Mesophotic Animal Forests of the Ligurian Sea (NW Mediterranean Sea): biodiversity, distribution and vulnerability

Francesco Enrichetti

Genova 2019

PhD Thesis

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Mesophotic Animal Forests of the Ligurian Sea (NW Mediterranean Sea): biodiversity, distribution and vulnerability

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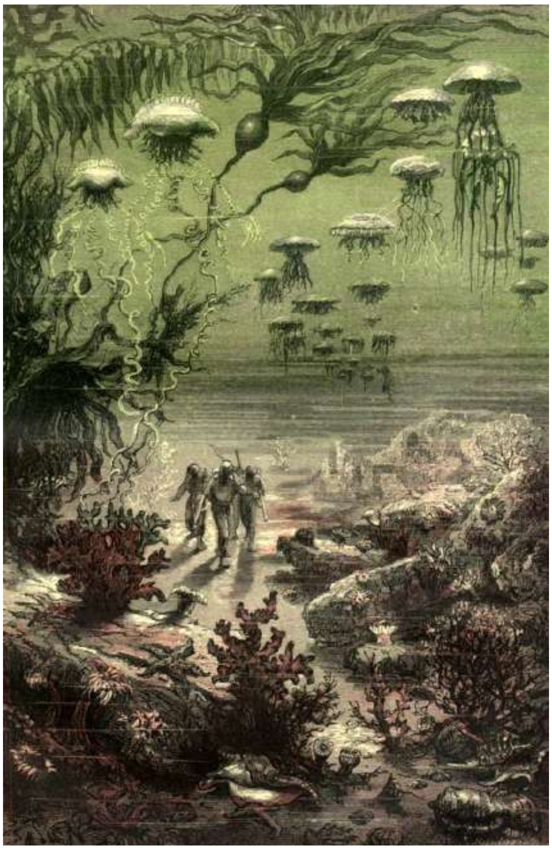
*Cover: Ligurian mesophotic animal forests with gorgonians (*Eunicella verrucosa*) and* Spongia (Spongia) lamella*. Photo by Simonepietro Canese (ISPRA).*



Mais, pendant quelques minutes,
je confondis involontairement les règnes entre eux,
prenant des zoophytes pour des hydrophytes,
des animaux pour des plantes.
Et qui ne s'y fût pas trompé ?
La faune et la flore se touchent de si près dans ce monde sous-marin ! […]
« Curieuse anomalie, bizarre élément,
a dit un spirituel naturaliste,
où le règne animal fleurit,
et où le règne végétal ne fleurit pas ! »

Jules Verne, Vingt mille lieues sous les mers

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Summary

Knowledge about deep-sea megabenthic communities has greatly increased during the last two decades thanks to the improvement of technical diving equipment, submersibles, remotely operated and autonomous vehicles. In the last 10 years, several studies, targeting the Mediterranean deep continental shelf or mesophotic zone (40-200 m), have reported the occurrence of rich hotspots of biodiversity, namely marine animal forests, dominated by habitat-forming species such as corals and sponges thriving in dim light conditions. This bathymetric range hosts the deepest extension of shallow-water animal forests as well as proper mesophotic assemblages thriving on the so-called *roche du large* ecosystems. These complex aggregations play a fundamental ecological role in the marine ecosystems (refuge effect, nursery area, benthic-pelagic coupling, biogeochemical cycles) and the characterization of their diversity and distribution is considered of primary interest worldwide. Explorations have depicted not only the diversity of these forests but also their vulnerability towards mechanical impacts inflicted by demersal fishing activities, especially in the overexploited Mediterranean basin. This leads to an urgent need to quantify the damages burdening on these sensitive ecosystems and to develop easy-to-apply tools to evaluate and monitor their environmental status, in order to provide effective conservation measures. This thesis aims to address these topics focusing on the marine animal forests of the Ligurian deep continental shelf.

The Ligurian Sea represents one of the most studied Mediterranean basins due to several extensive researches, which have characterized the benthic and pelagic fauna mainly through SCUBA diving (shallow waters) and trawl surveys (bathyal waters) since the beginning of the last century. On the other hand, a large knowledge gap still exists for this region regarding the deep circalittoral megabenthic communities and, overall, on the anthropic threats insisting on deep-sea communities. Indeed, considering the significant amount of professional and recreational fishermen operating at these depths in the Ligurian region, it was crucial to carry out an extensive investigation in this area.

In the present study, about 80 sites have been investigated during a four-years ROV survey conducted along the Ligurian deep continental shelf representing the most comprehensive study so far for the Italian waters. ROV footage was used to characterize the benthic biocoenoses thriving on soft and hard bottoms, determine the most relevant

environmental factors driving their spatial distribution, and quantify the anthropogenic disturbance. Twelve major assemblages have been identified by means of community analysis, including gorgonian and hydrozoan forests, dense keratose sponge grounds, a *Dendrophyllia cornigera* facies, bryozoan beds and soft-bottom meadows of sabellids and alcyonaceans, some of them reported for the first time. Forests of black corals and *Paramuricea macrospina* aggregations have also been reported from the video footage, although with a more scattered distribution. The distribution of these assemblages is determined by different environmental parameters, namely depth, substrate type and inclination.

The abundance of marine litter herein observed is among the highest ever recorded in Europe, with maximum values up to 7900 items ha^{-1}. Abandoned, lost or discarded fishing gears represent the 81% of the total sightings, with the highest abundance recorded on the deep rocky shoals facing the largest fishing harbors and fleets. The majority of the fishing gears observed is attributable to artisanal and recreational fishing activities, suggesting that these represent the main source of litter in the Ligurian Sea. Urban litter, mainly represented by plastic, has also been observed, generally with higher abundances nearby large cities and river mouths.

A multidisciplinary approach based on ROV explorations, background fishermen interviews and on-board bycatch monitoring has also been applied to obtain quantitative data on the impact of different fishing methods adopted within two Ligurian case studies. The study areas have been selected following a bathymetric point of view to study two different deep-sea ecosystems (a deep circalittoral coralligenous shoal exploited by a small-scale fishery and a trawling ground located nearby a cold-water coral thanatocoenoses), and the investigated parameters were designed to fulfil the criteria proposed by FAO for the identification of Vulnerable Marine Ecosystems (VMEs). A fine characterisation of the megabenthic assemblages and the fishing effort was obtained, as well as the quantification of no-target catches rates (with particular attention to structuring megabenthic invertebrates and rare species), and the frequency of gear entanglement and loss. This information allowed to initiate the process to delineate Fisheries Restricted Areas (FRAs), over these sensitive deep areas.

Finally, to evaluate the environmental status of megabenthic assemblages thriving on mesophotic coralligenous or rocky reefs at temperate latitudes, a multiparametric index (MACS) integrating all possible sources of ROV-based information regarding community structure and impacts has been developed and validated. This practical tool was thought to have a large-scale application within the on-going Marine Strategy monitoring programs of these ecosystems.

In conclusion, a georeferenced database of the location of diversity hotspots and sites of litter accumulation has been created, in order to set the baseline for the monitoring of Ligurian mesophotic sensitive animal forests. This dataset will provide managers and stakeholders the scientific information and the tools for the creation of an extensive network of deep-sea marine protected areas in the Ligurian basin.

Riassunto

Grazie alle innovazioni tecnologiche nel campo della subacquea tecnica, dei sommergibili e dei robot filoguidati (ROV) e autonomi, la conoscenza sulle comunità megabentoniche degli ambienti profondi è profondamente aumentata negli ultimi venti anni. In Mediterraneo numerosi studi incentrati sulla piattaforma continentale profonda, o zona mesofotica (40-200 m), hanno evidenziato la presenza di ricche oasi di biodiversità, note come foreste animali marine. Queste associazioni di organismi sono dominate da specie strutturanti come coralli e grandi spugne, che prosperano in condizioni di semioscurità. La caratterizzazione della diversità e della distribuzione spaziale di queste complesse aggregazioni è considerata di primaria importanza a livello internazionale, poiché esse svolgono ruoli ecologici fondamentali negli ecosistemi marini (aree di rifugio, foraggiamento, riproduzione e accrescimento; *pelagic-benthic coupling*; cicli biogeochimici).

Le esplorazioni condotte finora negli ambienti mesofotici mediterranei hanno anche rivelato che le foreste animali sono particolarmente vulnerabili all'azione meccanica degli attrezzi da pesca demersale. Vi è quindi l'urgente necessità di caratterizzare e quantificare questo tipo di impatto, sviluppando strumenti pratici da adottare per valutare e monitorare lo stato di salute delle biocenosi mesofotiche, permettendo di delineare efficaci misure di conservazione. Questa tesi si propone di affrontare tali tematiche concentrandosi sulle foreste animali mesofotiche della Liguria.

Il Mar Ligure rappresenta uno dei bacini mediterranei maggiormente studiati. I numerosi studi condotti sin dal secolo scorso hanno permesso di caratterizzare la fauna bentonica e pelagica, principalmente attraverso l'immersione subacquea e lo studio del pescato profondo. Tuttavia, ad oggi, solo poche informazioni sono disponibili sulle comunità megabentoniche del circalitorale profondo e, in particolar modo, sulle minacce antropiche che gravano su questi ecosistemi. Considerando l'elevata urbanizzazione delle coste e l'ingente numero di pescatori professionali e ricreativi che operano a profondità mesofotiche in Liguria, era necessario effettuare una estensiva esplorazione di quest'area per definire struttura e stato ecologico di questi ambienti.

Quattro campagne oceanografiche ROV (lo sforzo esplorativo più elevato mai fatto fino ad oggi per le acque italiane) hanno permesso di investigare oltre 80 siti mesofotici della piattaforma continentale ligure. I filmati ottenuti con veicoli filoguidati sono stati utilizzati per

caratterizzare le biocenosi bentoniche, identificare quali fattori ambientali determinino la loro distribuzione e quantificare l'impatto antropico. Dodici biocenosi principali sono state identificate dall'analisi di comunità, tra cui foreste di gorgonie e idrozoi, campi di spugne cornee, una facies a *Dendrophyllia cornigera*, campi di briozoi e, su fondi mobili, prati di sabellidi e coralli molli, alcuni dei quali vengono qui descritti per la prima volta. Sono state osservate anche foreste di coralli neri e aggregazioni della gorgonia *Paramuricea macrospina*, sebbene con una distribuzione più puntiforme. La distribuzione di queste comunità è legata a diversi parametri ambientali, come la profondità, la tipologia e l'inclinazione del substrato.

L'abbondanza di spazzatura osservata è tra le più alte mai registrate nelle acquee Europee, con valori massimi di 7900 oggetti per ettaro. Gli attrezzi da pesca abbandonati o perduti rappresentano l'81% del totale, con le più alte abbondanze osservate sulle secche rocciose del largo. La maggior parte degli attrezzi osservati sui fondali liguri, è attribuibile alla pesca artigianale e ricreativa, attività che rappresentano quindi le principali fonti di *marine litter*. La spazzatura urbana è principalmente rappresentata da diversi tipi di plastica, e raggiunge abbondanze maggiori vicino alle grandi città e alle foci dei fiumi.

Un approccio multidisciplinare, basato su esplorazioni ROV, interviste ai pescatori e monitoraggio delle catture a bordo delle imbarcazioni da pesca, è stato inoltre applicato per ottenere informazioni quantitative sull'impatto di due diversi metodi di pesca. Le aree di studio sono state selezionate considerando, da un punto di vista batimetrico, due diversi tipi di ecosistemi profondi: una secca coralligena profonda sfruttata dalla piccola pesca artigianale e una cala per la pesca a strascico del gambero rosso situata nei pressi di una tanatocenosi di coralli bianchi. I parametri indagati sono stati selezionati in modo da rispondere ai requisiti proposti dalla FAO per l'identificazione degli ecosistemi marini vulnerabili (VME), tra cui la caratterizzazione delle biocenosi e dello sforzo di pesca, la quantificazione delle catture non commerciali (con particolare riferimento agli invertebrati strutturanti e alle specie rare) e la frequenza di afferrature degli attrezzi sul fondo e di perdita. Questi studi hanno permesso di raccogliere informazioni molto utili per avviare un processo di gestione di questi siti sensibili profondi.

Infine, per valutare lo stato ambientale delle comunità megabentoniche dei fondi duri mesofotici a latitudini temperate, è stato sviluppato e validato un indice multi parametrico (MACS), in grado di integrare le numerose informazioni estrapolabili dalle esplorazioni ROV. Questo pratico strumento è ideato per una applicazione su larga scala, in linea con gli attuali programmi di monitoraggio della *Marine Strategy Framework Directive* (EU).

In conclusione, i risultati ottenuti in questo studio, hanno permesso di creare un *database* geo-referenziato dei siti ad elevata biodiversità e delle zone di accumulo della spazzatura, fornendo una linea di base per i futuri programmi di monitoraggio. Questo *database* inoltre fornirà, ai gestori ed ai soggetti interessati, le informazioni scientifiche necessarie per la creazione di una estesa rete di aree marine protette profonde nel Mar Ligure.

Introduction

1. Deep-sea scientific exploration

The scientific investigation of marine benthic assemblages can be traced back to the late 19th century when Möbius (1880), by studying the oyster beds of the Baltic Sea, introduced the term biocoenosis in order to indicate the interacting groups of organisms living in the same habitat. Few decades later, Petersen (1913) systematically employed its grab to describe the soft-bottoms invertebrate biocenoses of the Danish waters. The recurring combination of limited conspicuous species associations leaded to the classification of different benthic communities, each one characterized by different ecological settings. This approach laid the foundation for the development of benthonology, the discipline that investigates the zonation and structure of benthic communities and their ecological functioning.

Shallow benthic assemblages have received plenty of attention due to the easy accessibility of the intertidal and upper infralittoral zones. A further contribution was given after 1942, when J. Y. Cousteau and E. Gagnan designed the first safe open-circuit Self-Contained Underwater Breathing Apparatus (SCUBA). By contrary, deep-sea benthic communities were originally investigated only through indirect methods (grabs, dredgers, trawls), and scarce information was available due to the technical difficulties and high economic costs associated with working in deep-waters (Gori et al., 2017).

The firsts direct deep-sea explorations started in early 1930s, when Barton and Beebe performed a series of dives off the coast of Bermuda on board on the Bathysphere, a still sphere suspended from a surface vessel (Robinson, 1999). In the 1950s, A. Piccard developed the bathyscaphs FNRS III and Trieste, and investigated different areas of the western Mediterranean Sea between 100 and 2800 m depth (Martin, 1960), providing the results that still represent the main references for bionomic zonation of the Mediterranean Sea (Pérès and Picard, 1964). In the same years, J. Y. Cousteau and J. Mollard developed the diving saucer soucoupe plongeante, and explored several localities of the Mediterranean Sea at depths ranging from 70 to 400 m. The great improvement of photographic equipment adopted during these explorations, allowed for the first time to collect pictures and video clips, providing material for accurate descriptions of the benthic communities inhabiting the “silent world”, including coral forests (Laban et al., 1963; Vaissière and Carpine, 1964; Reyss and Soyer, 1965).

After the 1970s, very few direct observations were carried out within the

Mediterranean Sea, and most of the research technology development continued in other World regions. In 1964 the Woods Hole Oceanographic Institution (WHOI) and the U.S. Navy build the submersible Alvin, destined to become the leader of a class of numerous vehicles, much smaller and better manoeuvrable than bathyscaphes, able to reach deep-bathyal and abyssal depths. Alvin has dived the oceans more than any other research submersible in its five-decade career, and it has made some of the most important discoveries in marine biology of the last century, including deep-sea vents and seeps down to 4500 m (Corliss et al., 1979; Monastersky, 2012).

Remotely Operated Vehicles (ROVs) are unmanned devices, controlled and powered from a surface vessel by an operator via an umbilical (Fig. 1). The U.S. Navy initially developed them at the beginning of the 1960s, for inspection, construction and repair of oil and gas offshore platforms and pipelines and, as a consequence, their characteristics favored manipulative tasks. With respect to submersibles, they present greater endurance, the ability to perform in circumstances too hazardous for human intervention, and require narrower logistical necessities than human operated vehicles, strongly trimming the cost of a mission (Robinson, 1999). ROV equipment include several devices and sensors like sonars, submarine navigation systems, several typologies of hydraulic manipulators and sampling instruments. Since good video footages represent the core component of most deep-sea scientific researches, video and still imaging high quality systems as well as high-output lighting are present. The scientific community began using ROVs in mid-late 1980s; the first ROV to be developed and built by an academic institution was Jason, launched in 1988 by the Woods Hole Oceanographic Institution (WHOI) (Ballard & Hively, 2000). Jason can reach depth up to 6500 m and it has been used for hundreds of dives on hydrothermal vents of the Atlantic, Indian and Pacific Oceans, as well as in underwater archeology and wrecks explorations. In Europe, from 1999, France's IFREMER (*Institut Français de Recherche pour l'Exploitation de la Mer*) operates Victor, a 6000-m depth ROV that has been involved in several research programs targeting benthic and fish communities (Fabri et al., 2011). At present, additional instruments employed in deep-sea exploration are represented by Autonomous Underwater Vehicles (AUVs), that consent to preprogram their diving profiles and thus allowing to perform extended surveys and monitoring missions (Kunz & Murphy, 2008; Morris et al., 2014), and hybrid vehicles that can be switched from remote to autonomous control (Cressey, 2014).

As a consequence of the major availability of high-tech instruments for the exploration of the deep-sea, including progress in technical diving equipment, marine biologists and ecologists have now access to a large amount of information, often represented by high-definition pictures and video footages. Several efforts have been made in order to propose standardized methods for the analysis of such material (*e.g.* Mortensen and Buhl-Mortensen, 2004; Watanabe et al., 2009; Vertino et al., 2010; Gori et al., 2011; Cánovas-Molina et al., 2016a), generally targeting the megabenthos, the largest dimensional class of the organisms living on the seabed, easily detectable and identifiable from ROV footages.

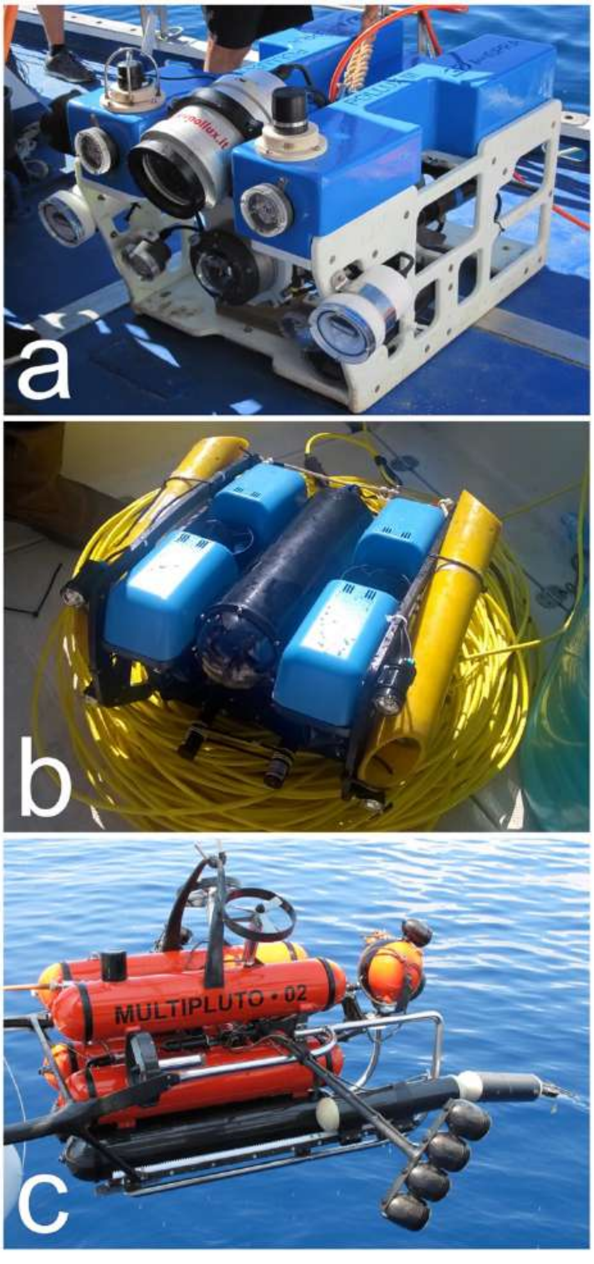

Figure 1. The remotely operated vehicles employed during the field work of this thesis. a) The ROV Pollux III, operated from the R/V *Astrea* by ISPRA (Rome), b) the BlueROV II, owned by COLMAR (La Spezia), and c) the ROV Multipluto, operated by Gaymarine (Switzerland) on board of *Daedalus* catamaran.

2. Mesophotic animal forests of the Mediterranean Sea

In the last two decades, several studies have been dedicated to the investigation of the Mediterranean megabenthic communities located at depths greater than 200 m, mainly represented by Cold-Water Coral (CWC) reefs (Tursi et al., 2004; Orejas et al., 2009; Mastrototaro et al., 2010; Chimienti et al., 2018; Orejas and Jiménez, in press) and sponge grounds (Bo et al., 2012; Maldonado et al., 2015). Such studies have been fundamental to depict the structure and functioning of deep benthic communities in the basin, their relationship with topographic reliefs and major hydrographic patterns, their biogeographic origin, their resilience to pressures as well as their value in terms of conservation (Mastrototaro et al., in press). This attention boosted a great number of parallel projects targeting side biological and ecological aspects, as well as less investigated areas or depths, including intermediated depths, located from 40 to 200 m, which received far less attention than aphotic habitats (Bo et al., 2009, 2014a, 2015, 2019; Salvati et al., 2010; Angiolillo et al., 2012; Giusti et al., 2012; Fabri et al., 2014; Cau et al., 2015, 2017; Gori et al., 2017; Grinyó et al., 2018; Dominguez-Carrió et al., 2018). This bathymetrical range represents the deepest sector of the continental shelf, corresponding to the circalittoral plan (*sensu* Pérès and Picard, 1964), and also known as twilight or mesophotic zone (Bo et al., 2009; Cattaneo-Vietti et al., 2010). The term mesophotic has been adopted mainly in order to identify similar depths at tropical latitudes with special reference to the distribution of hermatipic corals (Lesser et al., 2009; Soares et al., 2018).

Nevertheless, some authors employ this term also for temperate areas with special reference to the dim light conditions these ecosystems are adapted too (Cánovas-Molina et al., 2016a; James at al., 2017; Bo et al., 2019).

At these depths, the combined presence of factors typical of shallower ecosystems (*e.g.* penetration of light, organic material inputs from the coasts) with characteristic deep-water process (rich-in-nutrient upwelling currents, reduced competition for space) provides high and stable food supply, allowing the development of rich megabenthic aggregations (Buhl-Mortensen et al., 2010). Erect and massive sponges, arborescent cnidarians, bryozoans and other organisms, form complex three-dimensional structures (structuring or engineer species) that enhance spatial heterogeneity and provide new substratum and shelter for several associated species. For these reasons, these assemblages resemble their terrestrial counterpart, and have been named marine animal forests (Rossi et al., 2017) (Fig. 2). Such aggregations are not only important from a structural point of view, representing hotspots of biodiversity, but they influence the surrounding physic-chemical environment and play remarkable functional roles in different ecological processes, namely bentho-pelagic coupling and biogeochemical cycles (Buhl-Mortensen et al., 2010). Active (*e.g.* sponges, bryozoans, brachiopods) and passive suspension feeders (*e.g.* gorgonians, black corals, soft corals and pennatulaceans) capture plankton and particulate organic matter suspended in the water, and determine a significant flow of matter and energy from the pelagic to the benthic ecosystem (Cathalot et al., 2015). Additionally, the presence of such aggregations increases the larval settlement as well as the organic matter and the lipid content in the sediments, enhancing meiofaunal biomass and diversity (Cerrano et al., 2015).

An extend revision of the animal forests of the Mediterranean twilight zone, has been recently provided by Gori et al. (2017). On the basis of ecological, bathymetrical and topographical characteristics, four different categories of forests have been identified, namely those thriving on (i) deep coastal bottoms, (ii) continental shelf, (iii) shelf edge and upper slope, and (iv) offshore banks and seamounts. Within each proposed group, main differences in animal forests composition are observed in relation to the bottom type. Coralligenous reefs represent typical Mediterranean hard bottoms, whereas outcropping or sub-outcropping rocks (*roche du large*) become predominant at higher depths. On hard bottoms, marine animal forests present a distinct stratified structure including (i) a basal layer, constituted by encrusting or with limited (<1 cm) vertical growth organisms, (ii) an intermediate layer, dominated by species with moderate (1–10 cm) vertical growth, and (iii) an upper layer, characterized by canopy-forming organisms with considerable (>10 cm) vertical growth (Gatti et al., 2015). Coralline algae, encrusting sponges, serpulids, bryozoans and ascidians are some examples of organisms inhabiting the basal layer,

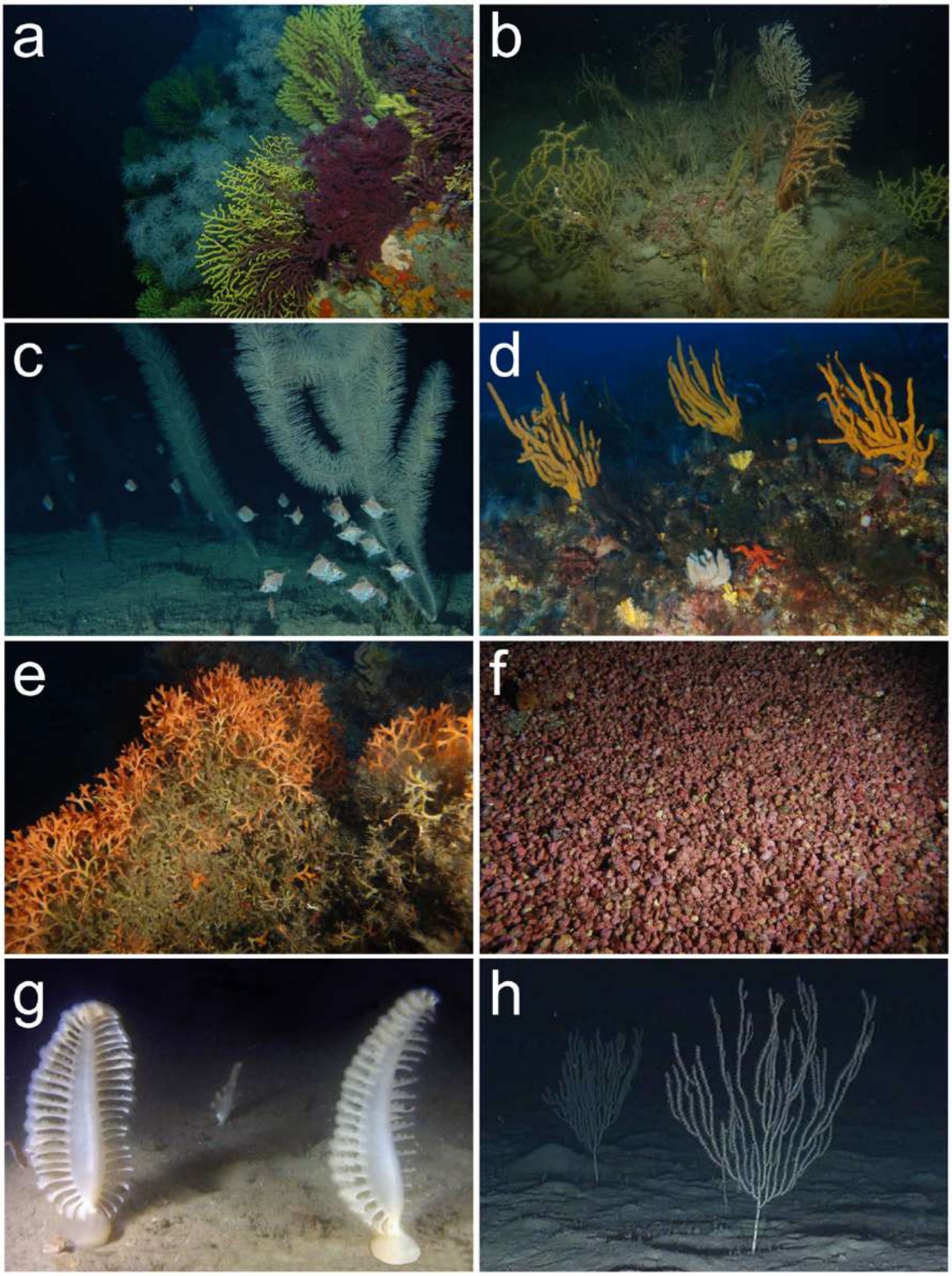

Figure 2. Images of marine animal forests from the deep continental shelf of the central Mediterranean Sea. a) Multi-specific forest of *Paramuricea clavata* and *Antipathella subpinnata* from Favazzina. b) Aggregation of *Paramuricea macrospina* from the Gulf of S. Eufemia. c) The black coral *Parantipathes larix* provides canopy for fishes (*Capros aper*) in the Tuscan Archipelago. d) Aggregation of the tree-like sponge *Axinella polypoides* from the Adventure Bank (Strait of Sicily). e) Facies of the bryozoan *Pentapora fascialis* from the Aeolian Islands. f) Marine animal forests are known to develop also on rhodolites and maërl; f) here the maërl beds of the Aeolian Islands. g) Aggregation of pennatulaceans (*Pteroeides spinosum*) and soft corals (*Alcyonium palmatum*) from Cap de Creus (Dominguez Carrió, 2018). h) Facies of *Isidella elongata* from the Balearic Sea (Mastrototaro et al., 2017). Photo credit (a-f): S. Canese (ISPRA).

whereas for the intermediate one, different massive or bushy morphologies of sponges, hydroids, soft corals, sabellids, bryozoans and ascidians are reported. Gorgonians dominate the upper layer, by forming mono and pluri-specific aggregations and showing important differences in species composition, colony density and size in relation to the habitat characteristics (Fig. 2a, b). So far, more than 20 species of gorgonians are considered structuring or habitat-forming species for the Mediterranean basin at these depths. Black corals are also important structuring species of the upper layer, due to their large size and to the ability to form large aggregations, even if they are less widespread and diverse than gorgonians (five forest-forming species in the Mediterranean Sea) (Gori et al., 2017; Bo and Bavestrello, 2019) (Fig. 2c). Large sponges, as the treelike *Axinella polypoides*, as well as arborescent bryozoans are also considered important components of the canopy layer (Lombardi et al., 2014; Coppari et al., 2016) (Fig. 2d, e).

On detritic and soft bottoms, animal forest are generally less structured, despite a large variability of situations is reported, including aggregations developing on rhodolites and maërl beds (Grinyó et al., 2018) (Fig. 2f). Large hydrozoan forests are reported from deep coastal detritic bottoms (Di Camillo et al., 2013), whereas soft corals and sea pens may dominate sandy and muddy areas of the continental shelf (Ambroso et al., 2013; Bastari et al., 2018) (Fig. 2g). At higher depths, corresponding to the shelf edge and the upper part of the continental slope, dense meadows of the bamboo coral *Isidella elongata* are known to occur, even if its widest distribution area is on the deepest plains (Bo et al., 2015; Mastrototaro et al., 2017) (Fig. 2h).

The recent explorations of Mediterranean animal forests also provided information on the fragility of such rich and complex ecosystems, highlighting the numerous threats affecting them. On one hand, still very little is known on the effects of climate change on the deep-sea communities (Danovaro et al., 2017) and very few evidences highlight the effects of natural threats such as bottom turbidities and hydrothermal phenomena leading to deep temperature anomalies and mass mortality events (Bavestrello et al., 2014). Twenty-years of large-scale exploration in the basin, however, have clearly identified anthropic pressures as the most relevant threat on mesophotic marine animal forests in the Mediterranean basin. These assemblages generally lie in close proximity to coastal areas, making them more vulnerable to pollutants coming from sewage discharge and rivers outflows near urban areas (Gori et al., 2017). Seafloor drilling activities for oil exploration and mining menace the integrity of several deep benthic communities, but the fishing activities represent the major source of threat on the highly exploited continental shelf (Aguilar et al., 2017) (Fig. 3).

3. Mediterranean fisheries and fishing impact on mesophotic animal forests

A total of about 86,500 operating fishing vessels have been censed within the Mediterranean and Black seas, even if such number should be considered an underestimate of the real size of the total fleet, given the lack of data regarding especially small-scale fisheries (FAO, 2018). Four countries give a major

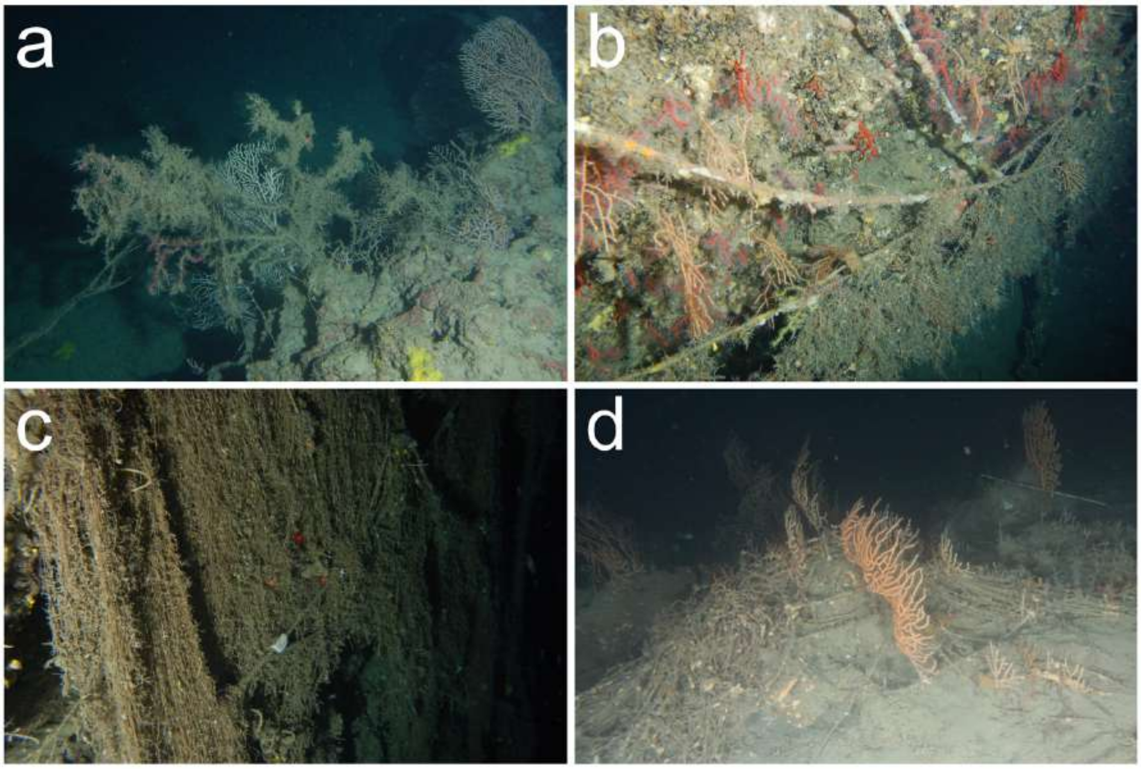

Figure 3. Images of fishing impact on Mediterranean mesophotic animal forests. a) Entanglement causes abnormal epibiosis and necrosis on large colonies of *Eunicella cavolini* in Calabria. b) Longlines and artisanal nets entangle a mixed aggregation of *Corallium rubrum* and *Eunicella cavolini* nearby Ischia Island; in this area some rocky elevation are completely covered by abandoned nets (c). Impact of abandoned fishing gears on a *E. cavolini* forest in North-western Sicily. Photo credit: S. Canese (ISPRA).

contribute, namely Turkey (17.8%), Greece (17.3%), Tunisia (15.1%) and Italy (13%). About 78% of the Mediterranean fishing fleet is represented by polyvalent vessels. This division includes boats using a combination of passive (*e.g.* post nets, longlines) and active gears (*e.g.* seiners, trawlers), none of which exceeding more than 50% of the time at sea during the year, and small-scale vessels with and without engine, using passive gears. Bottom trawlers represent 9% and purse seine and pelagic trawlers account for 5%. The remaining is represented by longliners, tuna seiners and dredges. Most of the categories here mentioned include demersal fishing methods, characterized by the use of gears that come into direct contact with the seafloor, and thus represent a concrete threat for benthic ecosystems, with trawl fishing considered the most diffuse and destructive one (Eigaard et al., 2016). Decades of chronic trawling have widely impacted large areas of the Mediterranean continental shelf and slope, constraining pristine animal forests to inaccessible or remote areas (Bo et al., 2015). Additionally, demersal small-scale fisheries, due to the large number of vessels involved (accounting for 83% of the Mediterranean fishing fleet), have been proven to determine significant impacts on megabenthic communities (Shester and Micheli, 2011; FAO, 2018).

Artisanal fisheries and small-scale fisheries are interchangeable terms adopted to broadly refer to a multi-faceted fisheries segment using low technology gears and vessels to target a variety of species for subsistence and local markets (Shester and Micheli, 2011). The main characteristics of the artisanal fisheries are the high diversification of gears, techniques, boats, target species, timing and fishing grounds, which increase the difficulties in the management of this sector. For this reason, the term *métier* has been introduced to refer to a group of a fishing operations defined by the combination of fishing gears, target species, area and season, allowing to describe fisheries at a level incorporating the largest part of their heterogeneity (González-Álvarez et al., 2016). In the Italian demersal artisanal fisheries, the main gears employed by fishermen are trammel nets, gillnets, combined gill-trammel nets, longlines and fishing pots (Cataudella and Spagnolo, 2011). Due to the small boats size, low horsepower and gross tonnage, artisanal fishing is essentially located on the continental shelf (0-200 m depth), exploiting fishing grounds, which can be reached in a few hours from the fishermen bases (Colloca et al., 2004; McCluskey and Lewison, 2008;).

Recreational fishing includes those fishing activities not conducted for commercial and subsistence purposes and plays an important social and cultural role worldwide (Pawson et al., 2008). Recreational fishing involves many different techniques (*e.g.* rod and line, hand-gathering, longlines, traps, spear gun), can be exerted from different locations (*i.e.* shore, boat, underwater) and targets a broad range of taxa (*e.g.* finfish, shellfish, crustaceans). Despite the general limited availability of data, several studies have demonstrated that recreational fishing has an important effect on fishing stocks (Arlinghaus et al., 2016; Hyder et al., 2017). Up to date, no data are available on the impact of recreational demersal gears on benthic communities, even if fragments of lines and accessories strictly associable to these techniques they are commonly identified in ROV surveys. Thus, the identification and quantification of the total population of recreational fishermen are of primary interest to depict a complete picture of the fishing impact on the continental shelf. Within the Mediterranean Sea some information have been obtained from national marine recreational fishing license systems and registration databases. Only fourteen states have a license system in force for recreational fishing, whereas Bulgaria, France, Greece, Israel, Libya, Malta and Tunisia do not (FAO, 2018). In Italy, up to 1,033,883 recreational fishermen have been censed by means of a fee-free online registration, which must be mandatory for every kind of marine recreational fishing technique (https://www.politicheagricole.it). About 70% fishes from support vessels often equipped with high-tech fish finders and scanning sonars and work on deep rocky elevations employing vertical lines and longlines in close proximity with the sea bottom.

Considering that species of commercial interest are often associated with animal forests (D'Onghia et al., 2010; Marliave et al., 2009; Baillon et al., 2014; Mytilineou et al., 2014; Cau et al., 2017), professional and recreational fishermen indirectly operate in areas characterized by rich megabenthic communities (Bo et al., 2014b; Gori et al., 2017). Demersal fishing gears may cause different type of impact on marine animal forests. Direct impacts include the breaking, the maiming and the direct removal of structuring organisms or seafloor. The characteristics of the fishing gears, the shape of the organisms, the flexibility of the skeleton and the way organisms aggregate on the sea bottom are the main factors influencing the catchability of a species (Mytilineou et al., 2014; Bo et al., 2014b). Gorgonians, black corals, scleractinians, bryozoans and sponges are extremely vulnerable due to their arborescent shape and large size, as they can easily remain entangled in nets and longlines, whereas trawling nets may collect also massive organisms and vagile fauna. Arborescent anthozoans are among the most abundant and frequent species in fishing bycatch representing to more than 70% of the catches (Sampaio et al., 2012; Mytilineou et al., 2014).

Indirect impacts of fishing activities include i) denudation of skeletal parts enhancing epibionts overgrowth, which in turn increases the friction of the colonies with water and leads to the rupture of the branches (Fig. 3a), ii) choking of the colonies and iii) burial caused by the resuspension of the sediments (Fig. 3b-d) (Bavestrello et al., 1997; Pham et al., 2013; Bo et al., 2014b; Sheehan et al., 2017). Fishing gears may continue to threat marine ecosystems once that they are lost at sea for long time. Abandoned, Lost or otherwise Discharged Fishing Gears (ALDFGs) may continue to catch organisms and, once collapsed, may continue to entangle and abrade structuring species shifted by bottom currents (Macfadyen et al., 2009; Sheehan et al., 2017). Their occurrence has been identified throughout the basin with areas subjected to a high incidence of marine litter (Bo et al., 2014b; Angiolillo et al., 2015; Cau et al., 2015; Pham et al., 2014). Furthermore, since lost fishing gears are mainly made of synthetic fibers, they persist in the marine environment (Macfadyen et al., 2009). The fouling organisms that accumulates on their surface progressively determine the burden of large nets that eventually sink on the sea bottom, potentially covering and suffocating large portions of megabenthic communities (Saldanha et al., 2003) shifting the assemblage to a low diversity one (Ponti et al., 2014).

The characterization and reduction of the impact of fishing activities on animal forests is a theme of major interest and not easy resolution, representing a complex issue related to several biological, physical, mechanical and social parameters. A large gap of knowledge is bounded to the large variability of gears and techniques employed by fishermen, especially concerning artisanal and recreational ones, making difficult to find standardised investigation methods. Furthermore, the impact is also strictly dependent to the topography and hydrology of the area and to the characteristics of the biocoenoses. As a matter of fact, only few information is available on the response of megabenthic communities to specific fishing activities (Bo et al., 2014b; Kaiser et al., 2018), whereas quantitative data on structuring invertebrate discard collected by fishing gears are scarce, especially for Mediterranean artisanal fisheries (Hawkins and Roberts, 2004; Mangi and Roberts, 2006; Gökçe and Metin, 2007; Batista et al., 2009; Maynou and Cartes, 2011; Sampaio et al., 2012; Mytilineou et al., 2014, Stephenson et al., 2017). Fishing effort linked to benthic ecosystem disturbance has been estimated through different approaches, including (i) the amount of resources devoted to obtain a certain fishing yield, (ii) fishermen interviews and daily sales' records, (iii) Vessel Monitoring System (VMS) data and (iv) Side-Scan Sonar (SSS) records (McCluskey and Lewison, 2008; Demestre et al., 2015). Additionally, ROV-Imaging related tools have been adopted to characterize benthic communities and quantify fishing disturbance, expressed as density of marine litter and percentage of damaged organisms (Bo et al., 2014b; Angiolillo et al., 2015; Díaz et al., 2015). These parameters give a well-defined picture of the health status of these ecosystems, but they do not depict the potential damage on the seafloor of a single fishing event, or the environmental sustainability of a certain *métier*, or the temporal scale of impact of fishing activities on a certain ecosystem.

4. Conservation of Mediterranean animal forests

Several national, regional and international organizations are commonly devoted to the reduction of the impact of fishing activities on marine benthic ecosystems. However, the rate at which tutelary measures are proposed and adopted is very low and strongly biased to the protection of habitat recognized as very productive and useful for humankind. As a matter of fact, several communities and ecosystems are completely ignored by conservation programs, because small, unattractive, largely unstudied, or unappealing to the general public (Aguilar et al., 2017). Nevertheless, several advances in the conservation of deep-sea ecosystems occurred in the last years at a global scale. The first measures were directed to the species level, mainly focusing on the safeguard of threatened species through the prohibition of their exploitation or trade. For example, the New Zealand Wildlife Act (1953) and the United States Endangered Species Act (1973) were national measures adopted for the protection of several species (including scleractinians, black corals and gorgonians), whereas the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention, 1976) and the Specially Protected Areas and Wildlife (SPAW) protocol of the Cartagena Convention represented international agreements. Most recently, the International Union for Conservation of Nature (IUCN) promoted the compilation of national and regional Red Lists for anthozoans targeting the identification of the most vulnerable species (Otero et al., 2018), while Food and Agriculture Organization of the United Nations (FAO) in collaboration with the General Fisheries Commission for the Mediterranean (GFCM) is promoting specific trainings for scientific observers on board of fishing vessels to detect sensible species and areas through the study of bycatch.

Despite the importance of species-specific protection, the conservation of animal forests is strictly dependent to the adoption of habitat and ecosystem approaches, including the spatial management of human activities. In this sense, it must be taken in consideration the important role played by Marine Protected Areas (MPAs), whether, so far, in the Mediterranean context they are limited to the 50m-bathymetry and off-shore areas face the limit imposed by distance on controls. Additionally, several

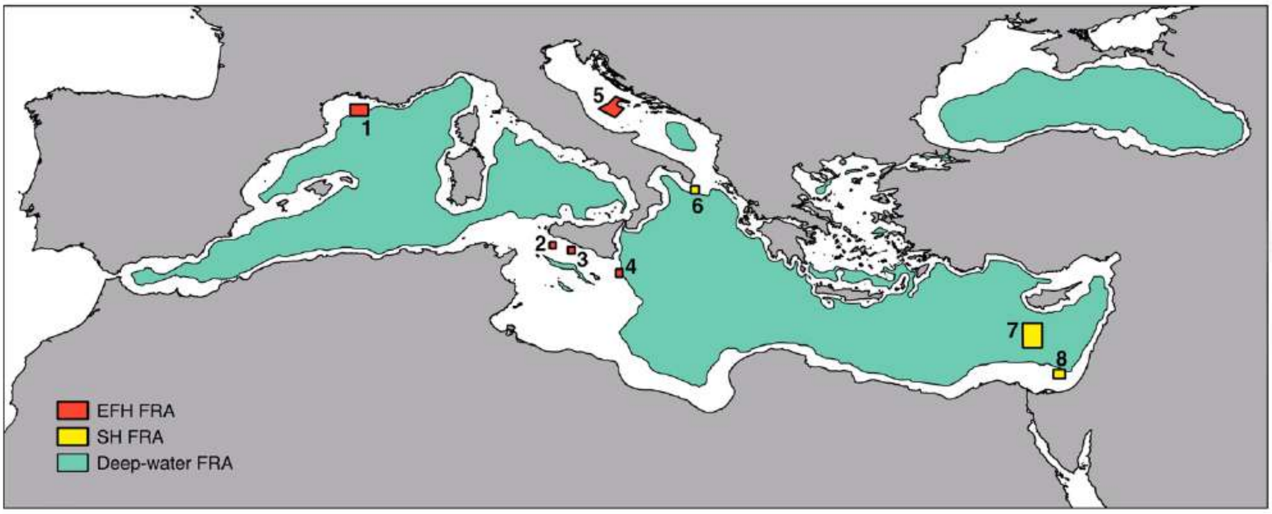

Figure 4. Map of the Mediterranean Fisheries Restricted Areas (FRAs). Five areas are recognized to be EFHs, namely Gulf of Lions (1), East of Adventure Bank (2), West of Gela Basin (3), East of Malta Bank (4) and Pomo Pit (5), whereas the *Lophelia* reef off Santa Maria di Leuca (6), the Eratosthenes Seamount (7) and the Nile delta area cold hydrocarbon seeps (8) are considered SHs. The Deep-water FRA protects unknow stocks and habitats below 1000 m (source: http://www.fao.org/gfcm/data/maps/fras).

international agreements provide not only lists of threatened species, but also lists of habitats or ecosystems. For example, in 2008, the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) created the List of Threatened and/or Declining Species & Habitat, including coral meadows, deep-sea sponge aggregations and mussel beds, whereas the Barcelona Convention approved two protocols for the protection of some deep-sea aggregations and upgraded various structuring anthozoan species in Appendix II (Aguilar et al., 2017).

The United Nation Convention on Biological Diversity (CBD), established in 1992, has considered the marine realm since its beginning, but it has been proven difficult to develop clear actions to face the loss of biodiversity. During the ninth Conference of the Parties, seven scientific criteria for the identification of Ecologically or Biologically Significant Marine Areas (EBSAs) were proposed (CBD 2008). To date, 204 EBSAs have been approved (fifteen of which lying within the Mediterranean Sea), despite the majority of them do not present detailed plans of conservation. Some of the criteria proposed for the identification of EBSAs, have been re-elaborated by the Food and Agriculture Organization of the United Nations (FAO), in order to identify Vulnerable Marine Ecosystems (VMEs). VMEs are groups of species, communities, or habitats that may be vulnerable to the impact inflicted by fishing activities and the term “vulnerable” reflects the fact that they are both easily disturbed and slow or unable to recover (FAO 2009). Examples of VMEs are given by several animal forests like CWC reefs, coral forests, deep-sea sponge aggregations, seeps and vents communities, which are often associated with peculiar features of the sea bed (*e.g.* submerged edges, seamount and canyons). Following the guidelines given by the United Nations General Assembly

(UNGA) and FAO, many states, non-government organizations, and Regional Fisheries Management Organizations (RFMO) have developed processes to identify VMEs and have adopted management measures to protect them, including the production of VMEs indicator species lists and encounter protocols, the employment of onboard observers, the requirement to collect information regarding bycatch, the banning of bottom contact gears and the direct closure of fishing areas (FAO 2016; OCEANA 2016; Aguilar et al. 2017).

In the Mediterranean Sea, the GFCM is in charge of the monitoring and managing of the fisheries since 1949, but despite its seniority, specific guidelines have not been yet adopted for VMEs. The GFCM has adopted Fisheries Restricted Areas (FRAs) as a multi-purpose spatial-management tool to restrict fishing activities in order to protect deep-sea Sensitive Habitats (SHs) and Essential Fish Habitats (EFHs). In order to create a FRA a specific procedure must be activated in which a detailed report of information is discussed by a GFCM commission evaluating various parameters such as occurrence of rare, fragile or unique biocoenoses as well as information on the fishing effort in the area (http://www.fao.org/gfcm/data/maps/fras). At present, the FRAs regulated by GFCM are represented by three deep-sea SHs and five EFHs (FAO 2016; GFCM 2017) (Fig. 4). Furthermore, GFCM has prohibited the use of towed dredges and trawl nets at depths greater than 1000 m, protecting fish stocks and VMEs in an area which cover about 59% of the Mediterranean Sea (GFCM 2005). Despite the huge area enclosed by this restriction, it does not confer any protection to many VMEs, because most of the Mediterranean VMEs occur shallower than 1000 m (Gori et al. 2017) and the majority of the Mediterranean fishing fleets does not operate beyond that depth limit (FAO 2016). Furthermore, the network of marine protected areas as well as the coastal trawling prohibition (GFCM 2012) cover only shallow littoral areas, leaving offshore and deep-sea habitats and species unprotected. For these reasons, the spatial protection of VMEs in the Mediterranean Sea is really weak and the implementation of Mediterranean VMEs, as well as the production of clear VMEs identification tools, are issues of major interest.

Within the European Union, the first policies established for the protection of the natural heritage included the Birds Directive (BD) (European Commission 2009), for the conservation of wild birds and their habitats through the designation of Special Protection Areas (SPA), and the Habitat Directive (HD) (European Commission 1992), for the protection and the conservation of natural habitats and wild flora and fauna through the establishment of Special Areas of Conservation (SAC). Existing SPA and SAC provided a network of protected sites across the European Union called Natura 2000, and Member States are required to report the state of their protected areas every six years. Additionally, in order to give a major protection to the inland and coastal waters, the European Union introduced the Water Framework Directive (WFD) (European Commission 2000), which introduced the concept of Good Environmental Status (GES). The WFD encouraged interest parties to achieve a good ecological and chemical status of the water bodies by the promotion of specific actions.

The Marine Strategy Framework Directive (MSFD) (European Commission 2008) represents at present the most comprehensive European Union regulation for the conservation and the sustainable use of the marine environments, including the deep-sea areas. It has been elaborated as a result of a growing international interest towards an ecosystem-based management of marine environments, including scientific-based reactive criteria and indicators defining the GES. In order to help Member States to assess the extent by which GES is achieved in the different marine areas under consideration, a list of eleven descriptors has been provided, aiming to summarize the way in which the whole system functions, to make criteria operational and allow subsequent progress (Borja et al., 2010). Among these descriptors, some are of major concern in the evaluation of the healthy status of the animal forests, namely Descriptor 1 (biodiversity is maintained), Descriptor 6 (the sea floor integrity ensures functioning of the ecosystem), Descriptor 10 (marine litter do not cause harm).

One of the key aspects of the ecosystem-based approach endorsed by the MSFD is the requirement of a more rational organization of the spatial distribution of human activities, in a way that balances the demands for development with the need to protect the marine ecosystem (European Commission 2008; Ehler & Douvere 2009). The fundamental requirement for the protection, the conservation and the restoration of species, assemblages and habitats is to know their distribution on the seabed. Despite several years of deep-sea exploration, much of the seafloor remains unexplored, making it impossible to provide maps of marine habitats also at intermediate depths. Bathymetrical data has been used to identify topographic features where animal forests may occur, but they often contain gaps and show a coarse resolution (Wessel et al., 2010). Habitat mapping based on environmental and biological variables could be a useful tool to fulfil information gaps for marine conservation plans (Althaus et al., 2012; Dunstan et al., 2012; Du Preez et al., 2016), although it is generally based on local ROV explorations and cannot be considered a replacement for finer-scale mapping based on detailed field surveys (Aguilar et al., 2017).

The identification of valid monitoring protocols for deep benthic ecosystems represents a theme of major interest, as there is a growing need to detect natural and human-induced changes and to attest the effectiveness of protection measures. At present, the majority of the long-term evaluations occurs in shallow areas, through the employment of a wide array of techniques, including visual censuses (Sala et al., 1996), photographs (Linares et al. 2012), Baited Underwater Videos (BUVs) (Stobart et al. 2015) and even underwater video transects performed by SCUBA divers (Tilot et al. 2008). On the contrary, very few monitoring programs currently assess the evolution of benthic assemblages dwelling in deeper areas. These programs are generally carried-out by means of highly destructive samplings, as in the case of MEDITS program in the Mediterranean Sea, which evaluates the state of benthic and demersal fish stocks through experimental bottom-trawling surveys (Bertrand et al. 2002). Anyway, the recent technological development is pushing through the employment of less destructive methods. Epibenthic communities and benthic habitats can be assessed by non-destructive imaging techniques, which collect quantitative data over relatively large areas in a cost-effective manner (Sheehan et al. 2010). Underwater video observations provide the necessary replication and scale (spatial and temporal), a relative quick data retrieval and can be used in outreach programs, where the effect of anthropogenic activities or the efficacy of protection measures can be shown to a wider audience of non-scientists (Bicknell et al. 2016). Even if the employment of ROVs or technical divers is still considered complex and expensive, some studies employed imaging technology to evaluate changes after the closure of marine areas to fishing practices (*e.g.* Huvenne et al. 2016) or even after an oil spill (Etnoyer et al. 2015). Furthermore, the last frontier in technology development leads to the continuous monitoring of deep-sea ecosystems, through the use of AUVs (Morris et al. 2014) and the implementation of a network of permanent deep-ocean monitoring stations (Danovaro et al. 2017).

5. Study area: the Ligurian Sea

The Ligurian Sea is located in the Northwestern sector of the Mediterranean Sea, and it is characterized by peculiar topographic and oceanographic features (Fig. 5). The western sector presents a steep and narrow shelf incised by several submarine canyons, whereas in the eastern sector, the continental shelf is wider, with a less pronounced slope and only few canyons (Fanucci et al., 1989; Cattaneo-Vietti et al., 2010; Würtz, 2010). The overall superficial water flow moves westward along the Ligurian coast, producing upwelling currents throughout the canyon systems. Productivity of the euphotic layer is enhanced by the common occurrence of mesoscale anticyclonic eddies (Casella et al., 2011), and by the several rivers flowing through the Alpine and Apennine chains, with predominantly torrential streams, (Cattaneo-Vietti et al., 2010).

This basin is relatively well studied from the biocoenotic point of view, especially for what concern littoral and bathyal communities (*e.g.* Brian, 1931; Issel, 1932; Tortonese, 1961; Morri et al., 1986; Relini et al., 1986; Bianchi and Peirano, 1995; Diviacco and Coppo, 2006). Mesophotic environments have been far less investigated in this region, but some preliminary investigations suggest the occurrence of rich megabenthic assemblages at these depths (Cerrano et al., 2010; Di Camillo et al., 2013; Bo et al., 2014b; Cánovas-Molina et al., 2016b). Due to the peculiar characteristics of the Ligurian Sea, mesophotic megabenthic communities are subjected to a great deal of anthropogenic disturbance mainly attributed to urbanization, commercial harbors and fishing fleets (Cattaneo-Vietti et al. 2010).

The Italian Ligurian coasts are among the most urbanized of the Mediterranean Sea with an estimated overall population of 1,262,000 residents (subjected to summer increase). About 63 towns are located along its coastline with four major cities (La Spezia, Genova, Savona, Sanremo) each accounting for more than 50,000 people (SM2, Chapter 4).

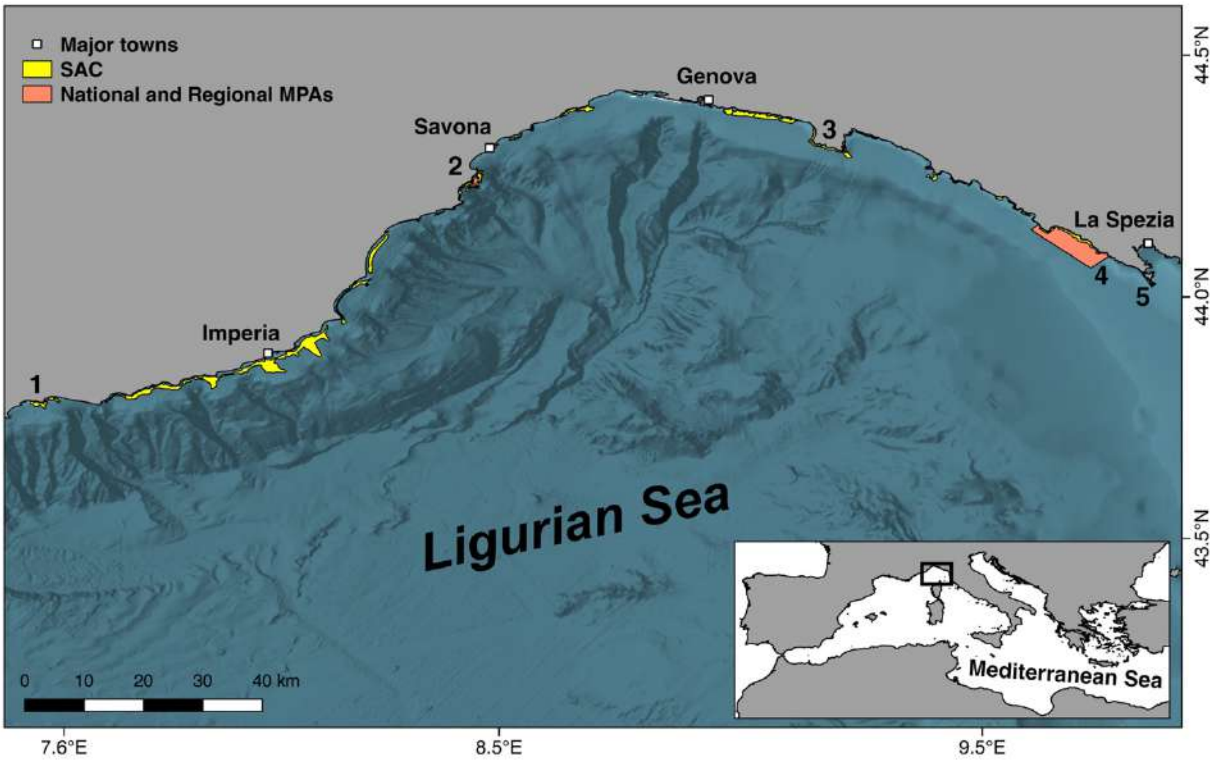

Figure 5. Map of the Ligurian Sea showing the four major towns and the tutelary areas along the Italian continental shelf. The three national MPAs are Isola di Bergeggi (2), Portofino (3) and Cinque Terre (4), whereas Capo Mortola (1) and Porto Venere (5) are regional MPAs. Insert: location of the Ligurian Sea within the Mediterranean basin.

Some of the Ligurian smaller town do not have a sewage system therefore blackwater discharges are occasionally present along the coast. Over 20,300 berths are present in Ligurian harbors, accounting for approximately 42 structures, with those of La Spezia, Genova and Vado Ligure being the largest commercial ports (SM3, Chapter 4). The Ligurian fishing fleet accounts for approximately 770 vessels (SM3, Chapter 4), mainly dislocated in the fishing harbors of La Spezia, Santa Margherita Ligure, Genova, Savona, Imperia, and Sanremo. Ligurian fleet shows distinct artisanal characteristics (Cattaneo et al., 2010) (Fig. 6) since, nowadays, the boats operating in the small-scale fisheries represent 80% of the regional fleet, the remaining being small trawlers (Cataudella and Spagnolo, 2011; Sturla and Trione, 2017). The most widely employed gear is the trammel net, followed by gillnets, then longlines and fishing traps. Due to the small boat size, low horsepower and gross tonnage, and considered the particular features of the Ligurian fishing grounds, which have very few exploitable shallow areas, artisanal fishing concentrates on the deeper continental shelf and upper slope. Fishing grounds are mainly represented by offshore rocky or coralligenous elevations, easily reached from the fishermen harbors.

Figure 6. The Ligurian fishing fleet shows distinct artisanal characteristics. Here some artisanal vessels from the characteristic harbor of Portofino.

Recreational fishing activities represent a consistent reality in this region, with over 220,000 fishermen as emerged from a recent census (https://www.politiche agricole.it). Such a huge number of users, increases in the summer time, and makes it hard to define concrete management policies and regulations.

Five MPAs (Capo Mortola, Isola di Bergeggi, Portofino, Cinque Terre and Porto Venere) and 27 SACs are reported for the Ligurian Sea, accounting for more than 15,000 hectares of tutelary surface (Fig. 5). However, all these protected sites are generally located within shallow, coastal areas, giving no protection to the majority of the megabenthic assemblages located on the deep continental shelf and upper slope.

6. General objectives of this thesis

The present thesis aims to describe the diversity, the distribution and the vulnerability of the megabenthic communities of the Ligurian Sea with special reference to the deep continental shelf. All the aspects covered in this thesis have been developed following the most recent comprehensive regulations for the conservation of marine benthic ecosystems, proposed by the EU and FAO. The results of this work are expected to represent a baseline for the identification of the main Ligurian mesophotic sensitive areas and the definition of management and conservation guidelines for these ecosystems.

This thesis is organized in five chapters, presented through three thematic sections.

Part 1- Biodiversity. In this section the natural heritage of the Ligurian deep continental shelf (40-210 m) has been investigated during a pluriannual, large-scale ROV exploration, following the demands proposed by descriptor 1 of MSFD, pointing through a knowledge-based monitoring of the biodiversity levels and filling the gap of knowledge for this region. The objectives of **Chapter 1** – "*Diversity and distribution of the megabenthic communities of the Ligurian deep continental shelf (NW Mediterranean Sea)*", were (1) to characterize the overall megabenthic biodiversity, (2) to identify the megabenthic assemblages through multivariate statistical methods, (3) to assess their geographical and bathymetrical distribution, and (4) to evaluate the overall effect of the selected environmental parameters over the distribution of the identified animal forests. Finally, the distribution of these assemblages has been organized into a GIS geodatabase, providing a valuable effective tool for future monitoring and conservation.

Part 2 - Fishing impact. The MSFD descriptor 6 considers the necessary measures to define standardized, effective methods to describe the sea floor integrity and its alteration by human activities. In consideration of the scarce information available regarding the impact of fishing techniques on Mediterranean mesophotic animal forests, the aim of Section 2 is to provide quantitative data on the impact of different *métier* adopted within two Ligurian fishing grounds, by following the criteria proposed by FAO for VMEs identification. The specific objectives were (1) to characterize the megabenthic assemblages and their health status following the MSFD protocols, (2) to make an inventory of the fishing fleets operating in the areas, (3) to quantify the uptake rates of no-target catches (with specific attention to structuring megabenthic invertebrates), (4) the frequency of gear loss and (5) the seabed entanglement events. A multidisciplinary approach was employed, including ROV explorations of the seabed, background fishermen interviews and on-boarding monitoring. Study areas were selected following a bathymetric point of view in order to study two different deep-sea ecosystems. In **Chapter 2** – "*Artisanal fishing impact on deep coralligenous animal forests: a Mediterranean case study of marine vulnerability*" are evaluated the effects of the small-scale trammel net fishery targeting spiny lobster on a 60-80 m depth coralligenous shoal. In **Chapter 3** – "Placogorgia coronata *first documented record in Italian waters: use of trawl bycatch to unveil vulnerable deep-sea ecosystems*" is evaluated the impact of the trawl fishery targeting red shrimps on a historical fishing ground, located at about 600 m depth nearby a CWC thanatocoenoses and hosting a rare deep-sea gorgonian.

