeting groundfish, including bottom trawlers and scallop dredges [90]. Monitoring of these no-trawl areas highlighted the importance of long-term spatial closures for meeting the multiple management objectives of recovering depleted fisheries, protecting benthic habitats, and restoring ecosystem structure and function. Protection of spawning stocks and juvenile haddock and cod has contributed to increase the abundance of these populations [31,89]. Scientific trawl surveys demonstrated that in four years since the closure (between 1994 and 1998), scallop total biomass and harvestable biomass increased by a factor of 14 and 15 respectively in the closed areas, and catches outside the closure boundaries increased through larval spillover from the no-trawl zones [31].

In addition to examples of recovery of individual size and abundance of fished stocks within the no/lightly trawled areas, and increased catches in adjacent fishing grounds, trawl bans can results in economic benefits through increased value of the catch (Table 3). In the Gulf of Castellamare no-trawl area, fishes reached larger sizes compared to adjacent fished areas, resulting in greater commercial value and increased reproductive output [91,92]. Increased catch of high value species, such as scallop, shrimp and crab has resulted in an increase of income for the Georges Banks fisheries [31,93]. Benefits can, in some cases, be repaid by other sectors and users groups. For example, a trawl ban off the coast of Java has resulted in more jobs for small-scale fishers [94].

Spatial gradients in fishing pressure across the Adriatic basin provide an opportunity to examine the impacts of trawling and make predictions about the possible future effects of its spatial management in areas that are currently intensely fished [22]. Along the western (Italian) Adriatic coasts, fishing effort has historically been greater whereas along the eastern (Croatian) side, fisheries have developed more slowly and in a less industrialized fashion. As an effect of this historical fishing pressure, in the last 60 years, catch rates and landings of elasmobranchs, declined by >94% and 80-89% respectively in the central and northern Adriatic Sea [22,95]. However, these declines were not homogeneous throughout the basin. Comparing catch rates between the two sides of the basin, Ferretti et al. (2013) found that a greater species richness and abundance of sharks and rays persisted on the eastern side of the Adriatic, reflecting the less intense fishing pressure on the Croatian side both historically and recently. Thus, the Croatian side acted as a refuge from intense fishing pressure. Data also indicate that mobile species like spurdogs, eagle rays and smooth-hounds that maintained higher abundances on the eastern side may have replenished the more intensely exploited western side and supported catches within this region [22].

Table 3. Examples of documented biological and economic effects of no-trawl zones.

Location	**Size of closure**	**Year of establishment**	**Type of management**	**Documented effects on fisheries**	**Effects on habitat/community**	**Economic effects**	**Reference**
Georges Bank and in Southern New England	22,000 Km^2	1994	Trawl ban in the 3 areas and complementary fishery regulation in the waters outside the closed areas (reduced effort, trip limits and increased mesh size)	- After about 4 years of closure, increase of spawning-stock biomass of cod, haddock, yellowtail flounder and other components of groundfish community; - Increase of total biomass of scallops; - Reduction in fishing mortality of the stocks	- Higher abundance of organisms, biomass and species diversity; - More complex habitat in undisturbed areas, formed by higher presence of fragile epifauna organisms (tube-dwelling polychaetes, bryozoans, hydroids)		[31,88]
Gulf of Castellammare (NW Sicily). It is an inshore area	200 Km^2	1990	Trawl ban	- Increase in biomass of eleven target demersal species (9 finfish and 2 cephalopods); - Increase in total catches	Not reported	Improved financial returns for the artisanal fishermen	[83,96]
Malacca Straits and the north coast of Java	Not reported	1980	Trawl ban	- Increase of demersal stocks	Not reported	Increase in the employment of the small-scale fishery	[94]
Eastern Florida (Deep-water *Oculina* coral reefs)	315 Km^2, expanded to 1029 km^2	first ban in 1984; expansion in 2000	Ban of trawling, dredging, bottom longlines and anchoring	- Fish populations have yet to recover from overfishing in the 1980s and 1990s	Healthier coral communities in no-trawl areas compared to areas where fishing is still present		[97]
Isle of Man	Nearly 2 Km^2	1989	Trawl ban	- The density of scallops above the minimum legal landing size (110 mm SL) was more than 7 times higher in the closed area than in the fished area by 2003; - Shift towards much older and larger scallops in the			[98]