Part 3 - Monitoring and conservation. In this third part, several aspects of monitoring and conservation of mesophotic animal forests have been discussed. Since the MSFD encourages member states to implement monitoring programs on marine debris and identify high-risk activities, **Chapter 4** – "*Distribution and assessment of marine litter on the Ligurian continental shelf (NW Mediterranean Sea)*" aims (1) to assess the composition and distribution of the marine litter on the Ligurian continental shelf, and (2) to identify the main sources of littering present in the area.

From an applied perspective, the achievement and maintaining of GES in the EU territorial waters, requires practice tools for the evaluation of the MSFD descriptors. The objectives of **Chapter 5** – "*Assessing the environmental status of temperate mesophotic reefs: a new, integrated methodological approach*", are (1) to employ part of the large-scale archive of ROV data collected within the Ligurian Sea in order to create a multiparametric index for assessing the conservation status of the megabenthic assemblages thriving on the deep continental shelf, (2) to validate the index on an independent dataset, and (3) to evaluate its performance through a comparison with other pre-existing indices.

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PART 1

BIODIVERSITY

*The Marine Strategy Framework Directive (MSFD) Descriptor 1 "Biological diversity" considers that a Good Environmental Status (GES) is met when "quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climate conditions" (European Commission 2010). Biological diversity could be assessed at different ecological levels (*e.g. *ecosystems, habitats, communities, species). Here, the diversity of megabenthic species and assemblages of the Ligurian deep continental shelf has been investigated at a regional scale. Up to eighty ROV dives have been carried out during a four-years campaign, performed between 30 and 210 m depth. Video footage was analyzed following* Gori et al. *(2011). The final dataset represents a large bulk of baseline information regarding all the invertebrate* *megafauna identifiable through ROV-Imaging as well as the characteristics of the sea bed, including bathymetry (obtained by means of a multibeam echo-sounder, and its derived metrics such as slope, orientation and rugosity) and substrate typologies (directly determined from the video footages). All this information allowed for a fine characterization of 12 Ligurian mesophotic megabenthic assemblages, highlighted with multivariate statistical methods, and in many cases representing the first description of such biocoenoses for the Ligurian or Mediterranean Sea. The role that the environmental factors play in determining the observed patterns has been deeply investigated, finding strong relationships between megabenthic assemblages and depth, sloping and substrate type. Finally, the distribution of megabenthic species and assemblages has been mapped, providing a pioneer and efficient management tool for future monitoring and conservation programs at a regional scale.*

Chapter 1

Megabenthic assemblages of the Ligurian deep continental shelf (NW Mediterranean Sea)

Abstract

The Ligurian Sea is one of the most studied Mediterranean basins thanks to many research expeditions, which have characterized its benthic and pelagic fauna through SCUBA diving and trawl surveys since the beginning of the last century. Nevertheless, a large knowledge gap still exists about the composition of deep circalittoral communities dwelling on the lowest part of the continental shelf, currently under intense pressure from professional and recreational fishing. Until now, deep circalittoral communities of the Ligurian Sea were regarded as a mixture of species belonging to the deepest extension of shallow-water habitats and deep mesophotic assemblages. A four-year ROV survey (2012, 2015, 2016 and 2017) was conducted along the Ligurian continental shelf and slope, between 30 and 210 m depth, in order to characterize the main benthic biocoenoses dwelling on this depth range and to determine the most relevant environmental factors that drive their spatial distribution. Twelve major assemblages were identified, including gorgonian and hydrozoan forests, dense keratose sponge grounds, a *Dendrophyllia cornigera* facies, bryozoan beds and soft-bottom meadows of sabellids and alcyonaceans. Forests of black corals and *Paramuricea macrospina* aggregations were also reported from the video footage, although with a more seldom distribution. A georeferenced database has been created in order to provide information to managers and stakeholders about the location of assemblages and high diversity areas, aiming to facilitate a sustainable long-term conservation of the Ligurian benthic biocoenoses.

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Additional Resources:
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1. Introduction

The Ligurian Sea, located in the northernmost part of the Western Mediterranean Sea, is a very well-studied area, with firsts descriptions of its benthic fauna dating back to the 1800s (Bertoloni, 1819; Risso, 1826; Verany, 1846). During the last century, our knowledge about the benthic ecosystems has increased significantly, leading to the production of many detailed faunal catalogues, both for fishes and benthic invertebrates (see references in Cattaneo et al., 2010). Several studies have focused on the diversity and distribution of megabenthic organisms in coastal areas (*e.g.* Tortonese, 1958, 1961; Balduzzi et al., 1994; Morri et al., 1986), paying particular attention to seagrass meadows (Issel, 1912; Bianchi and Peirano, 1995; Montefalcone et al., 2007) and shallow coralligenous reefs (Marchetti, 1965; Rossi, 1965; Cocito et al., 2002). As a result, shallow water megabenthic communities are nowadays fully described and spatially mapped (Diviacco and Coppo, 2006). The deep-sea megafauna (>200 m) has also been partially characterized, mainly through a long history of scientific observations of the trawling discards (Brian, 1931; Issel, 1932; Rossi, 1958; Relini-Orsi & Relini, 1972). Indeed, the coral frameworks located along the Ligurian continental slope, and formed after centuries of cold-water coral growth, was already tentatively mapped by Fusco in 1968 (Fanelli et al., 2017; Enrichetti et al., 2018).

Less attention, instead, has been given to the deep Ligurian continental shelf (40-200 m), corresponding to the "circalittoral" plane (*sensu* Pérès and Picard, 1964) and also known as the "twilight zone" (*sensu* Cattaneo-Vietti et al., 2010). The exploration of such environments in the Mediterranean Sea has increased in recent years thanks to the progress made in acoustic mapping techniques, the availability of HD video platforms (such as Remote Operated Vehicles) and deep SCUBA procedures (Bo et al., 2009; Cerrano et al., 2010; Di Camillo et al., 2013; Cánovas-Molinas et al., 2016; Gori et al., 2017). These studies highlight the presence of rich megabenthic communities on the continental shelf, some of which fall within the definition of "animal forests" (*sensu* Rossi et al., 2017a). Gorgonians, soft corals, sea pens, black corals, hydrozoans, sponges and large bryozoans constitute the main structuring components of such Mediterranean communities (Gori et al., 2017). Thanks to their complex three-dimensional shapes, which enhance spatial and functional heterogeneity (Buhl-Mortensen et al., 2010; Guizien and Ghisalberti, 2017), these associations favor the development of a rich associated fauna (Bo et al., 2015; Cerrano et al., 2010; Rossi et al., 2017a) and play a significant role in the pelagic-benthic coupling, providing a strong link between the water column and the seafloor environment (Gili and Coma, 1998; Rossi et al., 2017b).

Recent studies have identified a wide array of human activities that put at risk the survival of Mediterranean benthic communities, namely demersal fishing, pollution, construction of littoral infrastructures and seafloor drilling, among others (Hiddink et al., 2017; Marbà et al., 2015; Bo et al., 2014; Pham et al., 2014; Gori et al., 2017). Fishing pressure is widely distributed in the Western Mediterranean Sea and currently

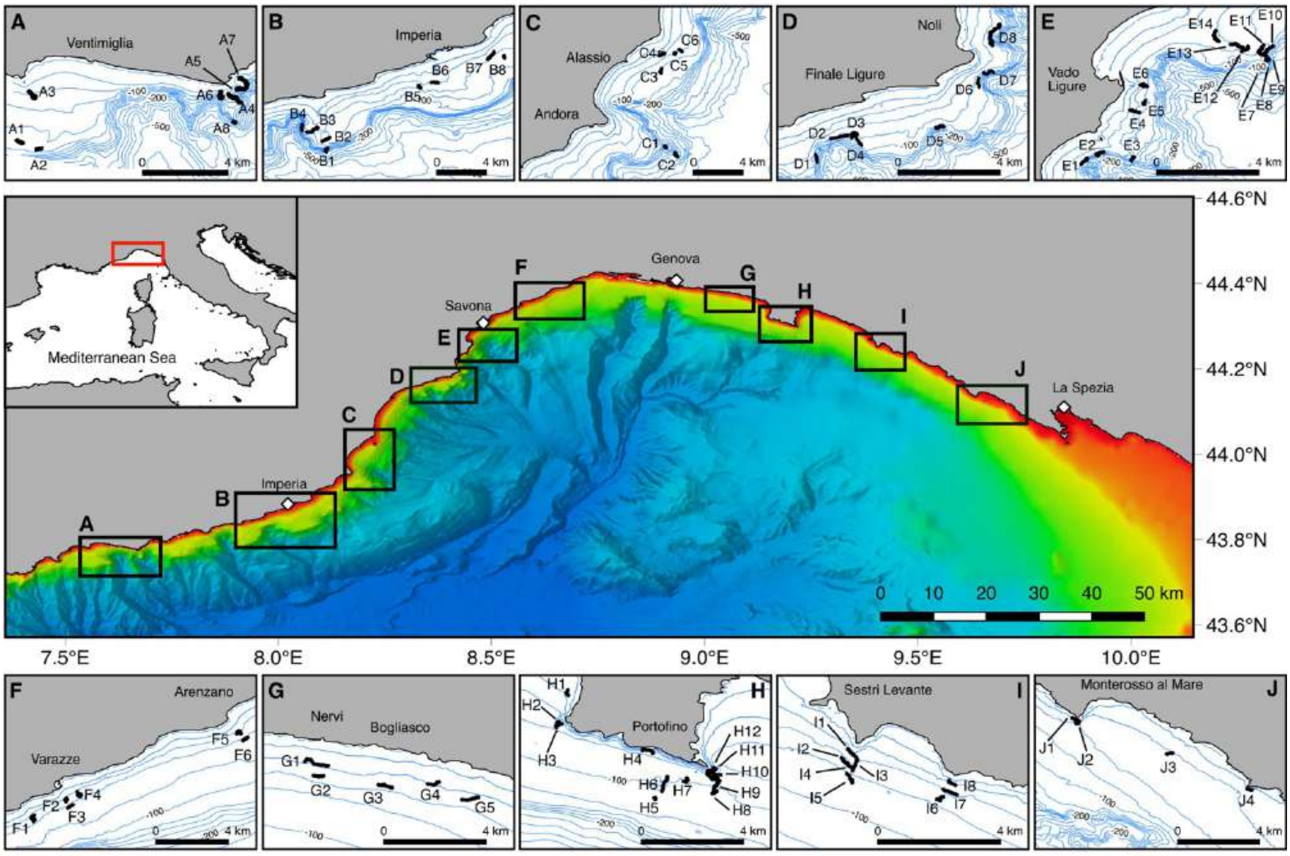

Figure 1. Map of the Ligurian Sea displaying the location of the 80 ROV dives carried out on the Ligurian continental shelf. The 10 study areas selected for this study are shown as black boxes (A-J).

represents the most critical threat for the long-term conservation of benthic habitats. Demersal gears in particular remove or damage benthic organisms, which due to their shape and size, can be dislodged from the seabed after getting entangled in nets and longlines (Cartes et al., 2013; Mytilineou et al., 2014; Deidun et al., 2015). Furthermore, demersal fishing also favors damage-caused diseases, the overgrowth of epibionts on coral structures and the burial of sessile organisms due to sediment resuspension (Bavestrello et al., 1997; Bo et al., 2014; Clark et al., 2015; Gori et al., 2017). Considering that fishes and crustaceans of commercial interest are often associated with complex, well-structured benthic communities (Marliave et al., 2009; D'Onghia et al., 2010; Baillon et al., 2014; Cau et al., 2017), the aggregation of professional and recreational fishermen in species-rich areas threatens their survival (Bo et al., 2014; Gori et al., 2017).

In this context, an extensive exploration of the Ligurian continental shelf megabenthic communities appears of primary interest, not only from a biocoenotic point of view, but also for management purposes knowing the peculiar characteristics and current threats of the basin. This study aims to fulfill the knowledge gap about the diversity of benthic communities found on the deepest part of the Ligurian shelf, between 30 and 200 m depth, based on a community analysis and a large-scale geographical and bathymetrical distribution, together with an assessment of the environmental factors that better explain such distribution. All this information will be of major relevance in order to determine the conservation status of the megabenthic communities and provide tools for the implementation of appropriate management policies to ensure their long-term conservation within the limits of the Ligurian basin.

2. Materials and Methods

2.1 Study area

The Ligurian Sea embraces the north-western sector of the Mediterranean Sea, extending from the Gulf of Lions to the northern Tyrrhenian Sea, and bordered on the south by Corsica Island. This study focuses on the deep continental shelf of the Liguria coastline, extending for over 350 km, from Ventimiglia to La Spezia (Fig. 1). The seabed topography of the Liguria continental shelf shows remarkable differences along its extent, creating two sub-basins. The western basin is deeper, reaching depths of 2600 m, and presents a steep and narrow shelf incised by 16 submarine canyons (Fanucci et al., 1989; Cattaneo et al., 2010; Würtz, 2012). Upwelling currents take place within this sector, enhanced by the common occurrence of mesoscale anticyclonic eddies, and resulting in a considerable input of nutrients in the euphotic layer (Casella et al., 2011). In the eastern sector, the continental shelf is wider, with a less pronounced slope and with only three major canyon systems. The majority of the Ligurian canyons are shelf-incising, with no clear bathymetric connection to a major river system (Harris & Whiteway, 2011). Due to the cyclonic circulation, the overall superficial water flow moves westward along the basin (Cattaneo et al., 2010). Intermediate Levantine waters and deep waters entering the basin from the Tyrrhenian Sea through the Tuscan Archipelago and along the western coasts of Corse follow a similar cyclonic path (Astraldi et al., 1994; Millot 1999; Cattaneo et al., 2010). More than 76 rivers, with predominantly torrential streams, flow through the Alpine and Apennine chains within the investigated portion of the Ligurian basin. Magra River, flowing in the easternmost part of the study area, represents the largest Ligurian river and causes the seawater to be turbid and rich in nutrients in the eastern sector (Bassano et al., 2000; Attolini e Coppo, 2005).

2.2 Video survey

Due to the large extent of the Ligurian coastline, video sampling was concentrated in ten major areas (from A to J moving westward) (Fig. 1), where a total of 80 underwater video transects were carried out (Tab. 1). Seventy transects were recorded during three surveys conducted on board of the R/V *Astrea* ISPRA (Rome) in 2012, 2015 and 2016 within a survey for the *Ministero dell'Ambiente e della Tutela del Territorio e del Mare* (MATTM) and the Marine Strategy Framework Directive (MSFD) monitoring program. ROV Pollux III was equipped with a digital camera (Nikon D80), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), underwater lights and a 3-jaw grabber. The ROV also hosted a depth sensor, a compass and the underwater acoustic

Dive	Date	Area	Site	ROV	Start position		End position		Depth (m)		Length (m)	Nº SU	% valid SU
					Lat. N	Long E.	Lat. N	Long. E	Start	End			
A01	01/09/16	Ventimiglia	Mortola Cape	PIII	7.552	43.743	7.555	43.742	89	85	545	54	87
A02	01/09/16	Ventimiglia	Mortola Cape	PIII	7.564	43.738	7.567	43.739	86	82	373	37	89
A03	01/09/16	Ventimiglia	Mortola Cape	PIII	7.563	43.769	7.559	43.772	45	30	1150	115	48
A04	02/09/16	Ventimiglia	Bordighera	PIII	7.675	43.770	7.682	43.766	39	98	1595	159	62
A05	02/09/16	Ventimiglia	Bordighera	PIII	7.680	43.774	7.682	43.774	69	55	186	18	44
A06	02/09/16	Ventimiglia	Bordighera	PIII	7.670	43.770	7.671	43.772	76	43	974	97	48
A07	07/06/12	Ventimiglia	Bordighera	PIII	7.682	43.780	7.680	43.773	38	75	1797	179	39
A08	09/06/12	Ventimiglia	Besughi Shoal	PIII	7.678	43.754	7.679	43.754	120	190	230	23	48
B01	07/06/12	Imperia	Gobbe del Cammello	PIII	7.918	43.784	7.916	43.781	96	156	778	77	39
B02	12/08/15	Imperia	Santo Stefano	PIII	7.920	43.796	7.912	43.792	63	58	874	87	57
B03	12/08/15	Imperia	Santo Stefano	PIII	7.907	43.806	7.897	43.801	46	66	1625	162	28
B04	13/08/15	Imperia	Santo Stefano	PIII	7.892	43.809	7.892	43.803	87	87	841	84	17
B05	13/08/15	Imperia	Porto Maurizio	PIII	8.010	43.848	8.011	43.847	47	48	789	78	15
B06	13/08/15	Imperia	Porto Maurizio	PIII	8.023	43.851	8.030	43.851	36	37	794	79	28
B07	14/08/15	Imperia	Diano Marina	PIII	8.086	43.882	8.080	43.875	49	53	1761	176	41
B08	14/08/15	Imperia	Diano Marina	PIII	8.096	43.878	8.097	43.876	65	70	273	27	78
C01	06/06/12	Alassio	Mele Cape	PIII	8.230	43.930	8.231	43.929	106	135	754	75	68
C02	06/06/12	Alassio	Mele Cape	PIII	8.240	43.923	8.243	43.919	179	212	674	67	30
C03	16/02/18	Alassio	Gallinara Island	BR2	8.226	44.009	8.226	44.004	80	89	736	73	95
C04	17/04/18	Alassio	Gallinara Island	BR2	8.223	44.022	8.229	44.024	40	39	756	75	85
C05	15/02/18	Alassio	Gallinara Island	BR2	8.240	44.023	8.240	44.024	66	59	474	47	94
C06	14/02/18	Alassio	Gallinara Island	BR2	8.247	44.026	8.244	44.028	60	55	390	39	100
D01	17/04/18	Finale Ligure	Pora Canyon	BR2	8.347	44.144	8.346	44.147	78	76	524	52	96
D02	17/04/18	Finale Ligure	Pora Canyon	BR2	8.362	44.157	8.354	44.155	88	78	822	82	93
D03	17/04/18	Finale Ligure	Pora Canyon	BR2	8.364	44.156	8.366	44.157	92	82	587	58	97
D04	18/04/18	Finale Ligure	Pora Canyon	BR2	8.369	44.152	8.365	44.157	92	85	639	63	90
D05	06/06/12	Finale Ligure	Lua Canyon	PIII	8.409	44.161	8.405	44.161	96	138	752	75	75
D06	18/04/18	Finale Ligure	Noli Cape	BR2	8.426	44.185	8.426	44.181	93	85	580	58	79
D07	21/04/18	Finale Ligure	Noli Cape	BR2	8.433	44.188	8.428	44.187	106	103	703	70	81
D08	10/08/15	Finale Ligure	Noli Canyon	PIII	8.432	44.201	8.435	44.211	71	72	1862	186	38
E01	10/08/15	Savona	Maledetti Shoal	PIII	8.434	44.222	8.437	44.224	53	83	819	81	46
E02	03/09/16	Savona	Maledetti Shoal	PIII	8.445	44.227	8.442	44.226	55	111	640	64	38
E03	05/06/12	Savona	Corallone Shoal	PIII	8.459	44.225	8.459	44.223	64	122	667	66	45
E04	09/08/15	Savona	Vado shoals	PIII	8.458	44.248	8.463	44.247	39	76	692	69	70
E05	09/08/15	Savona	Vado shoals	PIII	8.466	44.253	8.465	44.251	63	69	373	37	59
E06	09/08/15	Savona	Vado shoals	PIII	8.465	44.261	8.463	44.260	64	45	843	84	62
E07	05/06/12	Savona	Mantice Shoal	PIII	8.522	44.270	8.521	44.267	105	151	1013	101	45
E08	05/06/12	Savona	Mantice Shoal	PIII	8.525	44.273	8.524	44.271	84	80	399	39	54
E09	09/08/15	Savona	Mantice Shoal	PIII	8.526	44.273	8.524	44.271	106	81	844	84	33
E10	26/08/15	Savona	Mantice Shoal	PIII	8.528	44.279	8.525	44.273	78	106	1243	124	31
E11	03/09/16	Savona	Savona shoals	PIII	8.524	44.280	8.523	44.275	73	79	1289	128	57
E12	03/09/16	Savona	Savona shoals	PIII	8.516	44.279	8.513	44.277	65	70	732	73	81
E13	03/09/16	Savona	Savona shoals	PIII	8.507	44.280	8.514	44.279	55	59	924	92	51
E14	03/09/16	Savona	Savona shoals	PIII	8.500	44.287	8.502	44.282	56	48	987	98	68
F01	04/09/16	West Genova	Celle Ligure	PIII	8.551	44.331	8.551	44.327	44	52	1154	115	58

F02	08/08/15	West Genova	Varazze	PIII	8.573	44.340	8.574	44.342	47	44	628	62	68
F03	08/08/15	West Genova	Varazze	PIII	8.578	44.338	8.575	44.336	61	62	559	55	93
F04	08/08/15	West Genova	Varazze	PIII	8.582	44.347	8.582	44.346	40	47	839	83	100
F05	08/08/15	West Genova	Arenzano	PIII	8.695	44.387	8.692	44.387	41	43	848	84	62
F06	08/08/15	West Genova	Arenzano	PIII	8.700	44.385	8.697	44.383	62	62	536	53	91
G01	06/08/15	East Genova	Nervi	PIII	9.032	44.364	9.027	44.364	52	56	497	49	69
G02	06/08/15	East Genova	Nervi	PIII	9.034	44.369	9.023	44.371	34	34	1285	128	100
G03	06/08/15	East Genova	Bogliasco	PIII	9.066	44.358	9.059	44.360	57	55	839	83	66
G04	05/08/15	East Genova	Sori	PIII	9.089	44.361	9.083	44.360	35	37	614	61	77
G05	05/08/15	East Genova	Sori	PIII	9.100	44.353	9.108	44.354	53	43	958	95	60
H01	04/09/16	MPA Portofino	Punta Chiappa	PIII	9.148	44.337	9.149	44.334	34	34	625	62	87
H02	03/06/12	MPA Portofino	Isuela	PIII	9.144	44.320	9.144	44.320	29	31	245	24	50
H03	05/09/16	MPA Portofino	Isuela	PIII	9.145	44.320	9.143	44.318	52	73	939	93	69
H04	06/09/16	MPA Portofino	Cala degli Inglesi	PIII	9.190	44.305	9.185	44.307	73	50	806	80	69
H05	27/08/15	MPA Portofino	San Gorgio shoal	PIII	9.191	44.283	9.191	44.283	104	104	255	25	44
H06	27/08/15	MPA Portofino	San Gorgio shoal	PIII	9.197	44.294	9.195	44.287	96	104	1098	109	71
H07	03/06/12	MPA Portofino	San Gorgio shoal	PIII	9.205	44.292	9.207	44.293	98	92	544	54	59
H08	02/06/12	MPA Portofino	Punta del Faro	PIII	9.221	44.287	9.223	44.292	100	76	923	92	67
H09	25/08/16	MPA Portofino	Punta del Faro	PIII	9.222	44.287	9.220	44.286	101	104	503	50	72
H10	02/06/12	MPA Portofino	Punta del Faro	PIII	9.223	44.292	9.217	44.297	75	57	1494	149	38
H11	25/08/16	MPA Portofino	Punta del Faro	PIII	9.219	44.293	9.222	44.291	83	83	722	72	89
H12	25/08/16	MPA Portofino	Punta del Faro	PIII	9.223	44.295	9.217	44.297	86	63	747	74	86
H13	25/08/16	MPA Portofino	Punta del Faro	PIII	9.220	44.298	9.218	44.297	61	59	908	90	39
I01	03/08/15	Sestri Levante	Punta Manara	PIII	9.400	44.246	9.403	44.245	46	56	676	67	94
I02	03/08/15	Sestri Levante	Punta Manara	PIII	9.397	44.246	9.401	44.242	62	64	768	76	88
I03	03/08/15	Sestri Levante	Punta Manara	PIII	9.404	44.245	9.402	44.240	58	74	586	58	69
I04	23/08/16	Sestri Levante	Punta Manara	PIII	9.401	44.242	9.398	44.245	65	62	625	62	47
I05	03/08/15	Sestri Levante	Punta Manara	PIII	9.399	44.239	9.401	44.236	81	85	562	56	84
I06	03/08/15	Sestri Levante	Punta Baffe	PIII	9.436	44.229	9.439	44.230	73	66	512	51	86
I07	03/08/15	Sestri Levante	Punta Baffe	PIII	9.439	44.231	9.444	44.230	56	58	674	67	87
I08	03/08/15	Sestri Levante	Punta Baffe	PIII	9.444	44.234	9.441	44.236	37	33	383	38	76
J01	02/09/16	Cinque Terre	Punta Mesco	PIII	9.631	44.133	9.635	44.132	35	48	597	59	42
J02	23/08/16	Cinque Terre	Punta Mesco	PIII	9.635	44.130	9.636	44.131	51	47	665	66	21
J03	02/09/16	Cinque Terre	Corniglia	PIII	9.692	44.112	9.689	44.112	40	42	621	62	73
J04	23/08/16	Cinque Terre	Punta Montenero	PIII	9.741	44.090	9.738	44.090	29	35	539	53	30

Table 1. Main features of the 80 ROV dives carried out on the deep continental shelf of Liguria. GPS positions are provided in decimal degrees. The different areas explored can be identified in Fig. 1.

positioning system (Ultra-Short Baseline, USBL) LinkQuest TrackLink 1500 MA to obtain accurate georeferenced positions every second when underwater. Two parallel laser beams provided a scale for dimensional reference. The remaining 10 transects were recorded in 2018 by COLMAR S.r.l. (La Spezia) for the MSFD monitoring program, employing a BlueROV2, equipped with an HD video camera, underwater lights, parallel laser beams, USBL Sonardyne Scout Plus, a depth sensor and a compass. In order to guarantee a similar video quality for the whole video footage, the ROVs aimed to move at constant speed of less than 0.3 knots and a distance from the seabed lower than 1.5 m (Grinyó et al., 2017; Dominguez-Carrió et al., 2018). The average length of the video transects was 45 minutes and 776 m, with depths ranging between 28 and 216 m (Tab. 1, SM 1). Voucher samples for those species for which identification through the video footage was difficult were collected by means of a grabber, dried or fixed in absolute ethanol for further taxonomic identification.

2.3 Video analysis

Video images were evaluated using Apple's Final Cut Pro software following the methodology described by Gori et al. (2011). Loops and pauses during the ROV transect were erased in order to obtain a linear video track. After smoothing the ROV trajectory provided by the raw GPS positioning, track length was measured with the open-source software Quantum GIS (QGIS Development Team 2016). Sequences too far away from the sea floor or with poor image quality were considered unsuitable for the analysis. The valid footage accounted for approximately 83% of the recorded material, corresponding to a length of about 51 km. All megabenthic organisms observed in the images within a visual field of 50 cm wide were annotated to the lowest possible taxon. The time elapsed since the beginning of the footage was assigned to each organism, and time was later converted to distance using the speed of the ROV derived from the acoustic position system. Substrate type was also identified along the transect for each dive. Five substrate categories were used: (1) muds and fine sands, (2) sands and gravels, (3) cobbles and pebbles, (4) outcropping rocks and (5) coralligenous rocks. Each transect was then divided into a string of adjacent 5 m^2 sampling units (SU). The size of the sampling unit was chosen following the results of Grinyó et al. (2016) and Dominguez-Carrió (2018), which evaluated areas of a similar depth range. The number of organisms per species was assigned to each sampling unit, together with the percentage of each substrate category. Depth and slope values were derived from the multibeam bathymetry using the location of the middle point of each sampling unit.

2.4 Data analyses

2.4.1 Megabenthic diversity

In order to characterize the diversity of the benthic habitats along the whole study area, differences among sites were investigated in terms of species richness, diversity and biomass. Species richness was calculated as number of conspicuous animal taxa (excluding fish) per sampling unit, equally considering organisms identified as morphospecies under different taxonomic levels (species, genus or higher taxa). Megabenthic diversity was estimated using the Exponential of Shannon diversity index, which provides an indication of the effective number of species per SU and has the same linear metric as species richness (Jost et al., 2010). Finally, the number of organisms per SU was used as a proxy for biomass. Although there are attempts to quantify biomass for certain species based on visual observations (Durden et al., 2016), the complexity of applying such procedures over large species lists limited its applicability over our dataset.

2.4.2 Community analysis

Aiming to identify the major megabenthic assemblages of the Ligurian continental shelf, samples with less than five organisms were removed from the multivariate analyses in order to reduce background noise. Associations of species were determined by means of the clustering algorithm Ward's minimum variance method based on Bray-Curtis dissimilarity measures over square root transformed density data using the package stats in the R environment. The optimal number of groups was determined by selecting the highest average silhouette width from all cluster solutions using the silhouette function included in the cluster package (Maechler et al. 2017). Once groups were identified, a PERMANOVA was run to determine the statistical significance between groups using the adonis function included in the vegan package (Oksanen et al. 2016). The relative importance of all taxa in each assemblage was determined using the Indicator Value (IndVal), which measures the fidelity (relative frequency) and specificity (relative abundance) of species within groups to identify those that can be considered characteristic of the association (Dufrêne and Legendre, 1997). IndVal measures were computed using the function indval included in the labdsv package (Roberts, 2016). The capacity of the environmental parameters (depth, slope, substrate type and seabed roughness) to determine the composition of the different assemblages was explored by means of a distance-based redundancy analysis (RDA) using the function capscale included in the vegan package.

2.4.3 Geographic distribution

Based on the geographical position of each sampling unit, the spatial distribution of all assemblages was displayed using the open-source software Quantum GIS. To provide a better representation of the importance of structuring species within the diversity of the megabenthic assemblages, the density and geographical distribution of the most relevant species observed in the video footage was also represented in distribution maps.

3. Results

3.1 General results

The 80 ROV dives performed on the Ligurian continental shelf provided a total of 63 hours of video footage, corresponding to an explored area of 0.031 km^2 of seabed (Tab. 1). Dives depth ranged approximately between 30 and 220 m, over a very fragmented seafloor in which soft substrates, mainly represented by mud and fine sands, were the most widespread, occupying 63% of the total explored area. The remaining 37% corresponded to hard bottoms, that being cobbles and pebbles (1.4%), coralligenous rocks (9%) and outcropping

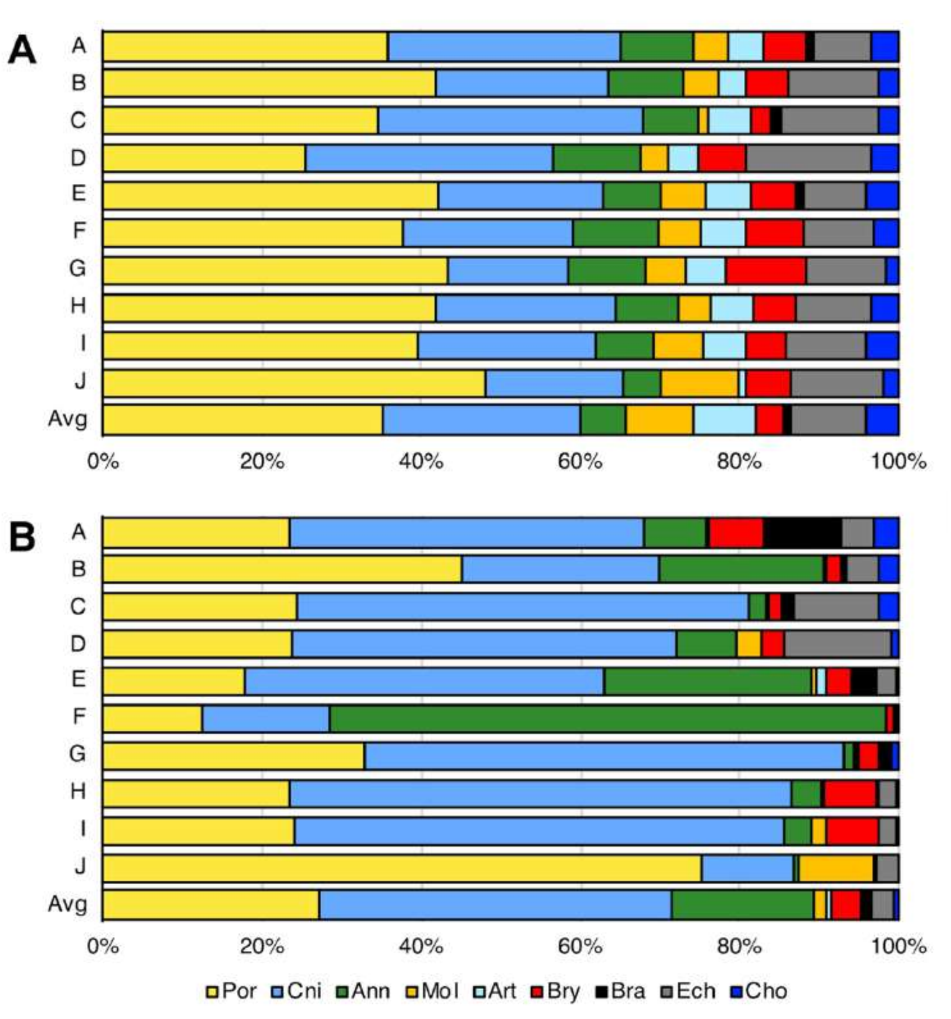

Figure 2. Percentage of the diversity in each of the 10 study areas (A: Ventimiglia; B: Imperia; C: Alassio; D: Finale Ligure; E: Savona; F: West Genova; G: East Genova; H: Portofino MPA; I: Sestri Levante; J: Cinque Terre MPA) according to their species composition (A) and abundance (B). Por: Porifera; Cni: Cnidaria; Ann: Annelida; Mol: Mollusca; Art: Artropoda; Bry: Bryozoa; Bra: Brachiopoda; Ech: Echinodermata; Cho: Chordata.

Area	nº dives	Area explored (m^2)	Depth range (m)	Av. nº spp. per SU ± sd	Av. expH per SU ± sd	Av. nº org per SU ± sd	Number of species								
							Por	Cni	Ann	Mol	Art	Bry	Bra	Ech	Cho
A	8	1845	28-200	4.0 ± 4.0	2.9 ± 2.2	19.8 ± 32.0	40	33	10	5	5	6	1	8	4
B	8	1340	33-154	6.2 ± 4.4	3.9 ± 2.6	46.0 ± 82.7	48	25	11	5	4	6	0	13	3
C	6	1435	28-216	2.8 ± 3.3	2.2 ± 1.9	17.5 ± 34.3	26	25	5	1	4	2	1	9	2
D	8	2345	71-141	2.9 ± 2.7	2.3 ± 1.7	12.0 ± 16.8	21	26	9	3	3	5	0	13	3
E	14	2950	36-151	6.3 ± 5.2	3.5 ± 2.4	91.6 ± 240	52	26	9	7	7	7	1	10	5
F	6	1715	34-63	3.5 ± 3.9	2.6 ± 2.0	64.3 ± 281	35	20	10	5	5	7	0	8	3
G	5	1605	30-57	2.3 ± 3.1	2.0 ± 1.4	18.8 ± 42.5	26	9	6	3	3	6	0	6	1
H	13	3115	29-106	3.8 ± 4.0	2.5 ± 1.8	34.2 ± 74.0	48	26	9	5	6	6	0	11	4
I	8	1885	33-85	3.0 ± 3.3	2.5 ± 2.0	15.0 ± 31.1	50	28	9	8	7	6	0	13	5
J	4	500	28-55	3.6 ± 3.8	2.7 ± 1.8	33.8 ± 61.0	50	18	5	10	1	6	0	12	2

Table 2. Specificities of each area explored based on the taxonomic composition of its megafauna species. See Fig. 2 for acronyms; SU: Sampling Unit.

rocks (27%). A comprehensive description of the environmental features that characterized each ROV dive is provided in SM1.

A total of 219,029 megabenthic organisms (excluding fish), belonging to 224 different taxa, were identified in the video images. From such list, 78% of the taxa could be identified to species (57%) or genus level (18%), whereas the remaining 67 morphospecies had to be classified in higher taxonomic levels: family (3%), order (3%), class (4%) or phylum (15%). The complete list of identified taxa is provided in the SM2. Sponges and corals played a major role in Ligurian biodiversity, contributing to almost 60% of the total number of taxa identified (Fig. 2A). In a lesser extent, echinoderms (9.5%), mollusks (8.6%), crustaceans (7.7%), annelids (5.9%) and ascidians (4.1%) also contributed to the overall megafauna diversity of the study area. Cnidarians were the most abundant group in terms of number of organisms (44%), followed by sponges (27%) and annelids (18%) (Fig. 2B). The remaining phyla contributed to approximately 10% of the total number of organisms. The yellow sponge *Axinella* spp., often in association with the zoanthid *Parazoanthus axinellae* represented both the most abundant and the most widely distributed organism, with 34,314 records and appearing in 89% of the dives. The scleractinian *Leptopsammia pruvoti* and the annelid *Bispira viola* were also very abundant along the Liguria coast. In terms of spatial occurrence, the gorgonians *Eunicella verrucosa* and *Paramuricea clavata* were the most widely distributed species besides the complex *Axinella* spp./*Parazoanthus axinellae*.

3.2 Regional differences

The 10 study areas were unevenly sampled. As a result, the amount of investigated area ranged from 500 m^2 in Area J (Cinque Terre) to about 3000 m^2 in Area E and H (Savona and Portofino, respectively) (Tab. 2). Depths within the four areas located in the easternmost sector of the Ligurian Sea were generally more homogeneous and shallower than in the other areas. An accurate description of the biological features, with also the characteristic species for each ROV dive, is provided in SM3. Overall, the average number of megabenthic organisms per SU was 38 (± 138), with an average number of species of 3.97 (± 4.14). The number of species identified in each dive was very inconsistent throughout the whole Ligurian continental shelf, varying between 8 (Pora Canyon) and 80 (Bordighera). Areas B (Imperia) and E (Savona), both located in the westernmost sector, presented on average the highest number of species per SU and also the highest species richness and diversity based on the Exponential of Shannon index (Tab. 2). The lowest values of species richness and diversity were observed in Area G (East Genova), which is located in the eastern sector. Area E (Savona) presented, on average, the highest number of organisms per SU (92 ± 240), with 2758 organisms identified in one single dive (Vado shoals).

In general terms, sponges played a major role in species composition, whilst cnidarians were the most abundant in the 10 investigated areas (Fig. 2). Large differences in species diversity and abundance were observed between Area C (Alassio) and D (Finale Ligure) (where

ID Ass.	Characteristic species	% SU	Av. n° spp. per SU ± sd	Av. n° org. per SU ± sd	Av. expH per SU ± sd	Substrate type (%)					Depth (m)	
						Mud/ Sand	Sand/ Gravel	Cobb./ Pebbl.	Rock	Cor. Rock	Min	Max
1	*P. fascialis*	9.59	6.2 ± 3.8	9.7 ± 19.7	4.0 ± 2.7	55	8	0.5	25	7	30	106
2	*L. myriophyllum*	3.46	3.6 ± 2.2	4.7 ± 3.1	2.3 ± 1.2	87			11	1	49	102
3	*Axinella* spp.	24.45	8.0 ± 3.4	12.1 ± 8.6	4.9 ± 1.9	25	3	0.5	40	30	28	83
4	Serpulidae	9.85	6.7 ± 2.7	8.9 ± 18.8	4.2 ± 1.8	16	0.2	1	79	4	34	208
5	*E. cavolini*	15.35	9.2 ± 3.4	15.0 ± 10.6	4.8 ± 1.7	11	0.3	1	83	5	31	129
6	*P. clavata*	13.41	7.4 ± 3.0	9.7 ± 6.3	4.6 ± 2.0	17	2		50	31	29	89
7	*L. pruvoti*	5.46	12.1 ± 5.9	63.8 ± 44.7	3.9 ± 1.8	8	5	2	50	36	30	85
8	*E. verrucosa*	9.65	6.7 ± 3.3	5.2 ± 2.6	4.4 ± 2.3	50		3	45	2	30	92
9	*D. cornigera*	1.88	3.0 ± 1.5	6.5 ± 3.4	2.0 ± 1.0	74			26		84	209
10	*P. spinulosum*	2.12	3.9 ± 2.1	19.1 ± 14.2	1.6 ± 0.8	96			4		68	106
11	*H.* cf. *med.*	3.28	6.6 ± 2.7	10.2 ± 7.6	3.4 ± 1.6	14	72		8	7	46	88
12	*B. viola*	1.40	4.5 ± 3.4	263.9 ± 183	1.3 ± 0.7	79			19		56	69

Table 3. Biological features of the 12 assemblages identified in this study. Characteristic species correspond to those scoring higher in the IndVal analysis, with the exception of the assemblage 3 where the most abundant species was selected as characteristic (see main text for further information). SU: Sampling Unit.

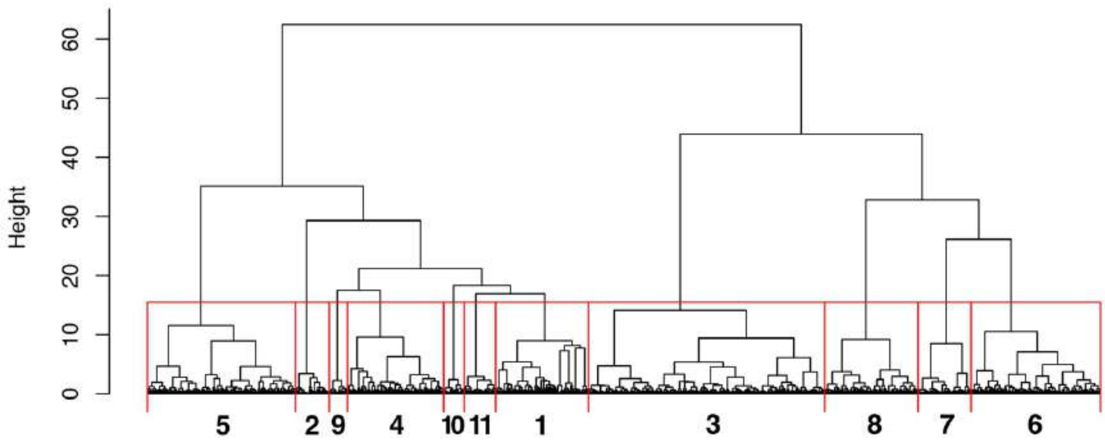

Figure 3. Dendrogram using Ward's clustering method constructed over Bray-Curtis dissimilarity measures of square-root transformed density data for all megabenthic species.

cnidarians contributed equally or more than sponges to the total biodiversity of the region, Fig. 2A), Area B (Imperia) and J (Cinque Terre) (where sponges were the most abundant organisms), and Area F (West Genova) (where annelids represented up to 70% of the total number of organisms, Fig. 2B).

3.3 Community structure

Excluding those sampling units (SU) characterized by bad visibility or excessive distance to the seabed, of the 6169 5-m^2 SU that derived from the video footage, 3747 (61%) were suitable for statistical analyses, corresponding to a total usable area of about 1.9 ha. Among those SU, 829 (22%) did not present a single megabenthic organism. The clustering of the remaining 2918 SU in a hierarchical dendrogram provided a total of 12 megabenthic associations (Tab. 3, Fig. 3), significantly different from each other based on species composition (p-perm<0.01). A comprehensive list of the most important species characterizing each assemblage is provided in Tab. 4. A species/taxa name, generally based on the characteristic species, was used to designate each community in order to facilitate the reading. A description of each assemblage identified follows.

Assemblage 1: Bryozoan beds (Fig. 4A). This assemblage mainly occurred on horizontal soft bottoms located between 50 and 80 m depth (Fig. 5). Its characteristic species was the erect bryozoan *Pentapora fascialis*, with maximum densities of 14 colonies·m^{-2} (Tab. 4). The bivalve *Neopycnodonte cochlear* also reached its highest densities within this assemblage. It was found growing on wrecks and large metallic objects randomly distributed on the seabed, which in some cases were completely covered by those bivalves. Serpulids of the species complex *Filograna/Salmacina* were also predominant in certain spots, forming large fields (*e.g.* site of Savona, Fig. 6A).

Assemblage 2: Hydrozoan forests (Fig. 4B). Large aggregations of the hydroid *Lytocarpia myriophyllum* (up to 3 col·m^{-2}) occurred on horizontal grounds covered by muds and fine sands, mainly between 70 and 80 m depth (Fig. 5). Some bryozoans and serpulids were often observed as epibionts of the large hydroid colonies.

Assemblage 3: Sponge grounds (Fig. 4C). These aggregations were encountered on various substrate types, with highest abundances on flat hard bottoms between 40 and 60 m depth (Fig. 5). This assemblage corresponds to a multi-specific sponge aggregation, dominated by the large massive keratose porifera *Sarcotragus foetidus* (densities up to 3.2 individuals·m^{-2}) together with *Dysidea* sp. (max. densities of 7.6 ind·m^{-2}). The zoanthid *Parazoanthus axinellae* displayed the highest IndVal value in this assemblage, although it was observed as an epibiont of the small yellow sponge *Axinella* spp. (Tab. 3 and 4, Fig. 6B). This sponge represented the most common organism along the Ligurian continental shelf, reaching densities of 25 ind·m^{-2}. Several other keratose sponges typify this assemblage, but their identification from the video footage was not always possible: six different morphotypes were recognized, with morphotypes 1, 2 and 4 being the most frequent (SM2). Rare and scattered large specimens of *Spongia lamella* were also observed within this assemblage, as well as the gorgonian *E. verrucosa*, the annelid *Bonellia viridis* and the ascidian *Halocynthia papillosa*.

Assemblage 4: Deep-rocky bottom communities (Fig. 4D). Steep rocks, mainly bare, usually colonized by unidentified serpulids worms and hosting several species of holothurians and sea urchins, were observed along the whole study area. Their distribution covered a wide bathymetrical range, although mainly concentrated between 85 and 105 m depth (Fig. 5). A large pseudo-encrusting unidentified sponge (resembling *Hexadella pruvoti*) was

Assemblage	Species	IndVal	Avg. density (org·m^{-2} ± sd)	Max. density (org·m^{-2})
1	*Pentapora fascialis*	0.16	0.89 ± 2.02	14
	Neopycnodonte cochlear	0.12	1.61 ± 6.73	49
	Filograna/Salmacina complex	0.09	0.62 ± 2.04	16.4
	Porifera sp. 57	0.09	0.32 ± 1.48	12.8
	Myriapora truncata	0.07	0.33 ± 1.24	7.8
	Reteporella spp.	0.05	0.14 ± 0.3	2
	Alcyonium acaule	0.05	0.09 ± 0.55	6.4
2	*Lytocarpia myriophyllum*	0.84	3.04 ± 1.55	7.2
	Filograna/Salmacina complex	0.06	0.33 ± 0.84	5
	Smittina cervicornis/Adeonella calveti	0.05	0.16 ± 0.26	1
3	*Parazoanthus axinellae*	0.36	3.75 ± 3.45	19.2
	Sarcotragus foetidus	0.21	0.39 ± 0.56	2.8
	Dysidea sp.	0.21	0.25 ± 0.65	7.6
	Axinella spp.	0.19	4.3 ± 3.73	25.4
	Axinella polypoides	0.11	0.09 ± 0.03	3.8
	Eunicella verrucosa	0.10	0.73 ± 1.22	8.6
	Halocynthia papillosa	0.06	0.21 ± 0.5	4.6
	Chondrosia reniformis	0.05	0.13 ± 0.56	8.2
	Bonellia viridis	0.05	0.08 ± 0.2	2
4	Serpulidae	0.27	1.23 ± 3.69	26.4
	Holothuria spp.	0.15	0.36 ± 0.48	2.8
	Cidaris cidaris/Stylocidaris affinis	0.14	0.47 ± 0.66	4
	Porifera sp. 49	0.11	0.21 ± 0.44	3
	Echinus melo/Gracilechinus acutus	0.08	0.05 ± 0.12	0.6
	Apomatus/Protula complex	0.07	0.06 ± 0.15	0.8
	Axinella spp.	0.07	1.33 ± 1.41	6.2
	Megerlia truncata	0.06	0.85 ± 4.39	40
5	*Eunicella cavolini*	0.60	4.95 ± 4.89	22.4
	Alcyonium coralloides	0.27	0.53 ± 1.04	8.4
	Axinella spp.	0.12	3.11 ± 3.77	20.4
	Hexadella racovitzai	0.08	0.13 ± 0.29	2.2
	Astrospartus mediterraneus	0.07	0.07 ± 0.21	1.6
	Holothuria sp.	0.06	0.21 ± 0.37	2.4
	Porifera sp. 49	0.06	0.19 ± 0.52	3.6
	Antedon mediterranea	0.05	0.22 ± 0.88	8.2
6	*Paramuricea clavata*	0.35	3.51 ± 3.33	18.8
	Aplysina cavernicola	0.09	1.05 ± 2.43	16.8
	Axinella spp.	0.08	1.42 ± 1.55	6.8
	Eunicella verrucosa	0.07	0.61 ± 1.36	15.04
	Filograna/Salmacina complex	0.06	0.34 ± 0.78	5.4
	Turbicellepora sp.	0.05	0.17 ± 0.41	2.6
7	*Leptopsammia pruvoti*	0.97	38.67 ± 33.62	143
	Corallium rubrum	0.49	3.98 ± 8.64	8.6
	Agelas oroides	0.26	0.39 ± 0.72	2.8
	Petrosia ficiformis	0.25	0.77 ± 1.89	12.6
	Paramuricea clavata	0.24	4.05 ± 4.47	17
	Axinella spp.	0.23	6.68 ± 4.35	24.2
	Aplysina cavernicola	0.22	1.51 ± 2.29	10.4
	Porifera sp. 4	0.20	0.47 ± 0.89	4.6
	Smittina cervicornis/Adeonella calveti	0.16	1.1 ± 2.11	11
	Parazoanthus axinellae	0.14	2.16 ± 3	11.8
8	*Eunicella verrucosa*	0.41	2.3 ± 1.51	7.2
	Crella sp.	0.14	0.09 ± 0.2	1
	Leptogorgia sarmentosa	0.11	0.15 ± 0.47	3.8
	Eunicella singularis	0.10	0.17 ± 0.54	3.4
	Turbicellepora sp.	0.06	0.15 ± 0.33	2.4
	Frondipora verrucosa	0.05	0.04 ± 0.14	1.4
	Sarcotragus foetidus	0.05	0.14 ± 0.35	3.2
	Reteporella spp.	0.05	0.12 ± 0.22	1.2
	Smittina cervicornis/Adeonella calveti	0.05	0.22 ± 0.44	2.6
9	*Dendrophyllia cornigera*	< 0.05	5.01 ± 3.69	14.8
	Cidaris cidaris/Stylocidaris affinis	< 0.05	0.57 ± 0.76	2.8
	Pachastrella monilifera	< 0.05	0.12 ± 0.39	1.8
	Gryphus vitreus	< 0.05	0.19 ± 1.04	5.8
	Myxicola sp.	< 0.05	0.05 ± 0.11	0.4
	Scleractinia sp. 1	< 0.05	0.13 ± 0.48	2.4
	Scleractinia sp. 4	< 0.05	0.01 ± 0.05	0.2
10	*Paralcyonium spinulosum*	1.00	16.91 ± 14.65	76.6

	Alcyonium palmatum	0.06	0.09 ± 0.22	1.2
	Acromegalomma sp.	0.06	0.02 ± 0.06	0.2
11	*Haliclona* cf. *mediterranea*	0.89	5.47 ± 3.94	15.4
	Amphiura sp.	0.18	0.29 ± 0.62	3
	Axinella spp.	0.12	2.1 ± 2.72	13.6
	Porifera sp. 20	0.11	0.05 ± 0.15	0.6
	Alcyonium palmatum	0.11	0.11 ± 0.23	1
	Sabellidae	0.11	0.07 ± 0.14	0.6
	Echinaster sepositus/Hacelia attenuata	0.10	0.13 ± 0.2	1
	Halocynthia papillosa	0.07	0.19 ± 0.43	2.2
12	*Bispira viola*	n/a	261.32 ± 185.21	551.6

Table 4. Characteristic species of each of the identified assemblages based on the score provided by the IndVal index. Only those species scoring higher than 0.05 are displayed.

observed within this assemblage, as well as the ubiquitous sponge *Axinella* spp. Dense patches of the brachiopod *Megerlia truncata* were spotted in seven SU belonging to this assemblage.

Assemblage 5: *Eunicella cavolini* forests (Fig. 4E). This community occurred on gently sloping rocky bottoms between 65 and 100 m depth (Fig. 5). Among the three gorgonian assemblages identified in the present study, *E. cavolini* forests (up to 22 col·m^{-2}) represented the richest in terms of number of species and expH diversity (Tab. 3, Fig. 5). The soft coral *Alcyonium coralloides* was often associated with gorgonian branches, whereas the sponges *Axinella* spp. and *Hexadella racovitzai* and some holothurians were found inhabiting the intermediate layer. Large ophiuroids of the species *Astrospartus mediterraneus* were also observed feeding at the top of the gorgonian colonies.

Assemblage 6: *Paramuricea clavata forests* (Fig. 4F). Assemblages characterized by the gorgonian *P. clavata* (densities up to 19 col·m^{-2}) occurred on horizontal and sloping outcroppings and coralligenous rocks generally between 45 and 65 m depth (Fig. 5). These forests were also characterized by the presence of dense aggregations of the sponge *Aplysina cavernicola* and *Axinella* spp., whereas in some cases other gorgonians such as *Eunicella verrucosa* contributed to forming multi-specific gorgonian aggregations. Serpulids and bryozoans occupied the intermediate layer or were found as epibionts on the gorgonian branches.

Assemblage 7: Coralligenous over-hangings (Fig. 4G). Sloping and vertical cliffs between 30 and 80 m depth hosted peculiar communities dominated by dense patches of the yellow scleractinian *Leptopsammia pruvoti* (very high densities of up to 143 ind·m^{-2}) and the precious red coral *Corallium rubrum* (up to 9 col·m^{-2}). This assemblage showed the highest average number of species per SU among all assemblages (Fig. 5). Several sponges (*Agelas oroides*, *Petrosia ficiformis*, *Axinella* spp., *Aplysina cavernicola*) and colonies of the gorgonian *P. clavata* were identified within this assemblage.

Assemblage 8: *Eunicella verrucosa* forests (Fig. 4H). This assemblage was characterized by monospecific or mixed aggregations, occurring on horizontal,

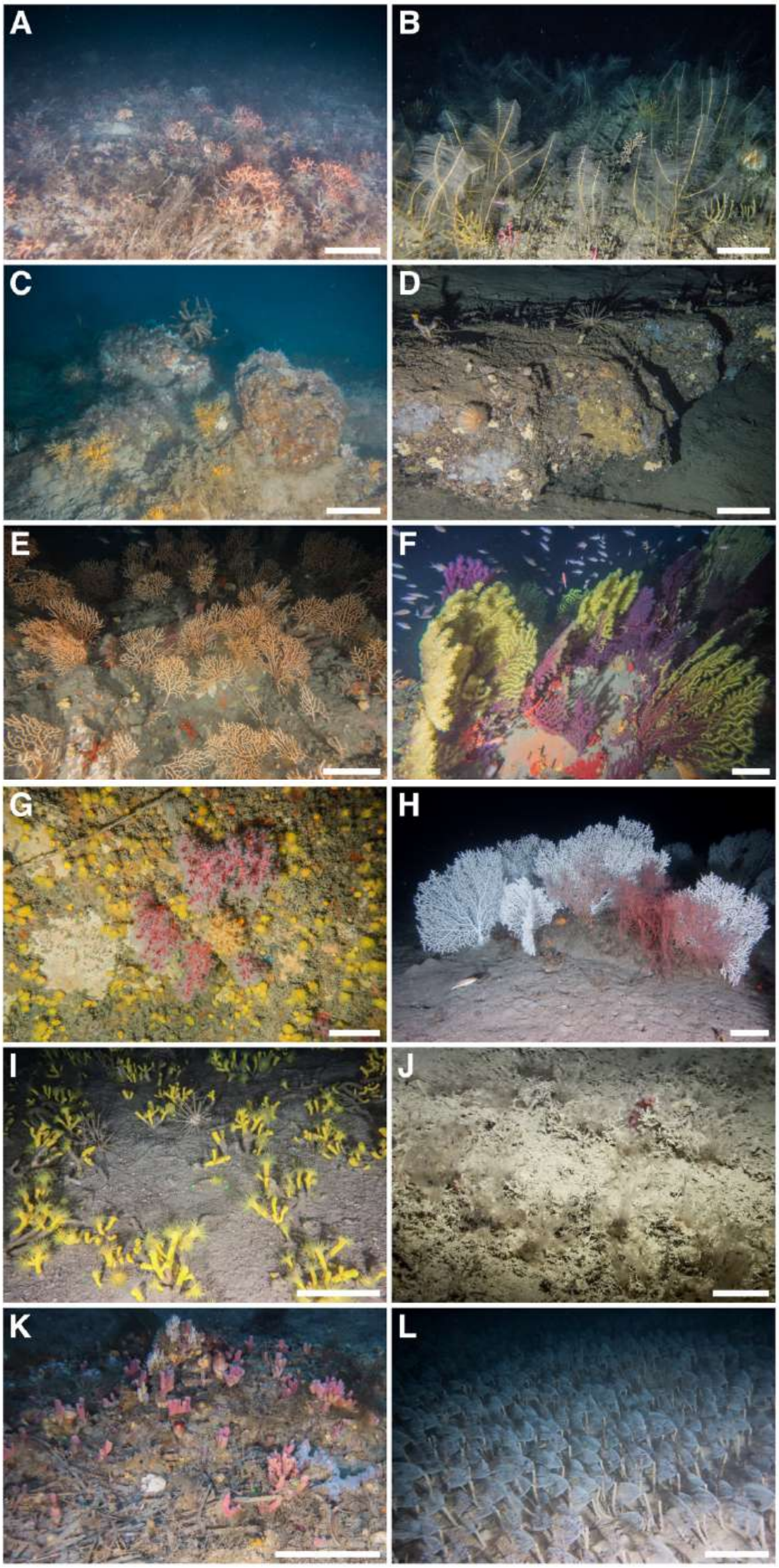

Figure 4. Selected images of the megabenthic assemblages identified in this study. (A) 1 - Bryozoan beds; (B) 2 - *Lytocarpia myriophyllum* forests; (C) 3 - Sponge grounds; (D) 4 - Deep-rocky bottoms with serpulids and echinoderms; (E) 5 - *Eunicella cavolini* forests; (F) 6 - *Paramuricea clavata* forests, (G) 7 - Coralligenous overhangs hosting *Leptopsammia pruvoti* and *Corallium rubrum*; (H) 8 - *Eunicella verrucosa* forests; (I) 9 - *Dendrophyllia cornigera* gardens; (J) 10 - *Paralcyonium spinulosum* fields; (K) 11 - *Haliclona* cf. *mediterranea* fields; (L) 12 - *Bispira viola* beds. Scale bar 20 cm, except for G and J of 5 cm.

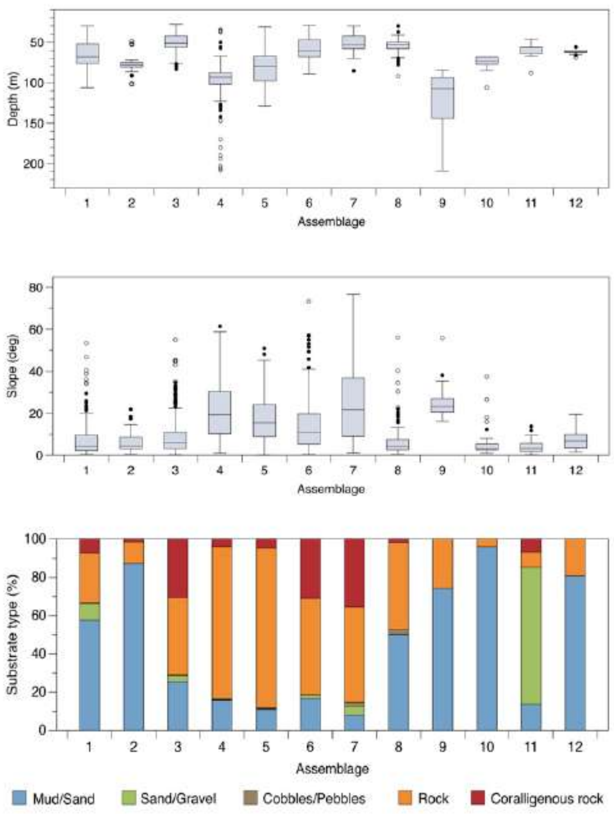

Figure 5. Range of the main environmental parameters for each assemblage identified in the cluster analysis based on the information gathered for all sampling units.

sub-outcropping rocks in high sedimented areas, where *E. verrucosa* reached densities up to 7 col·m^{-2}. Depth ranged from 30 to 90 m, but the majority concentrated in a narrow bathymetrical range around 50 m (Fig. 5). The sponges *Crella* sp. and *S. foetidus*, together with several species of bryozoans, were often observed. In some cases, *Leptogorgia sarmentosa* occurred in multi-specific patches, becoming predominant in some localities (4 col·m^{-2}).

Assemblage 9: *Dendrophyllia cornigera* gardens (Fig. 4I). Aggregations of *D. cornigera* (up to 15 col·m^{-2}) constituted the deepest assemblage observed in this study, being identified down to 210 m depth, although its main distribution was between 90 and 145 m. This scleractinian coral occurred mainly on muddy bottoms near rocky outcrops. Cidarids, the sponge *Pachastrella monilifera*, the brachiopod *Griphus vitreus*, the sabellid *Myxicola* sp. and some solitary scleractinians were observed associated with this assemblage. All species were characterized by low IndVal values, indicating a low specificity due to their presence in many other assemblages.

Assemblage 10: *Paralcyonium spinulosum* fields (Fig. 4J). Dense fields were found on horizontal soft bottoms located between 70 and 80 m depth (Fig. 5), with densities up to 77 col·m^{-2}. The soft coral *Alcyonium palmatum* and some sabellids were occasionally associated with this assemblage.

Assemblage 11: *Haliclona* cf. *mediterranea* grounds (Fig. 4K). Assemblage observed on horizontal detrital bottoms mainly ranging between 55 and 65 m depth (Fig. 5), where the sponge reached densities up to 15 ind·m^{-2}. Other species that contributed to this assemblage included the ophiuroid *Amphiura* sp., the sponge *Axinella* spp. and the soft coral *A. palmatum*.

Assemblage 12: Sabellid beds (Fig. 4L). This assemblage occurred on horizontal muddy bottoms, often nearby *E. verrucosa* forests, in a narrow bathymetrical range, around 60-70 m depth (Fig. 5). Here, the sabellid *Bispira viola* forms extraordinarily high-density aggregations (up to 550 ind·m^{-2}), hence displaying the highest average number of individuals per SU.

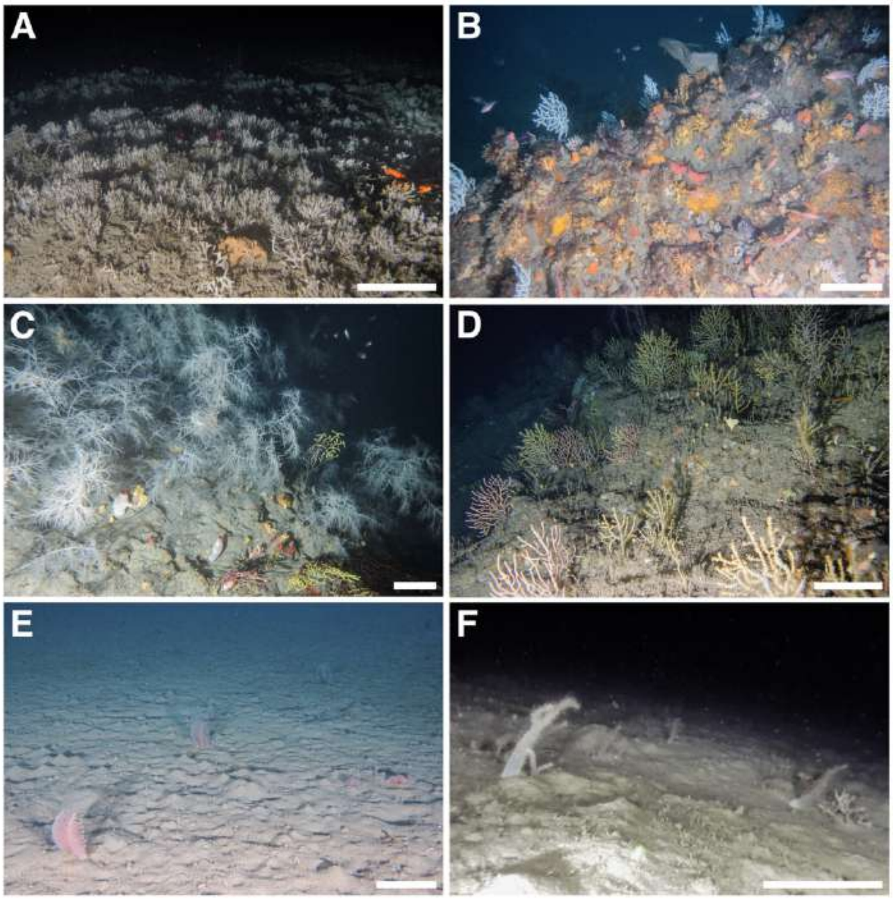

Figure 6. Additional benthic assemblages observed in the video footage recorded on the Ligurian Sea. (A) *Filograna/Salmacina* field. (B) A dense aggregation of *Axinella* individuals covered with *Parazoanthus axinellae*. Some gorgonians (*Eunicella verrucosa*) and a specimen of the keratose sponge *Spongia lamella* are also present. C) *Antipathella subpinnata* forest, with *Paramuricea clavata* and *Halocynthia papillosa*. D) Aggregation of *Paramuricea macrospina* (yellow), mixed with *Eunicella cavolini* (orange). E) Pennatula field. F) *Alcyonium palmatum* field. Scale bar: 20 cm.

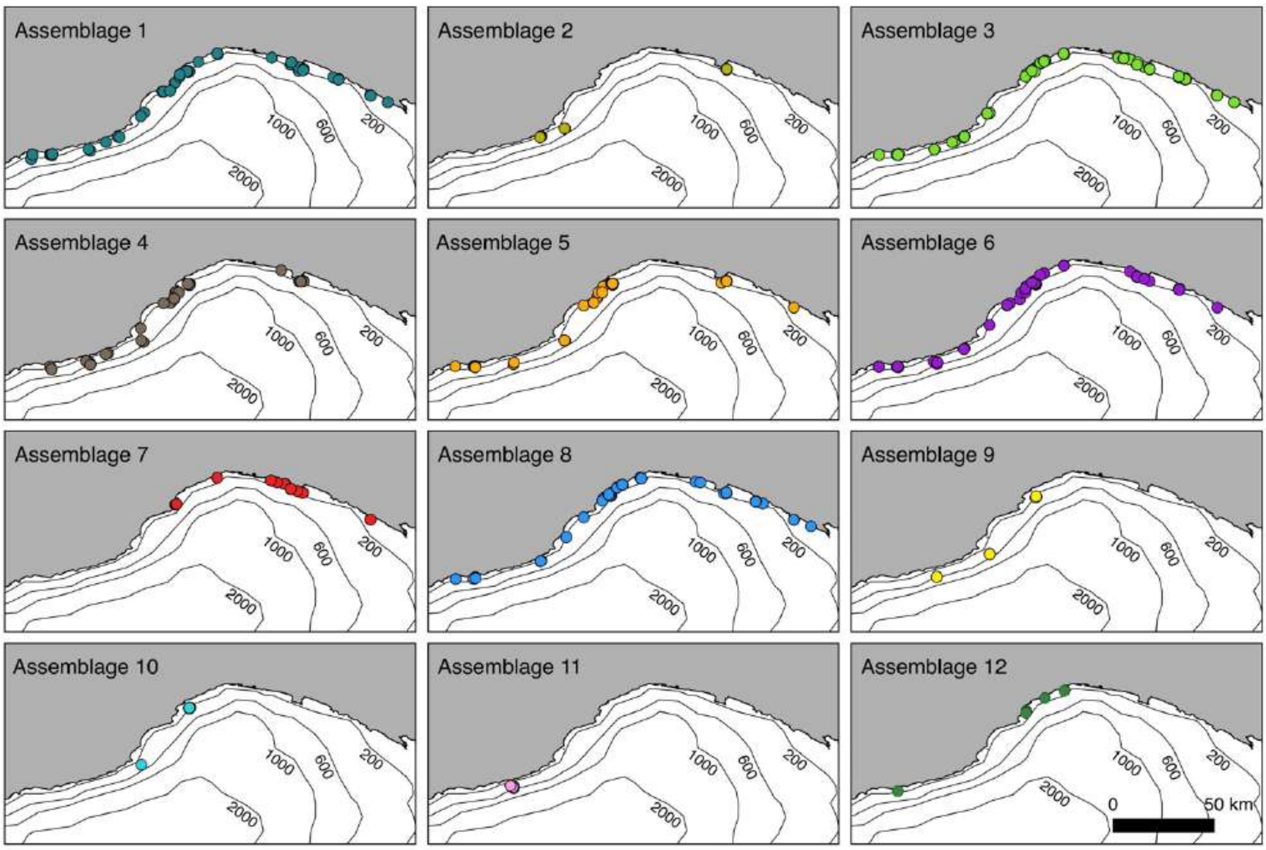

Figure 7. Maps showing the spatial distribution of the 12 megabenthic assemblages identified in the cluster analysis along the whole Ligurian coast.

3.4 Geographical distribution of megabenthic assemblages

The geographical distribution of the 12 assemblages identified in this study is shown in Fig. 7. Six assemblages, mainly represented by bryozoans, sponge grounds and gorgonian aggregations (Groups 1-3-4-5-6-8), had a widespread distribution along the whole study area, whilst the remaining assemblages showed a more scattered distribution pattern, being found in more specific locations.

Assemblage 1, with its characteristic species *P. fascialis*, occurred in all the investigated areas, but reached maximum densities in the sites of Savona and Portofino. Assemblage 3 occurred in 24% of the SU along the whole coast of Liguria, but the sponge grounds dominated by *S. foetidus* mainly occurred in western Liguria, in the sites of Santo Stefano, Diano Marina, Gallinara Island, Vado Ligure, Savona, Celle Ligure and Varazze. In general, *A. polypoides* showed lower densities than *S. foetidus*, but it could reach high abundances locally, as observed in Punta Manara, with densities of 4 ind·m^{-2}. *H. papillosa* appeared more abundant in the western sector of the Ligurian Sea, reaching highest densities in Santo Stefano and Porto Maurizio (5 ind·m^{-2}). Assemblage 5 mainly occurred in the areas located in the western sector of the Ligurian Sea, being the sites of Capo Mele, Mantice Shoal and Punta del Faro those that hosted the densest forests of *E. cavolini* (up to 22 col·m^{-2}). Assemblage 6 was also very widespread, occurring in all the investigated areas. The best developed forests of *P. clavata* (up to 19 col·m^{-2}) occurred in the sites of Maledetti Shoal, Savona shoals, Isuela and Punta Mesco. *E. verrucosa* was the most widespread gorgonian within the study area, with Assemblage 8 occurring in the 24% of the SU. Despite its wide distribution, *E. verrucosa* forests were mainly located in the western sector of the Ligurian Sea, reaching highest densities in the sites of Diano Marina, Finale Ligure and Arenzano (7 col·m^{-2}). Within this assemblage, *L. sarmentosa* became predominant in sites such as Bordighera and Punta Mesco. *Eunicella singularis* was also observed as part of Assemblage 8, with highest densities recorded in the site of Punta Manara (3 col·m^{-2}).

The best developed hydrozoan forests of Assemblage 2 occurred in the sites of Diano Marina, Capo Mele, Mantice Shoal and Punta del Faro. *L. pruvoti* and *C. rubrum* of Assemblage 7 reached highest densities in the sites of Maledetti Shoal and Portofino Promontory, where important deep vertical walls are present. Low-density populations of red coral were found in other sites of the western Ligurian Sea, such as Bordighera, Santo Stefano and Finale Ligure. Assemblage 9 was present in three sites, all of them located in the western sector of the Ligurian Sea, with the Mantice Shoal presenting the largest aggregation of *D. cornigera* encountered in the Ligurian Sea. Assemblage 10 occurred only on the deep shoals of Capo Mele and Mantice, both located in the western Ligurian Sea. The pink sponge *H.* cf. *mediterranea*, characteristic of Assemblage 11, was commonly observed on the horizontal detrital bottoms of Santo Stefano. Finally, sabellids fields of Assemblage 12 were observed in the sites of Bordighera, Vado Ligure, Varazze and Arenzano.

3.5 Relationship with environmental parameters

Based on the dbRDA, the selected factors explained 11.3% of the total variability in species density, with the first two axis explaining almost 74% of the constrained variance (CAP1: 46%, CAP2: 27%; Fig. 8). Depth was the best predictor among all used factors, placed alongside the first axis. The substrates "Mud/Sand" and "Rock", together with seabed roughness and slope were also important in explaining the spatial distribution of the fauna. The biplot shows that in shallow areas, the complex *P. axinellae*-*Axinella* spp. is dominant on coralligenous outcrops, together with *P. clavata* and *L. pruvoti*, whilst detritic flat bottoms are characterized by *E. verrucosa*, serpulids, keratose sponges and the bryozoan *Turbicellepora avicularis*. At intermediate depths, *A. cavernicola*, *C. rubrum* and *Haliclona poecillastroides* are the most representative species, although muddy and sandy bottoms are dominated by *B. viola*, *P. spinulosum* and *L. myriophyllum*. At greater depths, outcropping rocks are generally dominated by *E. cavolini*, serpulids and holothurians.

3.6 Rare and low densities assemblages

The density and distribution of the main structuring species encountered along the continental shelf of Liguria is provided in Fig. 9. In addition to the main species that emerged from the community analysis and that were commented above, other structuring organisms were also considered relevant and their ecology further evaluated. The selection of species was based on their importance within low-density populated environments (mainly soft bottoms) and/or because of their rarity. For instance, six forests of the black coral *Antipathella subpinnata* occurred between 60 and 100 m depth, on horizontal or sloping rocks (Fig. 6C). *A. subpinnata* was found in the sites of Santo Stefano, Finale Ligure, Corallone, Mantice Shoal and Punta del Faro, reaching maximum densities of 4.4 col·m^{-2} in Bordighera. With the exception of Punta del Faro, all forests are located in the western Ligurian Sea. Other antipatharians were observed in the site of Capo Mele, namely *Leiopathes glaberrima* (190-210 m depth) and *Parantipathes larix* (114-212 m depth). The octocoral *Paramuricea macrospina* was also reported in the western sector of the Ligurian Sea (Fig. 6D). The site of Capo Mele hosted the best developed *P. macrospina* aggregation (up to 9 col·m^{-2}), occurring on rocky bottoms at 100 m depth, where it forms mixed forests with *P. clavata* and *E. cavolini*. A shallower population of *P. macrospina* (75-80 m) was also reported from the site of Finale Ligure.

Soft bottoms, covered by mud and fine sands, appeared widespread within the Ligurian continental shelf. In those areas, pennatulaceans and soft corals were the most characterizing species, always observed with low densities. These organisms were found scattered along the entire study area, with higher densities in the western sector, especially in the proximity of canyons heads. *Pennatula* spp. (Fig. 6E) was observed between 40 and 130 m depth, with a maximum density of 3 col·m^{-2} in the sites of Bordighera and Capo Noli. The soft coral *Alcyonium palmatum* (Fig. 6F)

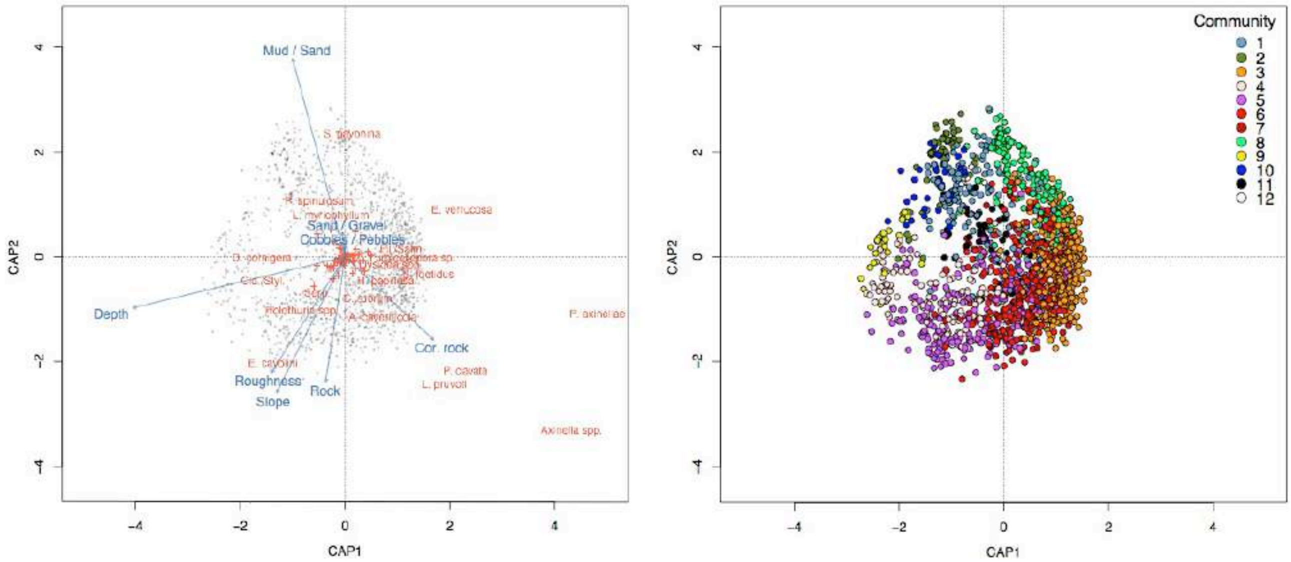

Figure 8. Results of the distance-based redundancy analysis (dbRDA) of Bray-Curtis dissimilarity matrix based on square-root transformed density data of megabenthic species. Samples are represented as grey or colored dots, environmental variables as solid lines with arrows and species as orange crosses. Names of species that showed a good fit in the first two canonical axes (>0.60) are shown in the plot.

occurred between 40 and 140 m, with maximum densities of up to 3 col·m^{-2} in the site of Finale Ligure.

4 Discussion

4.1 Megabenthic diversity on the Ligurian deep continental shelf

The recent development of marine technology allows direct observation of deep-sea ecosystems with great detail, providing invaluable data of their composition and main structuring species. In the Mediterranean basin, local or sub-regional studies evaluating the diversity of invertebrate megafauna based on video images have become popular in the last few years (Fabri et al., 2014; Grinyó et al., 2017; Dominguez-Carrió, 2018; Pierdomenico et al., 2019), but research providing quantitative data of benthic assemblages over large geographical extents is still scarce, with only a few exceptions (Pierdomenico et al., 2019). Our work represents the largest study of benthic megafauna using underwater imaging ever produced in the western Mediterranean, collected over 62 km of continental shelf.

Overall, the twilight zone of the Ligurian Sea should be considered as very rich due to the high number of megafauna species that it sustains. A large percentage of such species corresponded to canopy-forming organisms, like massive/erect sponges, large hydroids, gorgonians and black corals, as well as species of smaller sizes, including bryozoans, alcyonaceans and sabellids. Most of the identified species were suspension feeders, a functional group that dominated all the assemblages herein described. Such species composition, which favors the structuring of benthic communities, plays a key role in the maintenance of the overall benthic diversity of the area and must be considered a fundamental link for the energy transfer between the pelagic and the benthic ecosystem (Gili and Coma, 1998; Rossi et al., 2017b).

This study presents the most comprehensive characterization ever made for the different megabenthic communities that can be found on the mesophotic Ligurian continental shelf. Up to know, in fact, only few, local biocoenotic characterizations were carried out on these communities in this area (Cerrano et al., 2010; Di Camillo et al., 2013; Bo et al., 2014;). Some of the aggregations identified had previously been reported for the Ligurian Sea in the past, but their geographical and bathymetrical distribution had never been investigated over such a large spatial extent. Conversely, and quite surprisingly, the presence of *S. foetidus* assemblages, *P. spinulosum* meadows and *B. viola* fields was basically unknown for the Mediterranean basin, one of the best explored seas of the world.

Major differences in the spatial distribution of the benthic diversity were detected along the Ligurian Sea, with the western sector displaying higher number of species and assemblages, as previously reported by Cánovas-Molina et al. (2016). These patterns might be explained by the particular morphology of its narrow continental shelf, incised by several canyons, that probably enhances upwelling processes and promotes habitat heterogeneity, with the presence of several shoals, vertical walls and deep banks (Rovere et al., 2007; Cattaneo-Vietti et al., 2010; Casella et al., 2011). Conversely, the homogeneity of the wide continental shelf and large terrigenous supply introduced by River Magra on the eastern sector (Bassano et al., 2000; Attolini and Coppo, 2005; Cattaneo-Vietti et al., 2010) might explain the much lower diversity values recorded.

4.2 Assemblages interpretation

4.2.1 Hard substrate habitats

The most diverse assemblages identified in the video images responded to those characterized by the presence of gorgonian species. It is widely known that these organisms play a major role in the structuring of Mediterranean benthic communities, especially between 40 and 200 m depth (Gori et al., 2017). In the case of the Ligurian Sea, the octocorals *P. clavata*, *E. cavolini* and *E. verrucosa* constitute the dominant structuring species of three different assemblages, each of them displaying a strong preference for a specific depth ranges, a certain slope and a substrate type. With respect to the other mentioned gorgonians, *E. verrucosa* is certainly less studied and its distribution within the Mediterranean basin varies widely from one area to another (Sartoretto and Francour, 2011). Thus, the occurrence of large forests of this species in the Ligurian Sea is noteworthy. Maximum gorgonian densities were in line with those reported in other areas of the Mediterranean Sea (Linares et al., 2008; Gori et al., 2011; Sini et al., 2015; Grinyó et al., 2016), with the exception of the red coral *C. rubrum*, which appeared with densities consistently lower along the whole study area (Bavestrello et al., 2014, 2015; Angiolillo et al., 2016). It is known that the Portofino Promontory and the Maledetti Shoal hosted the most important red coral populations of the Ligurian Sea, once subject to a pluri-decennial harvesting pressure and now slowly recovering (Bavestrello et al., 2015; Cattaneo-Vietti et al., 2016). Regardless of this situation, our low values could relate to the size of sampling unit employed, since surveys of red coral generally use samples between 0.04 and 1.25 m^2 in order to investigate their patchy distribution. It is likely that the 5-m^2 sample unit adopted in this study is excessively large to detect small-scale differences in the density of this species, which usually grows in narrow crevices and interstices separated by areas with no specimens.

In the case of the sponge assemblages, one of the most interesting findings corresponds to the presence of dense aggregations of the large keratose sponge *S. foetidus*, with value never reported before. Keratose sponge grounds are considered rare in the Mediterranean Sea, with only few examples reported from the eastern basin (Bianchi et al., 2014; Idan et al., 2018), and were previously unknown from the Ligurian Sea. Nevertheless, Cánovas-Molina et al. (2016) reported from this area facies with massive/erect sponges including, among others, *Spongia lamella*, *Sarcotragus foetidus*, *Scalarispongia scalaris* and *Axinella polypoides*. Recent surveys conducted in 2018 enlarged the range of distribution of these sponge grounds that were reported also in the area of Sanremo (western sector), suggesting a vast occurrence of these biocoenoses in the NW Mediterranean basin and supporting a relevant ecological role for these structuring species. Overall, however, the small sponge *Axinella* spp., very common along the whole Mediterranean basin (Kefalas et al., 2003; Bertolino et al., 2013), was the most abundant species within Assemblage 3. This species has been observed in assemblages dominated by *A. damicornis* and *A. verrucosa* together with other semi-sciaphilous organisms dwelling on horizontal, coralligenous, rocky outcrops from the inner continental shelf, at depths of 50 to 100 m (Menorca Channel; Santín et al., 2018). In the Ligurian Sea, this sponge assemblage occurred on a similar substrate and slope, but mostly concentrated between 40 and 60 m depth, which might be explained by the most turbid waters of Liguria if compared to that of Mallorca island. The distributional and density data reported here, as well as the commonly observed symbiosis of *Axinella* spp. with the anthozoan *P. axinellae*, support the fact that sponges act as relevant ecosystem engineers also at a smaller scale of observation. The large water masses moved by the aquifer systems of the sponges could be enhancing the development of a rich array of associated species and epibionts, not only at the organism level but also at the population scale (Guizien and Ghisalberti, 2017). The ascidian *H. papillosa* was often present within this assemblage, also enhancing the water fluxes by active filter-feeding (Bo et al., 2011; Coppari et al., 2014). Furthermore, and from a structural point of view, other large sponges considerably contributed to increase the three-dimensionality of these sponge grounds, with examples such as the elephant ear *S. lamella* and the arborescent *A. polypoides*. As a matter of fact, this assemblage presented the highest average diversity per sample among all assemblages. Contrastingly, sponge aggregations of Assemblage 11 dominated by *H.* cf. *mediterranea*

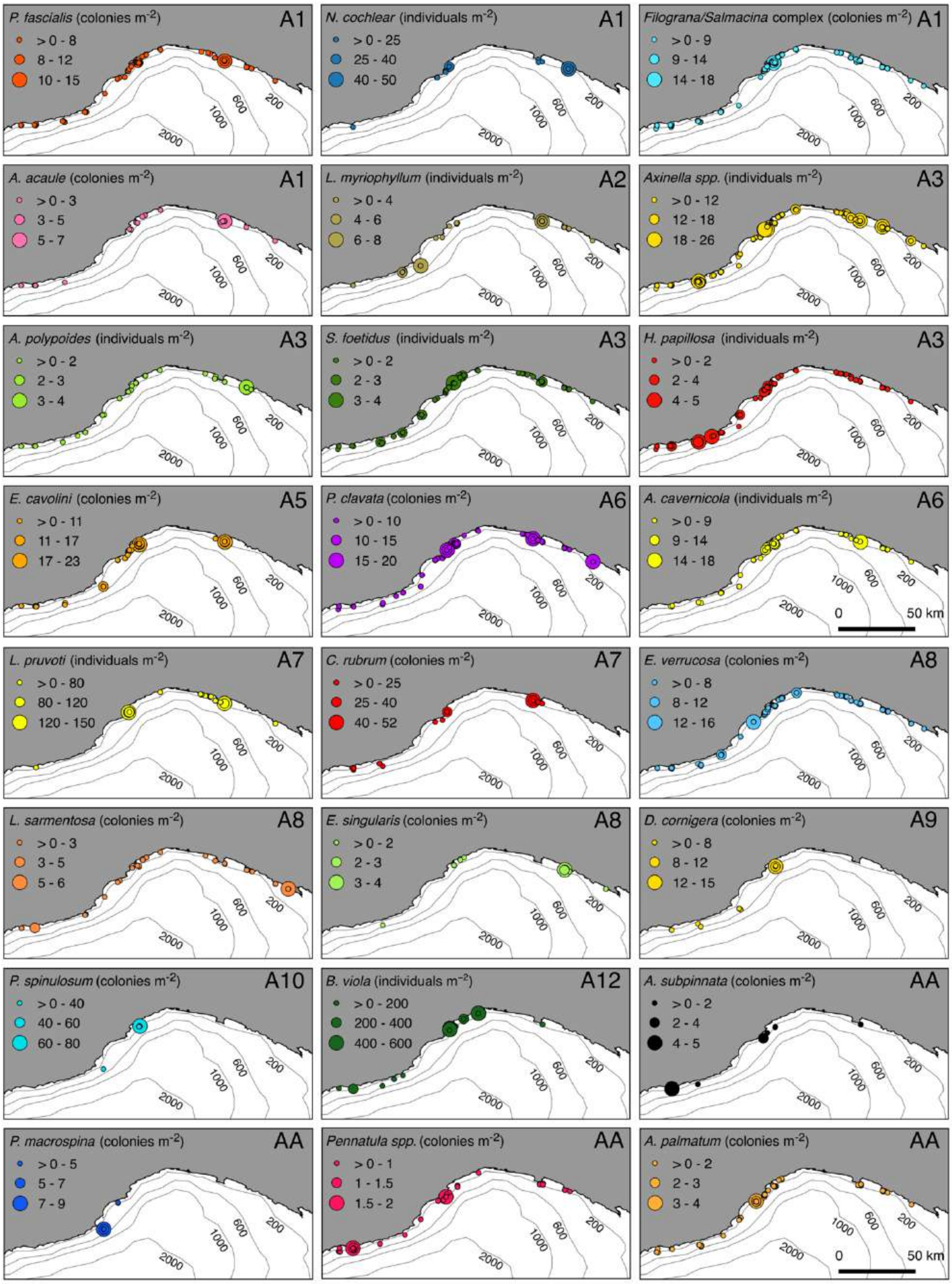
P. fascialis (colonies m-2) A1
> 0 - 8
8 - 12
10 - 15
N. cochlear (individuals m-2) A1
> 0 - 25
25 - 40
40 - 50
Filograna/Salmacina complex (colonies m-2) A1
> 0 - 9
9 - 14
14 - 18
A. acaule (colonies m-2) A1
> 0 - 3
3 - 5
5 - 7
L. myriophyllum (individuals m-2) A2
> 0 - 4
4 - 6
6 - 8
Axinella spp. (individuals m-2) A3
> 0 - 12
12 - 18
18 - 26
A. polypoides (individuals m-2) A3
> 0 - 2
2 - 3
3 - 4
S. foetidus (individuals m-2) A3
> 0 - 2
2 - 3
3 - 4
H. papillosa (individuals m-2) A3
> 0 - 2
2 - 4
4 - 5
E. cavolini (colonies m-2) A5
> 0 - 11
11 - 17
17 - 23
P. clavata (colonies m-2) A6
> 0 - 10
10 - 15
15 - 20
A. cavernicola (individuals m-2) A6
> 0 - 9
9 - 14
14 - 18
L. pruvoti (individuals m-2) A7
> 0 - 80
80 - 120
120 - 150
C. rubrum (colonies m-2) A7
> 0 - 25
25 - 40
40 - 52
E. verrucosa (colonies m-2) A8
> 0 - 8
8 - 12
12 - 16
L. sarmentosa (colonies m-2) A8
> 0 - 3
3 - 5
5 - 6
E. singularis (colonies m-2) A8
> 0 - 2
2 - 3
3 - 4
D. cornigera (colonies m-2) A9
> 0 - 8
8 - 12
12 - 15
P. spinulosum (colonies m-2) A10
> 0 - 40
40 - 60
60 - 80
B. viola (individuals m-2) A12
> 0 - 200
200 - 400
400 - 600
A. subpinnata (colonies m-2) AA
> 0 - 2
2 - 4
4 - 5
P. macrospina (colonies m-2) AA
> 0 - 5
5 - 7
7 - 9
Pennatula spp. (colonies m-2) AA
> 0 - 1
1 - 1.5
1.5 - 2
A. palmatum (colonies m-2) AA
> 0 - 2
2 - 3
3 - 4
200
600
1000
2000
0
50 km

Figure 9 (page 69). Maps showing the spatial distribution of 24 invertebrate species that are considered key structural organisms of the benthic assemblages identified in the cluster analysis. Codes A1 to 12 refer to the assemblage the species belongs to, and AA to the additional assemblages.

occurred only from the site of Santo Stefano, characterized by flat, detritic bottoms. A highly resembling assemblage has been reported from the inner continental shelf of the Menorca Channel (Santín et al., 2018). In this case, the sponge *Haliclona (Reniera) mediterranea* occurred on horizontal maërl beds between 60 and 110 m depth. It is yet to be understood what factors determine the occurrence of sponge grounds within the Mediterranean Sea, where until now, very few sponge grounds have been identified (Bo et al., 2012a; Maldonado et al., 2015; Idan et al., 2018; Santín et al., 2018). Ballesteros (2006) suggested that gorgonians might be dominant when high amounts of food particles are available, whereas active suspension feeders like sponges and bryozoans replace gorgonians under oligotrophic conditions. Similar considerations were made also to explain passive or active filter feeders-dominated communities on offshore mesophotic hardgrounds subjected to various hydrographical conditions (Bo et al., 2011). In any case, large gorgonian assemblages are known also from oligotrophic areas of the Mediterranean basin (Grinyó et al., 2016, 2018), and the Ligurian Sea should not be regarded as oligotrophic due to the topography of the seabed, the high urbanization of its coast, the presence of blackwater discharges and the large amount of river inputs as well as hydrological patterns. Furthermore, Casella et al. (2011) identified mesoscale eddies causing upwelling in the Ligurian Sea, contributing to increase the amount of food available in the water column. These eddies occur in the western sector of the Ligurian Sea, in correspondence to the densest assemblages of *S. foetidus*, *H. papillosa* and *B. viola*. All these evidences suggest that assemblages dominated by active suspension feeders might be dominating in areas where great amounts of food resources are available.

Assemblage 4 (characterized by a complete absence of large structuring species) occurred on hard bottoms in all the investigated areas, concentrating mainly between 80 and 100 m depth. This assemblage appears hard to justify because mesophotic rocky out-crops generally host dense gorgonian forests or sponge grounds, and competition for the substratum is high in marine ecosystems. Even if bare rocks could consistently occur at higher depths, this assemblage may represent zones of intense artisanal and recreational fishing activities, whose gears are known to efficiently remove structuring species (Mortensen et al., 2005; Sampaio et al., 2012; Mytilineou et al., 2014). The few species observed within this assemblage might be able to withstand the mechanical damage, like serpulids worms, which are not affected due to their life habits, or sea urchins and holothurians, which are subjected to large natural fluctuations in population size. Assemblage 4 is reported from all investigated depths, suggesting that mechanic impact might be occurring on a wide geographical and bathymetrical range. Signs of anthropogenic disturbance included damaged organisms and the presence of both marine litter and discarded fishing gears (see Chapter 2 in this thesis). On the basis of the high amount of lost gears observed, fishing activities might represent the main threat for the Ligurian megabenthic communities. Further efforts are required in order to characterize and quantify the main sources of pressure on these assemblages.

4.2.2. Soft-bottom habitats

The remaining assemblages mainly developed on detritic and soft-bottom areas, with only one assemblage displaying a wide geographical and bathymetrical distribution (Assemblage 1, bryozoan beds). In the Mediterranean Sea, bryozoans are important structuring species, considered most important animal group among coralligenous reefs builders (Ballesteros, 2006; Madurell et al., 2013; Lombardi et al., 2014). In Liguria, monospecific patches of the bryozoan *P. fascialis* had been previously reported from Tinetto Island and Alassio bay, at shallow depths (10-25 m) (Cocito et al., 1998, 2002; Lombardi et al., 2008, 2014). Due to the fragility of their carbonatic skeleton, their occurrence on horizontal detritic bottoms, can be considered an indicator of good environmental status especially with reference to shallow-water coastal trawling activities.

The remaining soft bottom assemblages showed more restrictive preferences in terms of environmental requirements. In the case of *L. myriophyllum* forests, substrate inclination and grain size could play a major role in their distribution, which until now was only reported near Imperia, Portofino and Monterosso (Rossi, 1950; Tortonese, 1958; Di Camillo et al., 2013). Our findings indicate that its occurrence is broader, including eight new sites (Fig. 10), where relatively dense aggregations (1.57 col·m^{-2}) were found near Portofino Promontory, between 60 and 70 m depth (Di Camillo et al., 2013; Cerrano et al., 2015). These observations suggest that these forests develop on muddy bottoms mixed with biogenic mineral debris at the base of rocky cliffs, characterized by a high terrigenous supply. Similar conclusions can be drawn for the three aggregations of *D. cornigera* here reported, which develop on soft-detritic bottoms near rocky shoals. *D. cornigera* aggregations were also reported from the canyons of the Gulf of Lions (Fabri et al., 2014), but only the Ligurian population and a smaller one reported on the Amendolara Seamount (Bo et al., 2012) are distinctively on soft bottoms. The aggregation lying at the base of the Mantice Shoal (Bo et al., 2014) represents the largest and northernmost record of *D. cornigera* within the Mediterranean basin.

In the case of the soft coral *P. spinulosum* (Assemblage 10), fields of this species had never been reported before from the Mediterranean Sea. Only similar patches of the soft-coral *Nidalia studeri* (Koch, 1891) had been reported from gently sloping rocky outcrops in the Menorca Channel (Grinyó et al., 2018), with densities up to 30 col·m^{-2}, comparable to those recorded for *P. spinulosum* at the Mantice Shoal. All these assemblages mainly occurred in the western sector of Liguria, where a more complex topography and peculiar hydrographic conditions enhance the amount of nutrients in the water. Finally, sabellid beds resembling those of Assemblage 12 have only been reported from a detrital bottom at 90 m depth in front of Cap Ferrat (western Ligurian Sea) during the MEDSEASCAN surveys (Pedel and Fabri, 2013). In that case, the exceptional abundance of annelids was explained by the high levels of organic input derived from blackwater discharges nearby. Within Liguria, all the sites reporting dense fields of *B. viola*, including some additional recent observations nearby Sanremo, are close to highly urbanized coasts where polluted water discharges are known to occur.

4.2.3. Rare and low-density assemblages

Multivariate analyses did not detect certain assemblages that could be visually identified directly form the video images, mainly due to low density values of the key structuring species. This is the case of largest part of the soft bottoms located between 40 and 140 m depth, which were dominated by the soft coral *Alcyonium palmatum* and the sea pen *Pennatula* spp. Both species showed similar densities to those reported in other shelf areas of the Mediterranean, such as Cap de Creus (Dominguez-Carrió, 2018). In the case of *Pennatula* spp., the density observed in the Ligurian Sea appears three orders of magnitude higher than that observed in the Adriatic and Ionian Seas, where abundance data is derived from trawling bycatch. Differences in the quantification method could underestimate local patches of very high densities, which can only be accurately determined using video images (Petović et al., 2016; Chimienti et al., 2015, 2018). Despite the ecological role of these soft-bottom engineers and their resilience to mechanical impact is far from being fully understood, their occurrence is extremely relevant for the conservation of areas highly subjected to trawling activities (Bastari et al., 2018).

The aggregations of black corals were not identified as independent assemblages either, although three species of large antipatharians were observed in the images. *A. subpinnata* represented the most common black coral within the basin at mesophotic depths with a total of six forests with two additional populations found on wrecks (Bo et al., 2008). On the other hand, *L. glaberrima* and *P. larix* occurred only in one deep dive supporting previous observations regarding the distribution of these two species mainly along the continental break and upper slope (Bo and Bavestrello, 2019). As gorgonians, large antipatharians act as major structuring species in Mediterranean benthic habitats (Bo et al., 2009; Gori et al., 2017), and their presence must be considered significant for the Ligurian biodiversity. Given their slow growth rates, their vulnerability to human impacts and their key role as nursery areas (Bo et al., 2015), the geographical and bathymetrical distribution of the three species of black corals within the Ligurian Sea was investigated, aiming to provide a consistent georeferenced record to aid in their conservation.

The gorgonian *P. macrospina* has been widely reported from the western Mediterranean Sea (Bo et al., 2012b; Grinyó et al., 2016), while records within the Ligurian Sea were scarce until now (Pica et al., 2018). A small fragment was reported in the trawling discard from the Gulf of Genova by Rossi (1985), and another specimen was described from the cliffs of Gallinara Island, below 50 m depth (Balduzzi et al., 1994; Bianchi et al., 2018). These last two records were based on the same single specimen (Bianchi, personal communication) for which taxonomic description is not available. In the present study, nor *P. macrospina* neither *P. clavata* were observed near the Gallinara Island, and the two populations of *P. macrospina* herein described were found deeper, lying below 75 m in an ecological setting similar to that described in the rocky coral oases of the South Tyrrhenian Sea subjected to moderate silting (Bo et al., 2012b).

4.3 Concluding remarks

This study highlights the rich megabenthic communities that can be found of the Ligurian deep continental shelf, filling an important knowledge gap about of the Mediterranean benthic biodiversity. Additional investigations will be necessary in order to investigate each megabenthic communities identified in this study, leading to a better interpretation of such assemblages, whereas site-specific studies will improve our knowledge and highlight their exclusive singleness.

Special protection measures are required in order to protect marine animal forests and thus, a large effort must be devoted to the identification and quantification of the major pressures threatening these ecosystems (Hinz 2015; Aguilar et al., 2017; Otero et al., 2017). In this sense, the present study provides a georeferenced database of the Ligurian megabenthic species and communities, that managers and stakeholders might use as a tool to identify potential sites of ecological importance eventually designing an effective network of MPAs along the Ligurian coast, to preserve the fragile diversity that dwells in its waters.

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Supplemental Material 1. Main environmental features of the 80 ROV dives carried out on the Ligurian continental shelf. Areas A-J can be identified in Fig. 1.

Dive	Depth (m)		Substrate type (%)					Slope (deg)		Roughness	
	Min	Max	Mud/Sand	Sand/Gravel	Cobbl/Pebbl	Rock	Cor. Rock	Min	Max	Min	Max
A01	81	89	96			4		0.0	16.6	0.02	1.61
A02	82	86	100					1.1	9.5	0.09	0.84
A03	28	47	55	1		28	17	0.3	34.8	0.03	3.37
A04	39	100	47			44	8	0.0	50.8	0.00	5.58
A05	53	69	79			21		13.8	57.6	1.17	7.37
A06	42	99	53		11	36	1	0.5	48.2	0.06	6.21
A07	38	80	63			35		4.8	15.6	0.05	7.36
A08	128	200	3			97		30.0	45.0	0.21	6.18
B01	88	154	13	2	11	74		8.7	55.0	0.03	3.02
B02	52	63	14	55		21	10	0.1	28.5	0.02	1.19
B03	45	67	1	74		10	15	0.2	55.1	0.01	2.97
B04	85	92	14			86		0.9	59.6	0.05	4.38
B05	45	52	42			8	50	0.3	42.8	0.02	2.02
B06	33	39	3	69		12	16	0.1	34.8	0.02	2.38
B07	49	54	43			10	47	0.0	47.1	0.00	2.54
B08	65	70	99			1		0.2	17.1	0.02	0.83
C01	99	135	4			96		30.0	45.0	0.05	6.39
C02	190	216	34			66		9.6	83.0	0.12	12.03
C03	80	87	95			5		1.5	18.1	0.19	1.62
C04	28	40	74			4	22	4.9	27.1	0.44	2.72
C05	58	66	62			38		0.7	35.8	0.12	3.62
C06	55	60	100					0.2	6.5	0.04	0.61
D01	74	89	40			60		8.1	57.3	0.77	7.86
D02	75	109	100					0.6	23.9	0.18	2.3
D03	78	92	80			18		0.6	50.5	0.08	5.71
D04	84	96	100					1.1	19.8	0.12	2.36
D05	91	129	15			85		11.4	75.0	0.18	6.68
D06	86	94	68			32		0.9	33.1	0.13	4.67
D07	93	108	53			47		2.6	70.0	0.38	16.1
D08	71	141	90			10		3.9	15.6	0.05	5.15
E01	51	85	41			59		1.1	79.4	0.08	11.1
E02	54	111	8			91	1	8.2	85.0	0.05	12.5
E03	65	110	59			41		9.6	38.0	0.03	8.22
E04	39	76	87			8	5	0.4	50.0	0.02	2.83
E05	59	69	78			20		0.1	63.5	0.06	3.97
E06	36	71	62			38		0.7	68.7	0.09	7.33
E07	79	151	61			39		1.3	70.0	0.01	3.08
E08	78	88	35			65		1.5	9.8	0.31	6.26
E09	85	121	92			8		3.1	49.7	0.23	6.26
E10	77	108	48			52		0.5	55.2	0.07	7.58
E11	65	85	58			34	9	0.1	49.3	0.03	5.88
E12	56	72	62			11	27	0.4	63.4	0.07	8.87
E13	51	60	70			18	12	0.2	17.8	0.03	1.72
E14	46	56	60			34	6	0.1	24.0	0.01	2.34
F01	39	52	64	1		31	4	1.8	2.3	0.04	1.08
F02	38	47	62			10	28	0.1	25.9	0.02	1.22
F03	60	63	98			2		0.1	11.1	0.02	0.49
F04	40	52	72			27	1	0.3	17.9	0.01	0.87
F05	34	42	74			6	20	0.4	44.1	0.02	2.29
F06	56	62	80			19		0.3	17.0	0.38	0.74
G01	52	56	80			20		0.2	9.0	0.04	0.40
G02	30	36		53		24	23	0.1	62.2	0.01	4.18
G03	53	57	69			18	13	0.3	29.8	0.05	1.40
G04	33	37		48		12	40	0.1	48.6	0.01	2.32
G05	43	53	64			11	25	0.1	28.3	0.02	1.52
H01	34	36	3	48	10	1	37	0.1	32.1	0.01	1.56

H02	29	44					100	5.3	81.8	0.31	15.3
H03	34	72	8	41		11	40	0.2	72.4	0.09	8.17
H04	34	73	10	27	50		12	1.0	52.6	0.32	1.55
H05	103	106	72			28		0.6	0.9	0.02	1.55
H06	93	104	90			10		0.4	0.9	0.01	1.48
H07	89	99	60			40		0.8	0.9	0.01	1.31
H08	73	101	85			15		0.5	12.0	0.02	2.13
H09	101	104	90			6		1.3	6.3	0.02	1.78
H10	55	90	53			44	3	0.2	10.0	0.03	4.36
H11	61	83	65			35		0.3	53.1	0.04	3.07
H12	60	86	88			12		0.1	64.8	0.03	4.88
H13	57	78	59		11	31		0.4	64.5	0.07	5.67
I01	42	54	56			35	9	0.3	49.3	0.02	2.79
I02	58	68	70			30		0.3	26.4	0.03	1.30
I03	58	74	68			32		0.2	49.2	0.02	3.17
I04	59	67	83			17		0.1	23.6	0.03	1.19
I05	79	85	100					0.1	12.5	0.03	0.56
I06	66	73	81			7		0.2	39.6	0.03	2.02
I07	51	58	61			38		0.3	21.5	0.05	0.98
I08	33	41	52	15	18	13	3	1.9	9.8	0.08	1.49
J01	36	49	2	51		14	34	0.2	67.4	0.02	5.38
J02	47	55	48			45	7	0.1	59.3	0.03	3.37
J03	40	42	100					0.0	32.9	0.00	1.81
J04	28	30	13	6		9	72	0.1	52.9	0.01	3.11

Supplemental material 2. Comprehensive list of the species identified in this study, with their abundance values and occupancy (SU, Sampling Unit).

Phylum	Subgroup	Species	Total nº of organisms	Max density (ind m²)	% of ROV dives	% of SU
Porifera	Calcarea	*Clathrina* sp.	63	0.12	18.75	0.77
	Demospongiae	*Acanthella acuta*	33	0.12	16.25	0.37
		Agelas oroides	617	0.56	48.75	3.58
		Aplysina cavernicola	5113	3.36	57.5	6.83
		Axinella polypoides	362	0.76	32.5	2.83
		Axinella spp.	34314	5.08	88.75	36.48
		Calyx nicaeensis	5	0.08	3.75	0.08
		Chondrilla nucula	3	0.04	1.25	0.03
		Chondrosia reniformis	780	1.64	57.5	3.52
		Cliona spp.	49	0.52	6.25	0.27
		Crella sp.	273	0.32	37.5	3.34
		Dysidea sp.	906	1.52	53.75	4.7
		Hexadella racovitzai	747	0.44	53.75	5.39
		Ircinia sp.	30	0.12	16.25	0.67
		Pachastrella monilifera	94	0.36	3.75	0.32
		Petrosia ficiformis	936	2.52	27.5	2
		Pleraplysilla spinifera	81	0.16	25	1.01
		Poecillastra compressa	36	0.16	3.75	0.29
		Polymastia sp.	10	-	1.25	-
		Raspailia (Raspailia) viminalis	6	0.16	1.25	0.03
		Rhizaxinella sp.	3	0.04	1.25	0.05
		Sarcotragus foetidus	1933	0.64	67.5	10.62
		Sarcotragus spinosulus	9	0.12	2.5	0.05
		Spongia (Spongia) lamella	85	0.16	32.5	1.25
		Tedania anhelans	2	0.04	1.25	0.05
		Tethya spp.	3	0.04	3.75	0.03
		Ulosa stuposa	21	0.12	7.5	0.16
		Haliclona cf. *mediterranea*	2447	3.08	16.25	2.46
		Haliclona sp. 1	112	0.12	22.5	0.64
		Haliclona sp. 3	77	0.28	22.5	0.72
		Haliclona sp. 5	2	0.04	1.25	0.03
		Keratosa sp. 1	242	0.48	42.5	2.8
		Keratosa sp. 3	196	0.28	26.25	2
		Keratosa sp. 4	39	0.08	18.75	0.45
		Keratosa sp. 5	176	0.24	26.25	1.73
		Keratosa sp. 8	18	0.08	15	0.24
		Keratosa sp. 9	11	0.16	5	0.11
		Porifera sp. 01	14	0.04	12.5	0.21
		Porifera sp. 02	89	0.2	31.25	0.96
		Porifera sp. 03	165	0.2	37.5	1.73
		Porifera sp. 04	1004	1.36	43.75	3.84
		Porifera sp. 05	122	0.24	28.75	1.07
		Porifera sp. 06	31	0.16	7.5	0.19
		Porifera sp. 11	26	0.08	10	0.21
		Porifera sp. 13	9	0.12	7.5	0.11
		Porifera sp. 15	56	0.2	20	0.75
		Porifera sp. 17	20	0.12	7.5	0.27
		Porifera sp. 19	803	0.8	57.5	4.03
		Porifera sp. 20	50	0.12	13.75	0.4
		Porifera sp. 24	30	0.24	11.25	0.35
		Porifera sp. 25	43	0.08	13.75	0.43
		Porifera sp. 27	24	0.2	8.75	0.11
		Porifera sp. 28	36	0.08	11.25	0.35
		Porifera sp. 29	12	0.04	2.5	0.03
		Porifera sp. 30	35	0.24	8.75	0.35
		Porifera sp. 40	1	-	1.25	-
		Porifera sp. 41	6	0.04	6.25	0.05
		Porifera sp. 45	110	0.12	40	1.6
		Porifera sp. 46	98	0.16	16.25	0.72
		Porifera sp. 47	92	0.32	32.5	1.07
		Porifera sp. 48	21	0.24	7.5	0.16
		Porifera sp. 49	1195	0.92	45	4.56
		Porifera sp. 54	7	0.08	2.5	0.03
		Porifera sp. 55	59	0.52	10	0.51

		Porifera sp. 57	306	2.56	18.75	0.99
		Porifera sp. 58	7	0.08	3.75	0.13
		Porifera sp. 59	72	0.52	7.5	0.4
		Porifera sp. 61	4	0.08	3.75	0.05
		Porifera sp. 62	2	0.04	2.5	0.03
		Porifera sp. 63	15	0.12	3.75	0.08
		Porifera sp. 64	2	0.04	1.25	0.03
		Porifera sp. 65	2	0.04	1.25	0.03
		Porifera sp. 66	2	0.04	2.5	0.05
		Porifera sp. 68	1	0.04	1.25	0.03
		Porifera sp. 69	1	0.04	1.25	0.03
		Porifera sp. 70	17	0.44	1.25	0.08
		Porifera sp. 71	21	0.28	1.25	0.16
	Homoscleromorpha	*Oscarella* spp.	655	1.52	37.5	3.63
Cnidaria	Anthozoa	*Adamsia palliata*	1	0.04	1.25	0.03
		Alcyonium acaule	242	1.28	32.5	1.71
		Alcyonium coralloides	1617	1.68	47.5	6.54
		Alcyonium palmatum	1205	0.64	46.25	10.76
		Alicia mirabilis	2	0.04	2.5	0.03
		Amphianthus sp.	1	0.04	1.25	0.03
		Anemonia viridis	2	0.04	1.25	0.03
		Antipathella subpinnata	303	0.88	12.5	1.07
		Arachnanthus sp.	37	1.16	1.25	0.11
		Caryophyllia spp.	254	0.28	38.75	1.71
		Cereus pedunculatus	1	0.04	1.25	0.03
		Cerianthus membranaceus	173	0.16	65	2.48
		Cladocora caespitosa	1	0.04	1.25	0.03
		Corallium rubrum	3302	10.16	20	2.05
		Corynactis viridis	1869	12.12	2.5	0.19
		Dendrophyllia cornigera	1449	2.96	13.75	2.7
		Eunicella cavolini	12398	4.48	38.75	10.01
		Eunicella singularis	307	0.68	15	2.38
		Eunicella verrucosa	7010	3.08	75	24.21
		Funiculina quadrangularis	12	0.04	7.5	0.19
		Hoplangia durotrix	75	2.6	2.5	0.03
		Kophobelemnon stelliferum	1	-	1.25	-
		Leiopathes glaberrima	8	0.08	1.25	0.08
		Leptogorgia sarmentosa	598	1.2	47.5	4.06
		Leptopsammia pruvoti	30599	28.6	22.5	3.07
		Paracyathus pulchellus	9	0.36	1.25	0.03
		Paralcyonium spinulosum	5042	15.32	7.5	1.17
		Paramuricea macrospina	209	1.72	2.5	0.67
		Paramuricea clavata	12186	3.76	66.25	15.35
		Parantipathes larix	21	0.08	2.5	0.08
		Parazoanthus axinellae	19318	4.08	76.25	22.36
		Pennatula sp.	152	0.4	31.25	2.24
		Phyllangia americana mouchezii	9	0.12	2.5	0.08
		Polycyathus muellerae	3	0.04	3.75	0.05
		Pteroeides spinosum	17	0.12	6.25	0.29
		Savalia savaglia	26	0.64	2.5	0.03
		Sagartiidae	6	0.08	3.75	0.08
		Scleractinia sp. 1	393	0.52	37.5	1.84
		Scleractinia sp. 2	390	1.36	33.75	2.35
		Scleractinia sp. 3	18	0.24	10	0.11
		Scleractinia sp. 4	160	1.76	6.25	0.27
		Scleractinia sp. 5	2	0.04	2.5	0.05
		Zoantharia	10	0.4	1.25	0.03
		Veretillum cynomorium	6	0.04	5	0.16
		Virgularia mirabilis	5	0.12	2.5	0.08
	Hydrozoa	*Eudendrium* sp.	84	0.28	6	0.32
		Halecium halecium	1	-	1.25	-
		Lytocarpia myriophyllum	2036	1.44	46.25	7.1
		Nemertesia antennina	5	0.04	2.5	0.03
		Sertularella grayi	78	0.64	2.5	0.32
		Hydrozoa sp. 1	816	0.72	72.5	5.82
		Hydrozoa sp. 2	547	1.48	23.75	1.79
		Hydrozoa sp. 3	17	0.16	6.25	0.08
		Hydrozoa sp. 4	11	0.16	3.75	0.08
		Hydrozoa sp. 5	66	0.12	7.5	0.24
Platyhelminthes	Rhabditophora	*Prostheceraeus roseus*	1	0.04	1.25	0.03
Annelida	Echiuroidea	*Bonellia viridis*	633	0.4	83.75	7.34

	Sabellida	*Acromegalomma* sp.	52	0.12	22.5	0.67
		Apomatus/Protula complex	260	0.24	48.75	3.02
		Bispira viola	28502	110.32	21.25	1.12
		Bispira sp.	3	0.08	2.5	0.05
		Dialychone sp.	2722	39.04	3.75	0.29
		Filograna/Salmacina complex	2599	3.28	78.75	12.57
		Myxicola sp.	189	0.12	63.75	3.15
		Sabella spallanzanii	116	0.12	47.5	1.47
		Sabella sp.	205	2	3.75	0.05
		Sabellidae	2363	38.28	38.75	2
		Serpulidae	2511	5.28	80	6.35
	Terebellida	*Lanice conchilega*	2	0.04	1.25	0.03
Mollusca	Bivalvia	*Atrina* sp.	12	0.24	3.75	0.16
		Neopycnodonte cochlear	1478	9.8	12.5	0.67
		Pecten sp.	10	0.12	6.25	0.21
		Pinna nobilis	1	0.04	1.25	0.03
		Pteria hirundo	9	0.04	8.75	0.19
		Spondylus gaederopus	1	0.04	1.25	0.03
	Gastropoda	*Aporrhais pespelecani*	1	0.04	1.25	0.03
		Bolinus brandaris	2	0.04	2.5	0.05
		Calliostoma zizyphinum	1	0.04	1.25	0.03
		Euthria cornea	1	0.04	1.25	0.03
		Facelina annulicornis	1	0.04	1.25	0.03
		Felimare sp.	18	0.12	15	0.27
		Flabellina sp.	15	0.12	6.25	0.13
		Galeodea echinophora	6	0.24	2.5	0.03
		Natica hebraea	1	0.04	1.25	0.03
		Peltodoris atromaculata	9	0.08	5	0.08
		Simnia spelta	1	-	1.25	-
		Tritonia nilsodhneri	1	-	1.25	-
		Vermetidae	12	0.32	5	0.13
Arthropoda	Crustacea	*Anamathia rissoana*	1	-	1.25	-
		Calappa granulata	1	0.04	1.25	0.03
		Corystes cassivelaunus	1	0.04	1.25	0.03
		Ethusa mascarone	1	0.04	1.25	0.03
		Galathea sp.	4	0.04	5	0.05
		Homarus gammarus	4	0.04	3.75	0.03
		Inachus sp.	12	0.04	12.5	0.27
		Lysmata seticaudata	5	-	1.25	-
		Maja squinado	2	-	2.5	-
		Munida sp.	21	0.08	20	0.32
		Mysis sp.	723	15.96	2.5	0.05
		Paguridae	67	0.28	27.5	0.83
		Palinurus elephas	108	0.24	32.5	1.04
		Pilumnus hirtellus	1	-	1.25	-
		Plesionika narval	512	0.56	6.25	0.03
		Scyllarus arctus	1	0.04	1.25	0.03
		Stenopus spinosus	1	0.04	1.25	0.03
Phoronida		*Phoronis* sp.	2	0.08	1.25	0.03
Brachiopoda	Rhynchonellata	*Gryphus vitreus*	86	1.16	1.25	0.05
		Megerlia truncata	1337	8	2.5	0.27
Bryozoa	Gymnolaemata	*Myriapora truncata*	2207	2.8	71.25	9.23
		Pentapora fascialis	1193	0.72	72.5	11.48
		Reteporella spp.	1	0.04	1.25	0.03
		Schizoporella sp.	2038	2.2	71.25	9.77
		Smittina cervicornis/Adeonella calveti	550	1.56	18.75	1.07
		Turbicellepora sp.	1169	0.76	65	8.81
	Stenolaemata	*Frondipora verrucosa*	165	0.28	51.25	2.62
		Hornera frondiculata	5	0.04	3.75	0.11
Echinodermata	Asteroidea	*Astropecten aranciacus*	1	0.04	1.25	0.03
		Astropecten sp.	4	0.04	2.5	0.11
		Chaetaster longipes	20	0.04	15	0.27
		Coscinasterias tenuispina	3	0.04	3.75	0.05
		Echinaster sepositus/Hacelia attenuata	522	0.2	73.75	7.47
		Marthasterias glacialis	7	0.04	8.75	0.16
		Ophidiaster ophidianus	2	-	2.5	-
		Peltaster placenta	49	0.08	16.25	0.59
	Crinoidea	*Antedon mediterranea*	408	1.64	6.25	0.99
		Leptometra phalangium	27	0.12	8.75	0.45
	Echinoidea	*Centrostephanus longispinus*	228	0.56	40	2.19
		Cidaris cidaris/Stylocidaris affinis	2044	3.96	46.25	8.17

		Echinus melo/Gracilechinus acutus	156	0.12	28.75	2.46
		Spatangus purpureus	1	0.04	1.25	0.03
		Sphaerechinus granularis	3	0.04	2.5	0.03
	Holothuroidea	*Holothuria* sp.	2438	0.92	65	12.12
		Parastichopus regalis	141	0.24	23.75	2.27
	Ophiuroidea	*Amphiura* sp.	613	1.56	27.5	1.84
		Astrospartus mediterraneus	263	0.32	30	2.46
		Ophiothrix fragilis	1	0.04	1.25	0.03
		Ophiura ophiura	1	0.04	1.25	0.03
Chordata	Aplousobranchia	*Aplidium* sp.	3	0.08	2.5	0.05
		Clavelina sp.	23	0.28	5	0.11
		Diazona violacea	1	0.04	1.25	0.03
		Rhopalaea neapolitana	1	0.04	1.25	0.03
	Phlebobranchia	*Ciona* sp.	37	0.32	22.5	0.48
		Phallusia fumigata	10	0.12	6.25	0.13
		Phallusia mammillata	1	0.04	1.25	0.03
	Stolidobranchia	*Halocynthia papillosa*	1850	0.92	72.5	10.27
		Microcosmus sp.	6	0.08	3.75	0.08

Supplemental material 3. Biological features of the 80 ROV dives carried out on the Ligurian continental shelf, with indication of characteristic species.

Dive	Nº of species	Av. nº spp. per SU ± sd	Max nº spp. in SU	Av. nº org. per SU ± sd	Max nº org. in SU	Av. expH per SU ± sd	Max. expH per SU	Dominant assemblage	Characteristic species
A01	33	1.96 ± 1.90	8	3.3 ± 4.2	20	1.96 ± 1.37	6.92	1	*A. palmatum*
A02	9	0.67 ± 0.82	3	1.4 ± 2.3	8	1.15 ± 0.39	2.65	n/a	*C. cidaris/S. affinis*
A03	70	4.49 ± 3.45	14	18.9 ± 21.2	95	3.28 ± 2.15	8.71	6	*Axinella* spp.
A04	80	6.93 ± 4.34	17	33.7 ± 28.9	129	4.37 ± 2.51	11.09	6	*P. clavata*
A05	17	1.00 ± 1.31	3	1.0 ± 1.3	3	1.47 ± 0.87	3.00	n/a	*S. foetidus*
A06	60	3.64 ± 3.10	12	14.5 ± 14.6	60	2.55 ± 1.58	6.60	8	*E. verrucosa*
A07	61	2.41 ± 3.67	12	14.2 ± 26.6	140	2.15 ± 1.79	6.46	5	*Axinella* spp.
A08	36	8.73 ± 3.23	14	92.9 ± 100.3	356	4.63 ± 1.83	7.68	4	*M. truncata*
B01	44	4.4 ± 2.84	11	49.9 ± 87.7	443	2.70 ± 1.68	8.24	4	*Dialychone* sp.
B02	58	6.68 ± 3.11	13	74.0 ± 146.7	1048	3.40 ± 1.88	8.53	11	*H.* cf. *mediterranea*
B03	66	6.26 ± 5.45	21	42.3 ± 66.3	319	3.59 ± 2.07	8.12	11	*Axinella* spp.
B04	51	9.29 ± 3.24	16	35.8 ± 20.3	84	6.05 ± 2.08	10.23	4	*Axinella* spp.
B05	63	7.33 ± 5.35	15	45.0 ± 41.8	134	3.97 ± 2.28	7.05	3	*Axinella* spp.
B06	32	2.14 ± 2.51	8	6.9 ± 11.8	45	2.09 ± 1.33	5.00	3	*Axinella* spp.
B07	73	8.53 ± 5.10	18	45.7 ± 33.0	130	5.56 ± 3.22	12.86	3	*Axinella* spp.
B08	21	2.90 ± 1.55	6	4.8 ± 2.3	11	2.67 ± 1.43	5.33	1	*L. myriophyllum*
C01	42	7.71 ± 3. 01	16	58.1 ± 56.5	347	4.58 ± 2.00	9.80	5	*E. cavolini*
C02	29	3.65 ± 1.63	6	14.9 ± 11.8	50	2.81 ± 1.23	5.12	9	*D. cornigera*
C03	15	1.10 ± 1.11	6	3.31 ± 8.4	55	1.23 ± 0.63	4.71	1	*A. palmatum*
C04	20	1.14 ± 1.88	7	8.20 ± 17.8	91	1.43 ± 0.83	3.64	3	*P. axinellae*
C05	22	3.41 ± 3.83	11	17.3 ± 23.3	87	2.80 ± 2.30	7.39	3	*E. verrucosa*
C06	9	1.03 ± 0.96	4	2.4 ± 3.1	12	1.29 ± 0.61	3.79	1	*P. fascialis*
D01	32	5.92 ± 3.42	14	33.6 ± 25.0	123	4.04 ± 1.98	8.48	6	*E. verrucosa*
D02	14	1.82 ± 1.04	5	3.9 ± 3.1	20	1.72 ± 0.83	4.75	1	*A. palmatum*
D03	31	2.18 ± 2.23	11	13.1 ± 19.3	101	1.67 ± 1.05	6.14	6	*Axinella* spp.
D04	8	1.49 ± 0.76	3	4.3 ± 2.6	13	1.41 ± 0.56	3.00	1	*A. palmatum*
D05	37	5.95 ± 2.42	10	20.7 ± 14.5	64	4.32 ± 1.89	8.49	4	*E. cavolini*
D06	18	1.72 ± 1.97	7	6.6 ± 10.0	38	1.60 ± 0.95	4.07	4	*Axinella* spp.
D07	24	2.53 ± 2.00	9	7.8 ± 8.2	32	2.25 ± 1.32	5.87	4	*C. cidaris/S. affinis*
D08	53	2.62 ± 2.59	14	10.8 ± 17.9	92	2.08 ± 1.29	6.65	1	*A. palmatum*
E01	70	9.62 ± 6.96	21	239.5 ± 251.4	794	2.78 ± 1.57	6.76	7	*L. pruvoti*
E02	70	18.63 ± 5.22	27	452. ± 224.	979	5.85 ± 1.73	10.23	7	*L. pruvoti*
E03	45	4.97 ± 5.03	17	46.7 ± 57.6	185	2.62 ± 1.87	6.92	5	*Axinella* spp.
E04	44	2.25 ± 3.80	12	32.5 ± 103.	578	1.83 ± 1.59	7.57	3	*P. axinellae*
E05	38	3.77 ± 4.14	12	275. ± 748.	2758	2.33 ± 1.65	5.97	6	*B. viola*
E06	58	4.83 ± 4.44	14	174.5 ± 472.2	2327	2.81 ± 2.18	8.27	3	*B. viola*
E07	49	4.91 ± 4.20	15	36.9 ± 44.9	153	2.82 ± 1.94	7.20	5	*E. cavolini*
E08	42	8.62 ± 3.28	14	81.7 ± 41.6	158	3.94 ± 1.72	7.35	5	*E. cavolini*
E09	30	3.29 ± 2.51	10	34.9 ± 22.0	83	1.92 ± 1.00	4.15	9	*D. cornigera*
E10	52	5.05 ± 3.59	15	63.6 ± 77.4	391	2.97 ± 1.80	6.75	5	*P. spinulosum*
E11	65	5.92 ± 3.30	14	50.3 ± 37.8	193	3.20 ± 1.88	8.91	10	*P. spinulosum*
E12	63	6.46 ± 4.10	18	42.3 ± 40.3	155	4.17 ± 2.40	10.38	1	*P. clavata*
E13	52	6.62 ± 4.19	15	26.1 ± 22.9	98	4.91 ± 2.97	10.65	1	*Axinella* spp.
E14	52	8.24 ± 3.72	16	36.5 ± 26.5	121	5.46 ± 2.39	11.12	8	*E. verrucosa*
F01	71	6.24 ± 5.48	15	32.6 ± 39.8	146	4.00 ± 2.67	10.66	3	*Axinella* spp.
F02	37	6.00 ± 4.72	15	36.4 ± 37.5	141	3.74 ± 2.49	11.98	3	*Axinella* spp.
F03	20	0.73 ± 1.37	7	32.5 ± 186.2	1324	1.19 ± 0.65	5.00	12	*B. viola*
F04	30	2.23 ± 2.30	8	11.9 ± 15.4	70	1.83 ± 1.14	5.40	8	*E. verrucosa*
F05	43	3.46 ± 3.25	11	28.4 ± 41.3	197	2.50 ± 1.54	6.56	3	*Axinella* spp.
F06	46	3.71 ± 2.74	16	294.4 ± 685.4	2595	2.28 ± 1.33	5.86	8	*B. viola*
G01	28	2.50 ± 2.73	8	5.74 ± 9.5	41	2.39 ± 1.82	7.19	3	*Axinella* spp.
G02	24	0.71 ± 1.17	5	4.2 ± 17.6	176	1.24 ± 0.56	3.79	7	*L. pruvoti*
G03	46	4.51 ± 3.47	11	30.7 ± 34.7	171	2.74 ± 1.36	5.72	3	*Axinella* spp.
G04	23	1.13 ± 1.64	7	2.3 ± 4.7	20	1.52 ± 1.04	5.99	3, 4	*Axinella* spp.
G05	50	5.05 ± 3.99	11	61.2 ± 74.8	291	2.85 ± 1.53	6.21	3	*L. pruvoti*
H01	26	2.44 ± 1.89	8	8.7 ± 11.8	50	2.09 ± 1.11	5.74	1	*M. truncata*

H02	36	9.75 ± 2.53	14	186.8 ± 136.7	471	4.27 ± 1.11	6.19	6	*L. pruvoti*
H03	46	5.19 ± 3.69	12	67.4 ± 95.5	496	2.92 ± 1.52	6.75	3	*Axinella* spp.
H04	37	1.98 ± 2.51	9	21.8 ± 55.2	318	1.59 ± 0.86	4.02	7	*L. pruvoti*
H05	30	2.36 ± 2.77	8	50.8 ± 103.1	283	1.63 ± 1.12	4.41	5	*C. viridis*
H06	39	1.18 ± 1.88	7	2.4 ± 5.0	28	1.56 ± 1.22	6.73	4	*Axinella* spp.
H07	33	3.25 ± 3.03	8	10.1 ± 10.9	37	2.56 ± 1.78	6.92	4	*Axinella* spp.
H08	46	2.71 ± 3.06	12	13.6 ± 18.1	66	2.11 ± 1.63	7.79	2	*L. myriophyllum*
H09	22	1.50 ± 1.66	7	2.2 ± 2.8	12	1.59 ± 1.23	5.86	1	Sabellidae
H10	69	8.12 ± 5.70	19	78.3 ± 77.5	237	4.10 ± 2.86	12.43	5	*Axinella* spp.
H11	53	5.19 ± 3.85	19	36.1 ± 42.7	175	3.04 ± 1.67	8.07	2	*E. cavolini*
H12	52	3.45 ± 3.26	14	34.9 ± 128.5	871	2.54 ± 1.52	6.61	3	*L. pruvoti*
H13	51	7.31 ± 4.47	14	58.6 ± 65.3	260	4.06 ± 2.08	8.26	5	*Axinella* spp.
I01	42	4.38 ± 3.24	12	33.6 ± 46.5	193	3.02 ± 1.70	7.31	3	*P. axinellae*
I02	31	2.49 ± 2.66	9	8.5 ± 13.7	60	2.31 ± 1.58	6.13	6	*P. clavata*
I03	39	3.40 ± 3.43	14	9.9 ± 11.3	40	2.90 ± 2.31	11.07	6	*P. clavata*
I04	40	2.97 ± 3.50	11	10.8 ± 16.5	54	2.59 ± 2.23	8.44	8	*Axinella* spp.
I05	9	0.40 ± 0.92	5	0.6 ± 1.3	6	1.14 ± 0.63	5.00	n/a	*A. palmatum*
I06	30	2.09 ± 3.04	11	22.1 ± 57.2	252	1.85 ± 1.88	8.45	1	*N. cochlear*
I07	43	4.72 ± 4.16	13	17.5 ± 19.1	89	3.65 ± 2.48	8.83	3	*Axinella* spp.
I08	26	2.76 ± 2.37	9	8.0 ± 9.0	31	2.50 ± 1.56	6.75	3	*P. axinellae*
J01	46	6.76 ± 3.07	14	90.2 ± 93.6	419	3.60 ± 1.45	7.03	3	*Axinella* spp.
J02	43	6.71 ± 3.91	12	37.3 ± 35.6	109	4.12 ± 2.01	8.21	8	*E. verrucosa*
J03	11	0.33 ± 1.00	4	0.5 ± 1.5	7	1.19 ± 0.61	3.79	n/a	*Pecten* sp.
J04	31	5.94 ± 2.21	8	34.6 ± 25.6	90	3.98 ± 1.23	5.83	3	*P. axinellae*

PART 2

FISHING IMPACT

Descriptor 6 of the Marine Strategy Framework Directive states that a Good Environmental Status is reached when "sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected" (European Commission 2010). In this sense, human pressure should not limit the capacity of the different ecosystem components to maintain their natural diversity, productivity and ecological processes. Thus, there is a driving need towards the identification of standardized methods to *describe and measure the sea floor integrity, especially in concomitance with vulnerable marine ecosystems, and their alteration by human activities. A significant documentation regarding the structure and functioning of the communities, the characterization of the fishing pressure burdening on the megabenthic assemblages, and the extent of the mechanical disturbance are essential elements in determining the integrity of benthic ecosystems, and are also required by FAO and GFCM for the identification of Vulnerable Marine Ecosystems (VMEs), Sensitive Habitats (SHs) and Essential Fish Habitats (EFHs).*

The second section of this thesis aims to provide such quantitative information for two case-study areas located within the Ligurian Sea, and representing diverse ecological settings impacted by different fishing techniques. The adopted multidisciplinary approach allowed integrating in a common picture the following aspects:

(i) the fine characterization of the structure, distribution and health status of the megabenthic communities inhabiting the selected area, obtained through a standardized ROV exploration (MSFD protocols for Coralligenous and deep-sea);

(ii) the depiction of the fishing fleet (and relative fishing effort) operating in the selected fishing ground, by means of an extended background research including fishermen and port authority interviews;

(iii) the quantification of the impact of the main métier adopted by fishermen in the investigated area, by considering the fishing efficiency (ratio between commercial and discarded catches), uptake rates of no-target catches (with specific attention to structuring megabenthic invertebrates), the frequency of gear loss and the sea bed entanglement events, during a long-term on-boarding monitoring.