				closed area and, lower estimates of total mortality			
Great Barrier Reef Marine Park (GBRMP)	≈33% of the area of the Marine Park (that covers a total area of about 344,400 Km^2)	2004	Trawling prohibited, large mesh gill netting allowed	- Increase mean size and abundance of fish (e.g. coral trout and stripey seaperch (*Lutjanus carponotatus*)) and reef sharks; - Provide ecosystem-wide larval supply - >20% predicted increase of biomass of seabed species	- Decrease in the frequency of starfish outbreaks, with positive effects on coral populations; - Increased abundance of corals	economic value of a healthy GBR to Australia is estimated to be about A$5.5 billion annually; 53,800 full time jobs	[99]

3.2 *Expected benefits for ecosystem functions and services*

Recovery of marine ecosystems from trawling impacts may provide additional benefits, beyond fisheries. The importance of soft bottom habitats, the most widespread bottom type in the Adriatic, is increasingly recognised [100]. Soft bottom benthos play important ecosystem functions such as controlling eutrophication and algal blooms by filtering large water volumes and stabilizing sediments [101–104]. Soft-bottom macrofauna can have profound influences on organic matter deposited on marine sediments [105]. Large-bodied species, such as sea urchins, influence sediment biogeochemistry through their burrowing activity [102]. Bioturbation, the disturbance of sedimentary deposits by living organisms, has a major influence on column fluxes of nutrients and oxygen between sediments and the water column by increasing nutrient remineralization [100,102].

Benthic filter feeders improve water quality through water filtration. They are involved in benthic-pelagic coupling, the cycling of nutrients between sediments and the overlaying water column [106], nutrient regeneration [103], and facilitation of surrounding communities by providing refuges from predation [107]. Bivalve filtration, in particular, can have a fundamental role in controlling phytoplankton communities and water quality. The filtering activity of bivalves and other filter feeders can control phytoplankton abundance, reducing algal blooms and consequent anoxic or hypoxic events [108]. For example, it was calculated that in oyster beds of Chesapeake Bay, USA, around 188,000 tons (dry tissue) of the oyster *Crassostrea virginica* would filter the whole water volume of the bay (around 70 x 10^9 m^3) in less than 1 week. The depletion of these oyster populations and reefs that have occurred from the beginning of 19th have caused an estimated 50-fold decrease in filtering activity [101,108]. These results raise the question of whether the once abundant population of filters feeders in the Adriatic may have contributed likewise to maintain its water quality.

The recent dramatic reduction in the Adriatic Sea of filter feeding organisms, such as clams [27], oysters [109], and sponges [110] due to intense fishing, habitat loss from bottom trawling, dredging, hypoxia, and climate change may have produced a functional loss similar to that documented in Chesapeake Bay. The disappearance or decrease of ecologically important filter feeders may in turn have led to dramatic ecosystem shifts [111], e.g. favouring the outbreaks of gelatinous plankton that impacts the communities in the water column by removing zooplankton and fish larvae [111]. The overexploitation of the north Adriatic *Ostrea edulis* reefs, whose landings decreased from ca. 57 t in 2002 to 1.5 t in 2012 [54], eventually caused their local extinction [112] and the consequent loss of their filtration efficiency in the basin, ultimately resulting in an ecological extinction (i.e., loss of their ecological function [113]). This decline, however, may be the tail end of a much larger historical reduction of any oyster species in the region. Lotze et al. (2006), estimated a reduction of oysters of around 90% since Roman time [114].