Results provided key upshots regarding the vulnerability and the conservation of the biocoenoses inhabiting the two investigated areas clearly identifiable as SHs. The protocol followed will be proposed as a standard method for data acquisition and data will be used to implement a Fisheries Restricted Area (FRA) procedure at GFCM for the definition of the first deep-sea Ligurian managed areas.

Chapter 2

Artisanal fishing impact on deep coralligenous animal forests: a Mediterranean case study of marine vulnerability

Abstract

Vulnerable Marine Ecosystems (VMEs) are characterized by prominent biological features susceptible to anthropogenic disturbances. Following international guidelines, the identification and protection of VMEs require a detailed documentation regarding both the community structure and the fishing footprint in the area. This combined information is lacking for the majority of the Mediterranean mesophotic rocky reefs that are known to host valuable animal forests.

A deep coralligenous site exploited by artisanal fishermen in the NW Mediterranean Sea is here used as a model to assess the vulnerability of animal forests and evaluate the sustainability of demersal fishing practices, particularly lobster trammel net. The Remotely Operated Vehicle (ROV) footage is used to document the biodiversity and health status of the megabenthic communities, while discard data are employed to quantify the entanglement risk, discard rates of fragile species and threats to sea floor integrity.

A multidisciplinary approach is proposed for the assessment of the vulnerability criteria of an EU Special Area of Conservation, leading to specific conservation measures, including the delineation of fishing restrictions.

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1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) "Deep-sea Fisheries Guidelines" volume describes Vulnerable Marine Ecosystems (VMEs) as areas that may be susceptible to impact from human activities, especially fishing. The term "vulnerable" reflects here the fact that they are both easily disturbed and slow or unable to recover (FAO, 2009). VMEs are often overlapped with Essential Fish Habitats (EFHs), identified as fundamental for critical life history stages of exploited fish species (*e.g.* nursery and spawning areas), and Sensitive Habitats (SH), internationally recognized as fragile, ecologically important habitats, which support relevant assemblages of commercial and non-commercial species. More specifically, within vulnerable ecosystems animal forests *sensu latu* (aggregations and reefs) often play an important role (Aguilar et al., 2017). Several species of sponges and cnidarians (hydroids, scleractinians, gorgonians, antipatharians) represent iconic taxa in these environments, thanks to their wide distribution and arborescent morphology, leading towards three-dimensional habitats capable of modifying the near-bottom conditions (Buhl-Mortensen et al., 2010; Guizien and Ghisalberti, 2017) and acting as important nurseries and refuges for invertebrates and fishes (Bo et al., 2009, 2015; Cathalot et al., 2015; Cerrano et al., 2015; Maldonado et al., 2017; Rossi et al., 2017; Mastrototaro et al., 2017).

In recent times, many non-government as well as National and Regional Fisheries Management Organizations (RFMO), have developed processes to identify VMEs and adopt management measures to protect them from bottom fishing activities including identification of indicator species, fishery monitoring protocols, employment of onboard observers, collection of information regarding discard rates, and closure of specific areas (Durán Muñoz et al., 2012; Ardron et al., 2014; Thompson et al., 2016; OCEANA 2016; Aguilar et al., 2017).

In the Mediterranean Sea, the General Fisheries Commission for the Mediterranean (GFCM) has adopted Fisheries Restricted Areas (FRAs) as a multi-purpose spatial-management tool to limit fishing activities hence protect deep-sea SHs and EFHs (GFCM, 2006, 2009, 2016, 2017). Furthermore, GFCM has prohibited the use of towed dredges and trawl nets at depths greater than 1000 m (GFCM, 2005), an area that covers more than 60% of the Mediterranean Sea, even if it is not reachable by any present fleet. Despite these limitations, no protection is still in place for the animal forests (as well as coralligenous bio-concretions and maërl beds) occurring along the continental shelf and upper slope (Kamenos et al., 2004; Gori et al., 2017; Chimienti et al., 2018) where the majority of the Mediterranean artisanal and recreational fishing boats operates. This is even more relevant as the network of marine protected areas, as well as the coastal trawling prohibition (GFCM, 2012), cover only shallow littoral areas leaving coastal offshore habitats completely unprotected.

Demersal small-scale fisheries represent 83% of the Mediterranean fishing fleet and have been proven to determine significant impacts on the megabenthic communities (Shester and Micheli, 2011; FAO, 2018). Artisanal fishing is essentially located on the continental shelf (0-200 m depth), exploiting fishing grounds, which can be reached in a few hours from the fishermen bases (Colloca et al., 2004; McCluskey and Lewison, 2008). Artisanal fisheries are characterized by a high diversification of gears, techniques, target species, seasons and fishing grounds, which increase the difficulties in the management of this sector. For this reason, the term *métier* has been introduced to describe the various combinations of features (González-Álvarez et al., 2016). Trammel nets are among the most employed gears in southern European countries (Erzini et al., 2006; Cataudella and Spagnolo, 2011). This gear is generally considered poorly selective with marked differences among the numerous trammel net *métiers* (Colloca et al., 2004; Erzini et al., 2006; Stergiou et al., 2006; Cataudella and Spagnolo, 2011; Catanese et al., 2018).

The FAO international guidelines for the management of deep-sea fisheries (FAO, 2009) laid out a combination of biological, ecological, and socio-economical characteristics for the classification of marine ecosystems as vulnerable. The identification of these features requires a significant documentation regarding the benthic community, the extent of the mechanical disturbance as well as the fishing effort, this latter referring to the amount and type of resources devoted to obtain a certain fishing yield in a specific site (Pascoe and Robinson, 1996; Ruttan, 2003; Del Valle et al., 2003; McCluskey and Lewison, 2008).

ROV-Imaging related tools have already been adopted to characterize benthic communities and quantify fishing disturbance, expressed as density of marine litter and percentage of impacted organisms (Bo et al., 2014; Angiolillo et al., 2015; Díaz et al., 2015). These parameters give a well-defined picture of the health status of these ecosystems, but they do not depict the potential damage on the seafloor of a single fishing event, or the environmental sustainability of a certain *métier* (aka the fishing efficiency calculated considering the commercial yield and the discard rate). This latter aspect can be investigated through the study of fishing benthic discard, but it has been relatively overlooked, especially for Mediterranean artisanal fisheries (Hawkins and Roberts, 2004; Mangi and Roberts, 2006; Erzini et al., 2006; Stergiou et al., 2006; Gökçe and Metin, 2007; Sartor et al., 2007; Batista et al., 2009; Catanese et al., 2018; Demestre et al., 2018; Gil et al., 2018).

A multidisciplinary approach targeting the characterization of a deep coralligenous Mediterranean site is here used as a reference for the assessment of the vulnerability criteria required by FAO for VMEs designation over mesophotic rocky reefs, with implementation of conservation measures regarding EU deep marine tutelary sites.

2. Materials and Methods

2.1 Study area

This study is focused on a deep coralligenous site known as “Maledetti Shoal”, located in the western Ligurian Sea (Fig. 1a). The Maledetti Shoal lies in close proximity to the large harbor areas of Vado Ligure (about 4 km south) and Savona (about 9 km south-west). It is a 1 km-long, almost vertical wall, parallel to the coastline and located 1.2 km off the coast, extending from Spotorno to the southern boundary of the Marine Protected Area (MPA) "Isola di Bergeggi". The cliff is not continuous for the presence of two large mudslides, occupying the central part of the shoal, whereas the western and eastern extremities gradually terminate in a highly silted slope (Fig. 1b, c). The vertical profile of the shoal is not even, and it consists of three regions: i) a flat, heavily silted rocky plateau at around 54-60 m depth, ii) a vertical rocky cliff extending down to 75-80 m depth, and iii) large collapsed rocky boulders interspersed with sandy patches down to 130 m depth (Fig. 1b, c). This site is known to host dense forests of the red gorgonian *Paramuricea clavata* (Risso, 1826) as well as the largest Ligurian deep population of the precious red coral *Corallium rubrum* (Linnaeus, 1758) (Parravicini et al., 2007; Cattaneo-Vietti et al., 2016; MIPAAF, 2013; Betti, 2017), although no specific characterizations were made so far. Harvesting of red coral was repeatedly reported in Liguria since ancient times; in the study area a relatively scattered employment of destructive methods such as the "*ingegno*" gear ceased before the beginning of the 19th century, while pouching by means of diving is known to have stopped in the 1980s (Cattaneo-Vietti et al., 2016), such that direct impact on red coral is not considered a problem in this specific site.

2.2 Tutelary regime

The Maledetti Shoal is included within the Special Area of Conservation "Fondali Noli - Bergeggi" (SAC, IT1323271) of the Natura 2000 Network (Fig. 1a). It is an area of 380 ha, including four Habitat types (following the Directive 92/43/CE): sand banks (1110), *Posidonia oceanica* (Linnaeus) Delile, 1813, meadows (1120), marine caves (8330), and coralligenous cliffs (1170), the latter covering about 46 ha.

The body responsible for the site management is the municipality of Bergeggi through the MPA "Isola di Bergeggi". With special reference to Habitat 1170b (deep coralligenous cliffs), the site is considered important at regional level due to its relative topographic uniqueness and was given high priority of conservation. The protection measures proposed for this area aim at enhancing the environmental status of the ecosystems suffering the effects of three main anthropic pressures, namely dredging, anchoring and fishing. Potential impacts related to dredging and anchoring operations concern destruction and sedimentation of habitat, as well as removal of arborescent species, although these effects are known only at very local scale. Potential impacts related to fishing gears operated on or nearby Habitat 1170b mainly concern the loss or abandoning of gears. Despite this latter phenomenon is diffusely reported, there are no quantitative information on this pressure or correlation with the conservation of the habitat, hence, differently from dredging and anchoring, no regional legislation is active on this purpose.

No Management Plan still exists for this SAC but some Conservation Measures (sensu Directive 92/43/EEC) have been delineated by the Liguria Region (DGR 1459/2014).

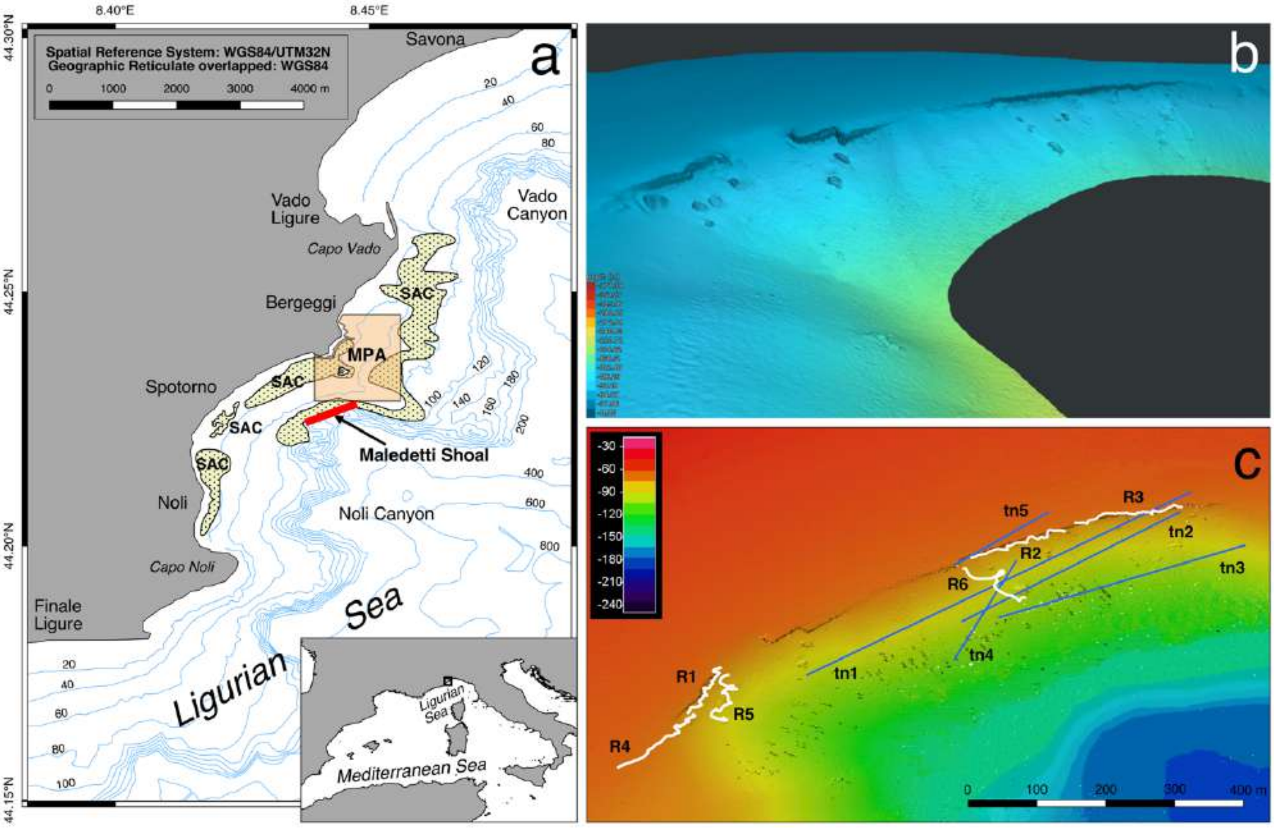

Figure 1. Map of the study area. a) Geographic localization of the Maledetti Shoal in relation to the main cities of the area, the Marine Protected Area "Isola di Bergeggi" (light orange), the Special Area of Conservation "Fondali Noli-Bergeggi" (SAC, dotted yellow), and the main features of the sea bed topography. Insert: localization of the Ligurian Sea and the study area within the western Mediterranean basin. b) Three-dimensional elaboration of the Maledetti Shoal. The vertical wall, the rocks at the cliff base, and the muddy landslides in-between are visible. c) Multibeam map showing the position of the six ROV transects (R1-R6, white) and the five fishing sets (tn1-tn5, blue).

2.3 Fishing pressure

The SAC "Fondali Noli – Bergeggi" lies in close proximity to the harbors of Noli and Vado Ligure (Fig. 1a). These two harbors include the majority of the professional fishing fleet operating in the area comprised between Vado Ligure and Capo Noli, accounting for 29 vessels following the official register (http://ec.europa.eu/fisheries/fleet/index.cfm). Ten fishermen interviewed during this study report that, nowadays, the real number of artisanal vessels operating in the area does not exceed 20 vessels, and that this number has been gradually decreasing since the 1980s.

At present, only four artisanal fishermen are known to fish on the Maledetti Shoal. Boats, 5-9 m in total length, 1-5 gross tons, 13-85 kilowatt, are all operated by only one person. This site is considered easily accessible (2 and 3 NM from the harbor of Noli and Vado Ligure, respectively). Here, the traditional trammel net called "*aragostara*" represents the main *métier*, targeting the spiny lobster *Palinurus elephas* (Fabricius, 1787) and the European lobster *Homarus gammarus* (Linnaeus, 1758). Gillnets are occasionally employed on the flat muddy plateau at the base of the vertical wall, down to 100 m depth.

Fishermen declared to frequent the site no more than 10-30 times per year, mainly during summertime, when sea conditions are optimal. Indeed, due to the complex topography of the shoal and the strong bottom currents, fishermen consider difficult to operate on this fishing ground. The name "Maledetti", which means "damned" in Italian, refers to the easiness to entangle on the sea bottom with the net and to the high likelihood to break it or lose it. Interviewed fishermen declared to strongly entangle on the bottom 2-5 times per years (up to 20) and at least three fishermen reported losing the gear on the site.
In addition to artisanal boats, several recreational fishermen frequent the site, using trolling lines, vertical lines ("*bolentino*") and longlines, but no quantitative data are available on this fleet. Professional trawlers, accounting for four vessels from the harbor of Savona and one from the harbor of Finale Ligure, are known to avoid the Maledetti Shoal.

2.4 ROV exploration and ROV-Imaging

Data were recorded on board of the R/V *Astrea* (ISPRA, Rome) in August 2015 and September 2016. A high-resolution Multibeam Echo Sounder (MBES, Kongsberg EM2040) has been employed in order to obtain the topography of the site. The resulting multibeam map has been used to localize the geographical position of the cliff and other relevant seabed features. These areas have been subsequently investigated by means of a ROV Pollux III, equipped with a digital camera (Nikon D80), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), and a 3-jaw grabber. An underwater acoustic positioning system (Ultra Short Baseline Linquest Tracklinq 1500 MA), a depth sensor, a compass and two parallel laser beams providing an 8-cm scale for the measurements, were also present.
A total of six 200 m-long video transects (R1-R6) have been carried out, accounting for approximately two hours of analyzed video, corresponding to 600 m^2 of explored sea bed and including both vertical cliffs and bottom boulders (Fig. 1c; Tab. 1). During video recording, ROV moved close to the sea bottom at a constant speed of 0.2 m s^{-1}. Laser beams were always included in the frame, providing a scale to define a fixed visual field within transects (0.5 m) for a total investigated area, per transect, of 100 m^2. Based on time, percentage of hard bottom and vertical slope was calculated for each transect. The frequent presence of trammel nets' signals during sampling operations prevented the exploration of the central portion of the shoal, both in 2015 and 2016 (Fig. 1c). Qualitative observations for this area were obtained from previous surveys conducted in 2012 by Centro Carabinieri Subacquei (Genova) by means of a Pluto ROV.
The Coralligenous Habitat Monitoring working protocol of the Italian Marine Strategy Framework Directive (MSFD) was applied to assess the structure and the environmental status of the benthic communities thriving on the Maledetti Shoal (MATTM-ISPRA, 2016). The working protocol adopts a landscape-based approach centered on remotely collected data gathered from standard video-transects and high-resolution photographs and focused on the megabenthic organisms, especially structuring species. The recorded videos were edited by means of Final Cut Pro software. Five parameters have been

extrapolated from the analysis: i) megabenthic species richness, ii) basal bio-cover, referring to the mean percentage of hard bottoms covered by organisms of the basal and intermediate layer, iii) abundance (n° individuals m^{-2}) and size (cm) of the structuring species (SS), iv) health status of SS anthozoans, considering the percentage of structuring anthozoans showing signs of necrosis and epibiosis or directly entangled with lost fishing gears, v) abundance and typology of marine litter encountered over 100 m^2.

These parameters have been elaborated with the Mesophotic Assemblages Conservation Status (MACS) Index (Enrichetti et al., 2019) in order to give a picture of the environmental status of the studied areas.

2.5 Discard analysis

A scientific observer was employed to evaluate the average fishing impact of the "*aragostara*" in the site through the analysis of the discard. Five fishing sets (tn1-5) were carried out on the Maledetti Shoal in summer 2016 and 2017 (Fig. 1c; Tab. 2). Data were collected on board of a local fisherman boat. The boat was made in fiberglass with a total length corresponding to 8.55 m, gross tonnage 5 tons and power 84.6 Kw. The boat was equipped with a sounder and a net hauler. The employed trammel net was a polyamide (nylon) monofilament, with an inner mesh size of 10 cm. Soak time varied between 2 and 4 days. The total fishing set was composed by an aligned series of panels of trammel net (from 4 to 10) each one 100 m long and 1 m high. The final length of the fishing set varied from 400 to 1000 m (Tab. 2).

In order to quantify the species composition and abundance of the benthic discard of the "*aragostara*", all the catches gathered during the hauling were identified, weighted, and divided into the following categories (Catanese et al., 2018): i) marketable catches, including target catches and commercial bycatch (the retained catch of non-target, but still commercial species), and non-marketable catches, including ii) commercial discard (potentially marketable organisms returned to the sea because low priced, undersized, bitten, rotten or damaged, both invertebrates and fish), and iii) non-commercial discard (benthic organisms, both invertebrates and fish without commercial value). For every organism of the non-commercial discard, the status upon arrival was detected on board distinguishing between entire or broken specimens. Skeleton remains were considered overall as biogenic detritus. Species have been identified at the highest possible taxonomic level and all individuals were photographed and measured. Species difficult to identify on board were taken to the laboratory for detailed taxonomic analysis.

Considering that fishing sets may show variations in the length of the net, mean data of abundance and/or weight of main categories as well as single species or other Operative Taxonomic Units (OTUs) have been normalized to a standard 200 m-length, generally representing the length of a single panel of trammel net.

In order to evaluate the efficiency of the *métier* in the study site and its potential impact, percentage rates of collection of all categories were calculated in terms of diversity, abundance and biomass. Marketable catches were priced following the local fish market prices to estimate

Dive	Date	Start position		End position		Depth range (m)	Edited duration (min:sec)	% Hard bottom	% Vertical slope
		Lat. N	Long. E	Lat. N	Long. E				
R1	10.08.15	44.2242	8.4368	44.2250	8.4374	54-67	19:45	95	43
R2	04.09.16	44.2275	8.4436	44.2270	8.4420	53-65	23:34	100	94
R3	04.09.16	44.2280	8.4458	44.2277	8.4439	53-63	23:03	98	89
R4	10.08.15	44.2232	8.4356	44.2242	8.4369	52-70	15:05	59	12
R5	10.08.15	44.2249	8.4376	44.2241	8.4375	68-87	13:23	99	42
R6	04.09.16	44.2268	8.4419	44.2263	8.4430	52-112	13:39	51	48

Table 1. ROV video transects (R) general information.

Fishing event	Hauling Date	Start position		End position		Depth (m)		Soak time (days)	Fishing set total length (m)
		Lat. N	Long. E	Lat. N	Long. E	Start	End		
tn1	12.07.16	44.2282	8.4460	44.2249	8.4390	55	107	3	500
tn2	03.08.16	44.2279	8.4458	44.2259	8.4418	61	97	3	500
tn3	22.07.17	44.2273	8.4470	44.2260	8.4425	79	98	4	1000
tn4	12.08.17	44.2270	8.4429	44.2252	8.4417	65	89	3	400
tn5	25.08.17	44.2279	8.4434	44.2269	8.4417	48	52	2	400

Table 2. Fishing operations general information (tn: trammel net).

the average economic yield of a fishing operation in this site. In addition, the amount of substratum (rocks and coralligenous framework) collected by the gear in each fishing operation was weighted to depict the effect of the trammel net on the sea floor integrity. For the same purpose, the number of entanglements on the sea bottom, as well as the number of gear loss events were annotated. Similarly, the collected marine litter was quantified and described. Whenever useful, mean values were normalized for 200 m of trammel net.

2.6 Statistical analyses

A one-way ANOVA was carried out to test for differences among the species composition of the megabenthic assemblages of the six transects considering the entire ROV density dataset [transformed sqrt(x) data, Bray-Curtis similarity measure, density data distributed homogenously with n = 100 for each transect]. A similar approach was used to test for differences in species composition of the fishing sets considering the entire dataset [transformed sqrt(x) abundance data normalized for a 200 m-long net, Bray-Curtis similarity measure, data distributed homogenously with n = 100 for each fishing set, excluding dead catches].

Finally, to find out whether there was a significant difference in abundance, mean height, percentage of impacted colonies of the structuring species (PC, *Paramuricea clavata* (Risso, 1826), CR, *Corallium rubrum* (Linnaeus, 1758), EC, *Eunicella cavolini* (Koch, 1887), EV, *Eunicella verrucosa* (Pallas, 1766)) between the six transects, Kruskal-Wallis

tests were performed on individual datasets.
Analyses were performed using PAST for MAC version 3.07 (Hammer et al., 2001).

3. Results

3.1 Biocoenotic characterization of the shoal

The results of the video analysis are summarized in table 3. Biodiversity of the Maledetti Shoal is high, with 100 hard bottom megabenthic OTUs (corresponding to 33621 records) reported for the entire area (SM1). Sponges include the majority of the identified OTUs (Fig. 2a), but represent only the 21% of the observed individuals (Fig. 2b), with *Axinella* spp. (up to 11 individuals m^{-2}) (Fig. 3a), *Aplysina cavernicola* (Vacelet, 1959) (up to 5 individuals m^{-2}) (Fig. 3b, c), and *Petrosia (Petrosia) ficiformis* (Poiret, 1789) (up to 5 individuals m^{-2}) (Fig. 3c) being the most abundant. The most represented taxon in terms of abundance is that of cnidarians (Fig. 2b). Among them, gorgonians (Fig. 3d, c, e) reach high densities, but the most abundant species of the shoal is the yellow scleractinian *Leptopsammia pruvoti* Lacaze-Duthiers, 1897 with densities up to 64 individuals m^{-2} (Fig. 3c, e, f). Other relatively abundant organisms are mainly bryozoans and echinoderms (Figs. 2b, 3b, i). The ascidian *Halocynthia papillosa* (Linnaeus, 1767) forms only localized aggregations (Fig. 3j).
The analysis of variance reveals no significant difference among the megabenthic communities of the six investigated transect areas supporting homogeneity of the assemblages (SM2).
Nine structuring species are present in the study area (Tab. 3; Fig. 3c-h) including cnidarians, sponges and bryozoans. The red gorgonian *P. clavata* is significantly the most abundant structuring anthozoan (4.3 ± 1.7 colonies m^{-2}) together with *C. rubrum* (2.2 ± 0.1 colonies m^{-2}) (Kruskal-Wallis, $p<0.001$, $H=14.73$, [PC=CR>EC=EV]). *E. cavolini* (0.2 ± 0.1 colonies m^{-2}; Fig. 3f), *E. verrucosa* (0.1 ± 0.1 colonies m^{-2}; Fig. 3g) and *Leptogorgia sarmentosa* (Esper, 1789) (with only one colony) are also reported, together with a specimen of the zoanthid *Savalia savaglia* (Bertoloni, 1819). Massive keratose sponges, represented by *Sarcotragus foetidus* Schmidt, 1862 (0.1 ± 0.03 individuals m^{-2}) and *Spongia (Spongia) lamella* (Schulze, 1879) (Fig. 3h) are found together with the arborescent bryozoan *Pentapora fascialis* (Pallas, 1766).
Structuring anthozoans show a distinct zonation on the shoal (Fig. 4). *P. clavata* and *C. rubrum* reach maximum density values on the vertical wall (up to 7.9 ± 1.4 colonies m^{-2} and 3.9 ± 1.4 colonies m^{-2}, respectively). *C. rubrum* forms dense patches in the small crevices or overhangs of the cliff often in association with *L. pruvoti* and several encrusting or prostrate sponges (Fig. 3c, e, f). *E. verrucosa* forms dense forests on the high silted plateau just above the cliff (Figs. 3g), whereas the collapsed rocks at the cliff base host sparse aggregations of *E. cavolini*, sometimes mixed with other gorgonians (Figs. 3f).
The mean heights observed for the four gorgonian species are reported in Fig. 5a supporting *P. clavata* and *E. verrucosa* as the significantly tallest structuring anthozoans in the area (17.2 ± 3.1 cm and 15.9 ± 5.2 cm, respectively) (Kruskal-Wallis, $p<0.001$, $H=11.43$, [PC=EV>EC>CR]).

Dive	N° of OTU	N° of individuals	Basal cover (%)	N° of SS	TOT density SS (org. m^{-2})	Mean h dominant SS (cm ± se)	% Imp. SS antho.	% Ep/Ne SS antho.	% Entangl. SS antho.	Litter density (it. m^{-2})	MACS index value
R1	40	8264	78	6	7.25	21.7 (± 0.9)	53.8	11.1	51.7	0.54	Moderate (46)
R2	53	9771	91	8	13.26	11.1 (± 0.2)	27.8	13.2	23.8	0.81	Moderate (54)
R3	73	9723	92	5	15.46	19.7 (± 0.4)	33.0	16.5	28.1	0.88	Good (57)
R4	50	1759	72	6	1.93	22.5 (± 2.2)	40.8	9.7	39.8	0.12	Moderate (50)
R5	46	2739	74	6	2.15	*4.5 (± 0.5)	35.6	28.8	17.1	0.20	Moderate (47)
R6	38	1365	70	6	1.61	10.2 (± 1.2)	44.7	23.7	21.1	0.12	Poor (42)
Avg.	50 (± 5.2)	5604 (± 1657)	79.5 (± 3.9)	6.2 (± 0.4)	6.94 (± 2.52)	n/a	39.3 (± 3.8)	17.2 (± 3.1)	30.3 (± 5.3)	0.45 (± 0.1)	Moderate (49 ± 2.2)

Table 3. Results of the ROV video transects analysis. Within each transect, the dominant structuring species is *Paramuricea clavata*, with the exception of the transect R5, for which it is *Corallium rubrum* (*). Average values are reported with standard errors (± SE). SS: structuring species; h: height; Imp.: impacted; antho.: anthozoan; Ep/Ne: epibiosis and necrosis; it.: items; n/a: not applicable. MACS index ranges: ≤35 = Bad; 36-45 = Poor; 46-55 = Moderate; 56-65 = Good; ≥66 = High (Enrichetti et al., 2019).

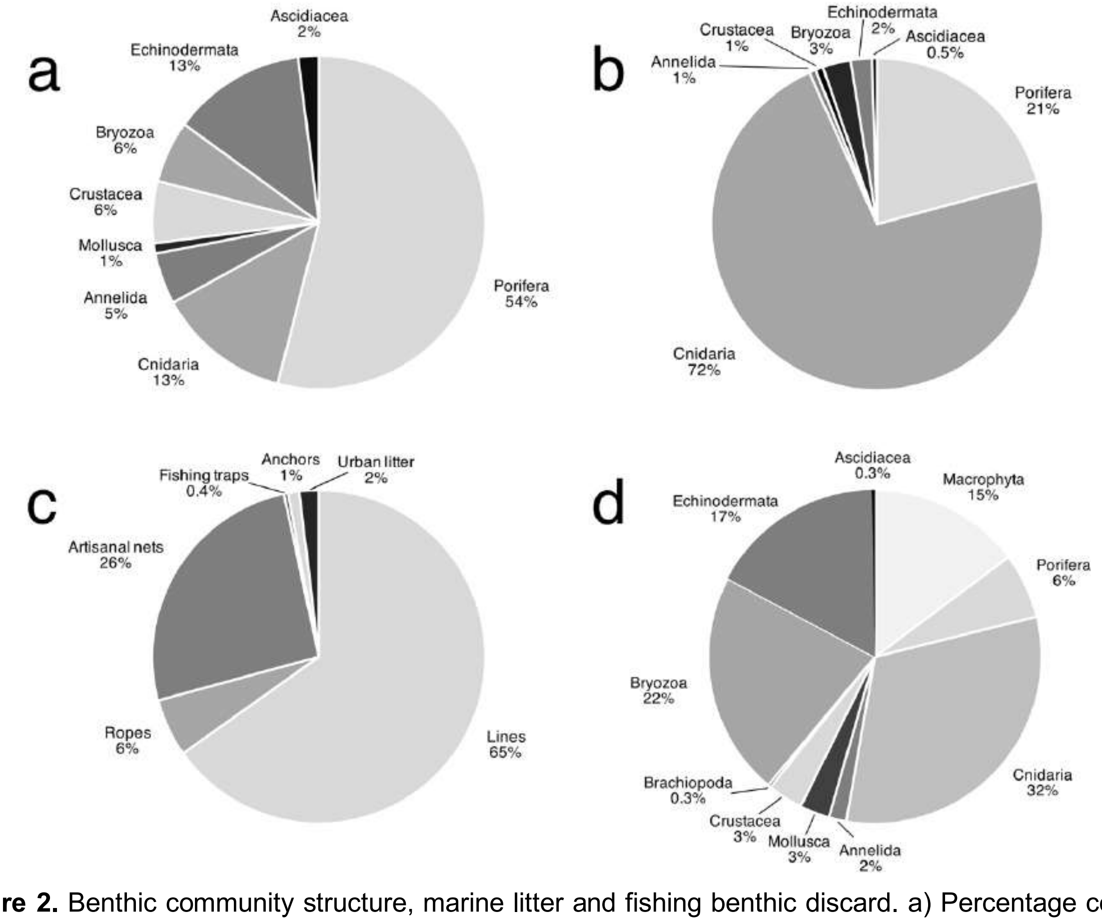

Figure 2. Benthic community structure, marine litter and fishing benthic discard. a) Percentage composition of megabenthic OTUs in the video footage. b) Percentage number of individuals per taxonomic category. c) Percentage composition of the marine litter on the sea bottom. d) Percentage composition of the benthic discard (as number of organisms).

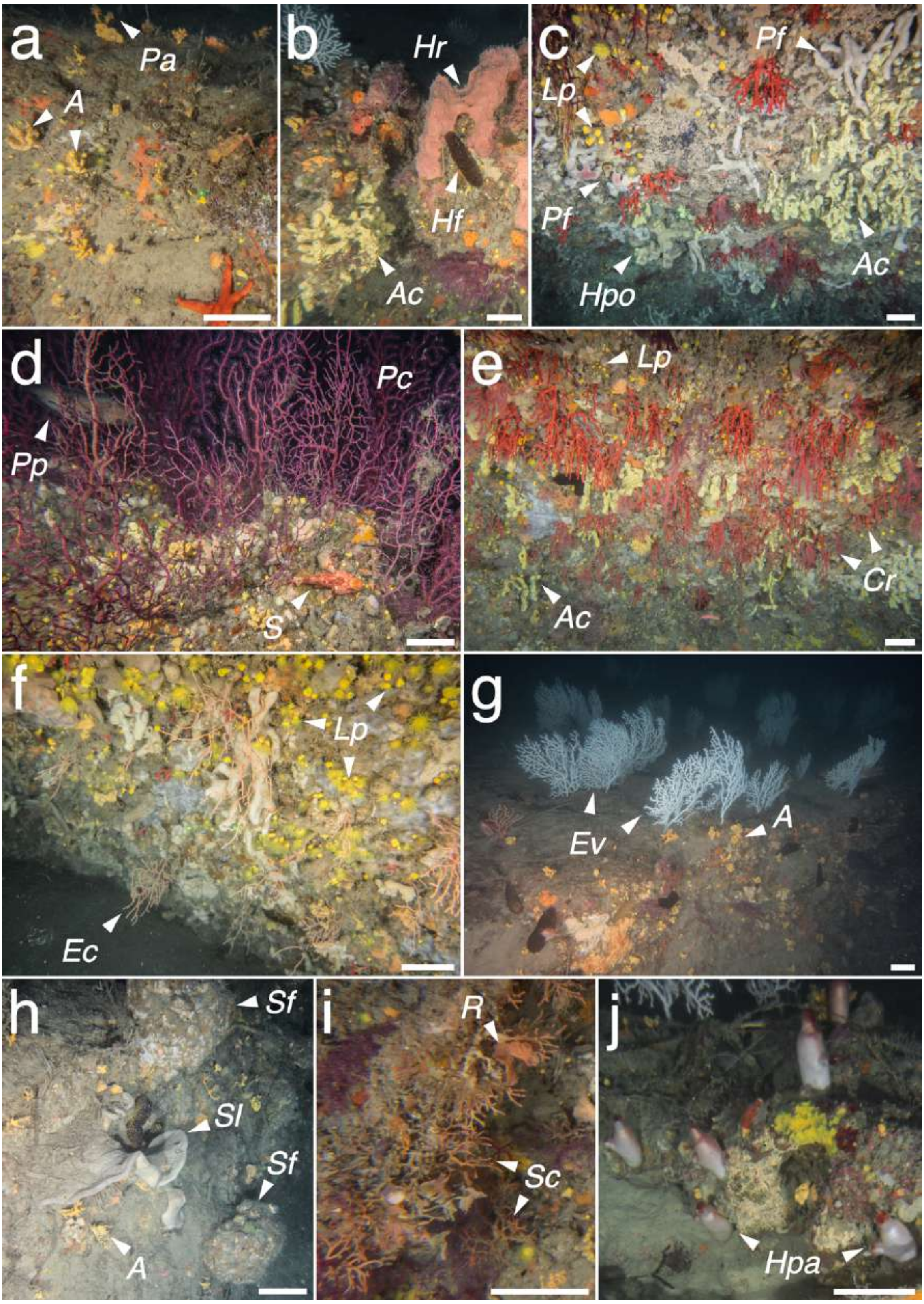

Figure 3. Biodiversity and major communities of the Maledetti Shoal. The shallower portion of the vertical wall hosts several sponge aggregations, including a) *Axinella* spp. (*A*) covered with *Parazoanthus axinellae* (*Pa*), b) *Aplysina cavernicola* (*Ac*) and *Hexadella racovitzai* (*Hr*). A specimen of *Holothuria (Panningothuria) forskali* (*Hf*) is also present. c) The sponge community associated with the red coral facies includes *Petrosia ficiformis* (*Pf*), *Haliclona poecillastroides* (*Hpo*) and *A. cavernicola*. d) *Paramuricea clavata* (*Pc*) forest acting as a refuge for *Phycis phycis* (*Pp*) and *Scorpaena* sp. (*S*). e) *Corallium rubrum* (*Cr*) forest pending from the

roof of a crevice, together with several sponges and the yellow scleractinian *Leptopsammia pruvoti* (*Lp*). f) The deepest portion of a rock at the base of the cliff shows a complete cover of sponges, *L. pruvoti* and *Eunicella cavolini* (*Ec*). g) *Eunicella verrucosa* (*Ev*) aggregation on the high silted plateau at the top of the shoal. h) Keratose massive sponges: *Sarcotragus foetidus* (*Sf*) and *Spongia (Spongia) lamella* (*Sl*). i) Calcareous bryozoans: *Smittina cervicornis* (*Sc*) and *Reteporella* spp. (*R*) j) Dense assemblage of the ascidian *Halocynthia papillosa* (*Hpa*). Scale bar: 10 cm.

P. clavata shows an unimodal distribution of the sizes with a peak in the second size class (height 10-20 cm) (Fig. 5b). Colonies taller than 30 cm account for less than 20% of the total population. The size-frequency distribution of *C. rubrum* shows a similar pattern, with the second class (height 5-10 cm) being the most represented (Fig. 5c).

The soft bottoms near the shoal host aggregations of the structuring hydrozoan *Lytocarpia myriophyllum* (Linnaeus, 1758) (with density up to 0.7 colonies m^{-2}). Other megabenthic organisms observed on the muddy bottoms close to the shoal included *Cerianthus membranaceus* (Gmelin, 1791), *Veretillum cynomorium* (Pallas, 1766), *Acromegalomma* sp., *Myxicola infundibulum* (Montagu, 1808), *Sabella pavonina* Savigny, 1822, and *Parastichopus regalis* (Cuvier, 1817).

The fish fauna of the Maledetti Shoal, observed by ROV, includes commercial taxa such as *Conger conger* (Linnaeus, 1758), *Phycis phycis* (Linnaeus, 1766) and *Scorpaena* spp. observed within the coral canopy (Fig. 3d). Other species include *Lappanella fasciata* (Cocco, 1833), *Serranus cabrilla* (Linnaeus, 1758), and schools of *Anthias anthias* (Linnaeus, 1758). One *Scyliorhinus stellaris* (Linnaeus, 1758) and several eggs laid on gorgonian branches were observed in the site.

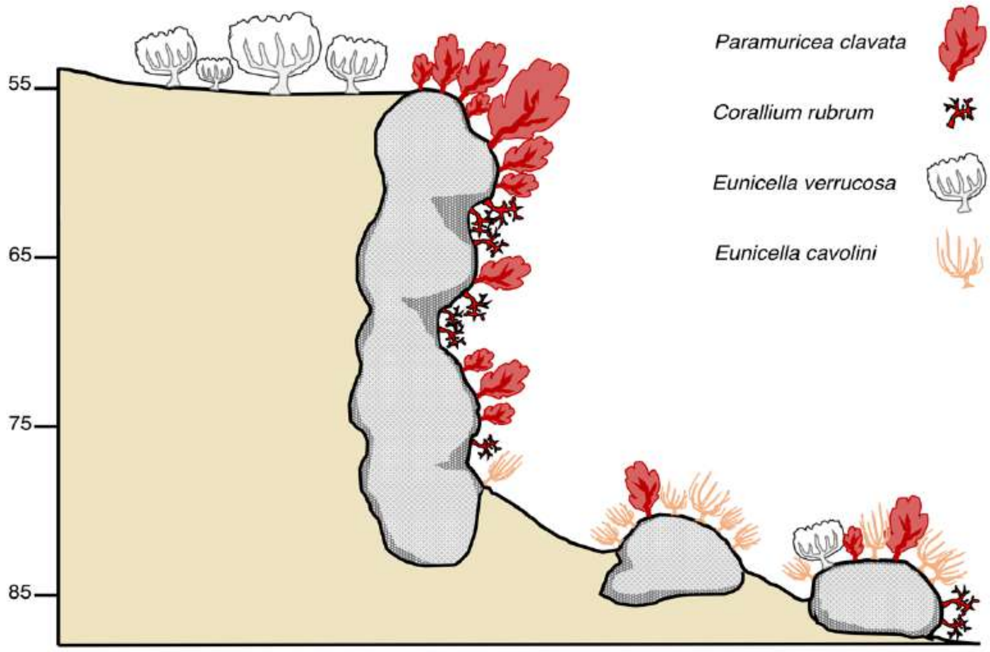

Figure 4. Schematic representation of the benthic zonation on the Maledetti Shoal.

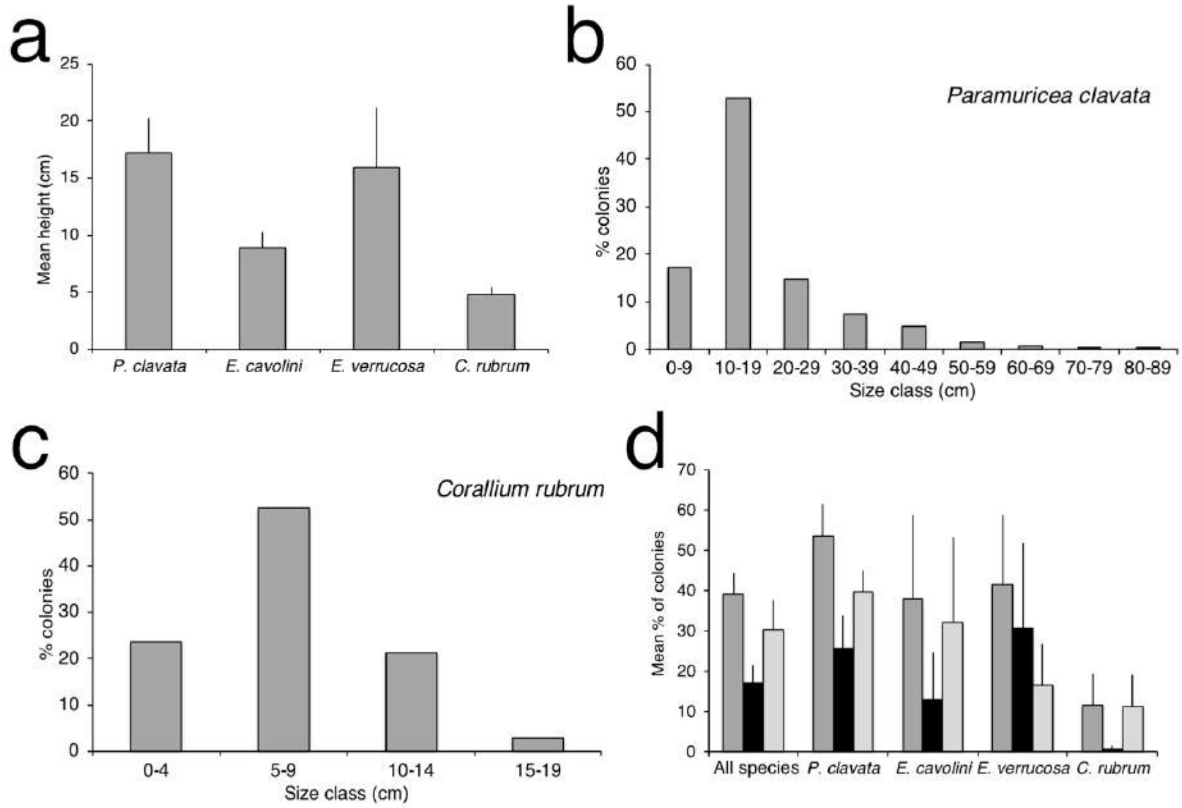

Figure 5. Structuring species on the Maledetti Shoal: a) mean density, b) mean height, c) size distribution of *Paramuricea clavata* and d) *Corallium rubrum*, e) mean percentage of impacted structuring anthozoan colonies, f) mean percentage of structuring anthozoan colonies showing epibiosys or necrosis, g) mean percentage of entangled structuring anthozoan colonies.

3.2 Health status of the benthic community

On average, 40% of the structuring anthozoans observed on the Maledetti Shoal shows signs of impact, with maximum values up to 54% for transect R1, located in the western sector of the vertical wall (Tab. 3). Signs of impact include presence of necrotic portions, overgrowth of epibiotic organisms, and direct entanglements of colonies by fishing gears (Fig. 6a-c). *P. clavata*, *E. cavolini* and *E. verrucosa* show the highest percentage of impacted colonies (from 38% to about 54%), whereas *C. rubrum* the lowest (about 12%) (Fig. 5d) (Kruskal-Wallis, $p<0.05$, $H=8.32$, [PC=EC=EV>CR]). Similarly, the three flexible gorgonians are significantly more often epibionted/necrotic (from 13% to 26% of the colonies) than *C. rubrum* (Tab. 3, Fig. 5d) (Kruskal-Wallis, $p<0.05$, $H=9.41$, [PC=EC=EV>CR]). Entanglement is the most common type of impact, involving on average the 30% of the structuring anthozoans (Tab. 3, Fig. 5d) (Kruskal-Wallis, p=ns, $H=5.61$, [PC=EC=EV=CR]).

The marine litter analysis shows a mean density of 0.5 ± 0.1 items m^{-2} for the entire study area (Tab. 3). Marine litter composition analysis shows a strong predominance of fishing material (98%) (Figs. 2c, 6c-i), with lines and artisanal nets being the most common. Only few objects are ascribable to the category urban litter, including mainly plastic items.

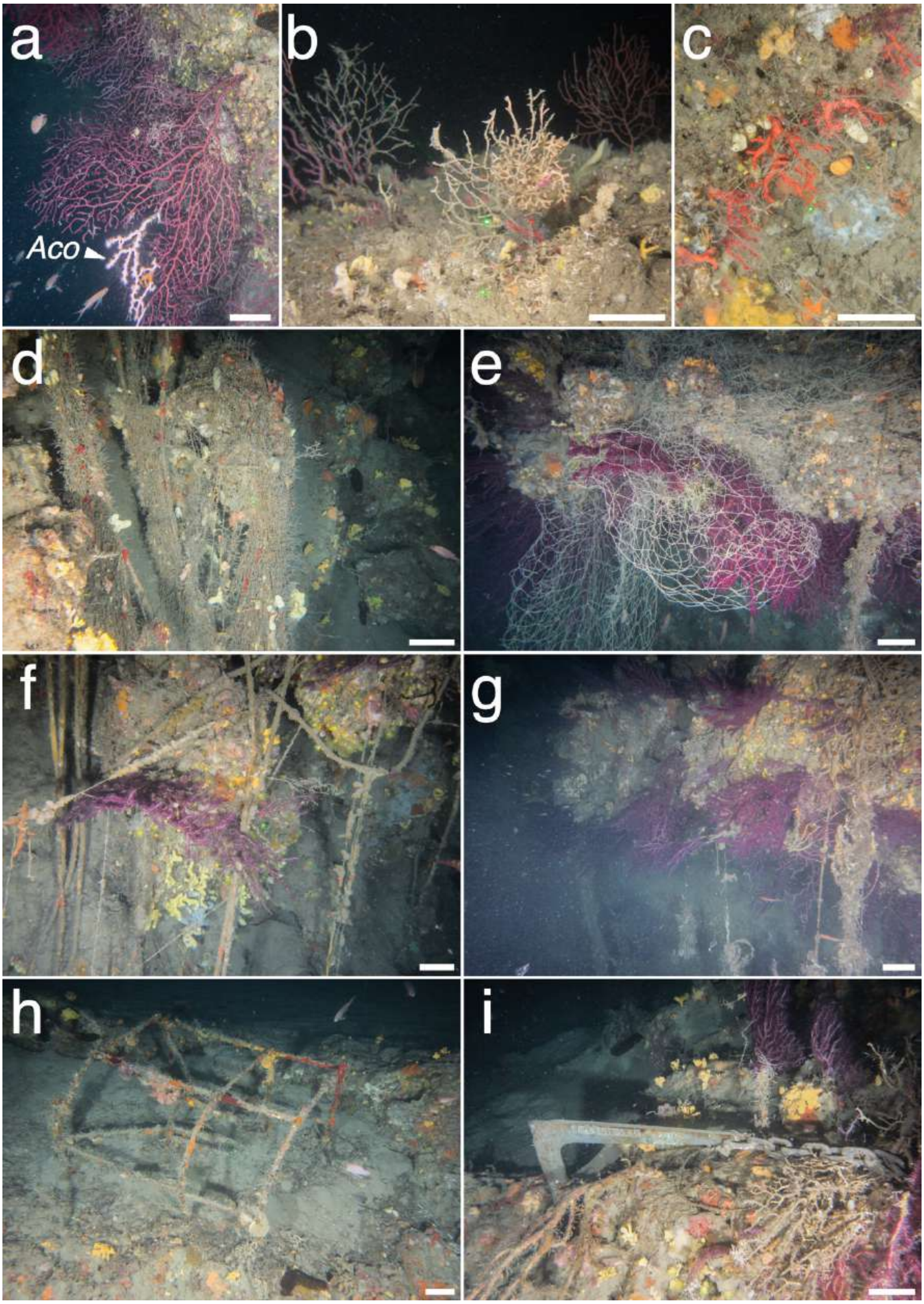

Figure 6. Fishing activities impacts on the Maledetti Shoal. a) *Alcyonium coralloides* (*Aco*) overgrowth on a colony of *Paramuricea clavata*, b) Signs of epibionts overgrowth and necrosis on *Eunicella cavolini* colonies, c) lines entangling colonies of *Corallium rubrum*. d) Highly encrusted lost trammel net on the vertical cliff, e) freshly entangled clean trammel net enveloping *P. clavata* colonies, f-g) numerous ropes and nets fragment entangled on the cliff and gorgonians, h) remains of a fishing pot, i) lost anchor and dead gorgonians. Scale bar: 10 cm.

The application of the MACS Index (Enrichetti et al., 2019) shows an overall moderate Environmental Status (average score 49 ± 2.3) for the Maledetti shoal (Tab. 3).

3.3 Trammel net catches

Results of the fishing-monitoring program (Fig. 7a-u) are summarized in Tab. 4 and SM3, for a total of 104 taxa and 685 records.

The analysis of variance reveals no significant difference between the species composition of the five fishing sets (SM2), which well represent the average content of the fishing catches in the study area.

The composition of the daily catch includes two target crustacean species, with *P. elephas* being more frequent than *H. gammarus* (SM3) and ten commercial bycatch species, with *Zeus faber* Linnaeus, 1758 and *Scorpaena* spp. being the most frequently collected, together with few other high-quality species such as groupers and seabreams (SM3). On the other hand, commercial discard catches include mainly *S. cabrilla*, *Helicolenus dactylopterus* (Delarche, 1809), and *Pagellus acarne* (Risso, 1827) (SM3).

In the non-commercial discard, cnidarians, bryozoans, echinoderms and macroalgae are predominant in terms of abundance (Fig. 2d), while cnidarians, sponges and crustaceans prevail in terms of diversity (SM4). 31 taxa found in the non-commercial discard were also detected by ROV investigation. In total, of all the recorded species, 13 are included in international conventions of protection (SM1, SM3).

Non-commercial discard catch rates for the most abundant species as well as the most representative morphological-taxonomic groups of the Maledetti Shoal's biocoenoses were considered (Fig. 8a, b). Calcareous bryozoans play a major role, showing the highest average catch rate (6.7 ± 2.7 normalized catches) (Fig. 8b), with *Turbicellepora* sp. and *Smittina cervicornis* (Pallas, 1766) being among the most abundant collected species (Fig. 8a). Catch rate refers to colonies or fragments alive when detached from the sea floor, however, bryozoans are frequently collected also as long-time dead fragments, composing the biogenic detritus at the base of the cliff (in the case of *Turbicellepora* sp., about 20% of the total catches).

Flexible gorgonians are the second-most collected group (6.2 ± 1.9 normalized catches) (Fig. 8b). About 30% of the collected specimens is completely necrotic suggesting that, similarly to bryozoans, they were already dead by the time of collection. Of the remaining catches, showing living tissue, 16% were entire colonies and 84% were fragments. Among the collected gorgonians, *E. verrucosa* is the most abundant, with 3.7 ± 2.1 normalized catches (colonies/fragments), followed by *P. clavata* (1.7 ± 0.7). Mean height of entire collected colonies is 18.8 ± 3.8 cm for *E. verrucosa*, and 19.5 ± 4.5 cm for *P. clavata*.

Holothurians are copiously collected by trammel net (6.1 ± 2.7 normalized catches) (Fig. 8b), with the soft-bottom species *Parastichopus regalis* (Cuvier, 1817) among the most abundant catches of all vagile species (3.0 ± 1.1) (SM3).

Hard-skeleton cnidarians are represented by five scleractinians species and by red coral (this latter with alive catches of 0.8 ± 0.6 and colonies 5.2 cm ± 0.2 in height) (SM3, Fig. 8a-b). Overall, about 92% of

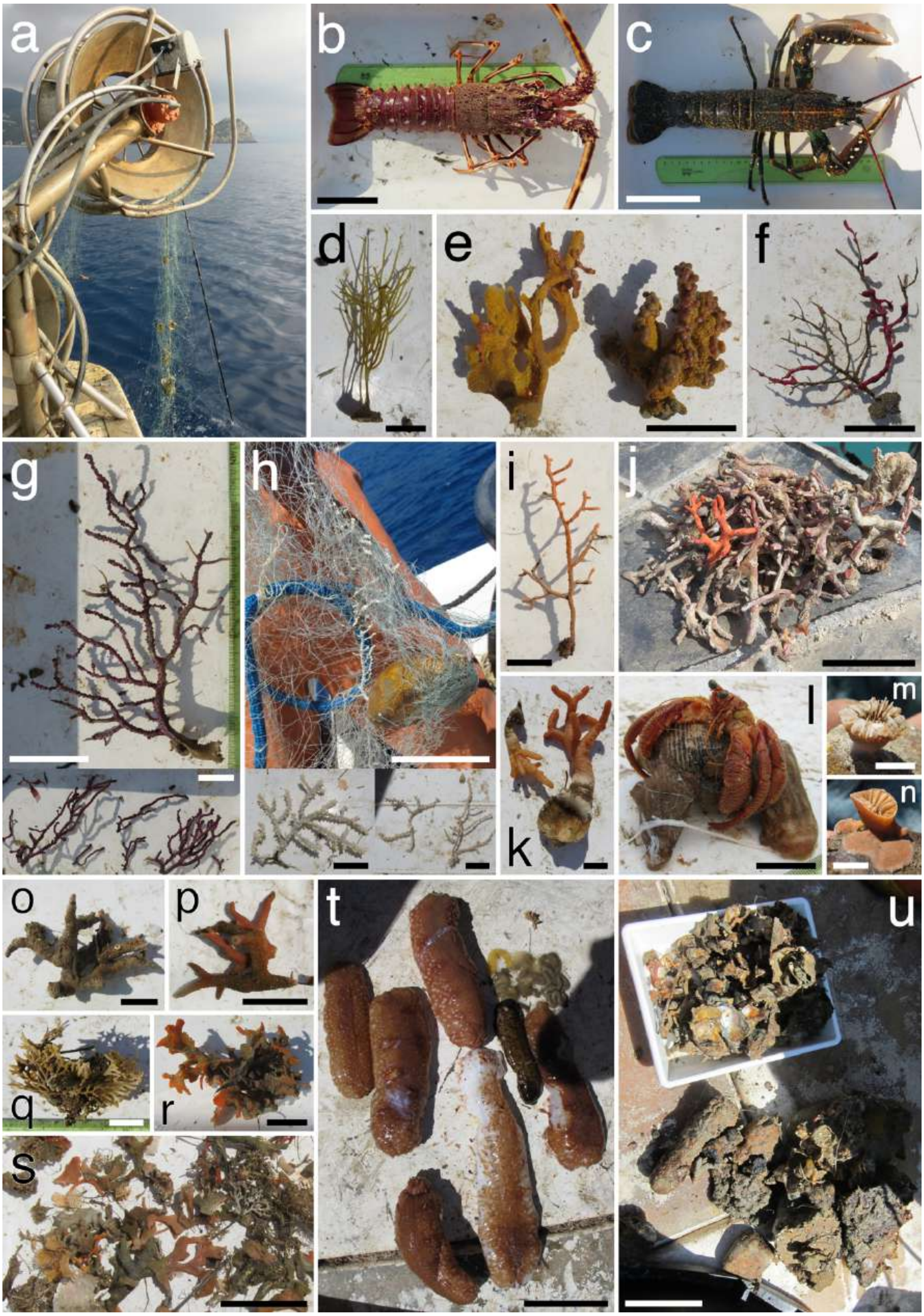

Figure 7. Discard of the trammel net from Maledetti Shoal. a) Hauling of the net. The target species *Palinurus elephas* (b) and *Homarus gammarus* (c). d-u) Non-commercial discard. d) Algae. e) *Axinella* spp. with *Parazoanthus axinellae*. f) Dead gorgonian with *Alcyonium coralloides* overgrowth. g) Colony and fragments (insert) of *Paramuricea clavata*. h) Entire colony of *Eunicella verrucosa* just picked by the net (inserts: *E. verrucosa* fragments). i) Colony of *E. cavolini*. j) Dead and living colonies of red coral. k) *Alcyonium palmatum* colonies. l) *Calliactis parasitica* over a hermit crab. m-n) The scleractinians

Caryophyllia sp. (m) and *Monomyces pygmaea* (n). o-r) Calcareous bryozoans. Dead (o) and living (p) colonies of *Turbicellepora* sp., *Frondipora verrucosa* (q), *Pentapora fascialis* (r). s) mix biogenic detritus with bryozoan fragments and shells. t) Several specimens of *Parastichopus regalis*. q) Substratum collected in one haul. Scale bar: m, n = 1 cm; d, h (inserts), i, k, l, o, p, q, r = 2 cm; e, f, g, g (insert), j, s = 5 cm; b, c, h, t, u = 10 cm.

the collected *C. rubrum* colonies is represented by long-time dead fragments, part of the biogenic detritus. Lastly, among sponges, only seven of the eleven collected OTUs are considered erect or massive, and thus more catchable; hence this sessile group remains overall poorly represented. *Axinella* species are among the most abundantly collected sponges with 1.4 ± 0.2 normalized catches (Fig. 8a).

Excluding long-time dead fragments, benthic invertebrate catches with evident signs of damages due to the mechanical impact of the net (breakages, wounds, …) represents here 30.4% of the overall alive catches.

3.4 Fishing impact

The efficiency and relative impact of the considered métier on this particular shoal can be evaluated considering three quantitative parameters (Fig. 9, Tab. 4).

In terms of diversity, marketable species represent on average 10% of the total number of species caught, while the discard rate is 90% of the taxa (77% attributed to non-commercial benthic OTUs - excluding those present only as biogenic detritus - and 13% to commercial bycatch taxa) (Fig. 9a). Similarly, in terms of abundance, the discard rate is on average 94% of the total number of individuals caught (82% attributed to non-commercial benthic OTUs and 12% to commercial bycatch) (Fig. 9b).

In terms of biomass, on average, one operation in this site brings on-board 16 ± 2.8 kg of material (6.3 ± 1.3 kg normalized for 200 m of net). Marketable catches account for 19% of the total average weight (3.3 ± 1.1 kg) while discard catches account for the 18% (1.1 ± 0.5 kg and 1.5 ± 0.3 kg, respectively for commercial bycatch and non-commercial

ID code	Marketable catches		Non-marketable catches		Amount marketable (kg)	Amount non-marketable (kg)	Revenue per fishing day (€)	Entang. event/ set	Gear breaking event/set	Biogenic detritus (kg)	Substra-tum collected (kg)	Marine litter (items)
	Target	CB	CD	NCD								
tn1	3 (1)	7 (4)	13 (8)	95 (35)	7.2	2.9	392	3	1	0.4	15	2
tn2	4 (1)	6 (4)	0 (0)	75 (34)	3.7	1.5	265	5	1	0.4	11	4
tn3	4 (2)	3 (3)	1 (1)	245 (41)	2.9	2.3	193	2	0	0.9	8	12
tn4	2 (1)	3 (3)	14 (5)	106 (28)	2.8	3.8	139	1	0	0.1	9	7
tn5	0 (0)	2 (2)	37 (9)	65 (19)	0.1	2.5	3	5	1	0.1	5	2
Mean norm. value	0.9 (± 0.3)	1.7 (± 0.4)	6.2 (± 3.4)	40.5 (± 4.5)	1.3 (± 0.5)	1.1 (± 0.3)	198 (± 65) *	n/a	n/a	0.1 (± 0.03)	3.8 (± 0.8)	1.9 (± 0.5)

Table 4. Summary table of the catches expressed as n° of individuals (in brackets n° of species) and other parameters of the fishing sets. Mean values are reported normalized for 200 m of trammel net (tn) with the exception of *. Mean values are reported with standard errors (± SE).

benthic discard) (Fig. 9c). The majority of the weight, though, is attributed to substratum (mainly represented by cobbles, pebbles and coralligenous framework) (Fig. 7u) and biogenic detritus (on average 63%, 10 ± 1.7 kg) (Fig. 9c, Tab. 4). The net, in fact, strongly entangled to the seabed 16 times while in place or during hauling, determining the breakage of the gear in three cases (Tab. 4). The biogenic detritus collected by the trammel net, plausibly at the base of the cliff, is mainly composed of long-time dead fragments of bryozoans, 41% (Fig. 7s), red coral branches, 28% (Fig. 7j), corallites, 20%, gorgonin skeletons, 6%, (Fig. 7f), shells, 5%, and coralline algae, 1%.
From an economic point of view, the average revenue of a fishing operation in the study site is 198 ± 65 €/haul (ranging from basically nothing up to 400 €) (Tab. 4).
Anthropic waste is consistently collected during the operations (1.9 ± 0.5 normalized items). Marine litter shows a predominance of material related to artisanal and recreational fishing (67%), with lines and ropes being prevalent (89%). Old, highly fouled gears are mixed with cleaner, recently lost ones. Recreational hooks and a cephalopod-fishing device represented the remaining fishing material. The urban litter included plastic bags and other plastic material, gloves and fabric.

4. Discussion

The Maledetti Shoal well represents a typical Mediterranean offshore mesophotic biogenic reef ecosystem (*roche du large sensu* Pérès and Picard, 1964) in which structuring species thrive

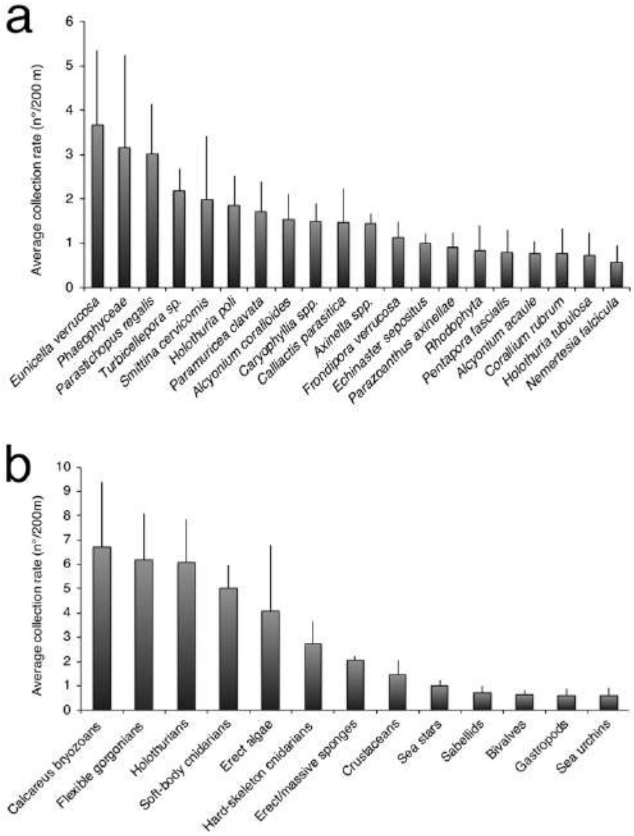

Figure 8. a) Average collection rate (normalized over 200m of net) of the 20 most abundant benthic OTUs of the discard, b) average collection rate (normalized over 200m of net) of the 13 most representative morphological-taxonomic groups of the benthic discard.

on deep continental hard grounds not directly connected to the littoral ones (Bo et al., 2012, 2015; Gori et al., 2017). The studied shoal shows exceptional features within the Ligurian underwater panorama. The site lies in close proximity to the Noli Canyon, source of upwelling deep waters, and its topographic features comprehend multiple inclinations gradients (plateau, cliff, boulders), allowing the co-occurrence of several typologies of animal forests (Fig. 3, 4), thus enhancing the biodiversity levels. The presence of a deep vertical cliff with crevices, in particular, explains the presence of a large population of red coral (MIPAAF, 2013; Cattaneo-Vietti et al., 2016). No such environment is present elsewhere in Liguria, hence this classifies as the most important Ligurian mesophotic population of this species, listed as endangered by the Mediterranean IUCN Red List for anthozoans (Otero et al., 2017). In addition, this area hosts 33 species of high ecological and/or commercial regional relevance, including cnidarians, crustaceans, sharks and marine mammals protected by international conventions (SAC, IT1323271).

Shallow-water populations of gorgonians are known from this area and nearby sites (Parravicini et al., 2007; Betti, 2017) as well as from the Portofino area in the eastern Ligurian Riviera (Bavestrello et al., 1999; Cerrano et al., 2000). Similarly, information is available on the distribution of shallow-water coralligenous assemblages along the Ligurian coast (Cánovas-Molina et al., 2016), but, with few exceptions (Cerrano et al., 2010), a large knowledge gap exists below 40 m depth. In this regard, this study represents the first fine characterization of a deep circalittoral site in this region.

Different proxies of vulnerability of the benthic biocoenoses have been considered in this study, namely i) population structure and health status of the structuring species and ii) damages inflicted by the gear to the megabenthic community through mechanical removal and entanglement. The ROV approach proved to be highly valuable in defining the community structure and the extent of the impact. The discard investigation, on the other hand, proved to be a useful tool in defining the catchability of the species (target and no-target) in this site as well as the punctual efficiency and revenue of the investigated *métier* as highlighted

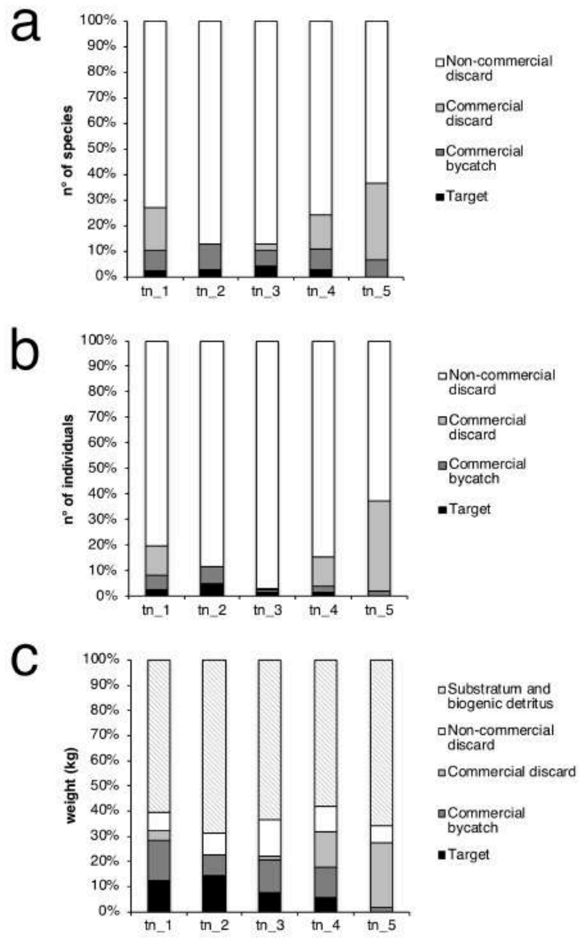

Figure 9. Fishing catch characteristics. Percentage composition of the trammel net catch expressed as n° of species (a), n° of individuals (b) and as weight (kg) (c) for the five analyzed fishing sets.

also in recent studies (Catanese et al., 2018; Gil et al., 2018).

Trammel nets are among the most common artisanal gears in the Mediterranean Sea (Cataudella and Spagnolo, 2011) and are known to be potentially highly impacting (Erzini et al., 1997; Gonçalves et al., 2007; Batista et al., 2009) depending on a wide array of parameters including their technical characteristics, the topography and hydrology of the area, as well as the experience of the fishermen and the weather. The *métier* herein investigated shows specific characteristics that make it particularly problematic in terms of incidence on vulnerable habitats. This net, in fact, is lowered directly on rocky elevations attracting target species thanks to the accumulation of carcasses of previously trapped fishes. The target catches increase with soaking time, which results to be the longest among trammel nets (Erzini et al. 2006; Stergiou et al. 2006; Sartor et al. 2007; Gil et al. 2018), determining abundant discard and a lower survival potential of caught species (Catanese et al., 2018).

Based on the analysis of the catches, this gear collects 9 and 12 times more non-commercial discard, respectively in terms of species diversity and number of organisms, than marketable catches, and a comparable amount in terms of weight, suggesting a high loss at three levels (diversity, abundance and biomass). In addition, this gear collects also a large amount of collected substratum, including coralligenous substrate (currently under EU's Habitats Directive - 1170 Reefs, Bern Convention and European Red List of Marine Habitats) (up to 15 kg per fishing set) (Tab. 4). The removal of large pieces of substratum is an evident modification of the seafloor integrity, one of the most relevant descriptors of the EU Marine Strategy Framework. The occurrence of biogenic detritus (as well as typically soft-bottom species) in the discard, on the other hand, is attributable to the scouring of the net over the bottom at the base of the cliff. Such detritus may result from the natural mortality of organisms rolling down the wall (Bavestrello et al., 1991), however, the high abundance of lost gears on the wall suggests that it is partially attributable also to previous mechanical impacts especially on fragile, carbonatic, erect species, such as bryozoans.

Structuring gorgonian species are highly aggregating (here up to 16 colonies m^{-2}) and are among the most conspicuous elements of deep coralligenous coral forests (Gori et al., 2017), potentially enhancing entanglement of gears (such as lines, hooks, weight) due to their branched, erect canopy and leading to high bycatch rates (up to a maximum of 12 living colonies/fragments per 200 m of trammel net in this study). On the Maledetti Shoal, gorgonians have been collected in 80% of the studied fishing sets, a percentage higher than what obtained by longlines from Mediterranean and Macaronesian areas, reporting gorgonians in 15% to 70% of the landings, respectively (Sampaio et al., 2012; Mytilineou et al., 2014).

The catchability of these species is dependent on various factors including the shape, size, breakability of the skeleton, and exposure of the colonies (Mytilineou et al., 2014; Bo et al., 2014; Kaiser et al., 2018). The high catch rate of *E. verrucosa*, for example, can be explained here by a more exposed habitus (the plateau above the cliff or the rocks at the base of the wall), which makes this species more susceptible to be captured by the net. The lower catch rate of *P. clavata*, instead, could be explained by the fact that on the vertical wall many colonies are already covered by lost gears or are confined to sheltered crevices. A similar situation is evidenced also for red coral: denser patches (250-300 colonies m^{-2}) of taller colonies (10 cm) are found in the cavities and overhangs (MIPAAF, 2013; Cattaneo-Vietti et al., 2016), while exposed patches are commonly formed by scattered, entangled colonies.

Overall, trammel net is selective neither on the target species nor on no-target ones, as demonstrated here and in other studies by the wide array of species in the discard using perfectly legal mesh size (Stergiou et al., 2006). Collection of benthos, in fact, affects not only structuring species, but also organisms thriving in the animal forest, both sessile (sponges, bryozoans) and vagile (echinoderms and crustaceans, many of whom attracted, as the target species, by the rotting material hanging on the net). One third of the collected species arrives onboard evidently damaged; mechanical impacts are likely to be caused during both the scouring of the net on the sea floor and the hauling operations. Some species, have been proven to survive if returned quickly to the sea, including spiny lobsters, the holothurian *P. regalis* (Catanese et al., 2018), and some gorgonians, such as *E. verrucosa*, which is settled on cobbles and is less damaged when eradicated. Despite this, the net is often hauled quickly in order to avoid entanglements and then cleaned in the harbor; in these cases, the catches are either not returned in their original location or dry out, including the more resistant ones determining a reduced survival chance. Small-scale fisheries are widely assumed to have a low impact on the benthic environment (Catanese et al., 2018), but the study herein presented concurs to demonstrate that some artisanal *métier* could be highly disruptive and scarcely sustainable.

Based on the entanglement events and the gear breakages occurred during the surveys, the loss risk frequency in this site is very high, around 60%. This can be explained by the tendency of fishermen to set nets or lines in close proximity to the wall or on the boulders at the wall's base, where the majority of the burrows are. Also, the daylong permanence of gears set in place, enhances the chance for current to move it towards the cliff, hence getting entangled during hauling. Additionally, in some areas of the wall, the dense gears coverage enhances the loss of new material. This density (about 45 items observed every 200 m of seabed explored) is not comparable to any other previously studied site (Bavestrello et al., 2014; Bo et al., 2014; Angiolillo et al., 2015; Yıldız and Karakulak, 2016; Cattaneo-Vietti et al., 2017).

The lack of an historical monitoring makes difficult to reconstruct the exploitation trend of the shoal. The prolonged disturbance is supported by the exceptionally high density of lost fishing gears, many of which heavily encrusted by fouling, suggesting accumulation over time and a long permanence on the sea floor. Additionally, the size structure of the gorgonian population is shifted towards the small-medium class (Kaiser et al., 2018) and the observation and collection of numerous epibionted and necrotic specimens supports a chronic mechanical stress (Bo et al., 2014).

Nowadays the artisanal effort is extremely low (four fishermen each spending 10-30 fishing day per years), but still highly risky, as demonstrated by the presence of abandoned fishing nets almost new, not covered by epibionts. It is plausible that recreational fishing also contributes with new fishing litter, but no characterization is yet possible. Considering the gasoline expenses, the risk of losing the net, the time spent in cleaning the gear from discard and litter, and the average revenue estimated (around € 200 per day), this activity would seem overall unprofitable and this might have contributed to the fishing effort reduction in the last decades.

5. Conclusions

Ultimately, in coastal mesophotic rocky environments, trammel net fishing concurs with other artisanal and recreational demersal gears in degrading the environmental status of benthic communities by: i) persistently altering the sea floor integrity by scouring or collapsing on the sea bed, ii) directly impacting the benthic assemblages by removing sessile and vagile fauna, and, ultimately, iii) re-shaping the three-dimensional coral canopy by progressively reducing its density, height and confining the colonies in protected niches. A reduced canopy influences its functioning, limiting its refugee, foraging and nursery effect (Guidetti, 2009; Rossi et al., 2017).

The multidisciplinary approach employed here allows fulfilling the international guidelines defining the criteria to assess VMEs in the context of mesophotic Mediterranean rocky shoals. GFCM, in fact, suggested five main ecological parameters supporting the definition of a vulnerable area (FAO, 2009): i) Uniqueness or rarity: the biological and topographic features of the Maledetti Shoal are unique in the Ligurian underwater panorama, including a deep coralligenous community dominated by a gorgonian forest of about 350,000 colonies and the largest mesophotic red coral population of the region, ii) Functional significance: the area represents an important site of refuge, foraging, breeding and nursery for commercial and non-commercial species, including chondrichthyes, iii) Fragility: highlighted by the catchability of the structuring species and the modification of the sea floor integrity, iv) Peculiar life-history traits: reflected in the occurrence of slow-growing canopy-forming species as well as deep coralligenous bioconstruction, v) Structural complexity: supported by a large variety of environments, the site hosts a complex three-dimensional forest enhancing high biodiversity levels.

Scientific and socio-economic evidence regarding the fishing fleet and the related activities are fundamental to define or implement specific conservation measures for EU Special Areas of Conservation. In the specific case of the Maledetti Shoal, based on the currently low frequentation levels by artisanal fishermen, on the typology of the fishing activity, on the risk of gears loss, on the accessibility of the area, on the characteristics of the biocoenoses, and on the present environmental status of the coral canopy, the following actions are suggested as complement to the existing measures: i) fulfilment of an environmental recovery program, through the systematic cleaning of the shoal by means of technical divers and ROV and successive disposal of the recovered gears, ii) definition of educative programs directed to the fishermen community (professional and recreational) delineating the importance and fragility of the shoal and nearby rocky areas, iii) definition of a Fisheries Restricted Area or a no-entry no-take zone including an area no less than 50 ha embracing all the environments of the shoal, iv) definition of fishing guidelines, including restricted permissions for professional and recreational fishermen, log books, maximal length of nets and longlines, obligation to return immediately living non-commercial discard within the collection area.

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Supplementary material 1 (next page). Hard-bottom invertebrate OTUs list resulting from the ROV video transects analysis. Aside numbers refer to the total number of individuals observed in the video footages. ° indicates species included in international conventions of protection (Barcelona, Berna, Bonn, Habitat Directive, Red List).

Porifera		*Spongia (Spongia) lamella* (Schulze, 1879) °	7
Acanthella acuta Schmidt, 1862	1	**Cnidaria**	
Agelas oroides (Schmidt, 1864)	272	*Alcyonium acaule* Marion, 1878	1
Aplysina cavernicola (Vacelet, 1959) °	1039	*Alcyonium coralloides* (Pallas, 1766)	153
Axinella spp.	3612	*Caryophyllia* sp.	5
Calyx nicaensis (Risso, 1826)	4	*Corallium rubrum* (Linnaeus, 1758) °	1252
Chondrosia reniformis Nardo, 1847	43	*Eunicella cavolini* (Koch, 1887)	107
Clathrina sp.	4	*Eunicella verrucosa* (Pallas, 1766)	47
Corticium sp.	1	*Leptogorgia sarmentosa* (Esper, 1789)	1
Dysidea sp.	1	*Leptopsammia pruvoti* Lacaze-Duthiers, 1897	20047
Haliclona poecillastroides (Vacelet, 1969)	136	*Paramuricea clavata* (Risso, 1826)	2470
Haliclona sp. 1	42	*Parazoanthus axinellae* (Schmidt, 1862)	252
Haliclona sp. 2	3	*Savalia savaglia* (Bertoloni, 1819) °	1
Haliclona sp. 3	1	Scleractinia sp. 1	27
Haliclona sp. 4	4	Scleractinia sp. 2	11
Hexadella racovitzai Topsent, 1896	125	**Annelida**	
Ircinia variabilis (Schmidt, 1862)	5	*Apomatus/Protula* complex	2
Keratosa sp. 1	25	*Bonellia viridis* Rolando, 1822	8
Keratosa sp. 2	4	*Filograna/Salmacina* complex	142
Keratosa sp. 3	25	*Sabella spallanzanii* (Gmelin, 1791)	5
Keratosa sp. 4	1	Serpulidae	58
Keratosa sp. 5	14	**Mollusca**	
Keratosa sp. 6	1	*Peltodoris atromaculata* Bergh, 1880	1
Keratosa sp. 7	1	**Crustacea**	
Keratosa sp. 8	2	*Galathea* sp.	1
Keratosa sp. 9	2	*Lysmata seticaudata* (Risso, 1816)	5
Oscarella sp.	170	*Munida rugosa* (Fabricius, 1775)	2
Petrosia ficiformis (Poiret, 1789)	646	Paguroidea	1
Pleraplysilla spinifera (Schulze, 1879)	1	*Palinurus elephas* (Fabricius, 1787) °	4
Porifera sp. 1	2	*Plesionika narval* (Fabricius, 1787)	239
Porifera sp. 2	8	**Bryozoa**	
Porifera sp. 3	44	*Adeonella calveti* (Canu & Bassler, 1930)	17
Porifera sp. 4	154	*Frondipora verrucosa* (Lamouroux, 1821)	13
Porifera sp. 5	10	*Pentapora fascialis* (Pallas, 1766)	5
Porifera sp. 6	11	*Reteporella* spp.	127
Porifera sp. 7	2	*Smittina cervicornis* (Pallas, 1766)	622
Porifera sp. 8	2	*Turbicellepora* sp.	131
Porifera sp. 9	1	**Echinodermata**	
Porifera sp.10	7	Asteroidea	6
Porifera sp. 11	4	*Astrospartus mediterraneus* (Risso, 1826)	1
Porifera sp. 12	136	*Centrostephanus longispinus* (Philippi, 1845) °	10
Porifera sp. 13	1	*Chaetaster longipes* (Bruzelius, 1805)	4
Porifera sp. 14	3	Cidaridae	24
Porifera sp. 15	18	*Echinaster (Echinaster) sepositus* (Retzius, 1783)	30
Porifera sp. 16	11	*Echinus melo* Lamarck, 1816	2
Porifera sp. 17	3	*Hacelia attenuata* Gray, 1840	17
Porifera sp. 18	1	*Holothuria (Panningothuria) forskali* Delle Chiaje, 1823	68
Porifera sp. 19	2	*Holothuria (Roweothuria) poli* Delle Chiaje, 1824	50
Porifera sp. 20	12	*Holothuria* sp.	421
Porifera sp. 21	327	*Holothuria (Holothuria) tubulosa* Gmelin, 1791	62
Porifera sp. 22	5	Ophiuroidea	1
Porifera sp. 23	1	**Ascidiacea**	
Porifera sp. 24	3	*Ciona* sp.	2
Sarcotragus foetidus Schmidt, 1862 °	49	*Halocynthia papillosa* (Linnaeus, 1767)	168

Supplementary material 2. Results of the one-way ANOVA tests carried out on the ROV and fishing discard datasets, respectively.

ROV transects dataset	SS	df	MS	F	p(same)
Between groups	5.21169	5	1.04234	1.95	ns
Within groups	317.487	594	0.53449		
Total	322.699	599			

Fishing discard dataset	SS	df	MS	F	p(same)
Between groups	1.12819	4	0.282046	0.83	ns
Within groups	168.447	495	0.340298		
Total	169.576	499			

Supplementary material 3. Catches list, in taxonomic order, resulting from the fishing monitoring. Aside numbers refer to the total number of individuals collected. * indicates species of the benthic discard only collected as a fragment, or ** both entire and fragments. Bold values indicate those species found also in the ROV benthic characterization. ° indicates species included in international conventions of protection (Barcelona, Berna, Bonn, Habitat Directive, Red List).

Targets	
Homarus gammarus (Linnaeus, 1758) °	4
***Palinurus elephas* (Fabricius, 1787) °**	**9**
Commercial bycatch	
Epinephelus marginatus (Lowe, 1834) °	1
Lophius budegassa Spinola, 1807	1
Pagellus acarne (Risso, 1827)	2
Phycis phycis (Linnaeus, 1766)	2
Scomber scombrus Linnaeus, 1758	1
Scorpaena elongata Cadenat, 1943	1
Scorpaena porcus Linnaeus, 1758	1
Scorpaena scrofa Linnaeus, 1758	2
Scorpaena sp.	4
Zeus faber Linnaeus, 1758	6
Commercial discard	
Boops boops (Linnaeus, 1758)	1
Citharus linguatula (Linnaeus, 1758)	1
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)	1
Engraulis encrasicolus (Linnaeus, 1758)	26
Helicolenus dactylopterus (Delaroche, 1809)	5
Merluccius merluccius (Linnaeus, 1758)	3
Mullus surmuletus Linnaeus, 1758	2
Pagellus acarne (Risso, 1827)	7
Phycis phycis (Linnaeus, 1766)	4
Scomber colias Gmelin, 1789	1
Scorpaena notata Rafinesque, 1810	1
Scyliorhinus canicula (Linnaeus, 1758)	2
Serranus cabrilla (Linnaeus, 1758)	7
Synapturichthys kleinii (Risso, 1827)	1
Todarodes sagittatus (Lamarck, 1798)	1
Trachinus draco Linnaeus, 1758	1
Zeus faber Linnaeus, 1758	1
Non-commercial discard	
Phaeophyceae Kjellman, 1891	**67**
Rhodophyta Wettstein, 1901	**23****
Chlorophyta Pascher, 1914	**2**
***Aplysina cavernicola* (Vacelet, 1959) °**	**2**
Axinella polypoides Schmidt, 1862°	1
***Axinella* spp.**	**19**
Bubaris sp.	3
***Dysidea* sp.**	**2**
***Haliclona* sp.**	**3**
Hymedesmia sp.	2
***Pleraplysilla spinifera* (Schulze, 1879)**	**1**
Scalarispongia scalaris (Schmidt, 1862)	6**
Suberites sp.	1
Sycon sp.	1
Actiniaria	1
***Alcyonium acaule* Marion, 1878**	**11**
***Alcyonium coralloides* (Pallas, 1766)**	**19**
Alcyonium palmatum Pallas, 1766	2
Calliactis parasitica (Couch, 1842)	17
***Caryophyllia* sp.**	**89****
Cladocora caespitosa (Linnaeus, 1767) °	3**
***Corallium rubrum* (Linnaeus, 1758) °**	**116****
Dendrophyllia cornigera (Lamarck, 1816) °	5*
Epizoanthus sp.	2
Eudendrium sp.	7**
***Eunicella cavolini* (Koch, 1887)**	**1**
***Eunicella verrucosa* (Pallas, 1766)**	**67****
***Leptopsammia pruvoti* Lacaze-Duthiers, 1897**	**3**
Hoplangia durotrix Gosse, 1860	4*
Monomyces pygmaea (Risso, 1826)	4**
Nemertesia falcicula Ramil & Vervoort, 1992	8
Nemertesia sp.	5
Paracyathus pulchellus (Philippi, 1842)	1
***Paramuricea clavata* (Risso, 1826)**	**20**
***Parazoanthus axinellae* (Schmidt, 1862)**	**12**
Phyllangia americana mouchezii M-E and H, 1849	1*
***Apomatus/Protula* complex**	**2**
***Filograna/Salmacina* complex**	**6****
Pontogenia chrysocoma (Baird, 1865)	1
Sabellidae	2
Aporrhais pespelecani Linnaeus, 1758	4*
Bolinus brandaris (Linnaeus, 1758)	9**
Galeodea echinophora (Linnaeus, 1758)	1
Neopycnodonte cochlear (Poli, 1795)	17**
Pecten jacobaeus Linnaeus, 1758	3*
Pteria hirundo (Linnaeus, 1758)	3
Trivia multilirata (G. B. Sowerby II, 1870)	1
Tethys fimbria Linnaeus, 1758	1*
Calappa granulata (Linnaeus, 1758)	6**
Dardanus arrosor (Herbst, 1796)	6
Dromia personata (Linnaeus, 1758)	3
Homola barbata (Fabricius, 1793)	1
Maja squinado (Herbst, 1788)°	3
Medorippe lanata (Linnaeus, 1767)	3
Neomaja goltziana (d'Oliveira, 1889)	1
Paguroidea	2
Parapenaeus longirostris (Lucas, 1847)	1*
Spinolambrus macrochelos (Herbst, 1790)	1
Scyllarus arctus (Linnaeus, 1758) °	1
Megerlia truncata (Linnaeus, 1767)	2
***Adeonella calveti* (Canu & Bassler, 1930)**	**4**
Cellaria salicornioides Lamouroux, 1816	1
***Frondipora verrucosa* (Lamouroux, 1821)**	**31****
Myriapora truncata (Pallas, 1766)	2**
***Pentapora fascialis* (Pallas, 1766)**	**24***
***Reteporella* spp.**	**9****
Schizomavella sp.	7**
***Smittina cervicornis* (Pallas, 1766)**	**49****
***Turbicellepora* sp.**	**165****
Antedon mediterranea (Lamarck, 1816)	3
Cidaridae	**8**
***Echinaster (Echinaster) sepositus* (Retzius, 1783)**	**13**
***Holothuria (Panningothuria) forskali* Delle Chiaje, 1823**	**5**
***Holothuria (Roweothuria) poli* Delle Chiaje, 1824**	**23**
***Holothuria (Holothuria) tubulosa* Gmelin, 1791**	**10**
Parastichopus regalis (Cuvier, 1817)	38**
Aplidium sp.	1
***Ciona* sp.**	**1**

Chapter 3

Placogorgia coronata first documented record in Italian waters: Use of trawl bycatch to unveil vulnerable deep-sea ecosystems

Abstract

Despite the pluri-decennial history of bottom trawling monitoring in the Italian Sea, limited attention has been given to the study of cold-water corals (CWCs) present in the bycatch. As a result, trawl samples still hold a great potential to reveal information about deep-water biodiversity. The rare Mediterranean CWC species *Placogorgia coronata* was repeatedly found in the bottom trawl discards of the red shrimp fishery in Santa Margherita Ligure (Ligurian Sea). The supposed rarity of this species is strongly biased by taxonomic problems within the genus as well as by the limited exploration of these deep habitats. Scientific literature as well as remotely operated vehicle surveys highlighted the presence, in the investigated trawled area, of a dead white coral framework, supporting the hypothesis that the CWC studied, probably thriving on coral rubble, occurs here due to the natural protection to trawling offered by the coral matrix. Trawlers, however, may accidentally affect these secondary hard grounds, as demonstrated by the discard content. The long-term monitoring of the catches highlighted a removal rate of up to one colony every two trawling operations; based on the anecdotal fishing effort in this area and the status of the discarded specimens, it is possible to estimate an average catch rate of about 18 live colonies per year per fisherman.

The occurrence of rare CWC species with low resilience to fishing disturbance is among the required information for the identification of fisheries restricted zones designed to protect deep vulnerable marine ecosystems.

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1. Introduction

The term cold water corals (CWCs) refers to a taxonomically heterogeneous group of azooxanthellate cnidarians, found below 200 m, acting as structuring species (Freiwald et al., 2004; Freiwald and Roberts, 2005; Roberts et al., 2006; Chimienti et al., in press), and thus enhancing the three-dimensional complexity of the sea bed (Buhl-Mortensen et al., 2010).

Mediterranean CWCs show peaks of diversity and abundance between 200 and 800 m (Chimienti et al., in press; Gori et al., in press). They broadly comprise about 30 species thriving between the continental shelf break and the bathyal slope both on hard grounds and soft bottoms, such as compact mud, sand, and biogenic rubble. About half of the species are CWCs *sensu strictu*, being the principal structuring components of complex deep benthic ecosystems, namely white coral facies (*e.g.* Tursi et al., 2004; Taviani et al., 2005; Freiwald et al., 2009; Orejas et al., 2009; Fabri et al., 2014; Fanelli et al., 2017;Chimienti et al., in press;) as well as forests of other arborescent anthozoans dominated by large gorgonians and antipatharians (*e.g.* Pardo et al., 2011; Giusti et al., 2012; Aguilar et al., 2013; Cartes et al., 2013; Bo et al., 2014, 2015; Fabri et al., 2014; Gori et al., 2017;). The remaining CWC species, mainly including gorgonians, soft corals, and pennatulaceans, are also known to thrive below 200 m; however, their role as potential habitat formers is still not well known due to their generally small size and limited field observations (Hebbeln et al., 2009; Mastrototaro et al., 2010; Freiwald et al., 2011; Chimienti et al., in press).

Among the various techniques employed to study the diversity and distribution of CWCs, remote operated vehicle (ROV) imaging is certainly the most widespread, while experimental bottom trawling is considered too destructive and largely targeted at soft bottoms. Large-scale trawling monitoring programs (*e.g.* MEDITS, GRUND, and CAMPBIOL for the Mediterranean basin) and other scientific studies are usually focused on target catches, and relatively limited data have been produced on structuring cnidarians (Relini et al., 1986; Gili et al., 1987, 1989; Massutí and Reñones, 2005; Schembri et al., 2007; Petović et al., 2016; Terribile et al., 2016). Nevertheless, fishing trawls do have an advantage, as they sample large areas of sea bed, and hence a wide variety of environments. The taxonomic investigation of the invertebrate discards from trawls, therefore, is a potentially rich source of biological information that would otherwise be wasted (Relini, 2007).

A good example of scientific use of the trawl discard material comes from the Ligurian Sea. This basin accounts, at present, for 10 main deep-trawling fishing grounds extending below 200 m (Relini, 2007). The fishing ground facing the Portofino Promontory (eastern Ligurian Sea) is considered among the longest exploited (having been actively trawled since the 1960s–1970s) and also among the best characterized in terms of deep benthic invertebrate biocoenoses (Brian, 1931; Issel, 1931, 1932; Rossi, 1958; Relini-Orsi and Relini, 1972a, 1972b; Relini-Orsi, 1974; Tunesi and Peirano, 1985; Relini et al., 1986; Relini et al., 2011). Various deep cnidarian species have been reported for this site between 200 and 700 m: the alcyonaceans *Isidella* *elongata* (Esper, 1788), *Paramuricea macrospina* (Koch, 1882), *Villogorgia bebrycoides* (Koch, 1887), *Bebryce mollis* Philippi, 1842, *Alcyonium palmatum* Pallas, 1766, the pennatulaceans *Funiculina quadrangularis* (Pallas, 1766), *Pennatula* spp., *Kophobelemnon stelliferum* (Müller, 1776), *Virgularia mirabilis* (Müller, 1776), and the hydrozoans *Nemertesia antennina* (Linnaeus, 1758), and *Lytocarpia myriophyllum* (Linnaeus, 1758) (Relini et al., 1986; Rossi, 1958). This area is also known to host dead white coral frameworks, generally avoided by the trawling operations except when accidentally or marginally hit, as demonstrated by the numerous available samples (Fusco, 1968; Rossi, 1958).

The possibility to analyze the discard content obtained from the fishing grounds of the Santa Margherita Ligure deep haul resulted in the description of the occurrence of a rare CWC species never reported before for Italian waters. The taxonomic position of the recorded *Placogorgia* species is discussed together with a characterization of its putative habitat carried out by means of ROV exploration. The conservation implications of this discovery are also presented taking into account the fishing effort in this site and the collection rate of this charismatic species.

2. Material and Methods

2.1 Study area

Trawling fishing grounds located along the eastern Ligurian Sea extend over 3,000 km^2, resulting in intense and profitable activities since the 1930s (Relini-Orsi et al., 2006; Relini, 2007). Major differences in the catches have been observed among epibathyal (250–450 m) and mesobathyal (450–700 m) depth ranges, with the former dominated by the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) and the latter targeting the red shrimp *Aristeus antennatus* (Risso, 1816) (Relini-Orsi and Relini, 1972b; Relini, 2007; Cattaneo-Vietti et al., 2010).

The study area, known as “Canyon di Terra le Rame” (literally referring to a fishing ground shore-side to a coral area) runs parallel to the coast for about 9 nm between the town of Sestri Levante and the Portofino Promontory (Relini, 2007) (Fig. 1). The sea bottom is mainly steep sloping compact mud (Fusco, 1968; Morri et al., 1986), with an irregular bathymetric profile culminating in the deepest portion of the Entella Canyon at the easternmost end of the fishing ground (Fig. 1).

Two major white coral frameworks (F1 and F2, covering about 9 km^2) have been historically reported along the southern border of the most exploited haul tracks, around 600–750 m, based on the information gathered from fishing discards (Rossi, 1958; Fusco, 1968) (Fig. 1). Rossi (1958) described these deep banks as mainly thanatocoenosis of *Lophelia pertusa* (Linnaeus, 1758) and *Desmophyllum dianthus* (Esper, 1794) covered in iron-magnesium oxide (Relini-Orsi, 1974). Additionally, patchy facies of living *Madrepora oculata* Linnaeus, 1758 were reported in shallower depths (Rossi, 1958; Morri et al., 1986), as later confirmed by direct observations carried out between 200 and 550 m depth along the outer shelf edge and the flanks of the Entella Canyon (Tunesi and Diviacco, 1997) (Fig. 1).

Nowadays, about 20 vessels are registered in the Santa Margherita Ligure

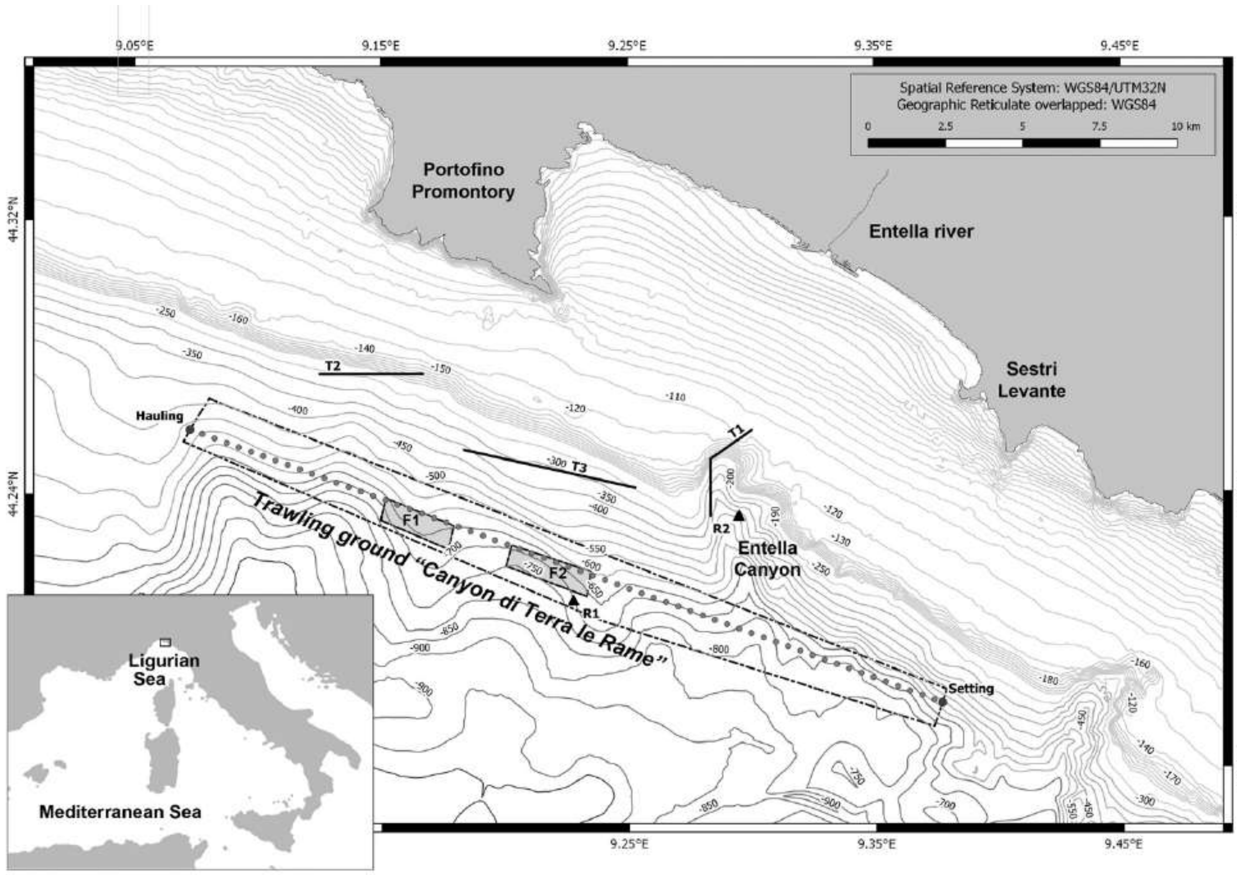

Figure 1. Map of the study area. The dotted–dashed box indicates the area vulnerable to trawling activities; nowadays, the principal trawl track is represented by the dotted line carried out to a maximum depth of 650 m. The grey boxes refer to the two coral areas reported by Fusco in 1968 (F1, F2). Black lines labelled T1, T2, and T3 refer to the dives carried out on board of submersible Cyana by Tunesi and Diviacco (1997). Living colonies of *Madrepora oculata* have been reported from T1 (516 m) and T2 (212–213 m). Black triangles labelled R1 and R2 refer to the ROV dives (initial coordinates) carried out during this study.

harbor, and 12 work here between 400 and 750 m. Anecdotal information from fishermen suggests that, of these 12 vessels, seven are occasional in this site, while five can be considered frequent, each carrying out 60–120 hauls per year.

2.2 Samples collection and analysis

During summer 2016 and 2017, 21 trawling operations, carried out onboard the M/P *Pinetto Giacomo*, were analyzed within a program focused on monitoring the non-commercial invertebrate catches coming from the red shrimp fishing ground of Santa Margherita Ligure, between 430 and 744 m depth (Tab. 1). Trawling operations are normally carried out tangentially to the coral areas identified on the Fusco (1968) map (Fig. 1).

All the collected gorgonian colonies were preserved for subsequent taxonomic identification. Skeletal architecture and polyps' arrangements were first examined under a stereomicroscope. Small branches and dissociated sclerites (obtained after hydrogen peroxide rinses) were then mounted on stubs covered with a carbon layer and sputtered with a 2 μm thick layer of gold–palladium.

Fishing operations	Latitude	Longitude	Depth (m)	Date	Tot time (h)	N°colonies
Set1	44.17881 N	09.37688 E	584	19.07.2016	7	1 alive
Haul1	44.25882 N	09.07234 E	560			
Set2	44.17676 N	09.37890 E	602	26.07.2016	7	2 dead
Haul2	44.25623 N	09.08639 E	610			
Set3	44.15183 N	09.41667 E	670	05.05.2017	7	2 dead
Haul3	44.26077 N	09.06383 E	475			
Set4	44.20965 N	09.26666 E	744	12.05.2017	3	0
Haul4	44.25933 N	09.05000 E	469			
Set5	44.21488 N	09.26982 E	716	10.08.2017	3	0
Haul5	44.25625 N	09.08192 E	552			
Set6	44.17928 N	09.37523 E	590	10.08.2017	7	1 dead
Haul6	44.25915 N	09.05677 E	430			
Continuous monitoring of the catches						
Set7			470-740	21.08.2017	7	0
Set8			470-740	22.08.2017	7	2D
Set9			470-740	23.08.2017	7	0
Set10			470-740	24.08.2017	7	0
Set11			470-740	25.08.2017	7	1D,1A
Set12			470-740	28.08.2017	7	1A
Set13			470-740	29.08.2017	7	1D, 1A
Set14			470-740	30.08.2017	7	0
Set15			470-740	31.08.2017	7	0
Set16			470-740	04.08.2017	7	0
Set17			470-740	05.08.2017	7	0
Set18			470-740	06.08.2017	7	0
Set19			470-740	07.08.2017	7	0
Set20			470-740	08.08.2017	7	0
Set21			470-740	11.08.2017	7	0
ROV dives	**Latitude**	**Longitude**	**Depth (m)**	**Date**	**Time**	**Tot time (h)**
R1 start	44.20900 N	09.22767 E	773	15.06.2017	11:05	2:00
R1 end	44.21000 N	9.226333 E	737	15.06.2017	13:05	
R2 start	44.23350 N	9.294833 E	465	15.06.2017	16:42	1:03
R2 end	44.23383 N	9.296667 E	425	15.06.2017	17:45	

Table 1 Setting and hauling information of the fishing operations with indication of the number and status (D: dead; A: alive) of sampled colonies. The lower part of the table gives a summary of the technical data of the ROV dives with indication of duration.

Arrangement, morphology, size, and ornamentation of sclerites were observed with a Vega 3_TESCAN microscope type LMU. Dead colonies were identified thanks to the presence of patches of tissue still hosting sclerites. The first six trawling operations, randomly monitored from the July 19, 2016, to August 10, 2017, were exclusively dedicated to the collection and identification of the gorgonian colonies. In order to estimate the removal rate of the colonies, 15 additional trawling operations, carried out over 15 consecutive working days, were monitored (from August 21 to September 11, 2017) (Tab. 1).

2.3 ROV exploration

In order to describe the white coral frameworks close to the fishing ground, representing a source of hard substrate, two exploratory ROV dives were conducted in summer 2017 on board the catamaran *Daedalus* with the ROV Multipluto (Gaymarine). The ROV was equipped with two strobe lights, a high-definition video camera (Sony RX100 II), a three-jaw grabber mounted on a manipulative arm, a depth sensor, a compass, and an acoustic tracking position system (USBL HDR Gaymarine).

A side-scan sonar (SSS, Gaymarine), operating at 50 kHz frequency, 2° × 60° beam width and maximum range 1500 m, was used to identify the occurrence of hard grounds in the coral areas identified by Fusco (1968) (F1, F2) as well as along the Entella Canyon. Three hours of video were then obtained in two areas adjacent to the trawl track. One track (R1) was carried out within Fusco's easternmost area (F2), while the second track (R2) was carried out in the deepest part of the Entella Canyon (Fig. 1).

3. Results

3.1 Morphological analysis of the Ligurian samples

Thirteen colonies were collected during the whole survey period. The best-preserved specimen, used as study reference, was 10 cm tall and 13 cm wide (Fig. 2a). The fan-shaped colony was irregularly branched with slender, thin branches (Fig. 2b), usually slightly thicker at the tips (Fig. 2c) and developing in the same plane. Anastomoses between branches were not observed. The stem had a basal diameter of 2 mm. The dry colony was greyish golden-brown (Fig. 2a) and this color was confirmed also by the analysis of the other live specimens. Basal plates were only collected for one specimen. All specimens analyzed shared a similar branching pattern for an average size (plus/minus standard error) of 13.9 ± 0.8 cm × 13.9 ± 1.0 cm.

The polyps were small, less than 1.5 mm high, and, depending on their contraction state, were either blunt when the operculum was withdrawn (Fig. 2d, f, g) or conical when the operculum was expanded (Fig. 2e, h). The polyps were well spaced, generally arranged on the lateral sides of the branches (Fig. 2b) or on the tips, where they were more crowded and irregularly distributed.

A schematic representation of the arrangement of the different types of sclerites is given in Fig. 3. The polyp wall was surrounded by large thornscales, which formed the calyx (Fig. 4a, b, c). Each thornscale showed a large apical thorn and a flat plate-like basal part with several downward root-like projections. A gradient of development complexity was visible in the thornscales of the calyx, with the apical thornscales smaller and slender with a more pronounced apical thorn, while the others showed progressively shorter thorns and larger and more branched basal plates (Fig. 3d-f, 4a-c). Thornscales measured from 0.7–0.9 mm high at the apex to 0.7–1.2 mm high at the base of the calyx.

The anthocodium showed a heavy armature of sclerites. Five or six rings of slender and curved sclerites (0.9–1.1 mm high) formed the collaret at the base of the anthocodium (Fig. 3c, 4d). The rods at the tentacle bases (0.5–0.9 mm high) formed the operculum (Fig. 3b, 4e), and other small sclerites

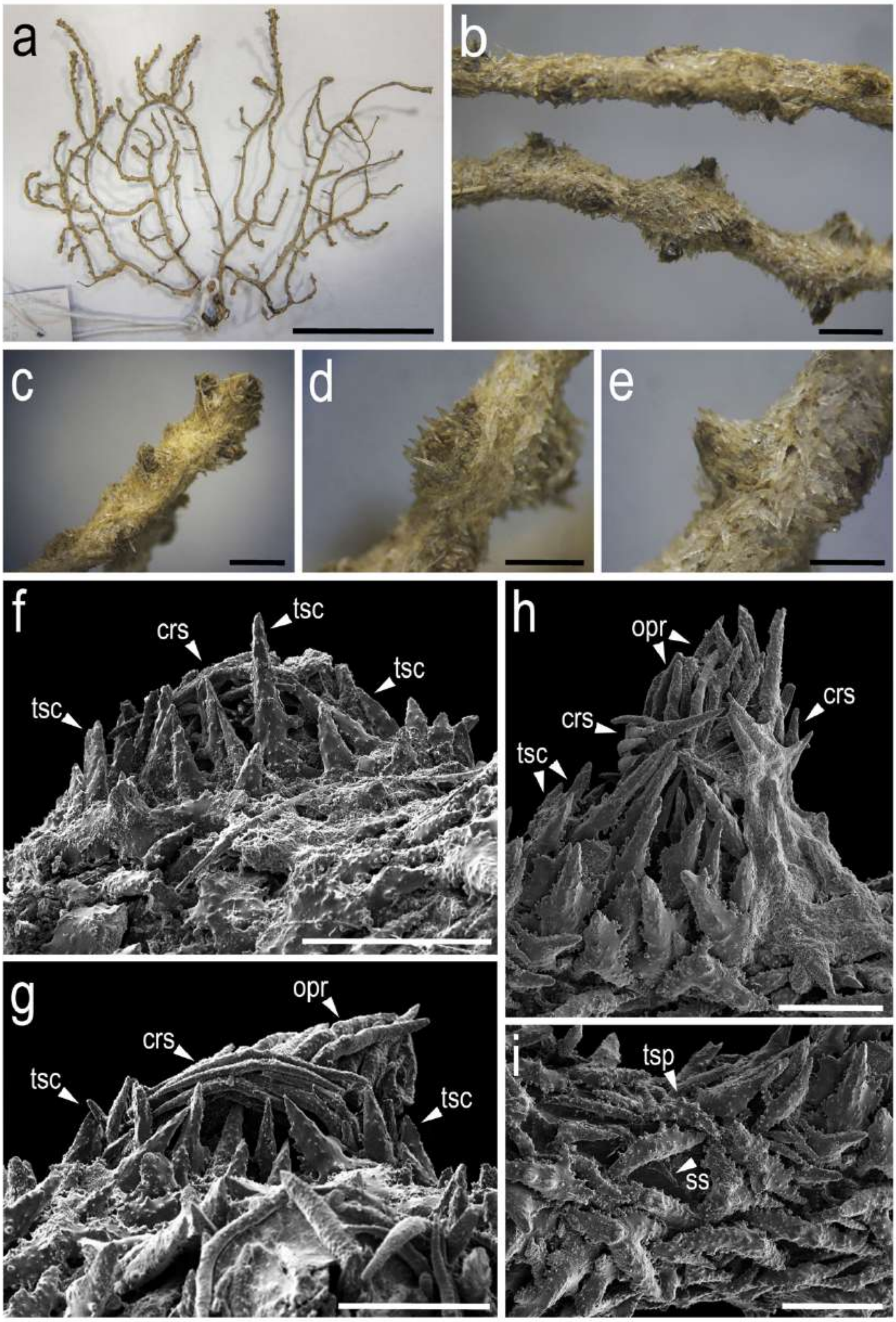

Figure 2. Morphological features of the best-preserved Ligurian specimen. (a) Dried colony. (b) Close-up of the branches showing the arrangement of the polyps. (c) Distal portion of a branch. (d, e) Two different polyps' morphotypes: a blunt one (d) and a conical one (e). (f) Lateral view of a blunt polyp at high magnification. Distribution of the sclerites (tsc, thornscales; crs, collaret rings sclerites) of the calyx wall is evident. (g) Collaret sclerites and operculum rods (opr) of the anthocodium are noticeable. (h) Arrangement of the sclerites in a conical polyp. (i) Close-up view of the coenenchyme sclerites. Some small simple sclerites (ss) of the inner layer are visible under the large thornspindles (tsp). Scale bars: (a) 5 cm; (b–e) 1 mm; (f–i) 0.5 mm.

(0.2–0.3 mm high) were present in the tentacles (Fig. 3a, 4f).
The coenenchyme had a dense layer of sclerites, which conferred a spiny aspect to its surface (Fig. 2b-e). These sclerites were arranged in two layers: the inner one contained simple or branched spindles (0.3-0.6 mm high) (Fig. 2i, 3h, 4g), while the outer layer contained thornspindles (Fig. 2i, 3g, 4h). These sclerites consist of a flat, enlarged, spindle-like basal part with root-like projections and one or rarely two thorns. In contrast to the thornscales, the thornspindles were flat to adapt to the calyx wall, much shorter (0.4–0.7 mm high), and more three-dimensional, due to the position of the thorn. The outer layer did not contain thornstars, which are an extremely modified type of thornspindle, made by three or more planar projections and a spine arising orthogonally (Carpine and Grasshoff, 1975).
All the sclerites (especially the apical spine of the thornscales) showed several tuberculate or spiny processes on their surface conferring respectively, a laciniate or echinulate aspect (Fig. 4).

3.2 Taxonomic and ecological remarks
The assignment to the genus *Placogorgia* Wright & Studer, 1889 poses various difficulties due to the critical distinction between this taxon and the other similar plexaurid genera, namely *Paramuricea* Koelliker, 1865 and *Echinomuricea* Verrill, 1869. These three genera share a particularly complex history of synonymies and poor descriptions, often based on a small number of specimens, that only an accurate morphological and molecular revision might solve. This situation is particularly exemplified by the description of the type species of the genus *Placogorgia*, *Placogorgia atlantica* Wright & Studer, 1889, lacking the main

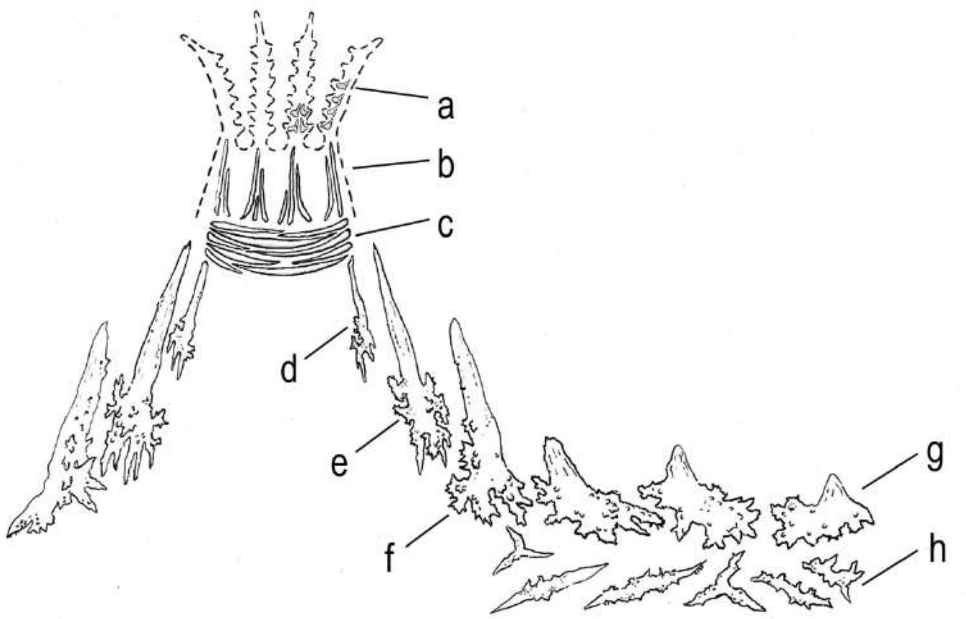

Figure 3. Schematic representation of the diversity and arrangement of sclerites' types in the Ligurian *Placogorgia coronata* (adapted from Grasshoff, 1977). (a–c) The anthocodium shows three different typologies of sclerites: the small sclerites of the tentacles (a), the opercular rods (b), and the spindles forming the collaret rings (c). (d–f) The calyx wall presents thornscales, whose thorns and basal plates differ gradually between apical (d), median (e), and basal sclerites (f). (g, h) The coenenchyme is organized in two layers. The outer layer presents thornspindles (g). The inner layer hosts simple and branched sclerites (h).

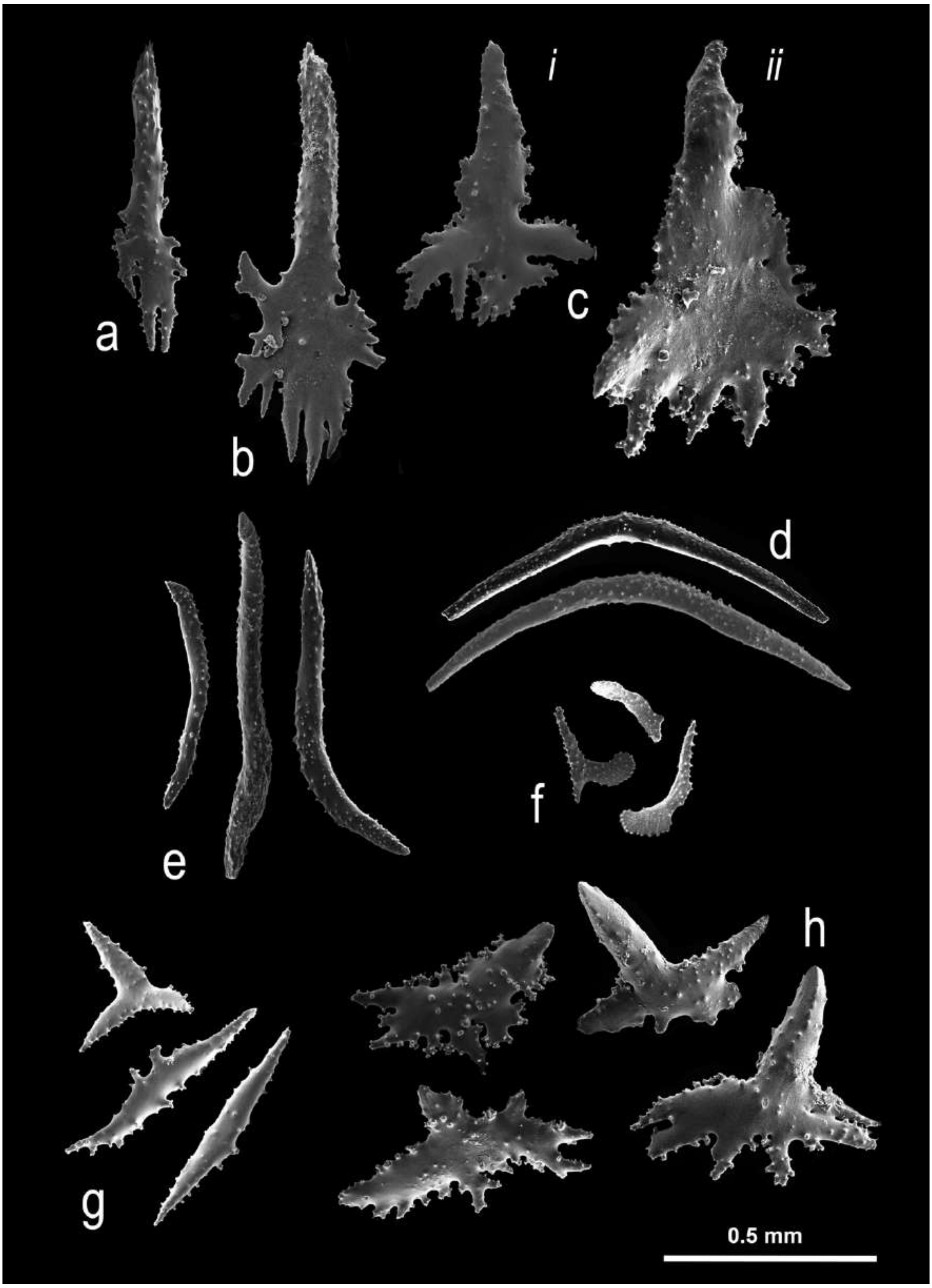

Figure 4. Sclerites set of the Ligurian *Placogorgia coronata*. (a–c) Thornscales from the calyx wall: (a) apical; (b) median; (c) basal, with i and ii being two different morphotypes of basal thornscales. (d–f) Sclerites from the anthocodium: (d) collaret sclerites; (e) operculum rods; (f) small sclerites from the tentacles. (g, h) Coenenchyme sclerites: (g) simple and branched spindles of the inner layer; (h) thornspindles of the surface layer.

character that Bayer (1959), Carpine and Grasshoff (1975), and Grasshoff (1977) considered crucial in distinguishing the genus *Placogorgia* from the genus *Paramuricea* (*i.e.* the presence of thornspindles in the outer layer of the coenenchyme).

At present, the genus *Placogorgia* accounts for about 30 species, showing a worldwide distribution, with 18 Indo-Pacific species and 12 Atlantic ones (Tab. 2 and references herein). Of these latter, six species inhabit exclusively the Caribbean area at depths ranging from 50 to 480 m, while the other six have been described from the eastern Atlantic–Mediterranean region at depths ranging from 170 to 2300 m (Tab. 2). Tables 3 and 4 summarize the characters of the Atlantic and Mediterranean *Placogorgia* species. Features related to size and ornamentation of the sclerites drive the taxonomic identity of these taxa; nevertheless, two subgroups, possibly reflecting genera affinities, can be delineated based on the colony morphology: one including species showing significantly taller colonies (up to 45 cm), with robust branches, occasionally fused, and irregularly arranged, with crowded polyps (*Paramuricea/Echinomuricea*-like), and another one including small (up to 15 cm), thin, fragile colonies with distant polyps arranged on one plane on ramifications showing no anastomoses (*Placogorgia*-like).

Among the six species present in the eastern Atlantic area, two are also currently considered valid members of the Mediterranean fauna: *Placogorgia coronata* (Carpine & Grasshoff, 1975) and *Placogorgia massiliensis* (Carpine & Grasshoff, 1975) (Tab. 2; Fig. 5). The only non-Mediterranean species showing an affinity with our specimens is the boreal species *Placogorgia graciosa* (Tixier-Durivault and d'Hondt, 1975), displaying a similar set of sclerites to *P. coronata*, but a remarkably different colony morphology. With regard to the two Atlantic-Mediterranean species, the main difference is based on the shape of the thornscales and the rare occurrence of a second projection in the thornspindles of the coenenchyme in *P. coronata*, whereas *P. massiliensis* shows only thornstars in the outer layer of the coenenchyme (Carpine and Grasshoff, 1975). Based on the thicker spine of the thornscales, especially in their central part (Fig. 4b, c), the more complex basal plate, and the more consistent similarity of the coenenchyme sclerites – especially thick thornspindles occasionally with two projections (Fig. 4h) – the Ligurian samples are identified as *P. coronata*.

3.3 Placogorgia coronata removal rate

Among the 13 retrieved colonies, six were collected during the random surveys, while seven additional specimens were collected during the continuous monitoring period (Tab. 1, Fig. 6a, b). Nine specimens were retrieved on-board dead (hence without polyps and almost completely deprived of coenenchyme) (Fig. 6c, d), while four specimens were alive (with polyps) (Fig. 6e).

Considering that the continuous monitoring period accounted for 15 working days, the removal rate is approximately 0.5 colonies per trawl operation, reducing to 0.2 for live colonies. This represents a 47% chance of a coral colony per trawl (or 20% if considering only living colonies).

Region	Species	N° of specimens	Depth (m)	Locality	Bottom type	Authority
W Atlantic region	***P. atlantica*** **Wright & Studer, 1889**	1	146	St. Paul's Rock (Atlantic ridge)	-	Wright and Studer (1889)
		-	Littoral	Barbados	-	Kükenthal (1924)
		-	72-300	Colombia	-	Chacón-Gómez et al. (2012)
	P. mirabilis **Deichmann, 1936**	Several	172-274	Straits of Florida	-	Deichmann (1936), Bayer (1957), Reed et al. (2005)
		-	53	Gulf of Mexico	-	Bayer (1957)
	P. polybrachis **Kükenthal, 1919**	-	Littoral	Barbados	-	Kükenthal (1919)
	P. rudis **Deichmann, 1936**	3	170	Barbados	-	Deichmann (1936)
		-	72-172	Gulf of Mexico	Mesophotic reef	Rezak et al. (1985), Etnoyer et al. (2016)
	P. tenuis **(Verrill, 1883)**	8	185	off Florida	-	Deichmann (1936)
		Several	76-479	Caribbean Sea	-	Deichmann (1936), Bayer (1957), Cairns and Bayer (2009)
		-	84-94	Gulf of Mexico	Mesophotic reef	Etnoyer et al. (2016)
	P. tribuloides **Bayer, 1959**	1	51-373	Gulf of Mexico, Straits of Florida	-	Bayer (1959), Cairns and Bayer (2009)
E Atlantic - Mediterranean region	***P. becena*** **Grasshoff, 1977**	1	599	Azores	Coarse sand	Thomson (1927)
	P. coronata **Carpine & Grasshoff, 1975**	2	990-1000	Madeira	-	Grasshoff (1977)
		Several	948-2080	Rabat Gulf	-	Grasshoff (1977, 1989)
		1	51	Cape Blanc (Morocco)	Coralligenous	Grasshoff (1977)?
		1	1000	Cassidaigne Canyon	Detritus	Carpine and Grasshoff (1975)
		-	1400-2200	Azores	-	Grasshoff (1981)
	P. graciosa **Tixier-Durivault & d'Hondt, 1975**	13	849-1331	SW Ireland	-	Stephens (1909)
		3	820-2282	Banc de la Chapelle	Detritus	Tixier-Durivault and d'Hondt (1975), Grasshoff (1977)
	P. intermedia **(Thomson, 1927)**	Several	350-1350	Azores	Rock, detritus, Telephone cable	Thomson (1927), Tixier-Durivault and d'Hondt (1975), Grasshoff (1992)
		1	Deep	Madeira	-	Johnson (1862)
	P. massiliensis **Carpine & Grasshoff, 1975**	1	675-718	E Gibraltar	Dead coral	Carpine & Grasshoff (1975)
		1	350-500	Cassidaigne Canyon	Rock	Carpine & Grasshoff (1975)
		-	488-510	NW Galicia	-	Grasshoff (1989)
	P. terceira **Grasshoff, 1977**	Several	238-2200	Azores	Coarse sand	Thomson (1927), Grasshoff (1981), Sampaio et al. (2012)
		2	170-300	Josephine Bank	-	Grasshoff (1977)
		1	1400-2200	Mid Atlantic ridge	Rock	Grasshoff (1977)
		1	2165	Mogador (Morocco)	Mud	Carpine & Grasshoff (1985)
		1	1311	Cape Verde	Sandy mud	Carpine & Grasshoff (1985)

Table 2. Geographical records of the 12 Atlantic–Mediterranean species of the genus *Placogorgia*.