Another bivalve that is currently present and harvested in the Adriatic is the clam *Chamelea gallina.* Based on laboratory estimates the filtration rate *of C. gallina* is 0.42 L h^{-1} [115] (a lower value than those measured for *Ostrea edulis* - 2.83 L h^{-1} g^{-1} [116] - and sponges - 1 - 6 L h^{-1} [117]). It is estimated that, to theoretically filter the entire Adriatic water volume, the currently depleted population of *C. gallina* would take 7 years longer than the clam population present in the 1950s (Bastari, unpublished data). Trawling and dredging have radically altered many epibenthic communities of the central and northern Adriatic Sea [118]. The consequences of this change in terms of filtration capacity have never been considered, but are expected to be important. Field and laboratory measurements, modelling, and historical reconstructions of ecosystem change are needed to assess and quantify changes in ecosystem function and services due to bottom trawling, and eventually make predictions about what might be recovered in LMPAs.

3.3 *Expected economic and political benefits*

A suite of marine sectors supports the marine economies of the Adriatic region. The most valuable sectors are aquaculture (250 million euros in 2012), coastal and maritime tourism (8 million euros), transport (5.2 million euros), fisheries (2.9 million euros), and offshore oil and gas activities (2.2 million euros). In terms of employment, tourism (198,760 jobs) is the most important sector, followed by fisheries (95,420 jobs), transport (55,860 jobs), offshore oil and gas (5,970 jobs) and aquaculture (4,030 jobs) [119].

The Adriatic region, producing an estimated 1 billion night accommodations annually [120], is one of the most visited sectors of the Mediterranean for tourism. In Italy, Adriatic tourism is worth almost three times the value generated by fisheries [119]. The environmental status of the sea is one of the most important factors influencing tourists' choices to vacation in Croatia [120]. A trans-boundary Adriatic LMPA could thus provide new opportunities to develop new economic sectors. An example could be well-regulated fishing activities where tourists can conduct a limited amount of fishing within the protected area for immediate consumption (charter fishing trips) with economic benefits and job opportunities for coastal communities in all neighbouring countries (transportation, accommodations, meals) [121,122]. In California, USA, recreational fishing within the four marine sanctuaries of the state generated more than $200 million in annual economic output and supported nearly 1400 jobs (http://sanctuaries.noaa.gov/news/press/2015/rec-fishing-california.html). Marine mammals, such as dolphins, whales, seals, as well as sea turtles and sharks, were abundant in the Adriatic basin in the past [6,22]. A no-trawl zone, by recovering prey species and food webs, could promote recovery of these top predators, which are important attractions for tourists interested in nature-related activities. It has been calculated that more than $300 million have been expended per year by shark-watchers, supporting 10,000 jobs [123].

There are also some potential political benefits derived from the creation of a shared no-trawl area [40,124]. A transboundary no-trawl area is expected to produce international cooperation between bordering countries, and may simplify the definition of their maritime boundaries (Table 1). A clear delimitation of marine regions will define responsibilities of each country in the management and surveillance of their areas of jurisdiction [124]. LMPAs in general could act as ‘peace parks’ [44] and create an important dialogue between states [40].

The third pillar of the Adriatic Ionian Macroregion EU Strategy for the Adriatic and Ionian Region (EUSAIR) focuses on environmental quality. One of the main goals is to reach a good environmental and ecological status of marine ecosystems, as also requested by the Marine Strategy Framework Directive (2008/56/EC). A trans-boundary no-trawl area would help to tackle the following ecological targets of GES: (i) the maintenance of sea-floor integrity, by restoring benthic communities and preserving sensitive species and their ecological functions; (ii) the maintenance of biological diversity, by the conservation of the habitats and species of the sandy bottoms; (iii) ensure the long-term abundance of species and all the food webs elements.