	COLONY								
	Color (dried)	**Colony height (cm)**	**Ramification pattern**	**Colony thickness**	**Basal diameter (mm)**	**Anastomosis**	**Polyps distribution and density**	**Calyx morphology**	**Contracted polyp height (mm)**
P. atlantica	White	33.6	Planar, irregular	Robust	4	No	Irregular, crowded	Conical	0.5-1
P. mirabilis	Greyish	20	Planar, irregular	Slender	1.5	Yes	Irregular, distant	Conical	0.5
P. polybrachis	White or grey	?	Planar, irregular	?	?	Yes	Irregular distant	?	?
P. rudis	White or grey	10	Planar, dichotomic	Slender	?	No	Irregular, distant	?	1
P. tenuis	Greyish	16x15 (up to 30)	Planar, irregular	Robust, thin branchlets	3-4	Yes	Irregular or biserial, crowded	Conical or blunt	0.5-1
P. tribuloides	White	5	Planar, dichotomic	Slender	2.5	No	Crowded	Cylindrical	1.9
P. becena	?	5x4	Non-planar, Irregular	Slender	?	No	Irregular, distant (2.5mm)	Conical	0.6-1.4
P. coronata	?	10	Planar, irregular	Slender	1.5	No	Irregular or biserial, distant	Conical	1-2.5
P. graciosa	Brown-white	28x13.5 18.5x10 (up to 33)	Planar, irregular	Robust	5-7	Yes	Irregular and crowded	Conical or blunt	1.3
P. intermedia	White	45x35 23x20	Planar, irregular	Robust	10	No	Irregular and crowded	Blunt	0.5
P. massiliensis	White	8	Planar, irregular	Slender	3.3	No	Biserial and distant	Conical	2
P. terceira	?	20	Planar, irregular	Robust, thin branchlets	4	No	Biserial and distant (2 mm)	Conical	1
Present study	Greyish-golden brown	10x13 13x8.5 18x15.5	Planar, irregular	Slender	2	No	Biserial and distant (2-3 mm)	Conical or blunt	0.5-1.5

Table 3. Taxonomic comparison of the Atlantic–Mediterranean species of the genus *Placogorgia* based on the features describing colonies. First six species are distributed in the western Atlantic Ocean, while the remaining six are found in the eastern Atlantic–Mediterranean region. The question mark indicates data not available.

	ANTHOCODIUM					CALIX				COENENCHYM		
	T length (mm)	O length (mm)	Number of O	C length (mm)	C rings	TS length (mm)	TS spine height	TS spine width	TS plate	TSP length (mm)	TSP n° of spines	S length (mm)
P. atlantica	0.1-0.2	0.37	At least 2 (2-3)	0.3-0.6	?	0.3-0.5	Short	Thick	From plate-like to branched	0.4-0.6	0	0.2-0.4
P. mirabilis	?	0.25-0.3	At least 2 (2-3)	0.25-0.3	Few	0.3	Short	Thick	Plate-like	Up to 4	0-1	0.4-0.5
P. polybrachis	?	?	At least 2 (2-3)	?	?	0.35	Tall	?	?	0.41	?	?
P. rudis	?	0.9	At least 2 (2-3)	0.5	Few	0.45-0.9	Variable	Thick	Plate-like	0.9	Several	NP
P. tenuis	?	0.4	1 (up to 2-3)	0.4	?	0.4	Variable	Thick	Plate-like	0.3-0.6	1	?
P. tribuloides	0.2	0.5	At least 2 (4-6)	0.5	3-6	1.8 (up to 2)	Tall	Slender	Plate-like	0.7-1.6	1 to several	0.3-0.4
P. becena	0.2-0.3	0.2-0.5	At least 2	0.3-0.4	5-6	0.4-0.6	Short	Thick	Plate-like	0.6-0.8	1 (rarely 2)	0.2-0.4
P. coronata	0.2-0.3	0.6-1.4	At least 2	0.8-1.2	5-8	0.6-1.6 (up to 2)	Tall	Thick (enlarged center)	Branched	0.4-1.0	1 (rarely 2)	0.3-0.7
P. graciosa	?	0.8-1.4	At least 2	0.6-1.2	5-6	0.9-1.8 (up to 2)	Tall	Thick (enlarged center?)	From plate-like to branched	0.6-1.2	1 (rarely 2)	0.2-0.5
P. intermedia	0.2-0.4	0.4-0.9	4	0.3-0.8	6-7	0.5-0.9	Tall	Thick	From plate-like to branched	0.6-1.2	Several	0.5-0.6
P. massiliensis	0.2-0.3	0.5-1.1	At least 2 (2-3)	1.1	4-5	0.3-1.2	Tall	Slender	Branched	0.3-0.4 (TST)	1 (TST)	0.3-0.5
P. terceira	0.3	0.5-1.1	At least 2	0.6-0.7	2-4	0.4-0.6	Tall	Slender	Branched	0.2-0.4 (TST)	1 (TST)	0.2
Present study	0.2-0.3	0.5-0.9	At least 2	0.9-1.1	6	0.7-1.2	Tall	Thick (enlarged center)	Branched	0.4-0.7	1 (rarely 2)	0.3-0.6

Table 4. Taxonomic comparison of the Atlantic–Mediterranean species of the genus *Placogorgia* based on the features describing the set of sclerites. First six species are distributed in the western Atlantic Ocean, while the remaining six are found in the Atlantic–Mediterranean region. The reported measures correspond to the longer side of each sclerite.

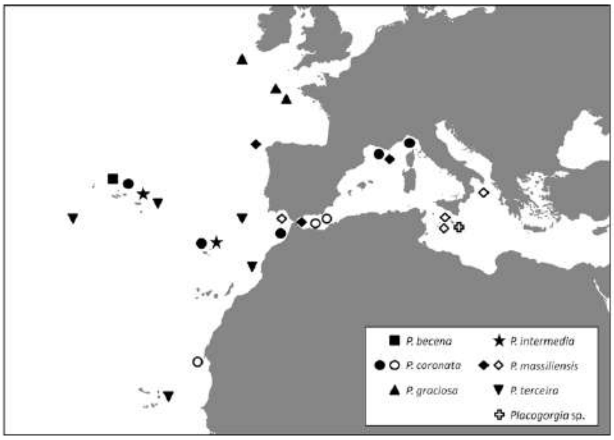

Figure 5. Distribution map of the genus *Placogorgia* in the eastern Atlantic–Mediterranean region. Filled labels indicate literature data and records from this study, whereas empty labels indicate dubious records or records based on pictures and grey literature

3.4 ROV characterization of the deep habitat

The SSS mapping of the two areas identified by Fusco (1968) highlighted the occurrence of hardgrounds only in the easternmost one, stretching from 600 to 800 m (Fig. 7a). Owing to the crossing of trawlers in the uppermost part of the area, between 500 and 650 m, the first dive (R1) was conducted in the southernmost part. The ROV footage showed an almost continuous crest of dead coral framework (Fig. 7b–d). Mounds, up to 2 m high, were characterized by a compact framework of heavily silted branches (Fig. 7d). Sparse blocks of dead corals were visible in the neighboring mud areas (Fig. 7e). Unsilted portions of hardgrounds were covered in iron-magnesium oxide. The observation of isolated fragments suggests that the framework is formed by *L. pertusa* (Fig. 7d). The presence of a few living unidentified corallites was also reported (Fig. 7b, f). Echinoids, foraminifera, scattered colonies of *Acanthogorgia* sp., and other unidentified, uncollected gorgonians were observed along the track (Fig. 7d, g, h). Alcyonaceans were found both on the framework and attached on dead coral rubble in the sediments nearby the mounds. Fish fauna included *Nezumia sclerorhynchus* (Valenciennes, 1838) and *Notacanthus bonaparte* Risso, 1840 (Fig. 6i, j). Litter, lost or discarded trawl nets, and entangled long lines were observed in the area (Fig. 7e).

The second dive (R2), carried out in the deepest part of the Entella Canyon, above the trawl track, was characterized by compact mud with cerianthids, echinoids, and the holothurian *Parastichopus regalis* (Cuvier, 1817). The fish *Paralepis coregonoides* Risso, 1820 and *Stomias boas* (Risso, 1810) were observed moving close to the bottom. No trawl marks were observed in the area.

4. Discussion

This study represents the first taxonomic record of *P. coronata* in Italian waters. The identification of the Ligurian samples posed various difficulties, mainly related to the fact that the taxonomic position of many *Placogorgia* species is uncertain, as evidenced by the numerous synonymies and genera changes over the years (Grasshoff, 1977). This is due to a lack of data for certain species and the fact that the sclerites, representing the main taxonomic features, are subjected to a high degree of plasticity both among species and among specimens. For this reason, the possibility that the other Mediterranean species *P. massiliensis* is a synonym of *P. coronata* is here highlighted in consideration of the few specimens so far analyzed of *P.*

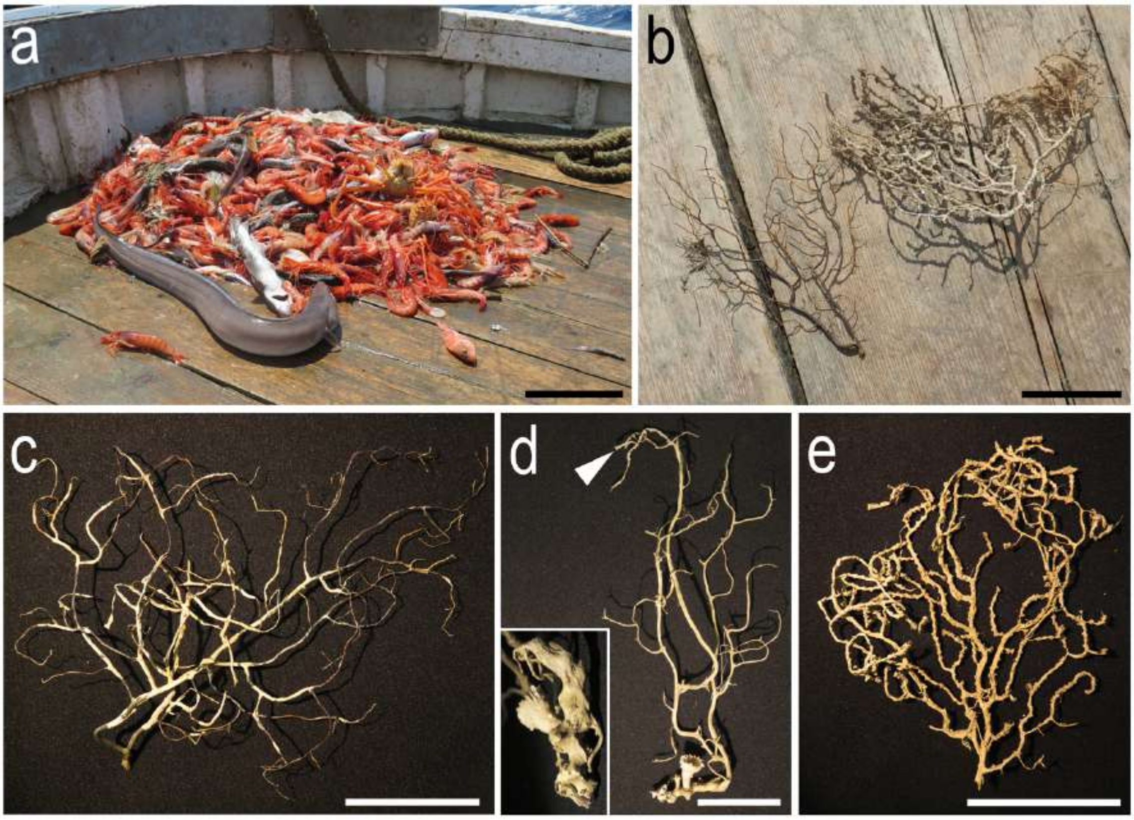

Figure 6. *Placogorgia coronata* in the trawl discard. (a) Overall catches of one haul. (b) Two colonies of *P. coronata* on the deck of the boat (shrimps' antennae are visible entangled in the top-right colony). (c–e) Colonies of *P. coronata* from the discard. (c) Dead colony almost completely denudated of polyps. (d) Dead colony with patches of polyps (arrow). The base has overgrown a dead fragment of *Madrepora oculata* showing the characteristic zigzag pattern (inset). A corallite is also visible in this portion. (e) Alive colony. Scale bars: (a) 10 cm; (b, c, e) 5 cm; (d) 3 cm.

massiliensis and the great variability of thornscales, stars, and spindles. Synonymy should be considered also from a biogeographical perspective: both species have been described in the Mediterranean basin from the French canyons at similar depth ranges (Carpine and Grasshoff, 1975), but *P. massiliensis*, originally considered an exclusively Mediterranean species, was later reported also outside of the basin (Grasshoff, 1989; Díaz del Rio et al., 2014) (Fig. 5).

The genus *Placogorgia* is considered rare in the Mediterranean Sea (Carpine and Grasshoff, 1975) (Fig. 5). However, following the first explorations of deep habitats, mainly related to CWC sites, additional records have appeared in the literature. Tursi et al. (2004) identified various specimens collected from 750 to 828 m in the white coral province of Santa Maria di Leuca (Ionian Sea) as *Placogorgia* sp. Freiwald et al. (2011), in the report of the Meteor Cruise M70 conducted in 2006 in the deep eastern Mediterranean Sea, classified three specimens as *P. cf. massiliensis*, collected between 460 and 786 m, on the Urania Bank, Southern Linosa Trough and Santa Maria di Leuca, while another specimen, photographed along the Malta

Trough, was identified from pictures as a white colony of *Placogorgia* sp. Occurrence of *P. coronata* was also reported on Chella Bank and Cabliers Bank in the Alboran Sea (R. Aguilar, personal communication, September 2017). Sclerites tables are not associated with these findings; therefore, in consideration of the great taxonomic difficulties related to this group of species and genera, they require further verification. However, they support the general picture describing a wider distribution of the genus, with the majority of the records found in the western basin.

In this regard, the fact that this species was never recorded before from the Santa Margherita Ligure fishing ground is surprising, especially considering the long history of studies conducted in the area based on trawl material (Rossi, 1958; Relini-Orsi and Relini, 1972a, 1972b; Relini-Orsi, 1974). A possible explanation could be that, today, trawls are conducted closer to the coral area than before; however, this is dubious, since accidental coral catches have always been reported here (Rossi, 1958). Therefore, this small species, remarkably similar to vegetal detritus when on board, would seem to have been overlooked.

Aside from taxonomy, some additional considerations can also be made on the bathymetric distribution and habitat of the genus *Placogorgia* in the basin. This taxon shows a distinctly deep distribution range in the Mediterranean Sea, with the majority of the records found in the mesobathyal interval (600–1000 m and 350–786 m respectively for *P. coronata* and *P. massiliensis*). Concerning the habitat, this genus seems often associated with the occurrence of scleractinian frameworks or rubble, as demonstrated by the only specimen collected with the base, perfectly covering *M. oculata* fragment (Fig. 6d, inset). This habitat is present not only in Santa Margherita Ligure, but also in the other localities where *Placogorgia* was reported; namely, Sicily, Santa Maria di Leuca, Alboran Sea, and Gulf of Lion (Tursi et al., 2004; Hebbeln et al., 2009; Orejas et al., 2009; Mastrototaro et al., 2010; Vertino et al., 2010; Pardo et al., 2011; Gori et al., 2013; Fabri et al., 2014; Lo Iacono et al., 2014). This association is not exclusive, as demonstrated by oceanic records that also describe *Placogorgia* on rocky bottoms, biogenic detritus, and anthropogenic substrates (Thomson, 1927; Carpine and Grasshoff, 1975; Tixier-Durivault and d'Hondt, 1975; Grasshoff, 1977).

The Ligurian records of *P. coronata* in the trawl discards highlight the fragility of this small CWC species as well as the risks to which the benthic community as a whole is subjected. The vertical development of the dead white coral crest offers natural protection against trawling (Bo et al., 2015; Fanelli et al., 2017). Nevertheless, the frequency of occurrence of the species in the trawl discards is surprisingly high. Based on the trawling effort in the fishing ground considered and on the observed collection frequency (0.5 colonies per haul and 0.2 living colonies per haul), the estimated removal rate per year for fishermen is, on average, about 90 colonies (or 18 living colonies), increasing to about 450 colonies (90 living ones) when considering the entire fleet operating here. Removal rates for CWC species are not frequently available in the literature. A study conducted on deep CWC forests within Greek artisanal fishing grounds

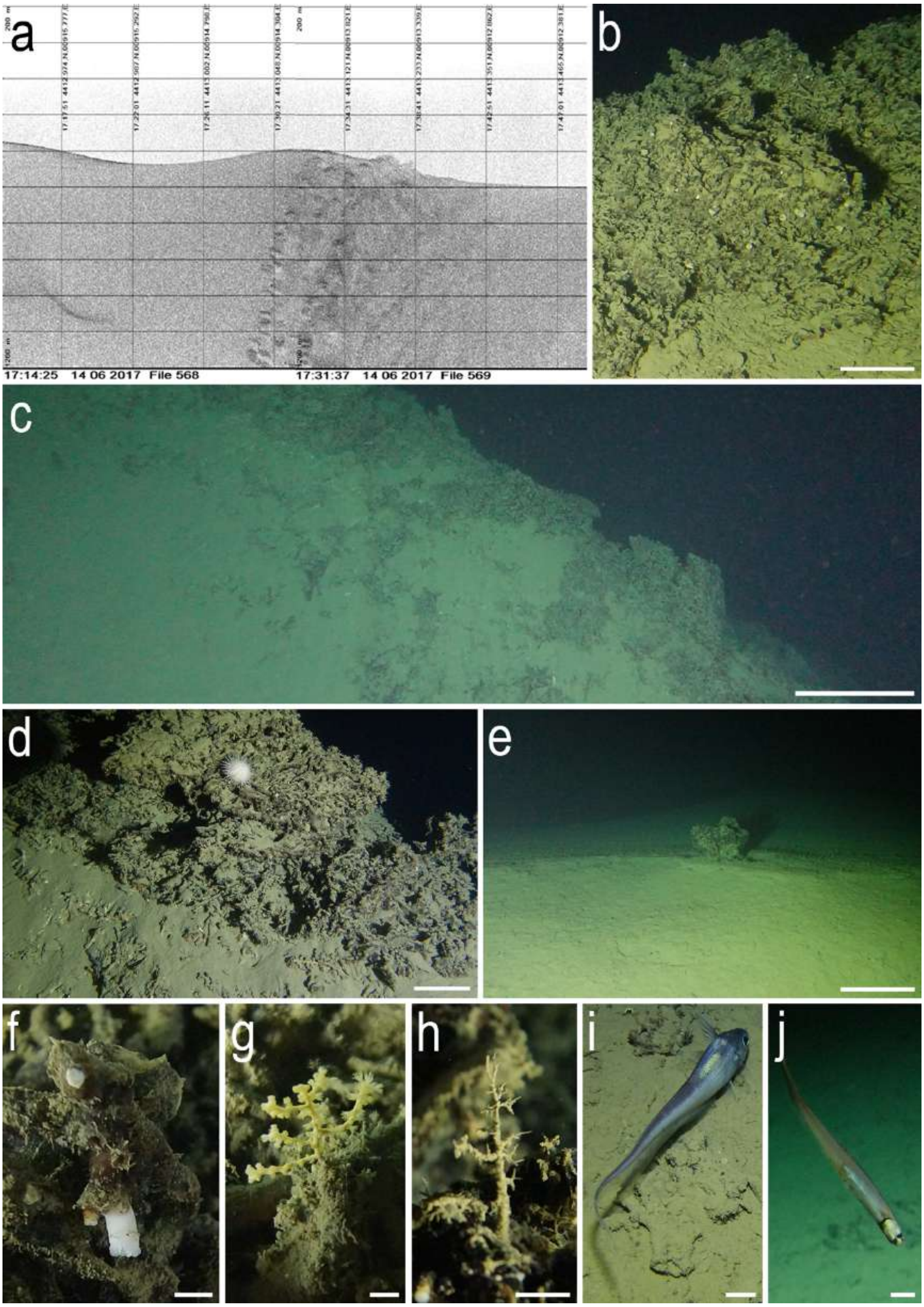

Figure 7. The coral framework of Fusco's area in Santa Margherita Ligure. (a) Side-scan sonar mapping of the area (R1) (distance between rows is 200 m). (b–j) Pictures of the coral thanatocoenosis and associated fauna observed by ROV at 737–773 m depth. (b) Dead coral framework. (c) Panoramic view of the reef crest. (d) Unidentified echinoid moving on the skeleton remains of *Lophelia pertusa*. Other dead, highly silted coral fragments lie on the crest's side. (e) Trawling net entangled on an isolated block of dead coral. (f) Close-up view of living unidentified scleractinian corallites. (g) Small colony of *Acanthogorgia* sp. settled on a dead coral branch. (h) Unidentified foraminifera. (i) The rat-fish *Nezumia sclerorhynchus*. (j) The shortfin spiny eel *Notacanthus bonaparte*. Scale bars: (b, d) 10 cm; (c, e) 50 cm; (f–h) 1 cm; (i, j) 2 cm

estimated collection rates varying from 30 to 130 colonies per fisherman per year for various arborescent anthozoan species (Mytilineou et al., 2014). In terms of percentage chance of collection, the monitoring of longline boats from Mediterranean and Macaronesian areas demonstrated that 15–70% of the landings contained a rich coral bycatch (Mytilineou et al., 2014; Sampaio et al., 2012). Bottom trawling has a far greater impact on the benthic communities than artisanal *métier* (Pham et al., 2014), and the catches are generally considered much larger. The removal rates for *P. coronata* in Liguria reflect the fact that this species is most likely found scattered on coral rubble adjacent to areas unsuitable for trawling such that its collection is more occasional. Additionally, collection rates are related to the fishing effort at this site, which, in turn, depends on the annual fluctuation of the commercial catches (red shrimps).

The high frequency of dead colonies in the catches (about 70% of the total collected colonies) suggests that individuals may have accumulated over time, due to the highly repetitive trawl tracks and possibly to the low survival rate of colonies once discarded overboard.

The population of this CWC species seems rather scattered nowadays, and the present situation reflects an historical disturbance. Mechanical impacts, in fact, do occur in the area, as demonstrated by overturned coral blocks, high levels of silting, lost gears - including fishing nets and long lines (Fig. 7e) - as well as by entanglement events on the coral framework followed by net loss (at least two cases in 2016 by one fisherman). Moreover, evidence of coral framework loss came from the SSS investigation that did not detect hardgrounds in one of the historical coral areas off Portofino appearing on the Fusco (1968) map. A similar situation was reported for another Ligurian bathyal coral area reported by the same author; recent investigations support the hypothesis that this area no longer exists, probably due to the intensive and destructive trawling action (Fanelli et al., 2017).

The particularly rich and diverse benthic and demersal fauna of the bathyal area off the Portofino Promontory was already highlighted based on the first trawl discards inventories, confirming the coexistence, in a relatively limited area, of CWC biocoenoses as well as soft bottom communities dominated by *I. elongata* and *Thenea muricata* (Bowerbank, 1858) (Relini-Orsi, 1974). These ecosystems, however, have been subjected to long-term fishing pressure, such that, as early as the 1970s, the area was considered overexploited with a low fishing yield and a visibly reduced abundance of benthic structuring species. In addition, large amounts of litter were already being recorded (Relini-Orsi and Relini, 1972a). The possibility to reduce the fishing pressure in an area adjacent to the coral framework off Portofino had already been suggested 40 years ago to allow fish stocks and benthos to recover (Relini-Orsi and Relini, 1972a; Relini, 2007). In this regard, the finding of rare CWC species in association with a structured ecosystem represents an important baseline toward protection measures, especially when coupled with information on fishing effort and impact. Indeed, since 2006, seven Fisheries Restricted Areas (FRAs) have been established in the Mediterranean basin to ensure the protection of sensitive deep-sea habitats and essential fish habitats. These areas include the Nile Delta and its cold hydrocarbon seeps, the Eratosthenes Seamount, the *Lophelia* reef off Santa Maria di Leuca, the eastern Gulf of Lion, and three banks in the Strait of Sicily. In addition, since 2005, the General Fisheries Commission for the Mediterranean Sea prohibited the use of bottom trawl nets below 1000 m, thus protecting a large portion of the basin unknown from the biological point of view and relatively unexploited based on the capacity of the Mediterranean trawling fleets.

The closure of areas along the Mediterranean continental shelf and slope poses greater socio-economic challenges due to the fact that the majority of the commercial activities are concentrated here, co-occurring with CWC sites that are mainly distributed over rough topographies (Chimienti et al., in press). In 2016, the Food and Agriculture Organization of the United Nations (FAO), in collaboration with the General Fisheries Commission for the Mediterranean Sea, organized a meeting focused on the definition and management of Mediterranean deep-sea fisheries and vulnerable marine ecosystems, within the framework of the International Guidelines for the Management of Deep-Sea Fisheries in the High Seas (FAO, 2016). A discussion was opened toward the creation of FRAs in mesobathyal areas (400–800 m) hosting red shrimp fishing grounds. Their designation requires information regarding the occurrence of vulnerable marine ecosystem indicator species (such as *P. coronata*) characterized by their rarity, fragility, relevant ecological role, and response to mechanical disturbances.

In this regard, an area stretching over the flanks of the Entella Canyon and including the easternmost part of the coral area F2 would represent a possible FRA for the Portofino area. A potential network of Ligurian deep coral areas could be created with the already established French FRA (Fabri et al., 2014) and embracing the northernmost distribution range for several CWC species. Such an area would give the possibility to monitor over time the recovery ability of benthic assemblages as well as potential positive responses of commercial species.

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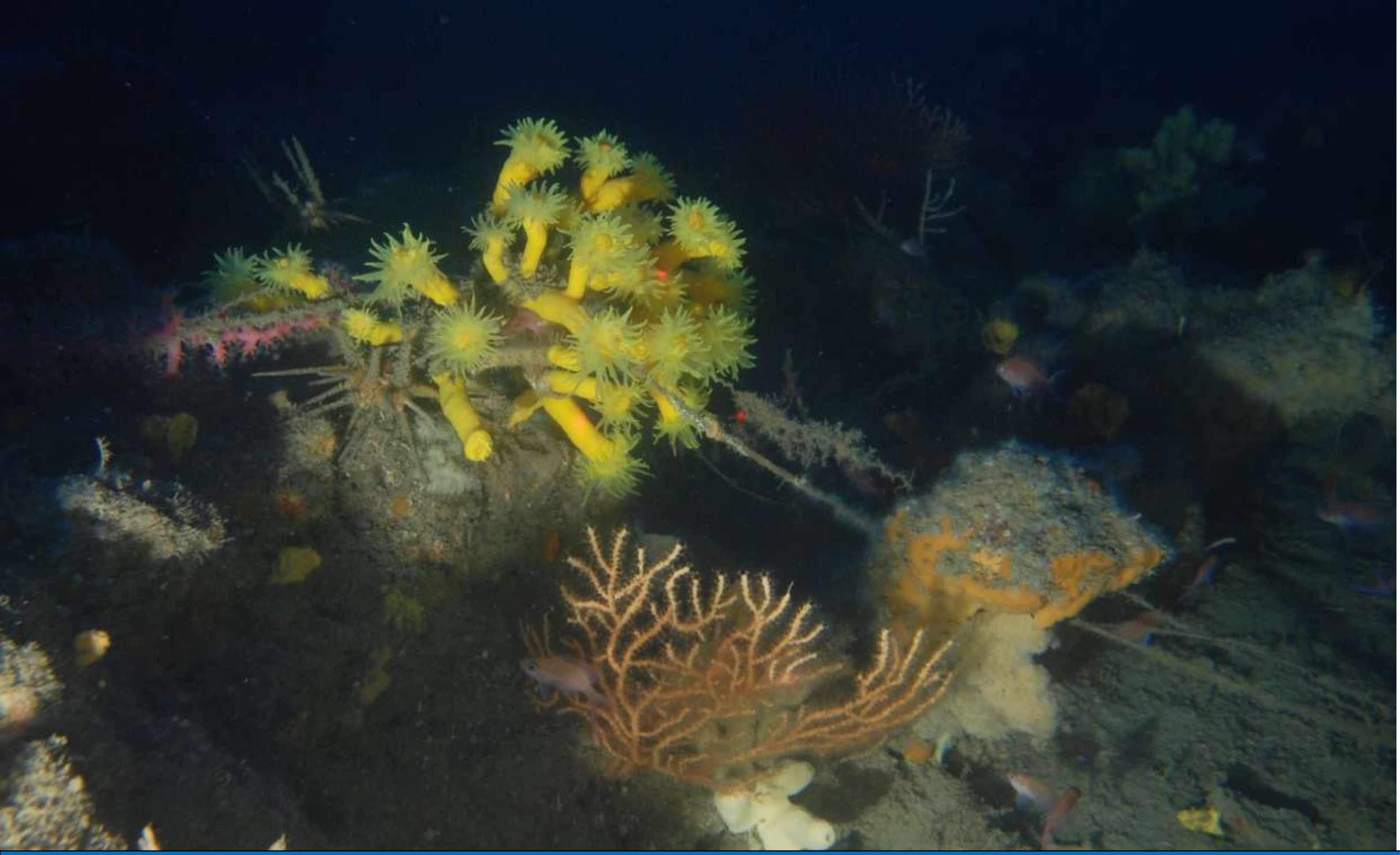

PART 3

MONITORING and CONSERVATION

At present, marine litter represents a common component of marine ecosystems worldwide. The Marine Strategy Framework Directive (MSFD) Descriptor 10 considers that good environmental status is met when "properties and quantities of marine litter do not cause harm to the coastal and marine environment" (European Commission 2010). Thus, European governments are required to characterize and monitoring marine litter in their waters, and are encouraged to identify, whenever possible, the activities responsible of its spread. This work provides a comprehensive catalogue of the marine litter present on the Ligurian deep continental shelf identified by means of ROV-Imaging *techniques. The distribution of different litter categories has been mapped, providing a useful tool for the identification of the main source of littering and a baseline for the implementation of restoration projects targeting removal and recycling of waste from VMEs and monitoring of the benthic ecosystem recovery.*

According to the MSFD, member states must identify and put into practice a series of management actions in order to achieve or maintain Good Environmental Status in their territorial waters. The assessment of the rate at which the environmental targets are achieved, requires adaptive approaches to management, including the availability of specific tools for the assessment of the environmental status of the ecosystems. Ecological indices represent effective instruments to evaluate and monitor the status of marine benthic ecosystems through time. Herein, the large-scale archive of ROV data collected from the Ligurian Sea has been employed in order to create a multiparametric index to assess the health status of the megabenthic assemblages thriving in temperate mesophotic rocky reefs. The parameters considered by the Mesophotic Assemblages Conservation Status (MACS) are easily achievable from video footages, and consider the criteria proposed by the MSFD for the evaluation of the descriptors biodiversity (1), sea-floor integrity (6) and marine litter (10), providing a useful, rapid device for the monitoring of mesophotic ecosystems dominated by animal forests.

Chapter 4

Marine litter assessment and distribution on the deep Ligurian continental shelf (NW Mediterranean Sea)

Abstract

Nowadays, litter represents a common component of marine ecosystems and the assessment and monitoring of waste are of primary interest worldwide. Here, we characterize and quantify the marine litter found along the Ligurian deep continental shelf, bordering one of the most urbanized coastlines of the Mediterranean Sea. Eighty sites were investigated by means of ROV within the mesophotic depth range to give the widest outlook ever produced on a basin scale. The environmental parameters and the anthropogenic pressures influencing the occurrence of debris were also explored. Litter is widely distributed in the region, with maximum densities of 7900 items ha^{-1}. Fishing gears represent the 81% of the total recorded waste, with the highest abundances found on the deep rocky shoals exploited by large fleets, mainly represented by artisanal and recreational boats. Urban litter, mainly in the form of plastic and glass bottles, is generally higher in the most coastal sites and nearby river mouths, especially in presence of large towns.

The densities of marine litter herein reported are among the highest recorded in Europe suggesting the occurrence of a critical situation in this region and the urgency for specific actions to prevent further spreading.

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1. Introduction

Marine litter is nowadays regularly observed in all offshore and coastal areas, embodying one of the most serious global environmental problems of the last decades (Galgani et al., 2015; Ryan, 2015; Thompson, 2015). According to the definition given by UNEP (2005), marine litter consists of "any persistent, manufactured or processed solid material discarded, disposed or abandoned in the marine and coastal environment". It consists of a wide range of materials, with plastic typically representing the most important fraction (Pham et al., 2014; Angiolillo et al., 2015; Buhl-Mortensen and Buhl-Mortensen, 2017). Jambeck et al. (2015) estimated that 4.8 to 12.7 million tons of plastic enter the oceans every year. Plastic bags, food or beverage containers and Abandoned Lost and otherwise Discharged Fishing Gears (ALDFG) are the most common items observed on beaches, floating on the sea surface or lying on the sea bottom (Galgani et al., 2000, 2013; Thiel et al., 2013; Topçu et al., 2013; Pham et al., 2014; Bergman et al., 2015). Land-based sources of marine litter include general public littering, poor waste management practices, industrial activities and sewage overflow with rivers and winds playing significant role in transporting litter towards the sea. Ocean-based sources of marine litter mainly include commercial shipping, leisure craft, fishing activities and off-shore installation (Rech et al., 2014; Sandri and Thompson, 2014; Galgani et al., 2015).

Once in the sea, about 50% of plastic sinks directly to the seafloor, whereas low-density polymers, such as polyethylene and propylene, may collapse at a later stage thanks to the additional weight of fouling (Engler et al., 2012). Floating marine litter may travel long distances before sinking, thus potentially invading remote areas. Several factors affect the final distribution of marine litter on the sea bottom, including hydrography, geomorphology and human factors (Galgani et al., 2000; Barnes et al., 2009; Pham et al., 2014). Densely populated coastlines, characterized by high levels of shipping and negligible tidal flows, generally show the highest densities of marine debris (Galgani et al., 2015). Despite submarine canyons can act as conduits for the transport of marine litter into the deep-sea (Galgani et al., 1996; Mordecai et al., 2011; Pham et al., 2014; Pierdomenico et al., 2019), the continental shelves are usually recognized as sites of accumulation, especially in enclosed seas such as the Mediterranean and the Black seas (Lee et al., 2006; Galgani et al., 2000).

Marine litter may threat the marine life in several ways, since it can kill or maim all sorts of marine organisms (UNEP 2009). Large attention has been devoted to the study of deleterious effects on charismatic organisms (such as sea turtles, seabirds, and marine mammals), often observed entangled in marine debris or with plastics in their digestive tract (Kühn et al., 2015; Gall and Thompson, 2015). Marine litter could also represent a way of dispersal for many organisms, acting as a potential vector for the introduction of allochthonous species (Kiessling, et al., 2015). The effects of debris on megabenthic communities include smothering and entanglement. Plastic bags, cloths, and other large items may cause widespread clogging phenomena within seagrass meadows, sponge grounds and coral gardens (Kühn et al., 2015; Richards and Beger, 2011), and their accumulation on the seabed can produce anoxia, which could lead to a change in the infauna community (Mordecai et al., 2011). Ghost fishing has been described as a process whereby lost or abandoned fishing gears continue to catch commercial species (Matsuoka et al., 2005). This phenomenon could also be applied to some urban marine litter and it may lead to mortality in gorgonians and other structuring species (Matsuoka et al., 2005; Brown and Macfadyen, 2007; Sheehan et al., 2017;). Derelict fishing gears can directly damage such species, which suffer broken parts and may be more susceptible to infections and epibiosis (Bavestrello et al., 1997; Asoh et al., 2004; Yoshikawa and Asoh, 2004; Chiappone et al., 2005; Pham et al., 2013; Fabri et al., 2014; Hinz et al., 2017).

Many efforts have been done at international, regional and national level scales in various areas of the world, in order to prevent, reduce and manage marine litter (Chung-Ling Chen, 2015). Within the European Union, currently, the most relevant action is represented by the Marine Strategy Framework Directive (MSFD), which provides a legal context within each Member State to describe and monitor the environmental status of the European marine waters (Council of the European Union, 2008). The ultimate goal is to achieve and maintain the Good Environmental Status (GES) in the marine environment by 2020, by taking proper measures. Marine litter has been elected as one of the eleven descriptors listed in Annex I of the MSFD for determining GES (Descriptor 10). The Commission Decision 2010/477/EU identifies some criteria and methodologies across the member states for a meaningful harmonization between different regions. Concerning Descriptor 10, the indicator 10.1.2 focuses on the amount of litter deposited on the sea-floor, including the analysis of its composition, spatial distribution and, where possible, source. For instance, the possibility to attribute some items to certain sources (for example fishing gears, sewage related debris, and tourist litter), provides valuable information for developing mitigation measures (Galgani et al., 2013).

The Ligurian Sea, lying in the northernmost part of western Mediterranean basin, represents one of the most urbanized basins within European and Mediterranean waters, and it occupies a central position within commercial maritime traffics (Cattaneo et al., 2010). The aim of this study was the investigation of the marine litter composition and distribution in this highly exploited area thanks to a large archive of ROV footages collected along the Ligurian deep continental shelf (30-220m). This depth range, commonly indicated as mesophotic, is a transition zone, which has been widely overlooked in the past in favor of shallower, more accessible areas (Cau et al., 2015). In order to explain the debris distribution and to identify the main sources of litter, different environmental parameters and anthropic pressures have been quantified within each studied site and relationships between these factors and marine litter densities have been explored.

2. Materials and Methods

2.1 Study area

The Italian portion of the Ligurian continental shelf extends for over 300 km, from Ventimiglia to La Spezia (Fig. 1). Its topography differs remarkably between the western and the eastern sector (Fanucci et al., 1989). The western shelf is deeper, steeper and narrower, cut by 15 major canyons, of which Roja, Nervia, Taggia, Verde, Mercula, Laigueglia, Cuenta, Pora, Noli, and Vado are within the areas investigated in this study (Würtz, 2012) (Fig. 1). In the eastern coast, the shelf is larger and with a gentler inclination; three major canyon systems, namely Polcevera, Bisagno, and di Levante, are known from the area (Fig. 1). The majority of the Ligurian canyons are shelf-incising canyons, with no clear bathymetric connection to a major river system (Harris and Whiteway, 2011). Ligurian rivers present predominantly a torrential character: about 76 streams flow through the Alpine and Apennine chains and enter the sea (http://www.pianidibacino.ambienteinliguria.it) (Fig. 1, SM 1). In the westernmost area, four major tributaries are known, the rivers Roja, Nervia, Argentina and Centa (representing 30% of the total outflows), whereas in the eastern area the rivers Polcevera, Entella, and Magra account for approximately 40% of the whole Ligurian hydrographic basins' extension. Once in the sea, rivers outflows move westward, driven by the overall cyclonic circulation of the basin (Pinardi and Masetti, 2000; Cattaneo et al., 2010).

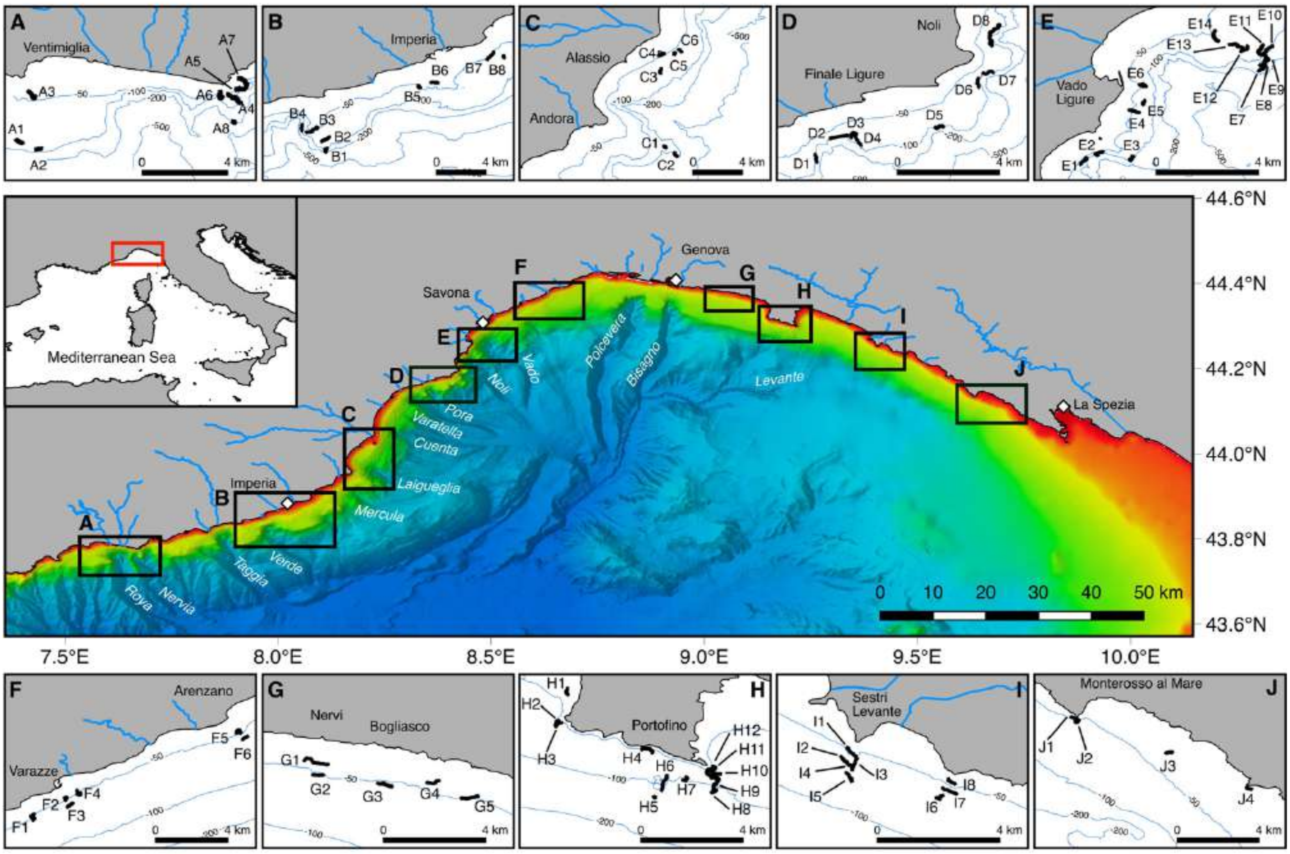


Figure 1. Map of the study area. The location of the investigated sites, as well as the 80 ROV dives with the respective codes are reported. The Ligurian major towns, rivers and canyons are also shown.

The Ligurian coasts are among the most urbanized of the Mediterranean Sea with 63 major towns and an estimated overall population of 1,262,000 residents (subjected to summer increase) (SM 2). Genova, with more than 580,000 inhabitants, represents the largest city center, followed by La Spezia, Savona, and Sanremo, each one accounting for more than 50,000 people. Even if the largest urban centers present sewage systems, blackwater discharges are occasionally present along the coasts. About 43 harbor structures are present, with those of La Spezia, Genova and Vado Ligure being the largest commercial harbors. Ligurian ports account for over 20,300 berths of which approximately 770 are registered for professional fishing boats (SM 3). The harbors of Sanremo, Imperia, Savona, Genova, Santa Margherita Ligure, and La Spezia host more than 50 fishing boats each (http://ec.europa.eu/fisheries; https://www.tuttobarche.it).

The Ligurian fishing fleet shows distinct artisanal characteristics: the 12% is represented by small trawlers and the 7% by purse seiners, whereas the remaining 81% operate in the small-scale fisheries, mainly targeting coastal demersal *métier* (Cataudella and Spagnolo, 2011). Yachting is common in the Ligurian Sea, where several touristic spots are present including five Marine Protected Areas. Recreational activities include, among others, fishing. Despite no precise census is available, this activity is extremely widespread and more than 220,000 licenses have been released in this region (https://www.politicheagricole.it).

2.2 Surveys

Ten major areas (namely A-J from East to West) were investigated by means of ROV video footages along the whole Ligurian continental shelf (Fig. 1). Within each area, from four to fourteen dives were carried-out, between 40 and 220 m depth, for a total of 80 video transects (Tab. 1). Seventy videos were recorded from 2012 to 2016, on board of the R/V *Astrea* ISPRA (Rome) employing ROV Pollux III, equipped with a digital camera (Nikon D80), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), lights and a 3-jaw grabber. The ROV hosted also an underwater acoustic positioning system ultra-short baseline (USBL) LinkQuest TrackLink 1500 MA, a depth sensor and a compass to obtain georeferenced tracks. Two parallel laser beams provided a scale for dimension reference. Ten additional videos were recorded in 2018 by COLMAR S.r.l. (La Spezia), employing BlueROV2, equipped with an HD video camera, lights, and laser beams for dimensional reference, USBL Sonardyne Scout Plus, depth sensor, and a compass. In order to guarantee the best quality for videos footages, the ROVs moved at constant slow speed ($<0.3 m\ sec^{-1}$) and height (<1.5 m) from the bottom, in continuous recording mode. Videos duration ranged between 10 and 120 minutes, corresponding to a linear distance ranging between 186 and 1862 m.

2.3 Video analysis

Video footages were analyzed by means of Final Cut Pro software. Loops and pauses were erased from the footages, to obtain a linear video track. Sequences too far away from the sea floor or with poor image quality were considered

Dive	Date	Area	Site	ROV	Start position		End position		Depth (m)		Time (min)	Length (m)
					Lat. N	Long E.	Lat. N	Long. E	min	max		
A01	01/09/16	Ventimiglia	Mortola Cape	PIII	7.552	43.743	7.555	43.742	81	89	26	545
A02	01/09/16	Ventimiglia	Mortola Cape	PIII	7.564	43.738	7.567	43.739	82	86	17	373
A03	01/09/16	Ventimiglia	Mortola Cape	PIII	7.563	43.769	7.559	43.772	28	47	55	1150
A04	02/09/16	Ventimiglia	Bordighera	PIII	7.675	43.770	7.682	43.766	39	100	83	1595
A05	02/09/16	Ventimiglia	Bordighera	PIII	7.680	43.774	7.682	43.774	53	69	10	186
A06	02/09/16	Ventimiglia	Bordighera	PIII	7.670	43.770	7.671	43.772	42	99	49	974
A07	07/06/12	Ventimiglia	Bordighera	PIII	7.682	43.780	7.680	43.773	38	80	53	1797
A08	09/06/12	Ventimiglia	Besughi Shoal	PIII	7.678	43.754	7.679	43.754	128	200	12	230
B01	07/06/12	Imperia	Gobbe del Cammello	PIII	7.918	43.784	7.916	43.781	88	154	52	778
B02	12/08/15	Imperia	Santo Stefano	PIII	7.920	43.796	7.912	43.792	52	63	36	874
B03	12/08/15	Imperia	Santo Stefano	PIII	7.907	43.806	7.897	43.801	45	67	97	1625
B04	13/08/15	Imperia	Santo Stefano	PIII	7.892	43.809	7.892	43.803	85	92	48	841
B05	13/08/15	Imperia	Porto Maurizio	PIII	8.010	43.848	8.011	43.847	45	52	48	789
B06	13/08/15	Imperia	Porto Maurizio	PIII	8.023	43.851	8.030	43.851	33	39	43	794
B07	14/08/15	Imperia	Diano Marina	PIII	8.086	43.882	8.080	43.875	49	54	108	1761
B08	14/08/15	Imperia	Diano Marina	PIII	8.096	43.878	8.097	43.876	65	70	13	273
C01	06/06/12	Alassio	Mele Cape	PIII	8.230	43.930	8.231	43.929	99	135	39	754
C02	06/06/12	Alassio	Mele Cape	PIII	8.240	43.923	8.243	43.919	190	216	30	674
C03	16/02/18	Alassio	Gallinara Island	BR2	8.226	44.009	8.226	44.004	80	87	47	736
C04	17/04/18	Alassio	Gallinara Island	BR2	8.223	44.022	8.229	44.024	28	40	46	756
C05	15/02/18	Alassio	Gallinara Island	BR2	8.240	44.023	8.240	44.024	58	66	37	474
C06	14/02/18	Alassio	Gallinara Island	BR2	8.247	44.026	8.244	44.028	55	60	29	390
D01	17/04/18	Finale L.	Pora Canyon	BR2	8.347	44.144	8.346	44.147	74	89	47	524
D02	17/04/18	Finale L.	Pora Canyon	BR2	8.362	44.157	8.354	44.155	75	109	50	822
D03	17/04/18	Finale L.	Pora Canyon	BR2	8.364	44.156	8.366	44.157	78	92	36	587
D04	18/04/18	Finale L.	Pora Canyon	BR2	8.369	44.152	8.365	44.157	84	96	42	639
D05	06/06/12	Finale L.	Lua Canyon	PIII	8.409	44.161	8.405	44.161	91	129	49	752
D06	18/04/18	Finale L.	Noli Cape	BR2	8.426	44.185	8.426	44.181	86	94	34	580
D07	21/04/18	Finale L.	Noli Cape	BR2	8.433	44.188	8.428	44.187	93	108	51	703
D08	10/08/15	Finale L.	Noli Canyon	PIII	8.432	44.201	8.435	44.211	71	141	100	1862
E01	10/08/15	Savona	Maledetti Shoal	PIII	8.434	44.222	8.437	44.224	51	85	60	819
E02	03/09/16	Savona	Maledetti Shoal	PIII	8.445	44.227	8.442	44.226	54	111	65	640
E03	05/06/12	Savona	Corallone Shoal	PIII	8.459	44.225	8.459	44.223	65	110	39	667
E04	09/08/15	Savona	Vado shoals	PIII	8.458	44.248	8.463	44.247	39	76	42	692
E05	09/08/15	Savona	Vado shoals	PIII	8.466	44.253	8.465	44.251	59	69	22	373
E06	09/08/15	Savona	Vado shoals	PIII	8.465	44.261	8.463	44.260	36	71	62	843
E07	05/06/12	Savona	Mantice Shoal	PIII	8.522	44.270	8.521	44.267	79	151	88	1013
E08	05/06/12	Savona	Mantice Shoal	PIII	8.525	44.273	8.524	44.271	78	88	32	399
E09	09/08/15	Savona	Mantice Shoal	PIII	8.526	44.273	8.524	44.271	85	121	54	844
E10	26/08/15	Savona	Mantice Shoal	PIII	8.528	44.279	8.525	44.273	77	108	71	1243
E11	03/09/16	Savona	Savona shoals	PIII	8.524	44.280	8.523	44.275	65	85	70	1289
E12	03/09/16	Savona	Savona shoals	PIII	8.516	44.279	8.513	44.277	56	72	44	732
E13	03/09/16	Savona	Savona shoals	PIII	8.507	44.280	8.514	44.279	51	60	57	924
E14	03/09/16	Savona	Savona shoals	PIII	8.500	44.287	8.502	44.282	46	56	64	987
F01	04/09/16	West Genova	Celle Ligure	PIII	8.551	44.331	8.551	44.327	39	52	78	1154
F02	08/08/15	West Genova	Varazze	PIII	8.573	44.340	8.574	44.342	38	47	35	628
F03	08/08/15	West Genova	Varazze	PIII	8.578	44.338	8.575	44.336	60	63	26	559
F04	08/08/15	West Genova	Varazze	PIII	8.582	44.347	8.582	44.346	40	52	52	839
F05	08/08/15	West Genova	Arenzano	PIII	8.695	44.387	8.692	44.387	34	42	47	848
F06	08/08/15	West	Arenzano	PIII	8.700	44.385	8.697	44.383	56	62	33	536

		Genova										
G01	06/08/15	East Genova	Nervi	PIII	9.032	44.364	9.027	44.364	52	56	27	497
G02	06/08/15	East Genova	Nervi	PIII	9.034	44.369	9.023	44.371	30	36	62	1285
G03	06/08/15	East Genova	Bogliasco	PIII	9.066	44.358	9.059	44.360	53	57	42	839
G04	05/08/15	East Genova	Sori	PIII	9.089	44.361	9.083	44.360	33	37	34	614
G05	05/08/15	East Genova	Sori	PIII	9.100	44.353	9.108	44.354	43	53	57	958
H01	04/09/16	MPA Portofino	Punta Chiappa	PIII	9.148	44.337	9.149	44.334	34	36	52	625
H02	03/06/12	MPA Portofino	Isuela	PIII	9.144	44.320	9.144	44.320	29	44	26	245
H03	05/09/16	MPA Portofino	Isuela	PIII	9.145	44.320	9.143	44.318	34	72	60	939
H04	06/09/16	MPA Portofino	Cala degli Inglesi	PIII	9.190	44.305	9.185	44.307	34	73	49	806
H05	27/08/15	MPA Portofino	San Gorgio shoal	PIII	9.191	44.283	9.191	44.283	103	106	15	255
H06	27/08/15	MPA Portofino	San Gorgio shoal	PIII	9.197	44.294	9.195	44.287	93	104	66	1098
H07	03/06/12	MPA Portofino	San Gorgio shoal	PIII	9.205	44.292	9.207	44.293	89	99	37	544
H08	02/06/12	MPA Portofino	Punta del Faro	PIII	9.221	44.287	9.223	44.292	73	101	67	923
H09	25/08/16	MPA Portofino	Punta del Faro	PIII	9.222	44.287	9.220	44.286	101	104	29	503
H10	02/06/12	MPA Portofino	Punta del Faro	PIII	9.223	44.292	9.217	44.297	55	90	121	1494
H11	25/08/16	MPA Portofino	Punta del Faro	PIII	9.219	44.293	9.222	44.291	61	83	41	722
H12	25/08/16	MPA Portofino	Punta del Faro	PIII	9.223	44.295	9.217	44.297	60	86	46	747
H13	25/08/16	MPA Portofino	Punta del Faro	PIII	9.220	44.298	9.218	44.297	57	78	57	908
I01	03/08/15	Sestri Levante	Punta Manara	PIII	9.400	44.246	9.403	44.245	42	54	36	676
I02	03/08/15	Sestri Levante	Punta Manara	PIII	9.397	44.246	9.401	44.242	58	68	46	768
I03	03/08/15	Sestri Levante	Punta Manara	PIII	9.404	44.245	9.402	44.240	58	74	37	586
I04	23/08/16	Sestri Levante	Punta Manara	PIII	9.401	44.242	9.398	44.245	59	67	54	625
I05	03/08/15	Sestri Levante	Punta Manara	PIII	9.399	44.239	9.401	44.236	79	85	29	562
I06	03/08/15	Sestri Levante	Punta Baffe	PIII	9.436	44.229	9.439	44.230	66	73	33	512
I07	03/08/15	Sestri Levante	Punta Baffe	PIII	9.439	44.231	9.444	44.230	51	58	40	674
I08	03/08/15	Sestri Levante	Punta Baffe	PIII	9.444	44.234	9.441	44.236	33	41	17	383
J01	02/09/16	Cinque Terre	Punta Mesco	PIII	9.631	44.133	9.635	44.132	36	49	43	597
J02	23/08/16	Cinque Terre	Punta Mesco	PIII	9.635	44.130	9.636	44.131	47	55	46	665
J03	02/09/16	Cinque Terre	Corniglia	PIII	9.692	44.112	9.689	44.112	40	42	35	621
J04	23/08/16	Cinque Terre	Punta Montenero	PIII	9.741	44.090	9.738	44.090	28	30	46	539

Table 1. Technical information for the 80 ROV dives carried out for this study.

unsuitable for the analysis. The remaining useful footages accounted for approximately 83% of the recorded material, corresponding to a total length of about 51 km. A visual census was carried out for each transect within a 50 cm wide visual field, based on the laser beams. All the anthropogenic items observed within the video transects were identified with the highest accuracy possible and grouped in two different classes: ALDFG and urban litter. The objects of the latter category were further subdivided in relation to their material in seven additional categories, namely plastic, glass, metal, ceramic, cloth, paper, and wood. Environmental parameters including depth, the percentage of hard bottom and slope were also characterized for each video footage.

2.4 Data analyses

2.4.1 Diversity, abundance, and distribution of litter

The length of the 80 video transects was measured by means of the software Quantum GIS. In order to consider only the analyzed portions of each video transect, the percentage of useful sequences was calculated from the video time codes and then converted in the final lengths of the videos. The analyzed surface was obtained by multiplying the final transect length for its average width (0.5 m). Litter density was calculated by dividing the number of items observed for the surface of each transect. Considering the heterogeneous seabed on this area mainly characterized by small elevations surrounded by mud or sand, the final values were normalized and converted into items ha^{-1}.

Differences among the ten study areas were investigated both in terms of litter density and litter composition. In order to depict the geographical distribution of marine litter along the Ligurian deep continental shelf, the values of densities for the different categories were plotted on a map by means of Quantum GIS software.

2.4.2 Relationship with environmental parameters

Five different environmental parameters were quantified for each video transect, in order to investigate relationships with the amount of marine litter: i) mean depth, ii) mean inclination of the sea bed, iii) percentage of hard bottom iv), distance from the coast, and v) cumulative surface of the catchment area of the rivers (considering those with a mouth within 5 km radius from each site). The catchment areas of the Ligurian rivers have been extrapolated from the Ligurian Region official website (http://www.pianidibacino.ambienteinliguria.it). Relationships between environmental parameters and the density of both ALDFG and urban litter were investigated by means of linear regression.

2.4.3 Relationship with anthropic pressures

Similarly to what was carried out with environmental parameters, three anthropic pressures were quantified within each site. The parameters considered were: i) the number of inhabitants of the coastal municipalities, obtained from the online database of the Italian national STATistical institute - ISTAT (http://dati.istat.it), ii) the number of berths for each Ligurian port, obtained from the EU Fleet register (http://ec.europa.eu/fisheries/), and iii) the number of fishing boats listed in each harbor, found on the online pilot's book for the Mediterranean Sea, (https://www.tuttobarche.it/). A range of 10 km instead of 5 km was considered for the parameters ii) and iii), due to the ability of the boats to move offshore.

Relationships between these parameters and the density of both ALDFG and urban litter were explored by means of linear regression.

3. Results

3.1 Litter diversity and composition

Marine litter has been observed in all the investigated sites, with 3546 items recorded overall. The majority of this material, accounting for 83%, was represented by ALDFG, whereas the remaining 17% was represented by urban litter (Tab. 2; Fig. 2).

Fishing lines were the most common items observed on the seabed (Tab. 2; Fig. 2). Nylon lines (Fig. 3a, b) accounted for 67.7% of the total ALDFG, whereas an additional 11.8% was represented by ropes. In terms of abundance, fishing lines were followed by fishing nets, almost completely represented by trammel nets (3.5%), gillnets (0.3%) and other unidentified artisanal nets (14.0%; Fig. 3c, d). Seventeen among trawling nets and purse seine nets (0.6%) were detected. The remaining part of ALDFG

		A	B	C	D	E	F	G	H	I	J	Total
Fishing lines	Line	102	228	167	109	515	184	75	258	89	65	1792
	Long line	8	26		3	78	7	1	53	15	10	201
	Rope	29	27	50	62	48	18	14	61	18	20	347
Fishing nets	Trammel net	6	7	1	7	35	2	7	24	10	3	102
	Gill net	1				4			4			9
	Unidentify Artisanal net	59	52	25	43	120	24	13	53	15	8	412
	Trawling and pourse sein nets	2	2	1	1	2	3		6		1	17
Other fishing gears	Fishing pot		1			2					2	5
	Mooring					6	1		14	2	1	24
	Unidentify fishing gear					1	12	5	7	6	3	34
Plastic	Bag	3	1			4	12		5	6		31
	Boat fender								2			2
	Bottle	1			2		4	1	3	1		12
	Chair					1			1			2
	Cotton bud	1										1
	Crate								2			2
	Cup					2			12	1		15
	Dive mask						1					1
	Latex glove		1									1
	Sheet	6	2	1	3	2	2		3	4		23
	Tank	2										2
	Tube						1					1
	Tyre	2	7	1	5	7	4		12	7	1	46
	Unidentified plastic	23	16	11	17	30	11	2	60	30	4	204
	Wheel rim				1							1
Glass	Bottle	9	11	2	2	8	12	12	48	6	2	112
Metal	Anchor	2				7			3			12
	Cable					1						1
	Can	1		1	1		7		4	1		15
	Chain					1						1
	Chair								1			1
	Sank boat	1							2	2		5
	Tube				1					1		2
	Unidentified metal	1	3			4	7	2	12	6		35
Ceramics	Archeological material				1		24					25
	Brick					1	5		5			11
	Toilet			1								1
Cloths	Shoes				1		1		1	3		6
	Unidentified fabric	7			1	2	12	2	1	3		28
Paper	Cardboard		1							1		2
Wood	Forniture									1		1

Table 2. Classification of all the litter items identified in the video footages. The number of items identified within each study area was reported.

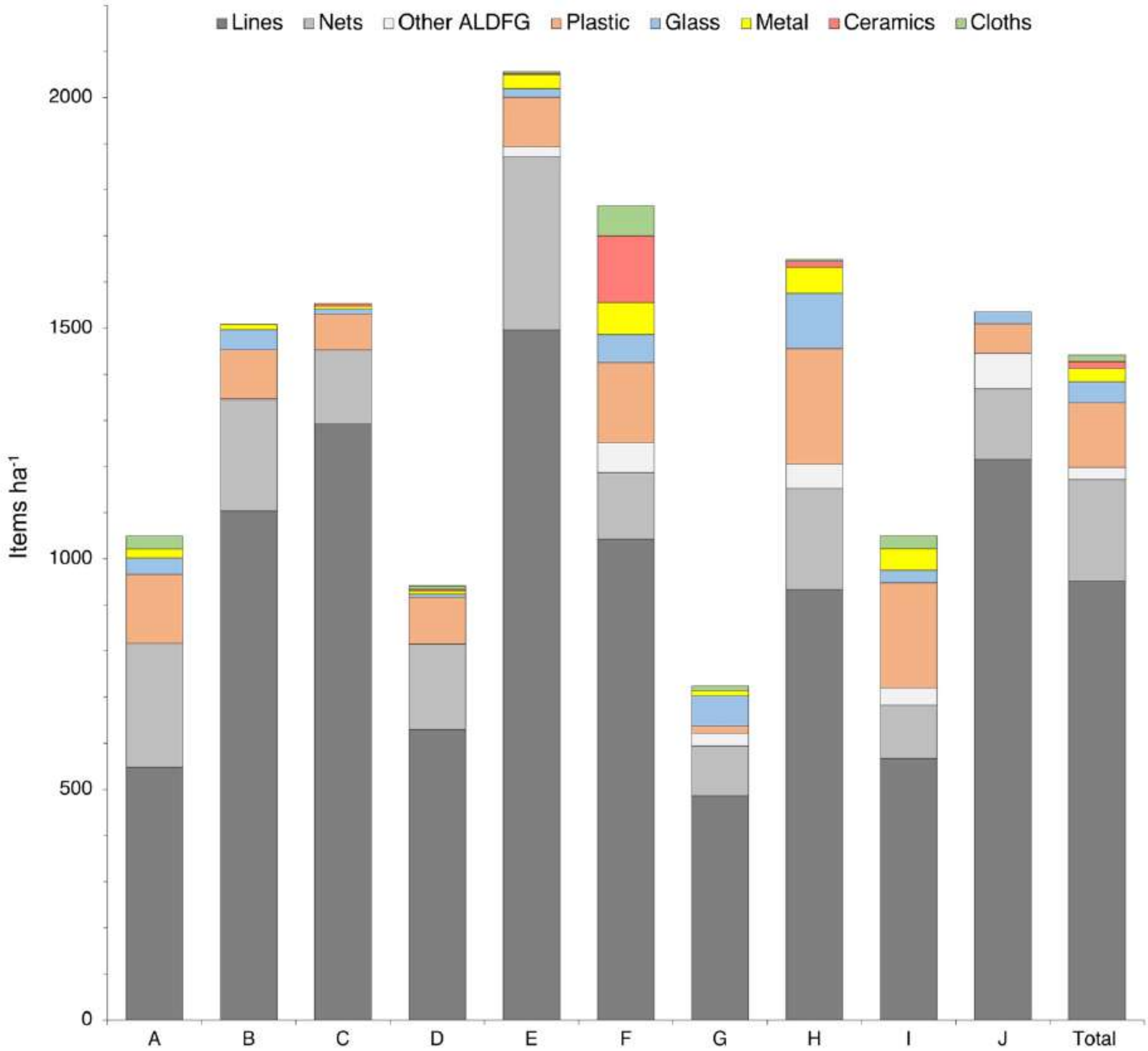


Figure 2. Density and composition of litter in the ten areas of the Ligurian deep continental shelf investigated in this study. Values of litter density have been reported in items ha^{-1}.

was represented by lost moorings (0.8%), fishing pots (0.2%; Fig. 3e) and other unidentified fishing gears (1.2%). About 57% of the urban litter was composed of plastics. Unidentified plastic items represented 33.9% of the urban litter, followed by tires (7.6%; Fig. 3f), bags (5.2%; Fig. 3g), sheets (3.8%), cups (2.5%), bottles (2.0%) (Tab. 2). Glass items accounted for 18.6% and were entirely represented by glass bottles (Fig. 3h). Metal cans, anchors, tubes and other metal objects, including five sank boats and other large unidentified items (Fig. 3i), represented 12% of the urban litter observed in this study. Ceramics accounted for 6.2% and were represented by amphorae, bricks and domestic items. Fabric pieces and shoes contributed with 5.7% to the urban litter (Fig. 3j), whereas the contribution of papers and machined wood was negligible.

3.2 Litter density and distribution

Mean densities of marine litter accounted for 1524 items ha^{-1}, with a maximum value up to 7983 items ha^{-1} in site E02 corresponding to the Maledetti Shoal, western Ligurian Sea. This site showed

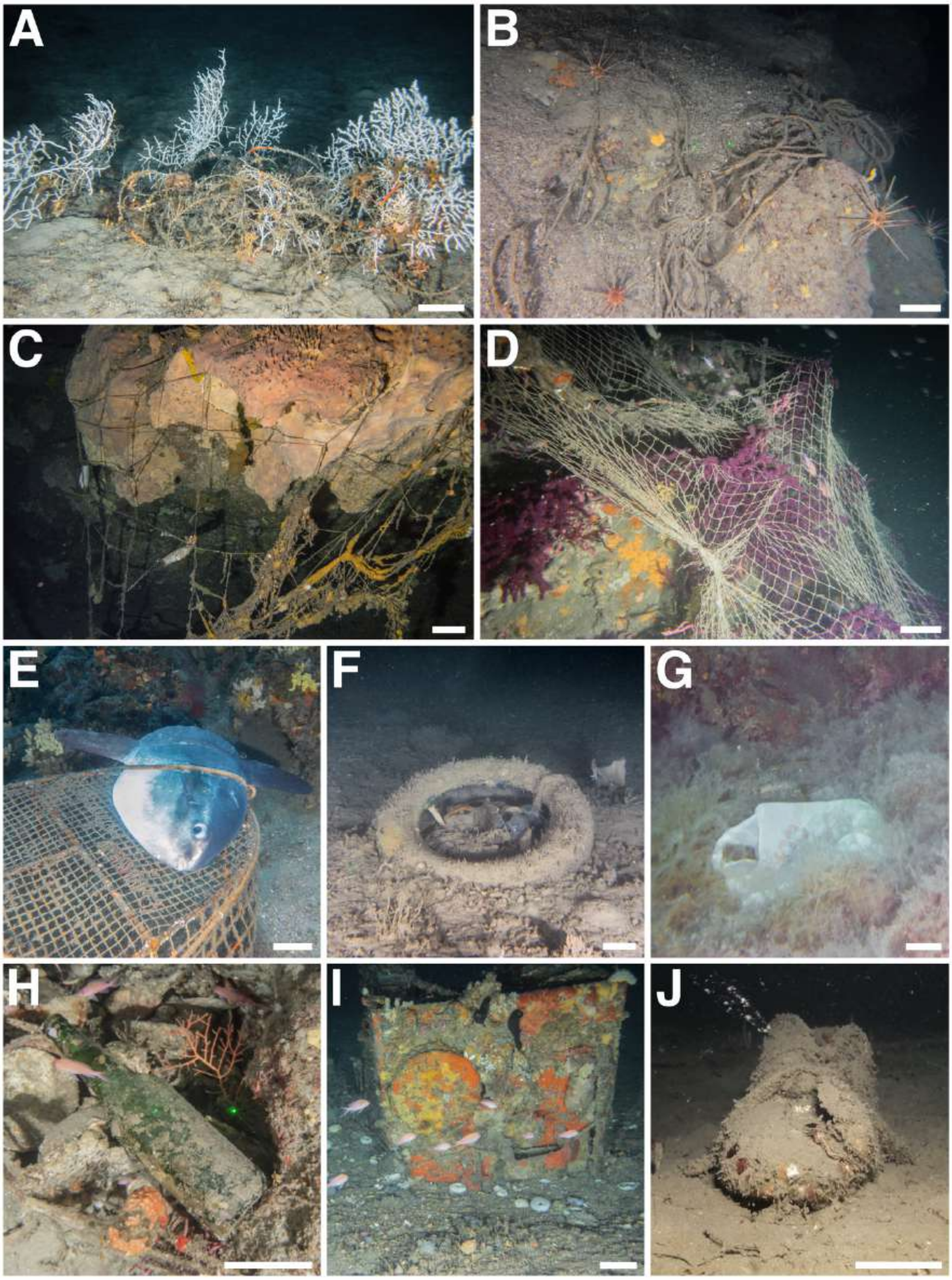


Figure 3. Images of marine litter from the Ligurian deep continental shelf. A-E: examples of lost fishing gears; F-J: urban litter. A: epibionted lines entangled on a gorgonian forest (*Eunicella verrucosa*) on a highly silted bottom. B: abandoned lines and ropes on hard bottom with cidarids. C: an old trammel net enveloping a rocky shoal. D: abandoned artisanal net covering and suffocating a gorgonian forest in the site of Vado Ligure. E: a sunfish entrapped in a fishing pot for spiny lobster. F: tire. G: plastic bag. H: glass bottle. I: a large unidentified metal structure highly encrusted by sponges and other organisms. J: an abandoned shoe. Scale bars: 10 cm (in J 5 cm).

the highest densities for both fishing lines (5575 items ha^{-1}) and fishing nets (2281 items ha^{-1}) (Fig. 4). Minimum values were reported from site D04 (Finale Ligure), located on the muddy bottoms of the Pora Canyon head, and accounting for 32 items ha^{-1}.

Some differences among the ten investigated areas were detectable in terms of litter density (Fig. 2). The areas E and F (Savona and West Genova, respectively) appeared the most polluted, with a density of litter up to 2056 and 1765 items ha^{-1}, respectively. Intermediate litter densities were reported for areas B (Imperia), C (Alassio), H (MPA Portofino), and J (MPA Cinque Terre), where values varying from 1649 to 1512 items ha^{-1} (areas H and B, respectively). Finally, areas A (Ventimiglia), D (Finale Ligure), G (East Genova), and I (Sestri Levante) presented the lower densities of marine litter, with area G showing 724 items ha^{-1}.

Litter composition appeared similar in the ten areas, with a strong predominance of ALDFG (varying from 68% to 94%) (Fig. 2). Fishing lines were recorded in all the investigated sites, with the only exception of site C03 (Isola Gallinara). The Maledetti Shoal (E02) and Capo Mele (C02), both from the western Ligurian Sea, showed the maximum densities of fishing lines, up to 5575 and 5048 items ha^{-1}, respectively. Fishing nets abounded mainly in the western areas A, D and E, whereas other ALDFG were recorded essentially in the eastern areas (Figs. 2, 4).

The main differences in litter composition regarded the urban litter. Plastics were always prevalent, with the exception of area G (East Genova), dominated by glass. The highest density of plastic (up to 1302 items ha^{-1}) has been observed in site H12, falling within the MPA of Portofino (eastern Ligurian Sea) (Fig. 4). Glass bottles reached higher densities in area H, with values up to 337 items ha^{-1} (site H12, P. ta del Faro), and in area B, with values up to 284 items ha^{-1} (site B05, Porto Maurizio). Metals were relatively abundant in sites E, F, H, and I with a maximum density of 192 items ha^{-1} in site F06 (Arenzano) and 168 items ha^{-1} in site H12 (P. ta del Faro). Ceramics contributed consistently only in area F, where 24 amphorae have been observed in the site F03 (Varazze). Finally, cloths were detectable in areas A, F, G and I, reaching maximum densities of 307 items ha^{-1} in site F06 (Arenzano).

3.3 Environmental parameters

Five environmental parameters have been quantified for the 80 investigated sites (Tab. 3). Mean depth ranged between 30 and 202 m, and slope varied between 0.6% and 46.6%. Percentage of hard bottoms within investigated sites varied from 0% to 100%. The areas A and C were the most influenced by river outflows, due to the presence of Roja and Centa rivers, representing, respectively, the second and third largest Ligurian streams in terms of hydrographic basin size (SM 1). Despite Magra represents the largest Ligurian river, it does not significantly affect the investigated sites, because its mouth lies more than 20 km from the nearest site. All the sites are relatively close to the coast, with the furthest one lying at 6.4 km of distance (Capo Mele).

No strong correlation has been detected among marine litter density and the investigated environmental parameters (Fig. 5). Only the factors “mean slope” and “percentage of hard bottom” presented a moderate correlation with the density of ALDFG (R = 0.7153 and R = 0.6617, respectively; N = 80). Very weak correlations have also been observed among ALDFG density and the factors “depth” (R = 0.2735) and “distance from the coast” (R = 0.2759). The presence of urban litter weakly increases nearby the coasts (R = 0.3237) and in the areas where large river mouths are present (R = 0.2579).

3.4 Anthropic pressures

The distance of 5 km radius was selected to measure the number of inhabitants facing each investigated site. Only two sites of Capo Mele (C01 and C02), both from the western Ligurian Sea, are known as offshore. Site H12 (P. ta del Faro) could also be considered lightly populated, whereas the number of inhabitants in all the other sites ranged from 1472 to 137,965.

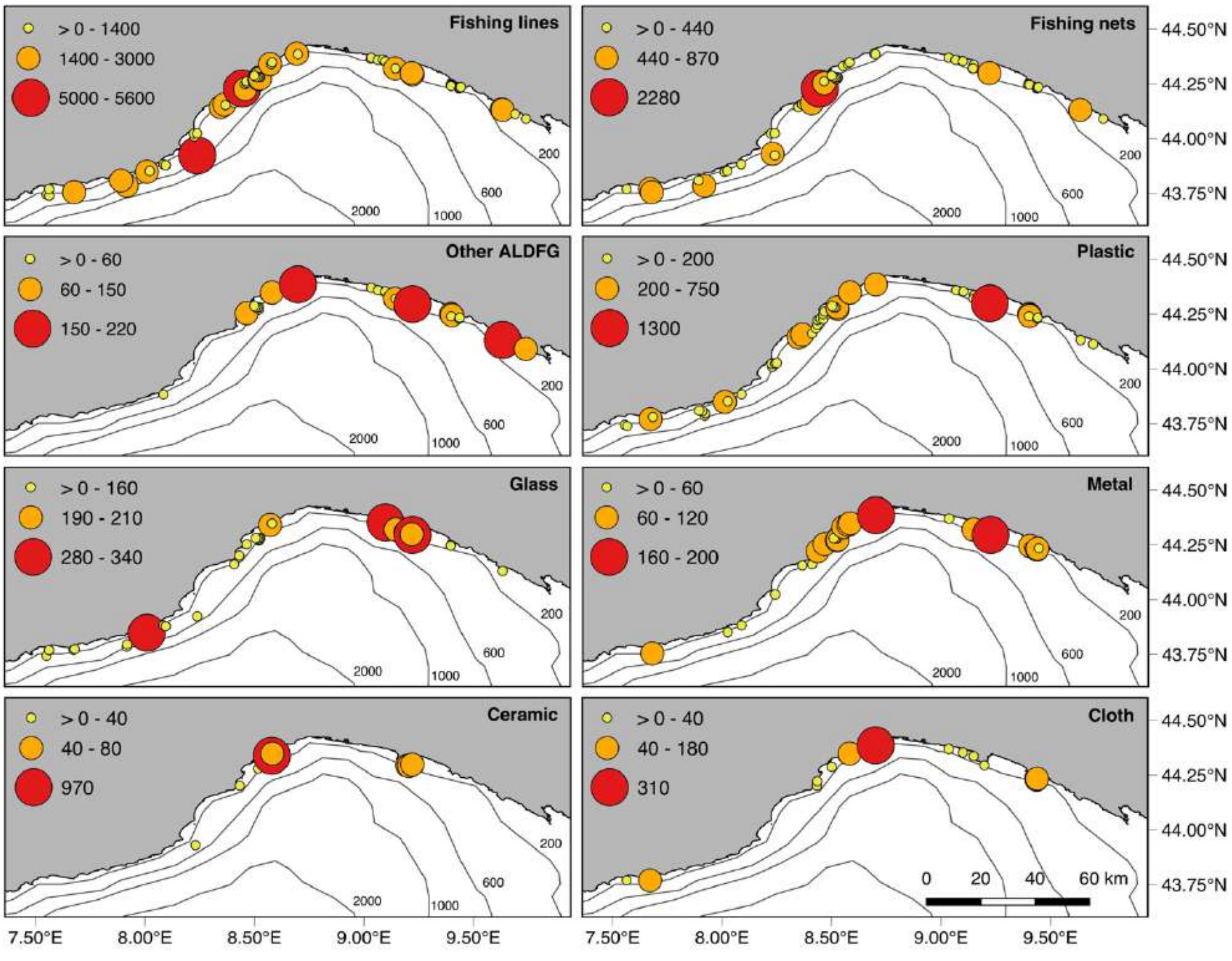


Figure 4. Densities for the main litter categories observed within the 80 sites investigated along the Ligurian deep continental shelf. All the values are reported in items ha^{-1}.

Dive	ALDFG density (items ha⁻¹)	UL density (items ha⁻¹)	Mean depth (m)	Mean slope (%)	Hard bottom (%)	River catchm. surface in 5 km radius (km²)	Distance from the coast (km)	N° of inhab. in 5 km radius	N° of berths in 10 km radius	N° of fishing boats in 10 km radius
A01	237	79	84	1.7	4	0	3.92	23926	573	19
A02	230	58	84	nd	0	3	4.4	23926	573	19
A03	656	47	36	7.1	45	686	1.46	36377	573	19
A04	1049	201	68	10.6	53	227	0.64	26253	1804	75
A05	473	158	59	nd	21	227	0.63	26253	1804	75
A06	1261	938	67	12.8	37	899	0.7	50179	1804	75
A07	519	130	62	27.5	37	227	0.43	26253	1804	75
A08	2974	110	156	31.7	97	39	2.5	26253	983	75
B01	3289	145	118	23.5	74	0	5.79	5100	2573	56
B02	397	142	57	4.5	31	0	4.39	6371	1696	0
B03	562	21	58	6.7	25	207	3.31	21206	1696	56
B04	1857	136	87	18.5	86	258	3.1	21206	2517	56
B05	1943	521	48	7.1	58	188	2.3	44498	3368	78
B06	621	83	36	7.1	28	188	2.42	43695	1378	78
B07	1770	235	52	7.0	57	141	1.42	52581	1798	78
B08	80	80	68	1.8	1	141	2.36	51453	1798	60
C01	2378	32	108	13.7	96	0	5.28	0	1585	98
C02	5134	86	202	30.0	66	0	6.39	0	2524	98
C03	28	111	84	9.4	5	215	2.9	36402	1415	22
C04	941	57	37	13.2	26	215	1.81	34602	1415	22
C05	695	217	60	7.9	38	453	2.34	40417	1415	22
C06	0	154	57	0.9	0	432	2.52	40417	1415	22
D01	2313	270	79	23.8	60	139	2.18	22931	1445	48
D02	50	101	94	6.1	0	93	1.51	14051	1445	48
D03	2126	349	82	17.6	20	93	1.53	14051	1445	48
D04	32	0	90	4.4	0	84	2.26	11724	1445	48
D05	1050	146	101	26.8	85	35	2.16	14525	1445	48
D06	835	114	90	8.9	32	27	0.83	14525	692	10
D07	621	0	101	28.4	47	27	1.01	2801	692	10
D08	395	103	93	12.7	10	27	1.34	6687	692	10
E01	3313	195	67	37.6	59	27	1.21	16045	1222	118
E02	7898	84	60	42.0	92	27	1.85	16045	1302	118
E03	1590	38	84	nd	41	27	1.99	7813	1222	118
E04	1257	68	55	7.3	13	99	0.7	13244	1222	118
E05	1342	141	63	8.2	22	99	0.97	13244	1222	118
E06	1418	118	55	12.9	38	125	0.75	13244	1222	118
E07	2320	145	107	17.5	39	43	4.74	68893	1419	142
E08	2313	183	83	17.1	65	43	4.41	68893	1419	142
E09	2027	229	96	21.5	8	43	4.39	74457	1419	142
E10	1406	165	90	20.2	52	47	4.12	66225	1419	109
E11	1309	293	75	12.9	42	47	3.77	84864	1419	109
E12	820	182	64	8.3	38	56	3.49	74457	1419	109
E13	1124	173	55	4.1	30	201	3.03	66225	1419	109
E14	882	162	50	3.5	40	201	2.09	84864	1419	109
F01	1192	397	46	2.8	35	110	0.95	95446	1317	103
F02	1905	305	41	6.0	38	106	0.94	29221	1317	141
F03	75	973	62	2.1	2	107	1.44	34785	1317	54
F04	1004	502	46	4.0	28	39	1.07	18814	1317	87
F05	2068	94	39	9.0	26	43	1.25	20729	1437	42
F06	1308	1001	59	4.9	20	101	1.71	137965	1437	85
G01	556	0	54	3.2	20	14	1.89	7068	260	43

G02	249	47	34	8.3	47	27	1.3	7068	260	43
G03	860	57	55	6.6	31	27	2.16	1472	260	43
G04	293	0	35	5.7	52	49	1.16	21578	280	39
G05	1287	360	48	5.6	36	40	1.78	27059	280	39
H01	1400	100	34	4.4	39	22	0.45	9991	1795	100
H02	3147	0	37	35.3	100	22	3.3	15587	835	100
H03	1155	442	47	22.9	51	22	2.8	16040	1795	100
H04	235	323	47	21.7	12	62	0.32	10162	1735	100
H05	1100	110	104	nd	28	26	2.36	453	1735	100
H06	603	344	96	8.9	10	62	1.07	10162	2194	100
H07	1103	501	93	13.1	40	62	1.06	10162	2194	120
H08	467	208	88	8.8	15	39	1.27	10162	1994	81
H09	272	317	103	2.1	10	185	1.28	10162	1994	81
H10	2267	543	70	17.0	47	185	0.73	10162	3594	139
H11	1224	175	73	10.5	35	185	0.53	10162	1994	81
H12	1492	845	74	13.0	12	185	0.53	39388	3594	139
H13	2116	1546	64	16.1	31	190	0.11	55573	3594	139
I01	1423	619	48	9.0	44	90	0.37	18172	2009	73
I02	418	195	63	9.6	30	90	0.82	18172	2009	73
I03	1200	600	63	7.4	32	84	0.72	18172	2009	73
I04	328	368	62	5.7	17	84	1.07	18172	2009	73
I05	152	152	82	nd	0	84	1.4	18172	2009	73
I06	999	291	69	8.1	19	61	1.14	21062	150	34
I07	556	327	54	5.5	38	61	0.71	21062	150	34
I08	660	60	36	10.7	16	61	2.35	21062	150	34
J01	2273	0	39	16.2	48	40	1.8	1951	173	62
J02	2412	161	52	8.7	52	40	1.62	2422	173	62
J03	157	157	41	nd	0	25	1.21	4091	113	19
J04	1365	0	30	12.2	81	25	1.17	1669	85	111

Table 3. Environmental parameters and anthropic pressures within the investigated sites. Density of ALDFG and urban litter are reported in items ha^{-1}. See text for additional explanations.

In order to quantify shipping in the investigated sites, the distance of 10 km radius has been selected. The number of berths per port varied from 85 to 3594 within 10 km radius for each site, whereas the number of fishing vessels varied between 0 and 146.

A very weak correlation has been observed between the number of berths and the density of urban litter (R = 0.5052) and between the number of fishing vessel and ALDFG (R = 0.4578). A weaker correlation has been also detected between urban litter and number off the fishing vessels (R = 0.2427) and the number of inhabitants (R = 0.3682).

4. Discussion

Marine litter investigation is now increasing worldwide, allowing comparison among different geographical areas (Galgani et al., 2000; Pham et al., 2014; Woodall et al., 2015; Buhl-Mortensen and Buhl-Mortensen, 2017). Despite several studies aim to characterize marine litter within different bathymetrical ranges, we specifically focused on the deep continental shelf (30-200 m) of the Ligurian Sea, a particularly poorly studied zone. In consideration of the various environmental and social factors

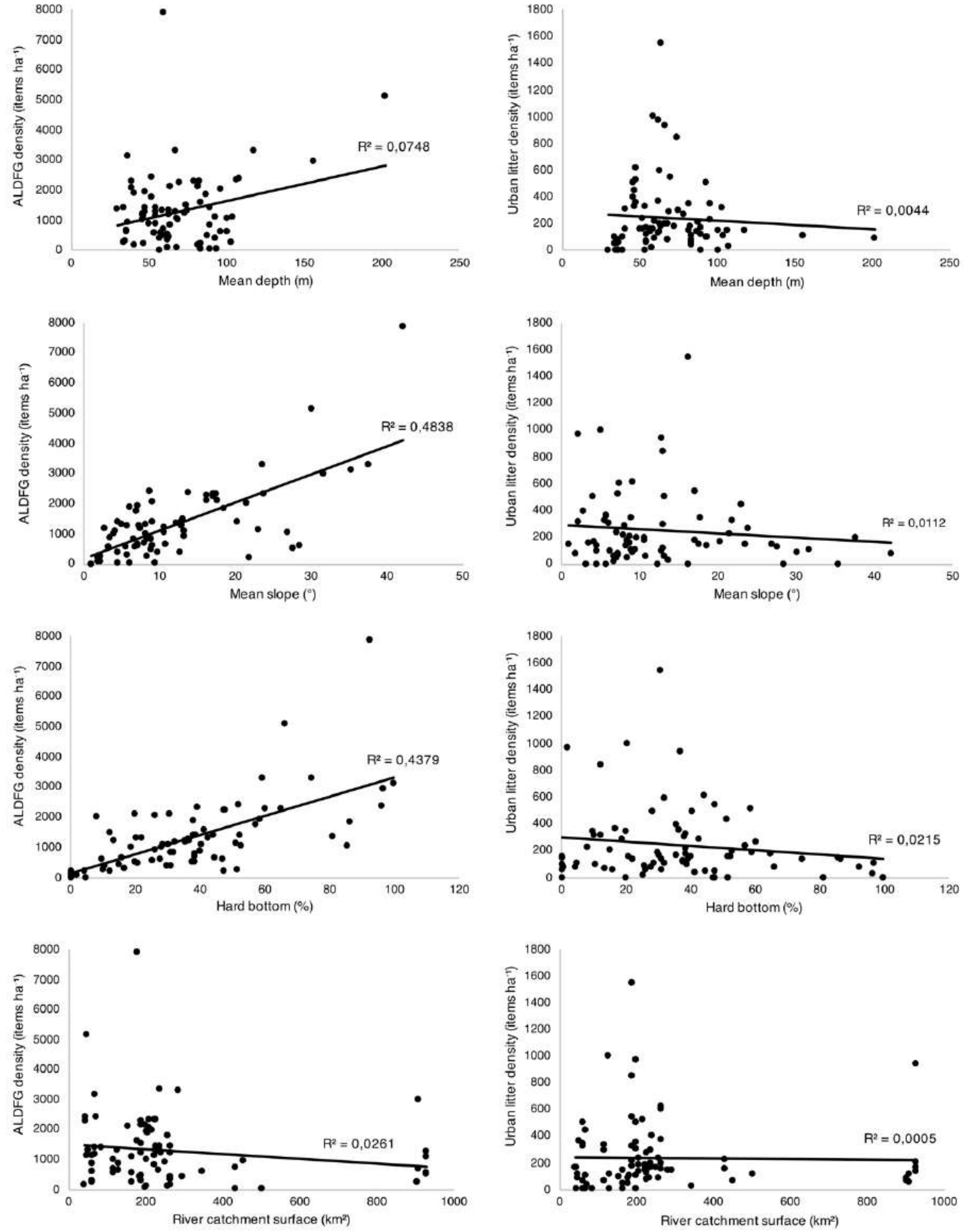
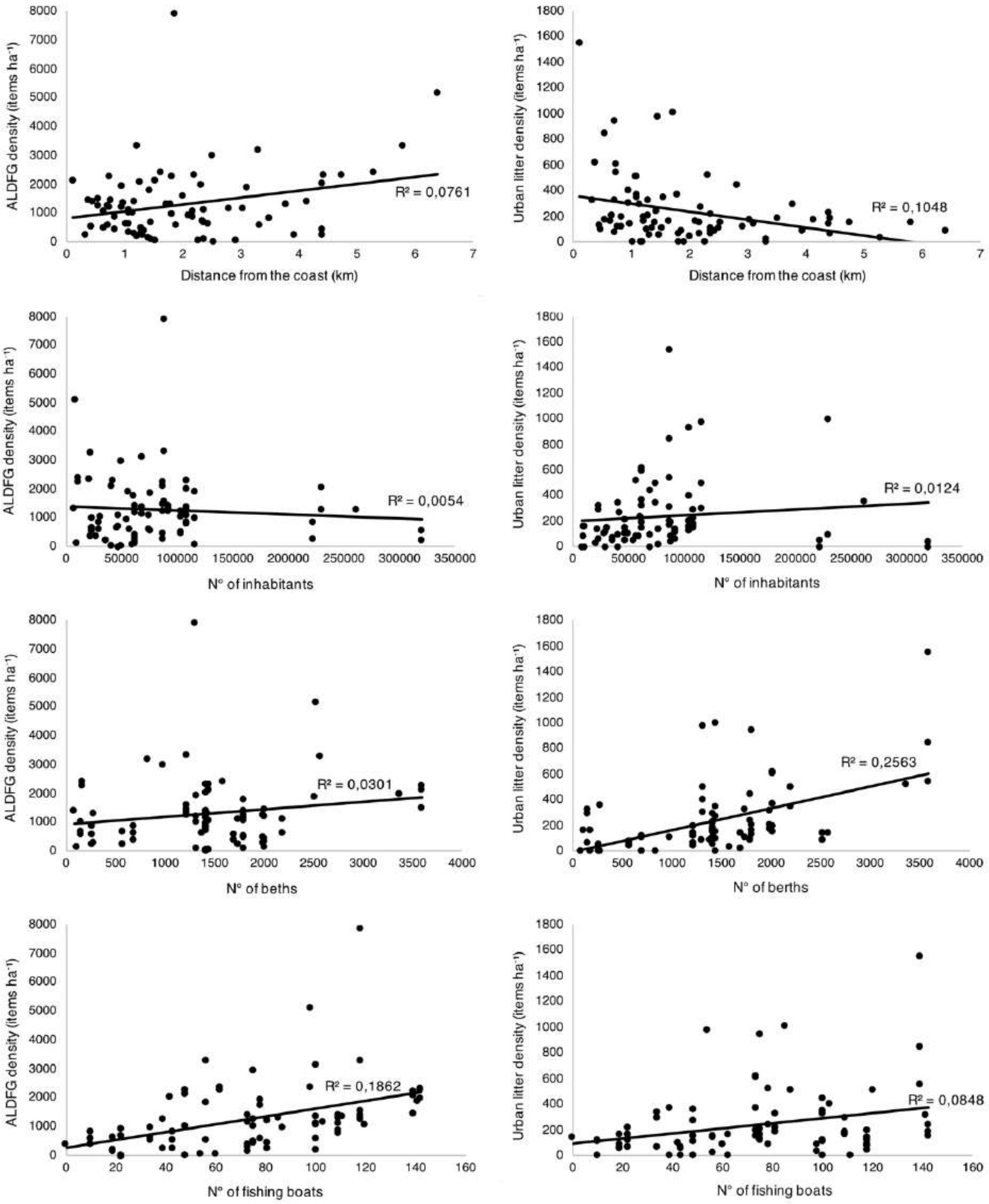


Figure 5. Relationships between marine litter density and environmental features (this page) and anthropic impact (next page) observed in this study

enhancing accumulation of marine litter in this basin, an extended survey of this area was opportune, resulting the widest census so far for the whole Mediterranean Sea.

The mean value of marine litter density observed in this study appears extremely high when compared to other values recorded in the Mediterranean and Atlantic waters (Tab. 4). A great



discrepancy is observed especially with the adjacent area of the Gulf of Lion and French Ligurian coasts, where the density of marine litter is two orders of magnitude lower than along the Italian Ligurian coasts. This finding was already reported by Fabri et al. (2014) who attributed this disparity to the different morphology of the continental shelf in the two basins. Nevertheless, since the majority of the Ligurian debris is represented by ALDFG, additional factors probably intervene in determining this situation. For instance, different socio-economic and cultural approaches to fishing activities between French and Italian fishermen could play a major role. This hypothesis is supported by the fact that other Italian sites are comparable in terms of litter density with what found along the Ligurian coasts (Tab. 4). Extremely high abundances of marine litter are reported from the Gulf of Naples (Angiolillo et al., 2015), especially for the Vedove Shoal where the debris is mainly represented by long lines and remains of disposable moorings, deliberately and continuously abandoned during purse seine fishing (Bo et al., 2014). Similarly, Marco Bank, in the Sicily Channel, results highly impacted by long line fishing (Bo et al., 2014). The density of marine litter observed along the North-western Atlantic coasts is generally three orders of magnitude lower than what registered in the Ligurian Sea. A sampling bias might concur in determining this situation, though, as ROV surveys generally report a higher abundance of marine litter than trawling (mainly employed in oceanic waters), probably because the two samplings strategy are conducted on different bottom types: trawl surveys are generally conducted on soft bottoms, whereas ROV is employed mainly on rocky bottoms. As reported by Pham et al. (2014) dissimilarities in sampling equipment implies that the results should be treated with caution. Surely, in this regard, ROV can be considered an optimal tool to quantify litter in mesophotic hard bottom environments, which are also those hosting the majority of the valuable Vulnerable Marine Ecosystems (VMEs). These latter are often dominated by arborescent species, which easily remain entangled in fishing gears (Sampaio et al., 2012; Bo et al., 2014; Mytilineou et al., 2014; Hinz, 2017).

Recent data on marine litter within the Ligurian Sea are considered scarce and scattered, however some historical information is available. Considerable amounts of litter were reported in the eastern Ligurian trawling grounds (400-700 m depth) already in the '70s and ’80s, with more than 50 kg of debris in one-hour hauling (Relini et al., 1989). Marine litter was described as ubiquitous in coastal areas, with higher abundance in the proximity of the river mouths. By that time, shipping was identified as the major source of litter, with the distribution of debris reflecting the main maritime traffics routes, with important accumulation areas between the harbors of Vado Ligure, Savona and Genova (Relini, 1972a, 1972b). Oil and chemical barrels, paint jars, food containers, cans, bottles and other materials related to the onboard activities, as well as lost fishing gears, were the most common items retrieved. Furthermore, military waste was sunk after WWII along the coasts (Fusco, 1968; Serena et al., 2011,) as well as residues (especially cars) from the common flooding occurring in Liguria (Relini, 1972a).

The anthropogenic marine debris stranded on the easternmost beaches of the Ligurian Sea has been recently investigated by Giovacchini et al. (2018), reporting densities higher than 10,000 items ha^{-1}. At twilight depths, some data have been collected by means of Remote Operated Vehicles (ROV) on the Santa Lucia Bank (140-210 m) and on the Mantice Shoal (70-150 m), respectively from the eastern and from the central Ligurian Sea (Bo et al., 2014). The density of litter in these two sites is comparable with the mean density obtained from the present study (Tab. 4). More specifically, the highest densities were reported from the Mantice Shoal, which is shallower and coastal and nearby the large harbor of Vado Ligure, whereas lower values were reported from the Santa Lucia Bank, a seamount about 50 km far from the coast, mainly used by recreational fishermen.

All these previous data are consistent with the present findings regarding the litter composition and the role of some environmental factors and anthropic pressures in determining the final litter distribution. The litter composition in Liguria reflects the situation observed in

	Location	Year	Method	Depth range (m)	Mean density (items ha⁻¹)	Reference
Ligurian Sea	Ligurian Sea	2012-2017	ROV	69	1524.3 (± 137.7)	**This study**
	Mantice Shoal	2012	ROV	70-150	1489.16	Bo et al., 2014
	St. Lucia Bank	2012	ROV	140-210	609.15	Bo et al., 2014
Gulf of Lion	Gulf of Lion	2009	Trawl	85	0.4 (± 0.1)	Pham et al., 2014
	Gulf of Lion	1992-1998	Trawl	0-800	1.43 (± 0.19)*	Galgani et al., 2000
	NW Mediterranean	1992-1998	Trawl	0-800	19.35 (± 6.33)*	Galgani et al., 2000
	Cape de Creus	2009	Trawl	40-80	97.6 (± 151.6)	Sanchez et al., 2013
Tyrrhenian Sea	Tuscany coast	2009	Trawl	40-80	60.0 (± 35.2)	Sanchez et al., 2013
	Eastern Corsica	1992-1998	Trawl	0-800	2.29 (± 0.72)*	Galgani et al., 2000
	Campania	2010	ROV	30-300	1200	Angiolillo et al., 2015
	Vedove Shoal (Campania)	2010	ROV	160-260	2248	Bo et al., 2014
	South Sardinia	2011	ROV	40-290	300	Angiolillo et al., 2015
	Sicily	2011	ROV	30-270	900	Angiolillo et al., 2015
Mediterranean Sea	Marco Bank (Sicily Channel)	2010	ROV	240-280	892	Bo et al., 2014
	Malta	2005	Trawl	50-700	1.02	Misfud et al., 2013
	Ionian coast (Greece)	2009	Trawl	40-80	25.2 (± 7.8)	Sanchez et al., 2013
	Adriatic Sea	1992-1998	Trawl	0-800	3.78 (± 2.51)*	Galgani et al., 2000
	Murcian coast	2009	Trawl	40-80	43.1 (± 65.1)	Sanchez et al., 2013
North-estern Atlantic	Norwegian margin	2007	MS	304	9.7 (± 3.8)	Pham et al., 2014
	Baltic Sea	1992-1998	Trawl	0-800	1.26 (± 0.82)*	Galgani et al., 2000
	North Sea	1992-1998	Trawl	0-800	1.56 (± 0.37)*	Galgani et al., 2000
	English Channel	1992-1998	Trawl	0-800	0.18 (± 0.07)*	Galgani et al., 2000
	Bay of Saine	1992-1998	Trawl	0-800	0.72 (± 0.06)*	Galgani et al., 2000
	Celtic Sea	1992-1998	Trawl	0-800	5.28 (± 2.47)*	Galgani et al., 2000
	Bay of Biscaye	1992-1998	Trawl	0-800	1.42 (± 0.25)*	Galgani et al., 2000

Table 4. Comparison of marine litter densities reported from different areas of the European and Mediterranean seas. Numbers in parenthesis indicate standard errors (or standard deviation when the asterisk is present).

other areas of the world, with plastic (including fishing gears) being always predominant (Galgani et al., 2000, 2013; Thiel et al. 2013; Topçu et al. 2013; Pham et al., 2014; Bergman et al., 2015).

Differences between the investigated Ligurian sites were detected both in terms of litter densities and composition and can be attributed to site-specific environmental characteristics or stressors present in the area. The occurrence of vertical rocky cliffs, for example, represents the main cause of fishing gears loss, probably because the rough hard bottom causes the snagging of the lines and nets (Ayaz et al., 2010; Yıldız and Karakulak, 2016). The highest densities of ALDFG are found on two rocky shoals characterized by a distinct topography. The Maledetti Shoal (site E02, almost 7900 ALDF ha^{-1}) is a 1 km long vertical wall, ranging from 55 to 80 m depth, highly frequented by artisanal and recreational fishermen employing trammel nets, longlines, and vertical lines. Fishermen consider very difficult to operate in this area, and the likelihood to entangle on the sea bed and to lose the gears is very high (see Chapter 2 of this thesis). Similar consideration could be made for the deep shoal of Capo Mele (site C02, more than 5100 ALDFG ha^{-1}). The absence of ALDFG from the site C06 (Isola Gallinara), mainly dominated by soft bottom, supports his hypothesis (Tab. 3).

The distance from the coast could be another factor influencing the final distribution of marine litter on the seabed, with offshore sites generally considered more pristine (Bo et al., 2011). In this study, however, all the sites laid within 6.3 km from the coast, hence could all be ascribable to the coastal range. This pattern is particularly evident for urban litter, while ALDFG is widely distributed also offshore due to the high exploitation of deep rocky shoals. Indeed, ALDFG density shows a feeble increment also with the depth, confirming this hypothesis.

As already reported for the Ligurian Sea by Relini (1989), Rech et al. (2014) and Sandri and Thompson (2014), rivers play an important role in transporting litter to the sea. In this study, only a weak correlation was observed between the amount of urban litter and the extension of the catchment surface of the rivers flowing in the sea within 5 km from each investigated site. Ligurian rivers present predominantly torrential streams, and the transport of debris into the sea could be extremely variable, with high peaks (generally in autumn with the rainy season) followed by long periods of quiet characterized by no plumes and hence no accumulation.

Among the anthropogenic pressures investigated in this study, we found that the number of inhabitants was not related with the amount of ALDFG on the seabed, and only a very weak correlation was observed with the presence of urban litter. This finding suggests that the major source of littering in the Ligurian Sea is not land-based. Consistent with this hypothesis, the number of berths and the number of fishing vessels in the nearby harbors are the factors that better explain the abundances of marine litter along the Ligurian deep continental shelf.

The composition of the debris allows recognizing the principal fishing categories responsible for the presence of litter at mesophotic depths mainly over rocky elevations, namely artisanal and recreative fishing. The observed fishing debris are often highly encrusted by

benthic organisms, with corallinaceous algae, sponges, hydrozoans, alcyonaceans, serpulids and bryozoans being the most recurrent (Fig. 3a, c). In these observations, all the degrees of colonization have been reported, with some gears almost completely clean (Fig. 3d). This fact suggests that the loss or abandon of gears is still in place, and urgent actions are required in order to mitigate the problem.
In this regard, this dataset represents an important regional georeferenced baseline to implement future monitoring as well as recovery projects. The removal of derelict fishing gears, for example, has already been adopted as a strategy to improve the environmental quality of the sea bottom (Macfadyen et al., 2009; Gilman, 2015). Furthermore, the stakeholders' involvement and the presence of well-organized services of litter disposal in all harbors are objectives of primary importance, as well as the application of fishing restrictions within the sensitive areas.

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Supplementary material 1. List of the 76 major Ligurian rivers with the size of each hydrographic basin reported in km^2.

Water body	Size hydrographic basin (km²)	Water body	Size hydrographic basin (km²)
Rio San Luigi	3	Quiliano	52
Rio Latte	10	Molinero	9
Roja	672	Letimbro	43
Nervia	185	Rio Podestà	4
Vallecrosia	21	Sansobbia	72
Borghetto	13	Rio Sanda	4
Rio Sasso	6	Rio Rianello	3
Rio Crosio	3	Teiro	28
San Romolo	11	Rio Portigliolo	5
San Francesco	7	Arresta	21
San Martino	10	Lerone	22
Armea	38	Cerusa	23
Rio Fonti	1	Leira	27
Argentina	207	Branega	5
Caravello	7	San Pietro	4
Santa Caterina	5	Varenna	22
San Lorenzo	26	Chiaravagna	11
Prino	45	Polcevera	140
Caramagna	22	Rivi di Genova	9
Impero	95	Bisagno	95
Varcavello	6	Sturla	13
San Pietro	18	Nervi	9
Cervo	22	Poggio	5
Merula	49	Sori	13
Centa	432	Recco	22
Carenda	21	Boate	26
Varatella	44	Rupinaro	13
Casazza	3	Entella	146
Nimbalto	23	Fravega	5
Barbena	4	Barassi	6
Ranzi	2	Gromolo	23
Maremola	46	Petronio	61
Bottassano	9	Castagnola	25
Pora	59	San Giorgio	4
Sciusa	25	Ghiararo	15
Noli	10	Bacino 5 Terre	25
Crovetto	17	Bacino La Spezia	61
Segno	21	Magra	1686

Supplementary material 2. Numbers of inhabitants for the 63 Ligurian coastal municipalities.

Coastal cities	Inhabitants	Coastal cities	Inhabitants
Ventimiglia	23,926	Albisola Superiore	10,407
Camporosso	5419	Celle Ligure	5353
Vallecrosia	7032	Varazze	13,461
Bordighera	10,416	Cogoleto	9145
Ospedaletti	3386	Arenzano	11,584
Sanremo	54,137	Genova	586,180
Taggia	14,032	Bogliasco	4486
Riva Ligure	2861	Pieve Ligure	2582
Santo Stefano al Mare	2239	Sori	4404
Cipressa	1271	Recco	10,106
Costarainera	803	Camogli	5481
San Lorenzo al Mare	1373	Portofino	453
Imperia	42,322	Santa Margherita Ligure	9709
Diano Marina	6004	Rapallo	29,226
San Bartolomeo al Mare	3127	Zoagli	2516
Cervo	1128	Chiavari	27,338
Andora	7470	Lavagna	12,579
Laigueglia	1800	Sestri Levante	18,172
Alassio	11,026	Moneglia	2890
Albenga	23,576	Deiva Marina	1438
Ceriale	5815	Framura	683
Borghetto Santo Spirito	5154	Bonassola	995
Loano	11,563	Levanto	5509
Pietra Ligure	8880	Monterosso al Mare	1481
Borgio Verezzi	2327	Vernazza	941
Finale Ligure	11,724	Riomaggiore	1669
Noli	2801	Portovenere	3702
Spotorno	3886	La Spezia	92,659
Bergeggi	1126	Lerici	10090
Vado Ligure	8232	Ameglia	4484
Savona	60,661	Sarzana	21,829
Albissola Marina	5564		

Supplementary material 3. Information on the number of berths and fishing vessels registered within the Ligurian harbors.

Harbour	N° of berths	N° professional fishing boats
Ventimiglia	323	19
Bordighera	250	
Ospedaletti	390	
Capo Pino	20	
Sanremo	821	56
Arma di Taggia	126	
Riva Ligure	120	
Santo Stefano al Mare	993	
San Lorenzo	457	
Imperia	1378	78
Diano Marina	250	
San Bartolomeo al Mare	170	
Marina di Andora	865	
Alassio	550	22
Loano	855	39
Finale Ligure	590	9
Noli	21	18
Vado Ligure	102	5
Savona	530	108
Celle Ligure	80	
Varazze	707	33
Arenzano	185	9
Sestri Ponente	545	
Genova	1760	85
Nervi	60	
Camogli	200	39
San Fruttuoso	20	
Portofino	260	6
Santa Margherita Ligure	355	54
Rapallo	900	1
Chiavari	459	20
Lavagna	1400	19
Sestri Levante	150	34
Bonassola	60	
Levanto	60	7
Monterosso - Porto di Ponente	53	12
Portovenere	32	
Le Grazie	15	
Marina di Fezzano	250	
La Spezia	2282	86
Lerici	1300	13
Porticciolo di Bocca di Magra	230	
Darsena – Compagnia Vela	170	

Chapter 5

Assessing the environmental status of temperate mesophotic reefs: a new, integrated methodological approach

Abstract

Temperate mesophotic reefs (circalittoral and offshore circalittoral hard/firm habitats), host complex three-dimensional animal forests and are vulnerable habitats protected by international agreements. In order to evaluate and monitor the environmental status of these ecosystems, the multi-parametric index Mesophotic Assemblages Conservation Status (MACS) was developed, including two independent components, namely Index of Status (I_s) and Index of Impact (I_i). The I_s includes six metrics, targeting conspicuous species diversity, basal layer and canopy composition, whilst the I_i is composed by six metrics targeting siltation level, canopy condition and marine litter occurrence. Underwater video transects recorded with a Remotely Operated Vehicle (ROV) along the Ligurian and Tyrrhenian Seas offered the chance to test the index on a large geographic scale and various ecological settings, for a total of 14 sites subjected to different human pressures. MACS index showed a high potentiality to distinguish among a wide range of environmental conditions, with local critical situations mainly related to high fishing pressure. With respect to other existing indices, MACS is based on a larger number of parameters, resulting in a higher ability to disentangle natural and anthropogenic factors, hence it leads to a better interpretation of local situations. This tool represents a contribution to the requirements for the monitoring of these marine environments, set by the EU Marine Strategy Framework Directive, the Habitats Directive, and the guidelines of the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast of the Barcelona Convention.

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Additional Resources:

Ecological Indicators (2019), 102, 218-229.

1. Introduction

Mesophotic environments, receiving less than 3% of the surface irradiance, embrace the continental shelf and the upper bathyal zone between 40 and 300 m depth and include both the “circalittoral” and the “offshore circalittoral” units of the revised European Nature Information System (EUNIS) habitats classification (Evans et al., 2016). Explorations with Remotely Operated Vehicles (ROVs) showed that they are extremely rich and diverse in terms of benthic ecosystems, hosting complex three-dimensional animal forests over biogenic and rocky reefs both in tropical (Lesser et al., 2009; Kahng et al., 2010) and at temperate environments (James et al., 2017; Bo et al., in press). These explorations highlighted the ecological importance of animal-dominated habitats, mainly dominated by arborescent, structuring anthozoans, sponges and bryozoans (Lombardi et al., 2014; Gori et al., 2017; Maldonado et al., 2017). Some studies also identified the main sources of vulnerability for these habitats and structuring taxa, proved to be important indicators of stress conditions (Bo et al., 2014; Gori et al., 2017; Kaiser et al., 2018). Since these ecosystems provide goods and services for the society (Folkersen et al., 2018), understanding their functioning and assessing their environmental status are topics of primary interest.

The European Union Marine Strategy Framework Directive (MSFD) (European Commission, 2008) has been elaborated as a result of a growing international interest towards an ecosystem-based management of marine environments, including scientific-based reactive criteria. Eleven descriptors have been identified to evaluate the reaching of the Good Environmental Status (GES) (European Commission, 2008, 2017; Van Hoey et al., 2010; Borja et al., 2011). Benthic ecosystems are crucial elements for many MSFD descriptors (in particular D1 – Biological diversity, D6 – Seafloor integrity, D10 – Litter), for the reporting under Article 17 of the Habitats Directive (Council of the European Union, 1992) and for the IMAP guidelines (Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coast) (UNEP-MAP, 2008) of the Barcelona Convention. In this regard, ecological indices are effective tools to evaluate and monitor over time the status of marine benthic ecosystems (Bianchi et al., 2012; Appolloni et al., 2018). In the last two decades many indices have been developed for the Mediterranean benthic ecosystems, including soft bottoms, seagrass meadows, underwater marine caves, as well as shallow-water hard bottoms and coralligenous reefs (for a review of the indices see Diaz et al., 2004; Borja et al., 2015).

Few efforts have been made to define indices for mesophotic environments based on ROV footages, resulting in two seascape approaches, namely MAES (Mesophotic Assemblages Ecological Status) (Cánovas-Molina et al., 2016) and CBQI (Coralligenous Bioconstructions Quality Index) (Ferrigno et al., 2017). These indices have been tested on a limited number of sites, with no replicates, focusing only on a limited geographic area, without reference conditions or taking into consideration only a partial set of the parameters obtainable from a detailed ROV census. The Italian MSFD protocol (MATTM-ISPRA, 2016) for monitoring mesophotic coralligenous and rocky reefs includes a standard sampling design conceived to gather various quantitative components, such as the occurrence and extent of the habitat (either biogenic or rocky reefs), the siltation level, and the abundance, status and population structure of habitat-forming megabenthic species (here animal forests), as well as presence and typology of marine litter.

This paper aims at proposing a new multi-parametric index that combines status and impact indicators following a DPSIR (Driving forces - Pressures - Status - Impacts - Response) approach (Elliot et al., 2017). We have named this index as Mesophotic Assemblages Conservation Status (MACS). The index integrates three descriptors included in the MSFD to define the environmental status of seas, namely biological diversity, seafloor integrity, and marine litter (European Commission, 2008, 2017). This is also in line with the IMAP guidelines of the Barcelona Convention (UNEP-MAP, 2008). The index has been calibrated on 14 temperate mesophotic reefs of the Ligurian and Tyrrhenian seas, all characterized by the occurrence of temperate reefs but subjected to different environmental conditions and levels of human pressures. Results have been validated through the application of an independent dataset of pressures, whereas the performance of MACS index was evaluated through a comparison with the two pre-existing indices (MAES and CBQI).

2. Materials and *methods*

2. 1 Study areas

This study focuses on fourteen rocky reefs located at circalittoral depths within the central-western Mediterranean Sea (Fig. 1, Tab. 1). The nine sites of the Ligurian Sea were selected because representative of different environmental settings. The sites of Santo Stefano and Diano Marina lie in the western sector of the Ligurian continental shelf, characterized by a narrow extension and a steep slope, cut by several canyons (Cattaneo-Vietti et al., 2010). The overall cyclonic circulation causes the upwelling of deep waters through the canyons, enhancing the productivity of the area. The eastern Ligurian continental shelf is characterized by a large extension and a gentle slope, and is interested by higher levels of siltation, partially due to the conspicuous inputs of the River Magra. The sites of Punta Manara, Punta Baffe and Punta Mesco lie within this area. The central sector is characterized by a high urbanization of the coast and the presence of large harbour areas hosting important fishing fleets. Within this sector, the sites of Vado Ligure and Savona are still interested by the presence of the western canyon systems, whereas the sites of Sori and Isuela are not. The sites of Punta Mesco and Isuela lie within the Marine Protected Areas of Cinque Terre and Portofino, respectively.

Five additional sites were selected from the Tyrrhenian Sea. The site of Vercelli Seamount lies in the middle of the Tyrrhenian Sea, far away from major sources of anthropogenic impact, and could therefore be considered as an off-shore semi-pristine reference area (Bo et al., 2011). The site of Favazzina, in Calabria, is known to host valuable animal forests subjected to low fishing pressures (Bo et al., 2009). This site was included in the analysis because it represents a coastal semi-pristine site. The site of Punta delle Oche, in South-

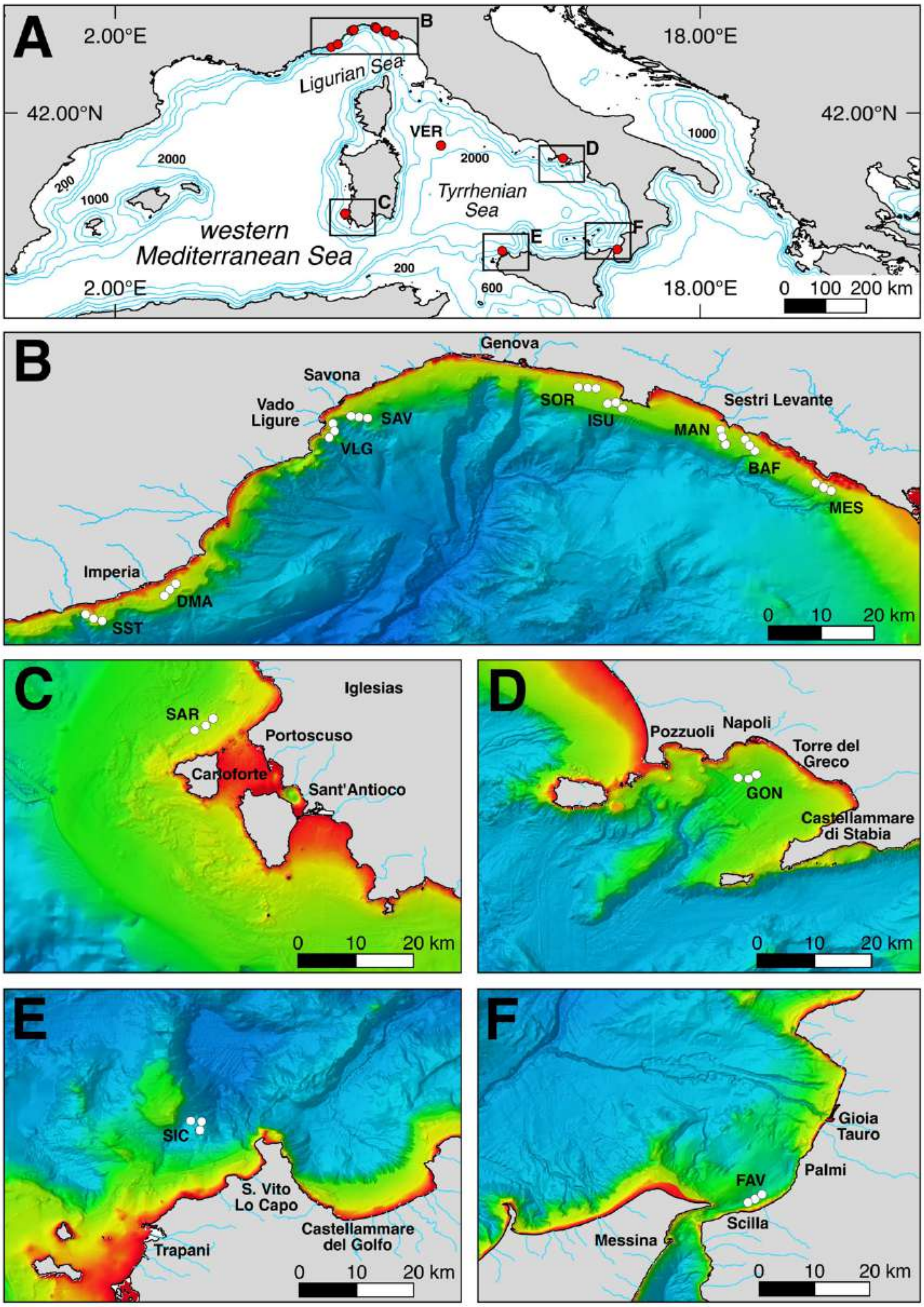

Figure 1. Map of the western Mediterranean Sea (A), showing the location of the 14 investigated sites (red dots). Rectangles and related close-ups indicate the position of the three ROV transects recorded within each site (white dots), the seafloor topography, the main rivers and urban centers of the area. B) Ligurian Sea; C) South-western Sardinia; D) Gulf of Naples; E) North-western Sicily, and F) South Calabria. SST, Santo Stefano; DMA, Diano Marina; VLG, Vado Ligure; SAV, Savona; SOR, Sori; ISU, Isuela; MAN, Punta Manara; BAF, Punta Baffe; MES, Punta Mesco; VER, Vercelli Seamount; SAR, Punta delle Oche; GON, Campo Scogli; FAV, Favazzina; SIC, Scuso Bank. Refer to Tab. 1 for the code of each site. Bathymetry has been downloaded from http://www.emodnet-bathymetry.eu.

Site	ID Code	Reference basin	Depth range (m)	Average slope (°)	Average hard bottom (m^2)
Santo Stefano	SST	Western Ligurian Sea	52-92	53	78
Diano Marina	DMA	Western Ligurian Sea	50-53	36	77
Vado Ligure	VLG	Western Ligurian Sea	48-67	49	55
Savona	SAV	Western Ligurian Sea	56-84	60	81
Sori	SOR	Eastern Ligurian Sea	34-53	34	74
Isuela	ISU	Eastern Ligurian Sea	32-54	59	77
Punta Manara	MAN	Eastern Ligurian Sea	55-71	25	24
Punta Baffe	BAF	Eastern Ligurian Sea	37-70	30	41
Punta Mesco	MES	Eastern Ligurian Sea	36-58	53	42
Vercelli Smt.	VER	Tyrrhenian Sea	81-106	58	74
SW Sardinia	SAR	Tyrrhenian Sea	86-73	23	69
Gulf of Naples	GON	Tyrrhenian Sea	119-144	41	52
Favazzina	FAV	Tyrrhenian Sea	69-84	66	89
Scuso Bank	SIC	Tyrrhenian Sea	108-139	43	67

Table 1. General information on the 14 sites investigated in this study.

western Sardinia, was selected as representative of a deep coralligenous area impacted by selective red coral harvesting (Cau et al., 2016). The last two sites included in the analysis, Scuso Bank in the Strait of Sicily, and Campo Scogli in the Gulf of Naples, are considered highly impacted, with the first one located off-shore and the second one coastal and located in one of the most polluted area of the Mediterranean Sea (Bavestrello et al., 2014; Bo et al., 2014).

2. 2 Data acquisition

The nine sites selected within the Ligurian Sea were investigated during two oceanographic surveys on board of R/V *Astrea*, conducted in August 2015 and September 2016 by ISPRA, the Italian National Institute for Environmental Protection and Research (Rome). Three replicated video-transects, each at least 200 m long, were collected in each site (SM1). Footages were obtained by means of the ROV Pollux III, equipped with a digital camera (Nikon D80, 10 megapixels), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), lights, and a 3-jaw grabber. The ROV hosted also an underwater acoustic positioning system (Ultra Short Baseline Linquest Tracklink 1500 MA), a depth sensor, and a compass to obtain georeferenced tracks overlapped to multibeam maps. Two parallel laser beams provided a 8 cm scale for size reference. In order to guarantee the best quality of video footages, ROV moved along linear tracks, in continuous recording mode, at constant slow speed (<0.3 m sec^{-1}) and at a constant height from the bottom (<1.5 m), thus allowing for adequate illumination and facilitating the taxonomic identification of the megafauna.

The additional five sites were investigated during previous oceanographic surveys conducted on board of R/V *Astrea* by ISPRA between 2009 and 2012, with the exception of the site of Vercelli Seamount, explored in 2009 with the ROV Pluto (Gaymarine, Switzerland) on board of *Daedalus* catamaran. The ROV Pluto was equipped with a digital camera (Nikon Coolpix 8700, 8 megapixel), a high definition video camera (Sony DV 3CCD mod 950), an underwater acoustic tracking position system providing records of its track along the seabed (HDR, Gaymarine ultrashort baseline operating with a 30 kHz responder), a depth sensor, a compass, and two parallel laser beams (90° angle) providing a 10 cm scale for measuring.

A total of 42 standard 200 m-long video transects were obtained from 26 ROV paths on average 806 ± 75 m long (from 373 to 1761 m). Transects were positioned along dive tracks by means of QGIS software editing. Each video transect was analyzed through ROV-imaging technique with Apple Final Cut Software, using starting and end time of the transect track as reference. Visual census of megabenthic species was carried out along the complete extent of each 200 m-long transect and within a 50 cm-wide visual field, for a total of 100 m^2 of bottom surface covered per transect.

Following the methodological Italian protocol on MSFD Habitat 7 (MATTM-ISPRA, 2016) regarding coralligenous and rocky reef habitats, the following parameters were collected:

I. Extent of hard bottom calculated as percentage of total video time showing this type of substratum (rocky reefs and biogenic reefs) and subsequently expressed in m^2;

II. Species richness, considering only the conspicuous megabenthic sessile and sedentary species of hard bottom in the intermediate and canopy layers (*sensu* Gatti et al., 2015a). Organisms were identified to the lowest taxonomic level and counted. Fishes and encrusting organisms have not been considered, as well as typical soft bottoms species. Some hard bottom species, especially cnidarians, can occasionally invade soft bottoms by settling on small hard debris dispersed in the sedimentary environment (Morri et al., 1991). For this reason, typical hard bottom species (*e.g.*, *Eunicella verrucosa*) encountered on highly silted environments have been considered in the analysis;

III. Structuring species were counted, measured (height expressed in cm) and the density of each structuring species was computed and referred to the hard bottom surface (as n° of colonies or individuals m^{-2});

IV. The percentage of colonies with signs of epibiosis, necrosis and directly entangled in lost fishing gears were calculated individually for all structuring anthozoans;

V. Marine litter was identified and counted. The final density (as n° of items m^{-2}) has been computed considering the entire transect (100 m^2).

Within each transect, 20 random high definition pictures targeting hard bottom were obtained, and for each of them four parameters were estimated, following an ordinal scale. Modal values for each transect were calculated. Evaluated parameters included:

VI. Slope of the substrate: 0°, <30° (low), 30°-80° (medium), >80° (high);

VII. Basal living cover, estimated considering the percentage of hard bottom covered by organisms of the basal (encrusting species) and intermediate (erect species but smaller than 10 cm in height) layers: 0, 1 (<30%), 2 (30-60%), 3 (>60%);

VIII. Coralline algae cover (indirect indicator of biogenic reef), estimated considering the percentage of basal living cover represented by encrusting coralline algae: 0, 1 (sparse), 2 (abundant), 3 (very abundant);

IX. Sedimentation level, estimated considering the percentage of hard bottom covered by sediments: 0%, <30% (low), 30-60% (medium), >60% (high).

2. 3 Data analysis

2.3.1 Methodological issues

In order to test whether the size of the sampling units adopted (200 m in length) was representative of the whole megabenthic community of mesophotic reefs, seven complete video paths, ranging from 600 to 1250 m in length, were analyzed. Each video track was divided into 50m-long segments. Species accumulation curves (sample-based rarefaction curves) were then traced for each transects (Bianchi et al., 2018). Species occurring with less than three individuals (or colonies) were not considered in the analysis. Additionally, the number of species found in each 200 m-long transect was compared to the total species richness of the dive track from which it was extracted.

In order to define the minimum percentage of hard bottom assuring the reliability of a 200 m-long path conducted in fragmented habitats, the correlation between species richness and percentage of hard bottom in each site was evaluated.

2.3.2 Index metrics

The MACS multi-parametric index for the assessment of the environmental status of mesophotic reefs is composed by two independent units, the Index of Status (I_s) and the Index of Impact (I_i) (Fig. 2, Tab. 2), following the DPSIR approach (Elliot, 2017). The I_s depicts the biocoenotic complexity of the investigated ecosystem, whereas the I_i describes the impacts affecting it. The MACS index indicates the resulting environmental status of the benthic community reflecting the combination of the two units and their ecological significance. It should be noted that I_s refers to the status of the main biological components of the benthic communities, while MACS can be considered as an evaluation of the environmental status in the sense of the MSFD, which asks for assessing GES (Boero, 2014). Environmental status is the outcome of the status of benthic communities plus the amount of impacts upon them.

A total of 12 metrics was considered for the MACS. Their selection reflects the most up-to-date knowledge regarding structuring habitats, both in terms of benthic community status as well as response and resilience to impacts. All the metrics adopted belong to the list of the MSFD parameters (Borja et al., 2011), and are easily inferable from the video analysis protocol previously described; therefore, their application on

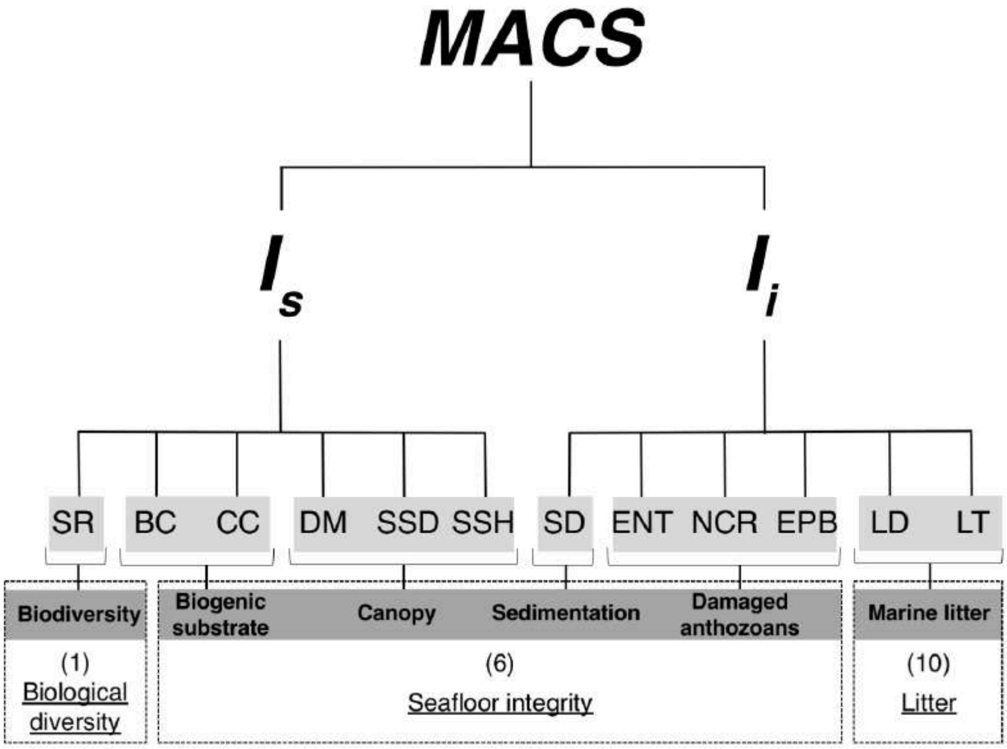

Figure 2. Scheme of the MACS index composed by two independent units, the Index of Status (I_s) and the Index of Impact (I_i). The twelve metrics composing the two units are reported in light gray. Below are represented the three MSFD descriptors and the relative indicators (dark gray). SR: species richness; BC: basal bio-cover; CC: coralline algae cover; DM: dominance; SSD: structuring species density; SSH: structuring species height; SD: sedimentation; ENT: entanglement; NCR: necrosis; EPB: epibiosis; LD: litter density; LT: litter type.

a large-scale monitoring is considered fully achievable. They are first order indices, and as such provide detailed information but also a quite dispersive array of numbers (Scianna et al., 2018). Aggregating them in second order indices concentrates the information and assures the necessary synthesis required by any index (Piazzi et al., 2017). The resulting second order indices, I_s and I_i, are both composed by six metrics. The final MACS index is a third order index, combining information from both the community status and the impact indices. For each metric, a score from 0 to 3 was assigned (Bianchi et al., 2004), following the ordinal ranks reported in Tab. 2.