4. Political opportunities and legal mechanisms for establishing a transboundary no-trawl area in the Adriatic Sea

There are historical precedents, current political opportunities and legal instruments for establishing a trans-boundary LMPA in the Adriatic Sea. In 2003, Croatia proposed the establishment of a 23,870 Km^2 Ecological and Fisheries Protection Zone (EFPZ) for marine biodiversity and fisheries conservation [125]. The EFPZ was approved by the Croatian government and enforced in January 2008. However, due to harsh opposition by Italy and Slovenia, fisheries restrictions within this area were applied only to non-European fleets [126]. In 2013, Croatia became a new member of the European Union. This membership has produced new opportunities to start negotiations for the establishment of an Adriatic transboundary LMPA between the two main Adriatic countries (Italy and Croatia) that could co-manage the area. The former European Commission's President Jose' Manuel Barroso has declared that the EU is willing to consider a special protection zone in the middle of the Adriatic [125], thereby demonstrating the Commission's intention to expand marine protection in this region. The EU is also currently funding a plethora of scientific projects on spatial planning within the Adriatic Sea [127–131], further highlighting the EU current effort to support spatial management aimed at promoting the recovery of Adriatic marine resources and economic sectors, and reduce conflicts among user groups.

Despite difficulties in international dialogue and possible stakeholder conflicts (Table 1), international laws provide the legal authority and support for establishing a transboundary LMPA in the Adriatic Sea that would protect its ecosystems and marine resources from bottom trawling, the main driver of their degradation [6,22,57]. The Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (namely, the Barcelona Convention), adopted in 1995 by all Mediterranean States, mandates the selection of *Specially Protected Areas of Mediterranean Importance* (SPAMIs) by each nation. In addition, more recently a list of Ecologically or Biologically Significant Marine Areas (EBSAs), including several of the existing SPAMIs, was identified and approved at the Extraordinary Meeting of the Focal Points for Specially Protected Areas (UNEP(DEPI)MED WG. 348/5 June 2010). The scientific criteria for the identification of the EBSAs, defined by the ninth Conference of the Parties to the CBD, are based on areas' uniqueness or rarity, special importance for life-history stages of species, importance for threatened, endangered or declining species and/or habitats, vulnerability, fragility, sensibility or slow recovery potential, biological productivity, biological diversity and naturalness [132]. The definition of the EBSAs has led to the identification of areas in offshore pelagic and deep-sea habitats in need of protection that are not included in established MPAs. The EBSAs have been endorsed by all the contracting parties of the Barcelona Convention [133].

In the Adriatic Sea, EBSAs have been identified in the northern, central and southern basins (Fig. 3), making these areas priorities for improved marine management and conservation [134]. The EBSA in the northern Adriatic basin, encompassing the area above the straight line linking Ancona (Italy) and the island of Ilovik (Croatia), was selected because of its high productivity, richness of benthic habitats, and the presence of breeding or feeding areas for dolphin and turtle populations. The central area encompasses the Jabuka-Pomo pits, but it is larger than the temporary no-trawl zone established in the Jabuka-Pomo pits in July 2015 (see above). Finally, the southern Adriatic-Ionian EBSA comprises particular features such as cold-water corals and sponge gardens [51,135,136].

Adriatic areas outside of the current EBSAs were identified as priorities for conservation by a suite of analyses and conservation plans [2]. Selection was based on information on the distribution of critical benthic habitats, importance to marine mammals and seabirds, and current distribution and intensity of threats to these ecosystem components [2]. Depending on the specific goals, data, and approaches, different areas were selected as top priorities, though areas within the central Adriatic emerge as priorities in most plans [2]. Systematic analyses, e.g. utilizing GIS approaches and MPA site selection algorithms, are needed to objectively and transparently identify candidate areas for the establishment of one or multiple no-trawl areas in the Adriatic, addressing and balancing different objectives and goals. The priority areas should include the protection of nursery habitats for demersal fish species, spawning areas for small pelagic, biogenic habitats, such as sponge and hydroid beds, supporting high biodiversity, and foraging areas for marine mammals, sea turtles and birds. The large amounts of data available for this region and numerous previous threat analyses and conservation planning exercises [5,57,76,78,129,137] provide a unique opportunity to inform the siting and configuration of a no-trawl area with sound scientific information.

Organizations such as the International Maritime Organization (IMO), or the General Fisheries Commission for the Mediterranean (GFCM) have specific mandates to regulate human uses of deep or offshore habitats, selected through the EBSAs process or independently, by NGOs or other stakeholders. In particular, the GFCM has the authority to adopt spatial management measures to effectively manage fisheries. In this role, GFCM has already declared four Fishery Restricted Areas (FRAs) covering a total area of 26,248 Km^2 or 0.15% of the Mediterranean Sea surface: the *Lophelia* reef off Capo Santa Maria di Leuca; the Nile delta area; the Eratosthenes Seamount; the Gulf of Lion. In these areas, the use of towed dredges and bottom trawl nets is prohibited. Following the GFCM's FRA protocol, a LMPA banning trawling could be established in the Adriatic Sea as well. Finally, the European Union has legal responsibility for fishery management in European waters. A no-trawl zone could also be implemented based on emergency measures foreseen by the Common Fishery Policy (CFP) [37]. Subsequent to the establishment of a FRA or a fish recovery area under the CFP, additional ecosystems conservation measures could be implemented through the establishment of SPAMI areas, thereby expanding regulation of activities from fishing, as in a FRA, to other marine uses.

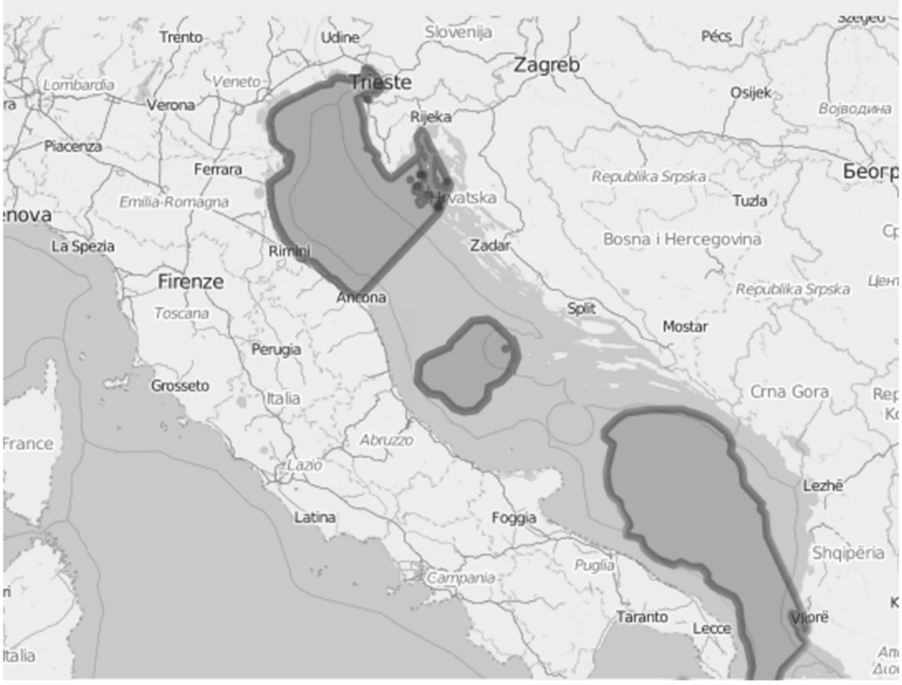

Fig.3. Adriatic Sea EBSAs. The delimited polygons show the proposed EBSAs of the Adriatic Sea (as reported by https://www.cbd.int/ebsa/)

5. Remaining challenges: governance and compliance

Establishing effective MPAs, including LMPAs, requires clear management objectives and the involvement and support of marine users, in particular fishers who generally are the category most directly affected by MPA establishment. The experience with the Pelagos Sanctuary (established in 1999 and entered into force on 2002 as an agreement between Italy, France and Monaco in the northern Tyrrhenian Sea to protect cetaceans, Fig. 1) [19] highlights the importance of setting processes and necessary resources for enforcement of regulations and stakeholder involvement [138]. In fact, twenty-four years after its establishment, Pelagos still lacks a management plan, systematic monitoring or enforcement measures, and there is no evidence that human threats to marine mammals have decreased within the area [139]. Cetaceans living in the sanctuary are toxicologically stressed, and still affected by fishing and potentially harmful military activities [138,140]. The absence of a governance and enforcement body are major reasons for Pelagos' lack of efficacy as a cetacean sanctuary [138].

Governance of a transboundary LMPA presents unique challenges. The Adriatic-Ionian Macroregion initiative provides a robust political and economic platform to promote cooperation among Adriatic countries in managing marine uses, and could represent the shared governance body for an Adriatic LMPA. The already existing Adriatic Protected Areas Network (AdriaPAN) represents an encouraging precedent of the willingness to develop a collaborative strategy between MPAs in the Adriatic region. This operational network of MPAs may serve as a facilitator of the process towards a shared governance body for an offshore Adriatic LMPA.

Monitoring and enforcement of regulations within large offshore areas require high economic investment [41,43]. New technologies and tools that are currently being developed and tested, such as the use of satellite imagery to counteract illegal fishing [141], will provide new opportunities for real-time, cost-effective surveillance and control of human activities in LMPAs. Involvement and participation of stakeholders, such as fishers, tourism operators, and supporters of the LMPA could or facilitate enforcement and foster compliance [142].

6. Conclusion

The establishment of a no-trawl area in the Adriatic Sea, possibly in the form of a Fisheries Restricted Area, could provide an unprecedented opportunity to promote the recovery of degraded Mediterranean marine ecosystems and fisheries, and to meet international commitments to expand marine conservation and improve fisheries management. We foster that the recognised limitations described for several LMPAs already established around the world could be overcome in the Adriatic basin. The available scientific knowledge identifies the central basin as representative of the main features of the whole Adriatic Sea including both ecological and biological important areas and overexploited bottoms. One of the main obstacles for the establishment of the no-trawl LMPA may derive from the different objectives and priorities of resource use and management of its bordering nations (most importantly Italy and Croatia). However, the critical status of the Adriatic marine resources and ecosystems, the inefficient results of their current management, altogether highlight the need of implementing new and urgent conservation actions. The EUSAIR process will provide a robust framework for the development of political, management and governance collaboration needed to establish an efficient transboundary LMPA in the Adriatic Sea. An Adriatic no-trawl area could benefit multiple marine users and economic sectors, in addition to fisheries. If successful, we foresee that the Adriatic process would provide an important precedent and a model for scaling up protection in other intensely used marine regions worldwide.

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Supplementary materials

Table S1. Very Large MPAs (VLMPAs) established to date in the world.

Name	Type of protection	Type of protection	Extension (Km²)	Year of establishment	Aim of protection	Environment
Galapagos Marine Reserve	Marine Reserve	Galapagos Islands; eastern Pacific Ocean	133,000	1998	Created to protect biodiversity of the islands and surrounding waters	Underwater volcanoes, seamounts, reefs, underwater cliffs, wetlands, and lagoons
Papahānaumokuākea Marine National Monument	MPA	Northern Hawaiian Islands	362,073	2006	Created to protect natural resources and Native Hawaiian culture	Islands and shallow water environments
Benthic protection areas	Ban of bottom trawling	New Zealand	1,200,000	2007	Created to protect seabed habitats	17 sites hosting seamounts and hydrothermal vents
Phoenix Island Protected Area (PIPA)	MPA	Central Pacific Ocean	408,250	2008	Created to maintain PIPA as a pristine set of islands	8 atoll and low reef islands. It comprises ocean floor and water column

						(average of 4,000 m deep, and maximum at 6,147 m)
Marianas Trench	Marine National Monument (MPA)	East Philippines	246,608	2009	Created to protect deep see habitats	Trench bottom, 21 undersea volcanoes, waters and submerged lands around 3 of the northern Mariana Islands
US Pacific Remote Island	Marine National Monument	Pacific Ocean	210,000	2009	Created to protect one wildlife and habitats	7 atolls and islands. It comprises coral reef, seabird, and shorebird
Sala y Gómez	No-take Marine Reserve	Chile	150,000	2010	Created to protect the undisturbed and relatively pristine area	The island and seamounts
Chagos Island	No-take marine reserve	Indian Ocean	640,000	2010	Created to safeguards diversity of marine life of the area	Deep water habitat and coral reefs

North-East Atlantic network of six high seas MPAs	Network of marine protected areas on the high seas	North-East Atlantic	286,200	2010	Created to establish an ecologically coherent network of well-managed MPAs	seamounts and sections of the Mid-Atlantic
South Georgia and South Sandwich Islands	MPA	Southern Ocean	1,070,000	2012	Created to protect marine life and allowing sustainable and regulated fisheries	Banks and troughs of South Georgia, bays, beaches and trench of the volcanic South Sandwich Islands
Coral Sea	No-take zone	Australia	989,842	2012	Created to protect a remote ocean ecosystem	Habitats for a range of species such as humpback whales, green turtles, white sharks, whale sharks and tuna. The East Australian Current forms here, which is a major pathway for

						mobile predators
Prince Edwards Islands	MPA	Southern Indian Ocean (South Africa territory)	180,000	Managed as a special nature reserve under since 1995. MPA since 2013	Created to protect offshore and deep ocean areas. It is the first South African offshore MPA	3 types of zones: A 12 nm sanctuary (no take) zone; 4 restricted zones, in which fishing effort is limited; A controlled zone, linking the 4 restricted areas.
Pacific Remote Islands Marine National Monument	Marine sanctuary	Central Pacific	1,271,500	2014	Create to protect biodiversity and to fight illegal fishing and seafood fraud	7 islands and reefs as well as the ocean around them

Table S2. List of Adriatic MPAs.

Name	Type of protection	Locality	Extension (Km²)	Year of establishment	Aim of protection	Environment
Brijuni	National Park	Croatia	33.95 sea area: 26.51	1999	Created to protect the high biodiversity	Brujuni island and surrounding sea
Kornati	National Park	Croatia	217.00 sea area: 167.00	1980	Created to protect biodiversity	Highly preserved communities. *Posidonia oceanica* meadows
Lastovo	Nature Park	Croatia	53.00 sea area: 143.12	2006	Created to preserve landscape and marine biodiversity	44 islands, islets, rocks and reefs
Limski Zaljev	Special Marine Reserve	Croatia	6.00	1970	Created to preserve biodiversity	
Malostonski Zaljev	Special Marine Reserve	Croatia	48.21	1983	Created to preserve biodiversity	
Mljet	National Park	Croatia	46.19 sea area: 15.19	1960	Created to preserve landscape and marine biodiversity	A portion of Mljet island. Forests and salt lakes
Telascica	Nature Park	Croatia	67.06 sea area: 39.72	1988	Created to preserve landscape	25 small bays and salt lakes

					and marine biodiversity	
Cres-Losinji	Special Nature Reserve	Croatia	525.76 sea area: 523.35	2006	Created to protect the bottlenose dolphin	Habitats and species found within the area
Miramare	Nature Reserve	Italy	0.30	1986	Conservation of species	Rocky bottoms, pebbly and sandy up to 8 m depth; mud bottoms up to 18 m
Torre del Cerrano	MPA	Italy	37.00	2009	Protection and valorisation of environment	Sandy bottoms and small rocky outcrops
Tremiti Islands	Nature Reserve	Italy	14.66	1989	Created to preserve landscape and marine biodiversity	Tremiti archipelago and its boundaries area
Torre Guaceto	Nature Reserve	Italy	22.27	1991	Created to preserve landscape and marine biodiversity	*Posidonia oceanica* meadows; coralligenous habitat
Rt Madona	Natural Monument	Slovenia	0.13	1990	Created to preserve landscape and marine biodiversity	Hard stone bottom, with scattered larger or smaller slabs

						of rock and monoliths
Debeli rtič	Natural Monument	Slovenia	0.25	1991	Created to preserve landscape and marine biodiversity	Representative cliffs on Slovenian coast; salt meadow
Strunjan	Nature Reserve	Slovenia	4.30	2004	Created to preserve landscape and marine biodiversity	Representative cliffs on Slovenian coast and the only two lagoons of Slovenia
Karaburuni Peninsula	National Marine Park	Albania	125.70	2010	Created to protect high biodiversity and natural habitats	Vlora Bay, the Peninsula of Karaburun and the Island of Sazani