The I_s analyses the status of the benthic communities by focusing on conspicuous species diversity, basal layer status and canopy condition (Tab. 2):

i) **species richness**. This indicator reflects the MSFD descriptor "Biological diversity", and considers: 1) the megabenthic conspicuous species richness (SR) as described in paragraphs 2.2.II;

ii) **biogenic substratum**. This parameter reflects the MSFD descriptor "Seafloor integrity". Here, the basal layer is investigated by ranking: 2) the basal living cover (BC); and 3) the

Components		Score 0	Score 1	Score 2	Score 3
Index of Status					
1.	Species richness (SR)	0	≤20	>20 ≤40	>40
2.	Basal bio-cover (BC)	0	<1.5	≥1.5 <2.5	≥2.5
3.	Coralline algae cover (CC)	0	<1.5	≥1.5 <2.5	≥2.5
4.	Dominance of structuring species (DM)	SSD ≤ 0.5 org. m^{-2}	1 sp.	2 spp.	≥3 spp.
5.	Density of all structuring species (SSD)	SSD ≤ 0.5 org. m^{-2}	≤2	>2 ≤5	>5
6.	Mean height of dominant structuring species (SSH)	SSD ≤ 0.5 org. m^{-2}	≤1/3 of ½ max H	>1/3 ≤2/3 of ½ max H	>2/3 of ½ max H
Index of Impact					
7.	Sedimentation (SD)	0	<1.5	≥1.5 <2.5	≥2.5
8.	Percent of entangled colonies (ENT)	0	≤20	>20≤40	>40
9.	Percent of necrotic colonies (NCR)	0	≤20	>20≤40	>40
10.	Percent of epibionted colonies (EPB)	0	≤20	>20≤40	>40
11.	Density of litter items (LD)	0	≤0.1	>0.1≤0.2	>0.2
12.	Litter typology (LT)	Absence of litter	Presence of GL	Presence of LFG	GL + LFG

Table 2. Reference scores for the 12 components used in the MACS Index. See the text for more details. GL: general litter; LFG: lost fishing gears.

coralline algae cover (CC) as mentioned in paragraphs 2.2.VII and 2.2.VIII.

iii) **canopy**. This parameter investigates the upper layer of the benthic community and reflects the MSFD descriptor "Seafloor integrity". The components involved are: 4) the dominance (DM) of the structuring species forming the canopy, classified as mono-, oligo- and polyspecific (Bianchi, 2001); 5) the density of all the structuring species (SSD); and 6) the mean height of the dominant structuring species (SSH), as mentioned in paragraph 2.2.III. The dominance of the canopy is evaluated considering the number of structuring species reaching abundance higher than the mean value per transect of the entire structuring assemblage. A lower score is given to monospecific benthic communities with respect to oligospecific and polyspecific ones (Tab. 2). The maximum height for each species was obtained from the literature (Tab. 3) and half of it was considered has a reference condition for the species population (Linares et al., 2008;

Sini et al., 2015). Scores were thus assigned considering one third and two thirds of the half of the maximum height (Tab. 3). For cumulative density of the structuring species equal or lower than 0.5 organisms m^{-2}, zero is assigned to the three metrics of the parameter canopy.

The I_i includes six components and focuses on the siltation level of the environment, the percentage of damaged structuring anthozoans, and the occurrence of marine litter (Tab. 2):

iv) **sedimentation**. This parameter reflects the MSFD descriptor "Seafloor integrity": 7) the percentage of hard bottoms covered by sediments (SD) is calculated as specified in paragraph 2.2.IX.

v) **damaged** anthozoans. This parameter reflects the MSFD descriptor "Seafloor integrity". Due to their documented response to anthropogenic mechanical pressures (Bavestrello et al., 1997; Bo et al., 2014), structuring anthozoans were chosen to depict the health status of the megabenthic communities. The investigated components include: 8) the percentage of colonies directly entangled with marine litter or fishing gears (ENT); 9) the percentage of colonies showing necrotic portions (NCR); and 10) the percentage of colonies showing portions with epibionts (EPB), as specified in paragraph 2.2.IX.

vi) **marine litter**. This section refers to MSFD descriptor "Litter" and considers two different aspects as mentioned in paragraph 2.2.V: 11) the density of litter items observed in each video-transect (LD); and 12) the type of garbage (LT), distinguishing the occurrence of general litter (score 1) and lost fishing gears (score 2). The highest score (score 3) is assigned when both typologies occur (Tab. 2).

2.3.3 Reference conditions definition

The identification of reference conditions is of fundamental importance when evaluating the ecological status of a habitat (Borja et al., 2012). Different methods are commonly used to this purpose, including: i) the identification of pristine situations, ii) the comparison with historical information, and iii) the development of a model (Gatti et al., 2015b). Previous studies have highlighted the rarity of pristine habitats in the Italian circalittoral zone: semi-pristine conditions persist only where the occurrence of fortuitous obstacles limits fishing activity (Bo et al., 2014, 2015; Angiolillo et al., 2015) or in particular off-shore sites (Bo et al., 2011). Historical data are not available for these ecosystems, so that only a conceptual model can be adopted. The Ecological Quality Ratio (EQR) has been commonly adopted in order to compare the observed situation with a hypothetical 'ideal' reference condition (Borja et al., 2010, 2011; Gatti et al., 2015a). Obviously, biological and environmental factors play a relevant role in defining the reference conditions. We can expect that, in the hypothetical reference condition, the parameters of the I_s reach the maximum value of 3 for each metric, corresponding to a total score of 18 (indicative of high biodiversity, high living cover in the basal layer, and well-developed canopy). Similarly, the metrics of the I_i are expected to have a zero score in the reference condition (hence, absence of siltation, of damaged structuring anthozoans, and of marine litter). Thus, each metric is normalized, by dividing for three (the maximum value expected) and multiplying for 100 (the scale of EQR).

For two metrics, namely coralline algae cover and sedimentation, EQR is defined exceptionally on a case-basis due to the fact that their natural trends are highly related to environmental factors. Coralline algae in the Mediterranean coralligenous bioconstructions, for instance, are known to develop up to 120 m depth (Ballesteros, 2006), although their maximum abundance is expected to be found at around 40-60 m in optimal conditions. For this reason, EQR for coralline algae cover considers that the maximum score of 3 can be potentially obtained only for transects up to 60 m, the score of 2 for those up to 90 m and the score of 1 is the maximum value that we can assign at depths greater than 90 m.

Shelf rocky reefs are exposed to natural sedimentation, but sedimentation level can be strongly influenced by anthropogenic uses of coastal land as well as by trawling activities (Tudela, 2000). In any cases, sedimentation level is related to substratum inclination. As the maximum angle of repose for sediment is 45° (Al-Hashemi et al., 2018), we adopted this value as the maximum inclination to score 3 for sedimentation. For more accentuate inclinations (>45°), the maximum score expected is 2. Additionally, in order to include the role of large rivers in enhancing natural sedimentation levels, score 1 is deleted to the sites lying downstream within 60 km from a river mouth with a hydrographical basin area > 1000 km^2.

Species	Score 1	Score 2	Score 3	Max size	Reference
Axinella polypoides Schmidt, 1862	≤13	>13 <25	≥25	70	Topsent, 1934
Spongia (Spongia) lamella (Schulze, 1879)	≤10	>10 <20	≥20	60	Pronzato 2018, pers. comm.
Sarcotragus foetidus Schmidt, 1862	≤8	>8 <15	≥15	50	Pansini et al., 2011
Dendrophyllia cornigera (Lamarck, 1816)	≤8	>8 <15	≥15	40	Fourt et al., 2017
Antipathes dichotoma Pallas, 1766	≤30	>30 <60	≥60	200	Bo et al., 2018
Antipathella subpinnata (Ellis & Solander, 1786)	≤25	>25 <50	≥50	150	Bo et al., 2009
Corallium rubrum (Linnaeus, 1758)	≤8	>8 <15	≥15	50	Fourt et al., 2017
Eunicella cavolini (Koch, 1887)	≤10	>10 <20	≥20	60	Sini et al., 2015
Eunicella singularis (Esper, 1791)	≤13	>13 <25	≥25	70	Linares et al., 2008
Eunicella verrucosa (Pallas, 1766)	≤13	>13 <25	≥25	80	Fourt et al., 2017
Leptogorgia sarmentosa (Esper, 1789)	≤18	>18 <35	≥35	100	Weinberg, 1976
Paramuricea clavata (Risso, 1826)	≤18	>18 <35	≥35	100	Linares et al., 2008
Paramuricea macrospina (Koch, 1882)	≤10	>10 <20	≥20	60	Grinyó et al., 2016
Acanthogorgia hirsuta Gray, 1857	≤5	>5 <10	≥10	30	Carpine & Grasshoff, 1975
Myriapora truncata (Pallas, 1766)	≤2.5	>2.5 <5	≥5	15	de la Nuez-Hernández et al., 2014
Pentapora fascialis (Pallas, 1766)	≤5	>5 <10	≥10	30	Cocito et al., 1998

Table 3. Size parameters for the 13-structuring species encountered in this study. Values are expressed in cm.

2.3.4 Combined MACS index and quality status

After transforming the scores of the 12 metrics in EQR, the two indices were aggregated for each transect using the following formulae (see paragraph 2.3.2 and Fig. 2 for acronyms explanation):

$$I_s = (EQR_{SR} + EQR_{BC} + EQR_{CC} + EQR_{DM} + EQR_{SSD} + EQR_{SSH})/6$$

$$I_i = (EQR_{SD} + EQR_{ENT} + EQR_{NCR} + EQR_{EPB} + EQR_{LD} + EQR_{LT})/6$$

The averaging approach was used because it is a simple way to aggregate multiple values as suggested in several reviews (Borja et al., 2014; Probst and Lynam, 2016), appreciable in routinely management applications.

The mean value of I_s and I_i for each site were obtained by averaging the values of the three replicated transects. A multivariate Correspondence Analysis was also carried out using the software PAST (Hammer et al., 2001), to identify which metrics were more important in distinguishing sites.

The final MACS index was then calculated for each site, combining information on community status and impact, using the following formula:

$$MACS = \frac{I_s + (100 - I_i)}{2}$$

The I_s, the I_i and the resulting index MACS are all numbers ranging from 0 to 100. Five classes of environmental quality status (following the Water Framework Directive) were defined (Tab. 4), and their boundaries selected by frequency distribution analysis (see Fig. 1 in Bianchi et al., 2012).

Index of Status	Index of Impact	MACS
≤ 35 = Bad	≤ 35 = Very low	≤ 35 = Bad
36-45 = Poor	36-45 = Low	36-45 = Poor
46-55 = Moderate	46-55 = Moderate	46-55 = Moderate
56-65 = Good	56-65 = High	56-65 = Good
≥ 66 = High	≥ 66 = Very high	≥ 66 = High

Table 4. Scores to define the categories for I_s, I_i and MACS.

2.3.5 Index validation

In order to evaluate how the three indices (I_s, I_i, and MACS) respond to different levels of human pressures, three main anthropogenic pressures were quantified within a radius of 25 km from the centroid of each site. i) The number of inhabitants of the costal municipalities, obtained from the online database of the Italian national STATistical institute – ISTAT (http://dati.istat.it), is expected to influence the levels of siltation, pollutants and marine litter in the nearby areas. Dredging activities, beaches nourishment and human use of coast and rivers, as occurs along the Italian coasts, could significantly enhance turbidity levels in the water. ii) The number of berths for each port, found on the on-line pilot's book for the Mediterranean Sea (https://www.tuttobarche.it/), represents an indirect indicator of the shipping traffic in the area, including recreational fishermen, and may represent a proxy for distribution and amount of marine litter on the seabed. Finally, iii) the number of fishing boats listed in each harbor, obtained from the EU Fleet register (http://ec.europa.eu/fisheries/fleet), provides information on the professional fishing pressure. The robustness of each index in relation to the mentioned pressures was assessed using linear regression.

2.3.6 Index performance: comparison with existing tools

The present dataset was analyzed also by means of the pre-existing tools MAES and CQBI, in order to compare outputs, and to highlight specific abilities and shortcomings of the three indices.

3. Results

3.1 Minimum length of the transect

The cumulative richness analysis of seven complete ROV paths showed that, on average, 55 ± 3.5% (SE) of the species were found in the first 200 m of the video transect (Fig. 3A). When the 200 m-long tracks were positioned in concomitance of the coral forests, as required by the Italian MSFD protocol, they contained on average 30 ± 2 species, representing 60 ± 2% of the total richness of the entire dive from which the transect was extracted.

The majority of the transects was highly fragmented, with hard bottoms occupying from 9% to 100% of the transect length (SM1); however, no significant correlation was found between the number of conspicuous species and the percentage of hard bottom in the 14 studied sites (Fig. 3B), which implies that only a limited amount of hard bottom within the transect (*e.g.*, 20%) would be enough.

3.2 General description of metrics

The sites examined exhibited a wide range of topographic characteristics (Tab. 1, SM1). The depth range investigated varied from 32 m (Isuela Shoal) to 144 m (Campo Scogli).

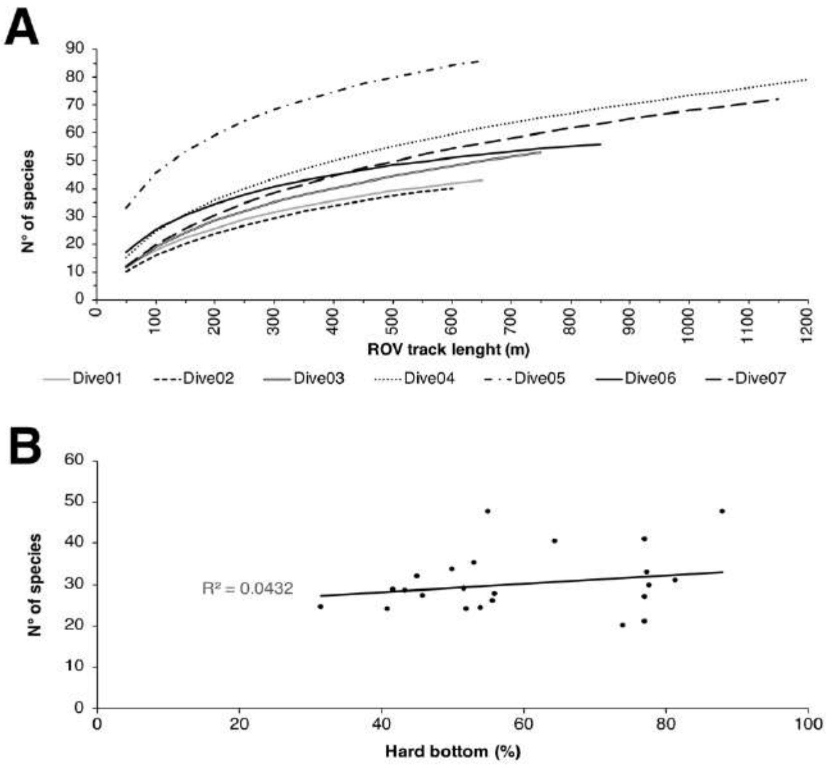

Figure 3. A) Species accumulation curves for seven full-length ROV dive paths. B) Correlation between species richness and percentage of hard bottom.

The sites with a gentler slope were those from South-western Sardinia (Punta delle Oche) and from eastern Ligurian Sea (Punta Manara and Punta Baffe), whereas the sites of Favazzina, Savona, Isuela Shoal and Vercelli Seamount exhibited a more complex topography. The mesophotic reefs of the eastern Ligurian Sea were highly fragmented, with on average only 24-42% of hard bottom. The site of Favazzina had, on average, the widest extension of hard bottom (89%).

Megabenthic species richness varied from a minimum of 12 species (Scuso Bank) to a maximum of 53 (Diano Marina). The basal living cover reached the highest values in the sites of Vercelli Seamount and Sardinia, and the lowest in the sites of Gulf of Naples and Sicily. Similarly, coralline algae cover was higher in the sites of Sardinia, Vercelli Seamount and Favazzina, very low in the eastern Ligurian Sea and completely absent in Campo Scogli (SM2).

A total of 16 structuring species (with over 8875 individuals or colonies counted) was recorded in the video-transects: three sponges (*Axinella polypoides*, *Spongia (Spongia) lamella*, *Sarcotragus foetidus*), one scleractinian (*Dendrophyllia cornigera*), two antipatharians (*Antipathes dichotoma*, *Antipathella subpinnata*), eight gorgonians (*Corallium rubrum*, *Paramuricea clavata*, *Paramuricea macrospina*, *Acanthogorgia hirsuta*, *Eunicella cavolini*, *Eunicella verrucosa*, *Eunicella singularis*, *Leptogorgia sarmentosa*), and two bryozoans (*Myriapora truncata*, *Pentapora fascialis*) (Tab. 3). Canopies were generally monospecific, with *E. cavolini* and *P. clavata* as the most commonly recorded species. Various site-specific differences were found in the metrics regarding the density and the height of the canopies (SM2).

Siltation levels were generally high in sites of Campo Scogli, Scuso Bank and central-eastern Ligurian Sea (SM2). The sites of Gulf of Naples and Scuso Bank exhibited the highest percentage of anthozoan colonies entangled by demersal fishing gears, as well as necrotic and epibiont-covered colonies. High values of epibiosis were observed also in the sites of Santo Stefano and Vado Ligure. Marine litter was virtually absent in the sites of Vercelli Seamount and Favazzina, whereas only the Sardinian transects were completely litter-free. Observed items included lost fishing gears (mainly artisanal trammel nets, longlines and ropes) and general litter (including plastic bottles and bags, tires, glass, rubber, textile and metal objects), with densities ranging from 0.01 (Vercelli Seamount) to 0.26 (Diano Marina) items per 100 m^2. Traces of lost fishing gear were present in 83% of the investigated sites, and 19% showed also general litter.

3.3 Indices outcomes

MACS ranged from 25 to 78, with I_s comprised between 30 and 81 and I_i between 15 and 80 (Fig. 4, 5, and SM2).

The highest values for the status of the benthic community were found in the sites of Vercelli Seamount, Favazzina, and Diano Marina (I_s = 81, 76, and 74, respectively), whereas the lowest values were found in the sites of Campo Scogli, Scuso Bank, and Sori (I_s = 30, 33, and 35, respectively). The highest impact was recorded in the sites of Campo Scogli, Scuso Bank, and Vado Ligure (I_i = 80, 76, and 70, respectively), while the less

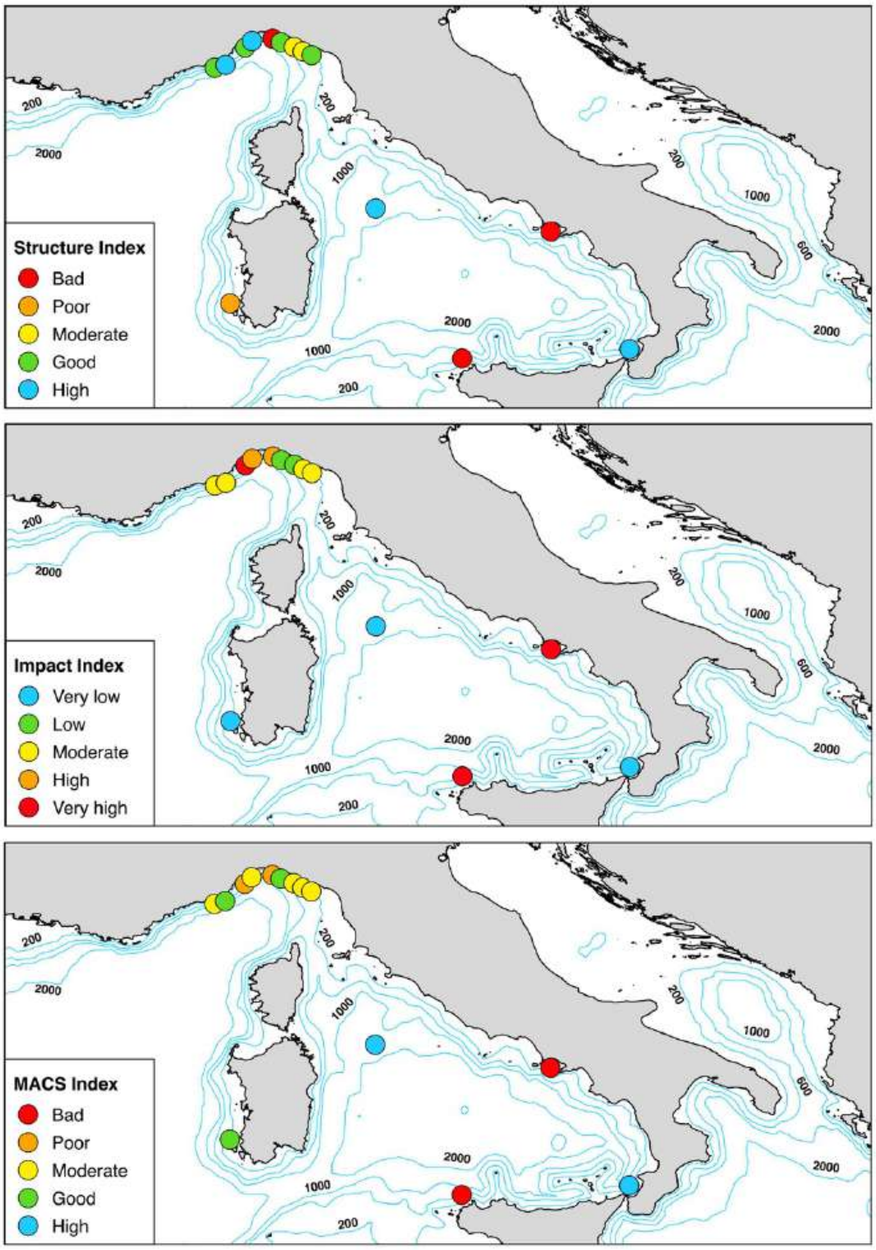

Figure 4. Results of I_s, I_i and MACS for the 14 investigated sites.

disturbed sites were Sardinia, Favazzina and Vercelli Seamount (I_i = 15, 24, and 25, respectively). Finally, the sites with the highest ecological status were Vercelli Seamount and Favazzina (MACS = 78 and 76, respectively), while the sites with the lowest values were Campo Scogli and Scuso Bank (MACS = 25 and 29, respectively).

All MACS classes were identified in the analyzed dataset (Fig. 6). Regarding the status of the community (I_s), up to 60% of the investigated sites were classified as very high and good, while up to 35% of the sites showed very bad or poor conditions in terms of anthropogenic impact (I_i).

A negative correlation between community status and impact indices was found (R^2 = 0.2867) (Fig. 5). The sites of Vercelli Seamount and Favazzina represent extreme positive conditions (very low I_i, high I_s) while the sites of Scuso Bank and Campo Scogli represent opposite situations (very high I_i, bad I_s). MACS application to the Ligurian sites highlighted three different situations (poor, moderate and good) with numerous combinations of I_s and I_i reflecting the high variability of environmental and human settings. Overall, the impact was generally moderate-high, whereas the community status ranged from bad to high. More specifically, sites characterized by a good environmental status (Diano Marina, Isuela) showed a combination of high-good I_s and moderate-low I_i. The sites characterized by a poor environmental status presented a combination of bad I_s and I_i (Sori) or a well-structured benthic community highly impacted by fishing gears (Vado Ligure). The sites characterized by a moderate environmental status might show a wide array of situations: moderate I_s and moderate-low I_i were found in two sites (Punta Manara, Punta Baffe); otherwise, such a moderate environmental status was generally given by good-high I_s combined with moderate-high I_i (Santo Stefano, Savona, Punta Mesco). The Sardinian site was the only one characterized by a poor I_s and a very low I_i, resulting in an overall good status.

In the Correspondence Analysis (Fig. 7), the first axis, mainly related to the presence of coralline algae according to depth, explained 45% of the variance, whereas the second axis explained 28% and was mainly related to the characteristics of the canopy and to entanglement, necrosis and epibiosis.

Generally, in the sites characterized by a high-good environmental status, diversity, basal living cover, and coralline algal cover represented the driving components, together with limited signs of damage on a well-structured canopy and scarce presence of litter. On the contrary, in sites characterized by a poor-bad environmental status, the benthic community was dominated by a poorly structured, highly impacted canopy, as well as by low bio diversity levels coupled with high occurrence of litter and siltation levels. Sites characterized by a moderate environmental status generally exhibited structured canopies with an abundant basal living cover, moderate siltation levels and high abundance of litter.

3.4 Indices validation

Number of inhabitants, berths and fishing vessels produced similar effects on the three indices (Fig. 8). Negative correlations with the I_s (R^2 = 0.253, R^2 = 0.214, R^2 = 0.519, respectively) and MACS index (R^2 = 0.314, R^2 = 0.310, R^2 = 0.241, respectively) were always observed, whereas positive correlations with I_i were found (R^2 = 0.233, R^2 = 0.261, R^2 = 0.034, respectively).

3.5 Index performance: comparison with MAES and CQBI

Performance of MACS was tested against the outputs of two pre-existing indices dealing with the use of ROV-derived metrics in mesophotic contexts (Fig. 6). MAES and CQBI outcomes resulted in a percentage of sites classified as moderate/medium of 71% and 57%, respectively, while with MACS this percentage was reduced to 36%. The MAES index identified a good ecological status for the sites of Vercelli Seamount and Favazzina, a bad ecological status for the sites of Campo Scogli and Scuso Bank, and a medium status for all the remaining sites. A similar output was obtained with the application of the CQBI, which reported a good ecological status also for the sites of Isuela and Sardinia.

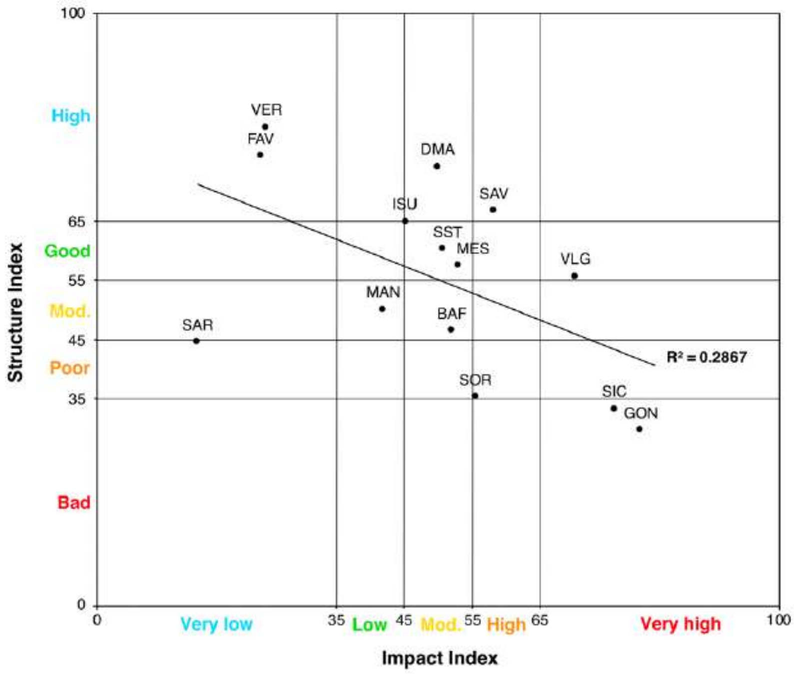

Figure 5. Correlation between I_s and I_i for the investigated sites.

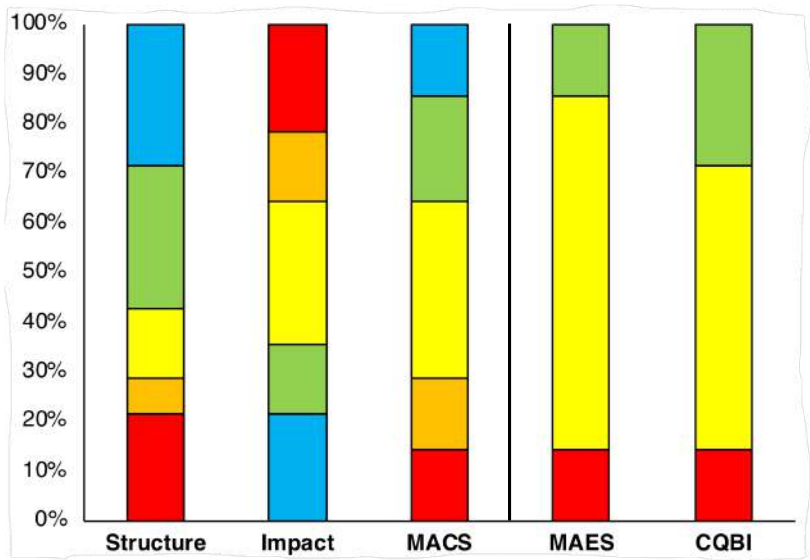

Figure 6. Indices comparison: the outputs of MACS (and related indices), MAES and CQBI for the 14 investigated sites are here presented as percentage composition of the various outcoming classes. Colors used for Status, Impact and MACS refer to the five categories presented in this study, while for MAES and CQBI, colors were adapted to the status classes reported in the respective papers (MAES, bad, moderate, good; CQBI, bad, medium, good, optimum).

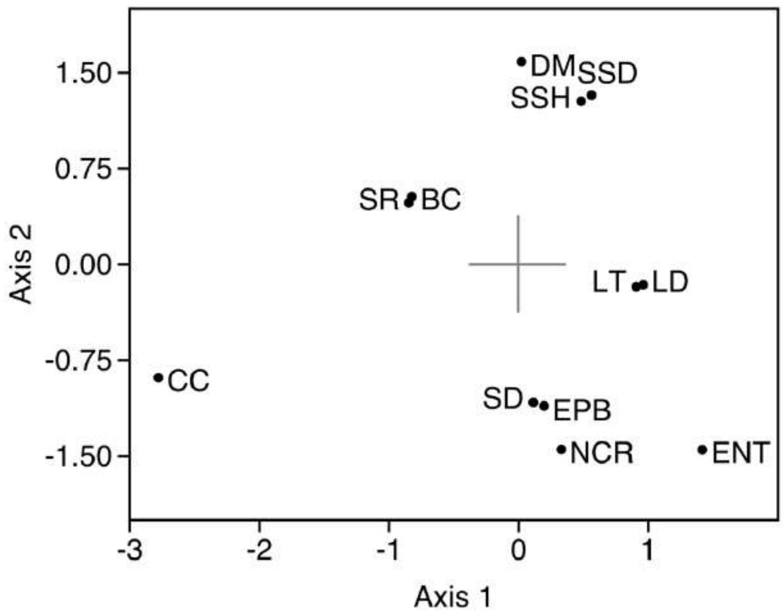

Figure 7. Multivariate Correspondence Analysis of the 12 MACS metrics in the 14 investigated sites.

4. Discussion

This study describes the structure and application of a multi-parametric index (MACS) for a large-geographic environmental status assessment of mesophotic temperate reefs. This index takes into consideration the most recent European legislation (European Commission, 2008, 2017) targeting, through a holistic functional ecosystem-based approach, the protection and restoration of marine ecosystems (Browman et al., 2004; Borja et al., 2008, 2010).

The MACS index is composed of metrics obtainable from the analysis of ROV or technical diving video footages. Both these methodologies have been largely employed in the last 10 years as a scientific tool for deep Mediterranean explorations (Gori et al., 2017), but only limited experiences used the ROV footage to elaborate ecological indicators (Cánovas-Molina et al., 2016; Ferrigno et al., 2017). ROV offers various advantages including a seascape approach, a wide geographic coverage, and virtually no bathymetric limits. The methodology herein proposed focuses on conspicuous species that are relatively easy to identify, therefore minimizing the observer effect and the specialist-oriented analysis, and maximizing the taxonomic resolution. The natural history of the target megabenthic species is usually well known, allowing for an easier identification of the responses to various natural and human pressures such as fishing, sedimentation, and mass mortalities. Disadvantages related to the employment of this technique include relatively high operative costs, low taxonomic resolution in the basal layer species identification, and influence of the resolution of the ROV footage on the quality of the output.

From a methodological point of view, the 200 m-long transects are representative of the overall diversity of the studied environments, and the survey proved to be efficient also in areas with highly fragmented hard bottoms. These results support the adaptability of the method underlying the index to a wide array of habitat situations.

The MACS index shows a high potentiality to distinguish among a wide range of ecological conditions, efficiently disentangling natural patterns from human-induced ones, hence leading to a better interpretation of local situations. Correlations were observed between the indices outputs (I_s, I_i and MACS) and the investigated anthropogenic pressures, as clearly demonstrated by the classification of the reference sites. In the case of the Ligurian continental shelf, four main pictures are highlighted: a reduced fishing effort, as well as a reduced urbanization, supports the overall moderate-good environmental status of the western sites. High siltation levels related to the vicinity to a major river outflow mainly drive the moderate status of the eastern Ligurian sites. In the central Ligurian Sea, large harbors and high fishing effort decrease the environmental status, which potentially could be higher due to heterogeneous topography and proximity to canyon systems, enhancing the resilience of the benthic communities to impacts. In this same region, high urbanization and highly fragmented hard-bottoms may also lead towards poorly structured benthic communities. Partially, the effect of Marine Protected Areas might lead towards higher environmental status (*e.g.* Isuela, Punta Mesco).

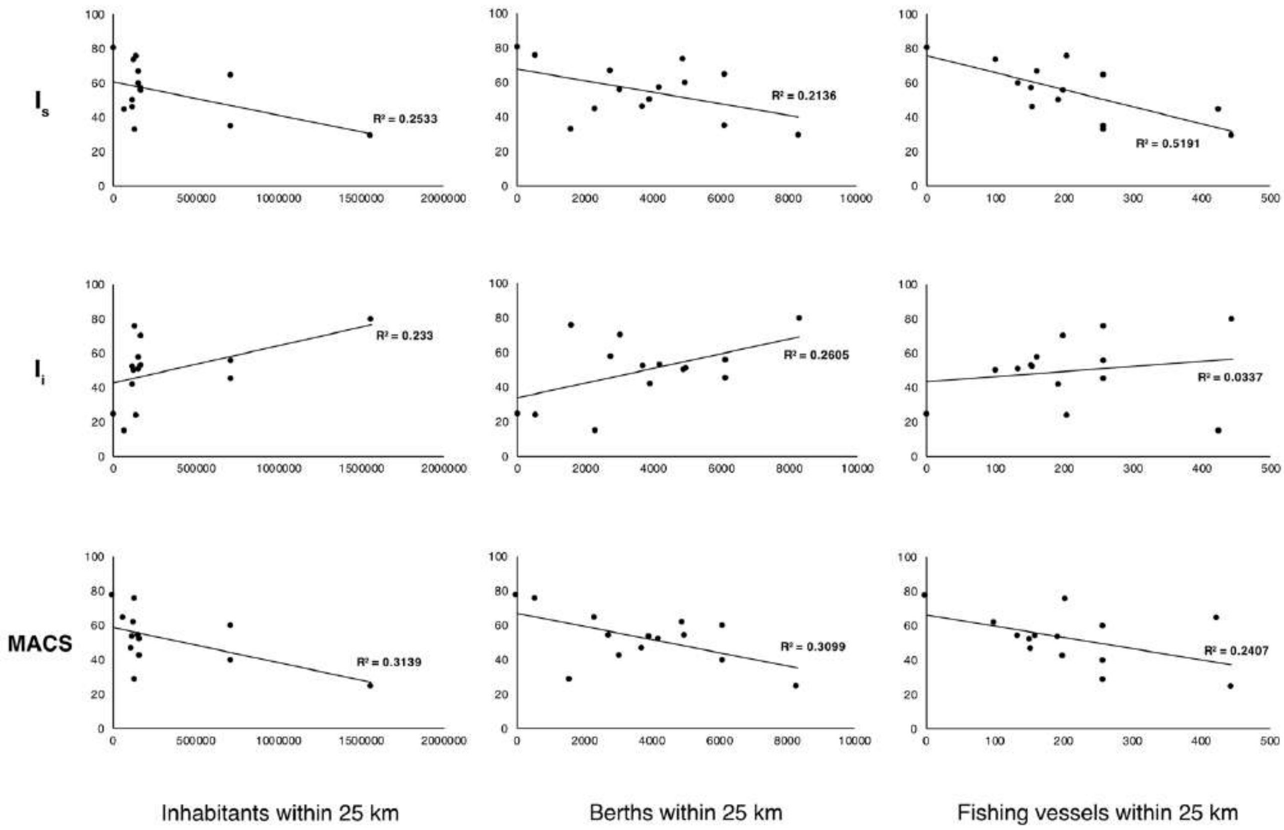

Figure 8. Linear correlation of I_s, I_i and MACS indices with the anthropogenic pressures in a 25-km radius around each investigated site.

Hard-bottom structuring species are a focal point in the MACS index. Focusing on the canopy-forming species is not related to methodological and taxonomic issues only, but also to ecological ones. The selected metrics involving structuring, long-living species (such as carbonatic organisms and arborescent gorgonians and antipatharians) are effective indicators of disturbance and recovery ability (Bianchi et al., 2017), in particular the species abundances and changes in size (Kaiser et al., 2018). With regards to the change in size, structuring anthozoans were chosen to depict the health status of megabenthic communities due to their documented response to anthropogenic mechanical pressures (Bavestrello et al., 1997; Bo et al., 2014). This approach only partially works for sponges and bryozoans, more actively responding to lost fishing gears (sponges are usually less sensible to the mechanical impact of lines and calcareous branched bryozoans generally break down, so the disturbance is not detectable from the video analysis). This information is lacking for the sites with no canopy, and the output of the MACS index should be taken with caution in these cases. The site of Punta delle Oche, in Sardinia, represents a good example of rocky reef without canopy. In this site, poor community status and very low impact coexist, resulting in an overall good environmental status. Indeed, ROV exploration highlighted low levels of siltation, absence of marine litter, high basal living cover and coralline algae cover. The absence of structuring species could be related to natural characteristics of the site, but also to the selective harvesting of red coral occurring in the area since several decades (Cau et al., 2016).

With respect to existing ROV-based indices developed to assess the quality of circalittoral Mediterranean rocky reefs, *i.e.* the MAES index (Cánovas-Molina et al., 2016) and the CQBI (Ferrigno et al., 2017), the MACS index here proposed integrates a larger number of metrics that better delineate the environmental and human variability of coastal sites. The DPSIR approach (Elliot et al., 2017) adopted by MACS index, in particular, allows for efficiently explaining site-specific situations by coupling different kinds of information. The outcomes of both MAES and CQBI indices result in a greater homogenization of the Ligurian sites, mainly classified as moderate/ medium, whereas MACS index results in a wider diversification of the classes better reflecting the local situations. This also thanks to the use of I_s and I_i in the identification of the main forces driving the final environmental status of each ecosystem.

While a unique index like MACS is understandably preferred by managers and administrators, when facing conservation issues the favorite option should be always accompanying it by the two second order indices I_s and I_i, in order to clearly see what is necessary to act on. These two descriptors are expectedly inversely linked, but the different level of resistance of different benthic communities (Montefalcone et al., 2011), may make the relationship not always straightforward, with healthy benthic communities occasionally persisting in the face of relatively high impacts. As illustrated by some Ligurian mesophotic reefs (*e.g.*, Savona), the resistance of the local benthic communities can allow for rather high values of the third order - and therefore highly aggregated - index MACS even in presence of significant impacts, which might lead to neglecting the precautionary need of reducing local impacts in order to assure sustainability of the good environmental status observed.

Benthic indicators are fundamental tools to implement MSFD requirements, as they allow gathering an effective and representative picture of ecologically complex situations, so to give the synthetic information essential to identify the areas where specific management measures must be undertaken to allow achieving GES. The MACS index is here proposed as a comprehensive, seascape-approached, monitoring tool with the potential to be employed by a wide array of marine technicians over hard-to-reach environments. Results represent valuable information to be employed to evaluate improvements of the environmental status and thus the effectiveness of the measures that must be undertaken in the monitoring sites and for future coastal planning and definition of protection zones.

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Supplementary material 1. General information on the 42 analyzed video transects.

Site	Transect code	Date	Start coordinates	End coordinates	Depth range (m)	Average slope (°)	Hard bottom (m^2)
Santo Stefano	T1	12.08.15	43.7949°N; 7.9191°E	43.7943°N; 7.9171°E	52-60	21	89
	T2	12.08.15	43.8021°N; 7.9034°E	43.8015°N; 7.9023°E	58-67	65	64
	T3	13.08.15	43.8073°N; 7.8926°E	43.8060°N; 7.8922°E	82-92	73	80
Diano Marina	T1	14.08.15	43.8821°N; 8.0867°E	43.8814°N; 8.0866°E	50-53	34	58
	T2	14.08.15	43.8804°N; 8.0856°E	43.8801°N; 8.0858°E	50-53	35	83
	T3	14.08.15	43.8778°N; 8.0834°E	43.8773°N; 8.0827°E	50-53	40	90
Vado Ligure	T1	09.08.15	44.2602°N; 8.4663°E	44.2602°N; 8.4650°E	48-62	55	79
	T2	09.08.15	44.2524°N; 8.4656°E	44.2514°N; 8.4653°E	60-67	46	42
	T3	09.08.15	44.2479°N; 8.4609°E	44.2474°N; 8.4625°E	52-64	47	44
Savona	T1	03.09.16	44.2787°N; 8.5233°E	44.2784°N; 8.5231°E	64-69	56	86
	T2	03.09.16	44.2756°N; 8.5229°E	44.2759°N; 8.5240°E	77-84	58	87
	T3	03.09.16	44.2794°N; 8.5163°E	44.2785°N; 8.5164°E	56-64	66	71
Sori	T1	05.08.15	44.3608°N; 9.0874°E	44.3604°N; 9.0859°E	34-35	39	77
	T2	05.08.15	44.3528°N; 9.1031°E	44.3532°N; 9.1047°E	52-53	33	84
	T3	05.08.15	44.3537°N; 9.1070°E	44.3541°N; 9.1085°E	43-47	31	61
Isuela	T1	05.09.16	44.3371°N; 9.1489°E	44.3361°N; 9.1485°E	33-36	46	57
	T2	06.09.16	44.3205°N; 9.1448°E	44.3210°N; 9.1446°E	32-49	76	100
	T3	06.09.16	44.3202°N; 9.1438°E	44.3196°N; 9.1435°E	47-54	56	74
P.ta Manara	T1	23.08.16	44.2423°N; 9.4017°E	44.2433°N; 9.4002°E	63-67	17	9
	T2	23.08.16	44.2438°N; 9.4002°E	44.2446°N; 9.3990°E	57-65	21	24
	T3	03.08.15	44.2429°N; 9.4031°E	44.2414°N; 9.4023°E	64-71	36	39
P.ta Baffe	T1	03.08.15	44.2324°N; 9.4412°E	44.2316°N; 9.4427°E	51-56	21	47
	T2	03.08.15	44.2292°N; 9.4381°E	44.2301°N; 9.4393°E	64-70	42	12
	T3	03.08.15	44.2349°N; 9.4442°E	44.2359°N; 9.4423°E	37-39	27	64
P.ta Mesco	T1	23.08.16	44.1308°N; 9.6339°E	44.1316°N; 9.6339°E	49-58	55	61
	T2	23.08.16	44.1316°N; 9.6342°E	44.1319°N; 9.6359°E	47-53	33	18
	T3	06.09.16	44.1333°N; 9.6332°E	44.1328°N; 9.6349°E	36-45	72	46
Vercelli Smt.	T1	01.07.09	41.1068°N; 10.9065°E	41.1079°N; 10.9074°E	81-97	62	84
	T2	01.07.09	41.1092°N; 10.9095°E	41.1102°N; 10.9100°E	90-106	52	72
	T3	02.07.09	41.1102°N; 10.9100°E	41.1113°N; 10.9116°E	83-90	59	65
SW Sardinia	T1	24.10.11	39.2410°N; 8.2822°E	39.2415°N; 8.2835°E	73-79	18	79
	T2	24.10.11	39.2415°N; 8.2835°E	39.2421°N; 8.2853°E	80-86	18	83
	T3	24.10.11	39.2421°N; 8.2853°E	39.2420°N; 8.2864°E	80-85	33	44
Gulf of Naples	T1	22.06. 12	40.7572°N; 14.2518°E	40.7569°N; 14.2503°E	132-140	39	70
	T2	22.06. 12	40.7572°N; 14.2519°E	40.7576°N, 14.2524°E	139-144	34	45
	T3	22.06. 12	40.7652°N; 14.2714°E	40.7651°N; 14.2712°E	119-127	50	41
Favazzina	T1	01.09.09	38.2638°N; 15.7376°E	38.2640°N; 15.7390°E	69-84	67	94
	T2	01.09.09	38.2640°N; 15.7390°E	38.2646°N; 15.7407°E	70-86	69	90
	T3	01.09.09	38.2646°N; 15.7407°E	38.2651°N; 15.7415°E	79-84	61	82
Scuso Bank	T1	24.09.11	38.2197°N; 12.5877°E	38.2224°N; 12.5879°E	108-112	30	80
	T2	24.09.11	38.2224°N; 12.5879°E	38.2210°N; 12.5875°E	108-114	50	57
	T3	24.09.11	38.2210°N; 12.5875°E	38.219619, 12.586750	115-139	50	64

Supplementary material 2. Outcomes of I_s, I_i, MACS, MAES and CBQI.

	Status Index								Impact Index							
Code	SR	BC	CC	DM	SSD	SSH	Final value	Condition	SD	ENT	NCR	EPB	LD	LT	Final value	Condition
SST	78	100	28	44	44	67	60	Good	61	11	22	78	44	89	51	Moderate
DMA	89	100	33	78	56	89	74	High	33	33	22	33	89	89	50	Moderate
VLG	67	78	0	56	78	56	56	Good	100	33	55	55	78	100	70	Very high
SAV	67	89	44	44	89	67	67	High	83	44	33	33	67	89	58	High
SOR	56	78	33	11	11	22	35	Bad	100	0	56	45	56	78	56	High
ISU	56	100	56	44	78	56	65	Good	83	22	22	22	44	78	45	Low
MAN	33	67	0	44	78	78	50	Moderate	22	33	44	33	33	89	42	Low
BAF	56	78	11	45	45	45	46	Moderate	67	11	44	56	33	100	52	Moderate
MES	56	78	11	67	89	44	57	Good	28	44	33	33	89	89	53	Moderate
VER	78	100	83	67	78	78	81	High	50	11	22	33	11	22	25	Very Low
SAR	67	100	100	0	0	0	45	Poor	33	0	33	22	0	0	15	Very Low
GON	33	33	0	22	45	45	30	Bad	100	100	67	67	67	78	80	Very high
FAV	100	100	100	78	44	33	76	High	44	11	11	33	22	22	24	Very Low
SIC	33	33	100	11	11	11	33	Bad	89	67	67	89	56	89	76	Very high

	MACS		MAES		CQBI	
Code	Final value	Environmental Status	Final value	Environmental Status	Final value	Environmental Status
SST	55	Moderate	13	Moderate	5	Medium
DMA	62	Good	14	Moderate	5	Medium
VLG	43	Poor	12	Moderate	5	Medium
SAV	54	Moderate	13	Moderate	4	Medium
SOR	40	Poor	11	Moderate	6	Medium
ISU	60	Good	14	Moderate	7	Good
MAN	54	Moderate	12	Moderate	6	Medium
BAF	47	Moderate	12	Moderate	5	Medium
MES	52	Moderate	12	Moderate	5	Medium
VER	78	High	17	Good	8	Good
SAR	65	Good	11	Moderate	7	Good
GON	25	Bad	9	Bad	2	Bad
FAV	76	High	15	Good	7	Good
SIC	29	Bad	7	Bad	3	Bad

Conclusions

This study presents the most comprehensive characterization of the marine animal forests of the Ligurian deep continental shelf, focusing on their diversity, distribution (Chapter 1) and vulnerability (Chapters 2 and 3). Essential information for their monitoring and conservation are herein provided, including the assessment of marine litter (Chapter 4) and the proposal of an ecological index to evaluate the conservation status of mesophotic assemblages at temperate latitudes (Chapter 5).

Most of the results presented in this thesis derive from the employment of non-destructive techniques, whereas the majority of the samples collected to quantify the impact of specific *métier* is based on by-catch data. ROV-video Imaging allows extrapolating quantitative information about species abundance and distribution over a large geographic scale, accompanied by the collection of a limited number of organisms to confirm species identification. Up to 224 invertebrate species (and morphotypes) have been identified in this study, validating the use of this technique for the characterization of mesophotic megabenthic assemblages. Video-based samplings are currently considered a valid alternative to traditional methods employed to study deep-sea benthic environments. This approach avoids the detrimental consequences of using destructive methods, and it is especially preferable for the study of vulnerable species, often characterized by slow growth and high longevity.

Part 1 – Biodiversity

Chapter 1 reports the most comprehensive characterization of the megabenthic communities ever made for the Italian coasts of the Ligurian deep continental shelf, revealing the extraordinary diversity of the invertebrate megafauna thriving in this area. By comparing these results with analogous investigations conducted within the Mediterranean Sea, the number of megabenthic morphospecies herein identified is significantly higher. Up to twelve megabenthic assemblages have been identified by means of multivariate statistical analyses, including: (1) bryozoan beds dominated by *Pentapora fascialis*, (2) dense hydrozoan forests of *Lytocarpia myriophyllum*, (3) sponge grounds dominated by the large massive keratose demosponge *Sarcotragus foetidus*, (4) deep rocky bottom communities colonized by serpulids worms and hosting holothurians and sea urchins, (5) gorgonian forests of *Eunicella cavolini* and (6) *Paramuricea clavata*, (7) coralligenous overhangings hosting dense patches of the yellow scleractinian

Leptopsammia pruvoti and the precious red coral *Corallium rubrum*, (8) forests of the gorgonian *E. verrucosa*, (9) coral gardens of *Dendrophyllia cornigera*, (10) fields of the soft coral *Paralcyonium spinulosum*, (11) assemblages of the sponge *Haliclona* cf. *mediterranea*, and (12) dense sabellid fields dominated by *Bispira viola*. Additional relevant assemblages emerged from the analysis of the video footages, but were not detected from the community analysis because of the low number and low densities of such aggregations. For instance, six black coral forests of *Antipathella subpinnata*, two meadows of the rare gorgonian *P. macrospina*, and soft-bottom fields of pennatulaceans and soft corals were also reported from the Ligurian deep continental shelf. The distribution of these assemblages is strongly determined by different environmental parameters, namely depth, substrate type and inclination, and major differences are observed on a sub-regional scale between the western and eastern sectors, mainly related to the different topography and extent of nutrients inputs.

All the information obtained from this study has been mapped, providing a georeferenced database of the Ligurian mesophotic megabenthic species and their communities. This archive offers to managers and stakeholders the firsts necessary tools for marine spatial management initiatives required by the MSFD, leading to the identification of potential sites of ecological importance and to the designation of an effective network of MPAs along the Ligurian coast.

Part 2 – Fishing impact

A detailed quantification of the fishing effort and impact has been provided for two areas elected as representative Ligurian case studies of two different socio-economic and environmental settings hosting deep animal forests. The Maledetti Shoal (Chapter 2) is a vertical coralligenous wall lying along the mesophotic continental shelf and hosting dense gorgonian forests subjected to recreational and artisanal fisheries. By combining the data obtained from ROV monitoring with the analysis of the commercial and discard catches of the commonly employed spiny lobster trammel net, a detailed characterization of the biocoenoses as well as their fragility was achieved based on the actual efficiency of the *métier* for the site as well as the fishing effort insisting here. At present, in fact, four artisanal fishermen operate in the area (each spending 10-30 fishing days per year), whereas the number of recreational fishermen is highly underestimated. Benthic invertebrate non-commercial discard, mainly represented by erect bryozoans and flexible gorgonians, characterizes the catches both in terms of the overall diversity and number of individuals, whereas biogenic substratum prevails in terms of weight, with up to 4 kg of coralligenous framework collected on average every 200 m of trammel net. Based on the recorded entanglement events and the breakages occurred during the surveys, the loss risk frequency in this site is very high, around 60%, which valued the site its name, Maledetti (“damned”).

Regarding the bathyal case study, the red shrimp trawling ground of Santa Margherita Ligure (Chapter 3) is one of the most exploited in the Mediterranean basin, historically surveyed since the 1960s. The analysis of the discard lead to the finding of the rare octocoral *Placogorgia coronata* highlighting the high biological value of this area, characterized by the occurrence of an extended white coral thanatocoenosis working as a secondary substrate for many benthic species surrounded by mud plains. As in other cases, these hard elevations usually work as deterrents towards the dredging action of trawling, however, decades of exploitation is forcing the local fishing routes to shift closer and closer to the area, dislocating occasionally large portions and spreading nearby coral rubble, which is the preferential substrate for various species, including *P. coronata*. About 12 vessels operate in the fishing ground, of which seven can be considered occasional and five are frequent, each carrying out 60–120 hauls per year. The long-term monitoring of the catches stressed a high impact, with a removal rate of 1 colony every 2 trawling operations as well as frequent hard-bottom removal and gear entanglement and loss.

Following the demands of the MSFD, this chapter presented effective multidisciplinary methods to describe the sea floor integrity and its alteration by human activities, providing quantitative data describing the impact of different *métier* on distinct ecosystems. The work conducted here helped to fulfill all the criteria proposed by FAO for the identification of VMEs and thus these areas will be proposed as valuable candidates for specific conservation measures.

Part 3 – Monitoring and conservation

The abundance of marine litter on the Ligurian deep continental shelf is extremely high, with an average of 1524 items ha^{-1} (Chapter 4). This value appears even more impressive when compared with similar studies conducted within Mediterranean and European Atlantic waters. Despite the differences in abundance, the other investigated parameters, including litter composition, show strong similarities with previously investigated sites. Up to 83% of the reported items were represented by abandoned, lost or otherwise discarded fishing gears (ALDFG), with fishing lines being the most common items observed on the seabed. Urban litter accounted for 17%, and was mainly represented by plastic items and smaller quantity of glass, metal, ceramic and fabric.

Differences in litter density and composition between the Ligurian sectors were mainly related to the occurrence of vertical rocky cliffs representing the main cause of fishing gear loss. The highest density of ALDFG, indeed, was observed on two rocky shoals characterized by a distinct rough topography, namely Maledetti Shoal and Capo Mele, exceeding 7900 items ha^{-1}. Fishing activities (especially artisanal and recreative) and shipping are the major sources of litter in the Ligurian Sea, and the number of berths and fishing vessels in the nearby harbors are the factors that better explain the abundances of marine litter on the deep continental shelf. The data obtained from this study have been georeferenced and mapped, and will provide baseline information for the future monitoring of marine litter on the Ligurian deep continental shelf, as required by MSFD descriptor 10.

In this regard, benthic indicators are fundamental tools to implement MSFD requirements, as they provide synthetic and representative information of ecologically complex situations, allowing identifying the areas where specific management measures must be undertaken to allow achieving the Good Environmental Status. The multi-parametric index Mesophotic Assemblages Conservation Status (MACS) developed in this study represents a comprehensive, seascape-approached, monitoring tool (Chapter 5), providing valuable information to evaluate the ecological status of mesophotic temperate reefs dominated by animal forests, and thus the effectiveness of the measures that must be undertaken for future coastal planning and definition of protection zones. This index is based on underwater video transects recorded by ROVs or technical divers, with the potential to be employed by a wide array of marine technicians, to study hard-to-reach environments. The MACS index has been elaborated following a DPSIR (Driving forces - Pressures - Status - Impacts - Response) approach and thus it includes two independent components, namely Index of Status (I_s) and Index of Impact (I_i). The I_s includes six metrics, targeting conspicuous species diversity, basal layer and canopy composition, whilst the I_i is composed by six metrics targeting siltation level, canopy condition and marine litter occurrence. This combined structure provides a high potentiality to distinguish among a wide range of ecological conditions, with local critical situations mainly related to high fishing pressure. The index has been tested on fourteen selected sites from the Ligurian and Tyrrhenian Sea, characterized by various ecological settings and subjected to different human pressures. Furthermore, it has been validated on an independent dataset including the number of inhabitants, berths and fishing vessels present within a radius of 25 km from the centroid of each site. Negative correlations with the I_s and MACS index were always observed, whereas positive correlations with I_i were found. With respect to other existing ROV-based indices, MACS showed a stronger performance, being based on a larger number of parameters. This resulted in a higher ability to disentangle natural and anthropogenic factors, hence to a better interpretation of local situations.

Future perspectives

Results obtained in this thesis highlight the biocoenotic richness of the Ligurian mesophotic ecosystems. Their species composition, overall diversity and distribution are described here for the first time, but further studies are required to provide a better characterization of the population structure of the main forests as well as a more detailed insight into their health status. For instance, gorgonians result to be the most relevant mesophotic megabenthic assemblages and a detailed analysis of the size frequency distribution for the main species, as well as a large-scale investigation of the canopy condition (including traces of epibiosis, necrosis and entanglement) would provide important information for their conservation. Furthermore, some of the assemblages herein reported are basically unknown for the Mediterranean basin, thus it is mandatory to carry out specific characterizations focused on the Ligurian keratose sponge grounds, *Dendrophylla cornigera* bed, and sabellids fields to evaluate their structure and extent.

Results should then be employed to generate a predictive habitat distribution modelling for the whole Ligurian deep continental shelf. The production of predictive maps would provide an additional reliable tool that can be readily used for management purposes, whereas the ground truthing of so many sites would ensure high confidence of the final maps. Additionally, the wide geographic extent of the investigated area and relative dataset would allow implementing methodological studies on predictive habitat accuracy and reliability.

This thesis provides also key upshots regarding the vulnerability and the conservation of the biocoenoses inhabiting two investigated areas clearly identifiable as Sensitive Habitats. The Maledetti Shoal arises as the largest deep coralligenous cliff of the Ligurian Sea, representing a unique case of *roche du large* ecosystem, hosting the largest deep population of red coral, whereas the deep trawling ground of Santa Margherita Ligure is characterized by high topographic complexity and occurrence of rare cold-water coral species. The collected information will be used to implement a Fisheries Restricted Area procedure at GFCM for the definition of the first deep-sea Ligurian managed areas, whereas the adopted protocol is proposed as a standard method for data acquisition. As the two case-studies herein presented, other Ligurian mesophotic sites emerge for the richness of their megabenthic communities, the presence of peculiar topographical features, and show various degrees of vulnerability (Fig. 1). From West to East, the site of Bordighera hosts the best-preserved black coral forest of the Ligurian Sea, together with dense forests of *L. sarmentosa*. In the sites of Santo Stefano and Diano Marina, several types of community coexist, including gorgonian forests, sponge grounds and aggregations of hydrozoans, black corals and ascidians. The deep shoal facing Capo Mele (also known as “Secca delle Vedove”) is certainly of primary interests, due to the richness of its dense forests of *E. cavolini*, *L. myriophyllum* and beds of *P. spinulosum*, showing also the highest diversity of black corals. This site hosts the Ligurian largest population of *P. macrospina* and is also characterized by abundant marine litter. In the central area, the Mantice Shoal presents the northernmost aggregation of *D. cornigera* within the Mediterranean Sea, together with forests of *E. cavolini*, *A. subpinnata* and *P. spinulosum* beds. The shoals facing the city of Savona host gorgonian forests, sponge grounds and bryozoans and serpulids beds. In the central eastern sector, the site of Punta del Faro facing Portofino appears as a biodiversity hotspot where coralligenous overhangings coexist with deep gorgonian and black coral forests, while the nearby soft bottoms host dense aggregations of *L. myriophyllum*. Finally, in the easternmost sector of the Ligurian Sea, subjected to particularly high silting conditions, the site of Punta Manara hosts aggregations of *A. polypoides* and *E. singularis*, and the sites of Monterosso and Montenero present dense *P. clavata* and *L. sarmentosa* forests.

All these sites display remarkable features and require a distinctive characterization of their megabenthic

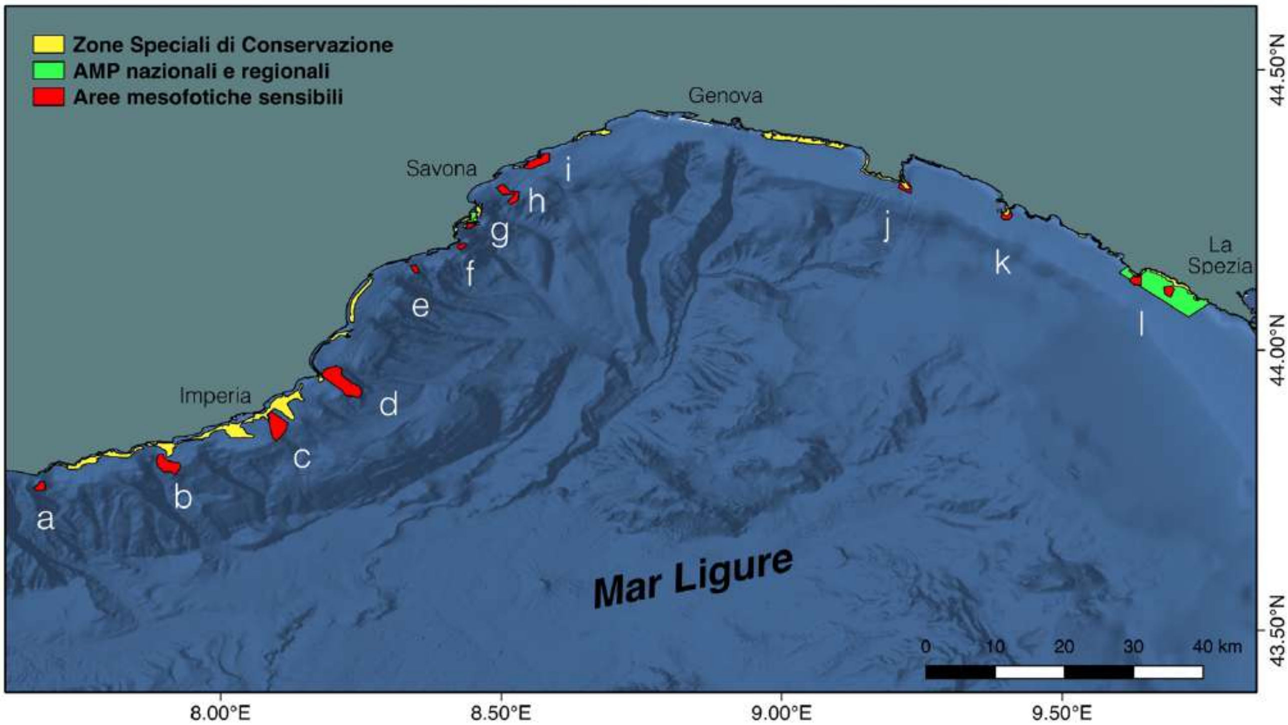

Figure 1. Spatial distribution of the mesophotic animal forests of the Ligurian Sea. a: Bordighera; b: Santo Stefano; c: Diano Marina; d: Capo Mele; e: Finale Ligure; f: Capo Noli; g: Maledetti Shoal; h: Mantice Shoal and Savona shoals; i: Celle Ligure-Varazze; j: Punta del Faro; k: Punta Manara; l: Punta Mesco and Corniglia.

communities and vulnerability. The application of the MACS index to the whole Ligurian dataset as well as the use of other indicators (including for example the occurrence of protected species) will help providing a clear picture of the overall condition of the Ligurian mesophotic animal forests, leading to the identification of the most relevant vulnerable sites where to target conservation actions. As a matter of fact, the results presented in this thesis, together with data coming from bathyal areas, embody the scientific base for the future identification of the Ligurian deep sensitive areas that will shape an extensive network of deep-sea marine protected areas.

Annexes

LIST OF PUBLICATIONS

Enrichetti F., Bo M., Morri C., Montefalcone M., Toma M., Bavestrello G., Tunesi L., Canese S., Giusti M., Salvati S., Bertolotto R.M., Bianchi C.N. (2019) Assessing the environmental status of temperate mesophotic reefs: A new, integrated methodological approach. Ecological Indicators, 102: 218-229.

Enrichetti F., Toma M., Bo M. (2018). Discovering the extraordinary animal forests of the Ligurian Sea: a fragile treasure of Mediterranean biodiversity. The Marine Biologist, 11: 13-14.

Enrichetti F., Bavestrello G., Coppari M., Betti F., Bo M. (2018). *Placogorgia coronata* first documented record in Italian waters: use of trawl bycatch to unveil vulnerable deep-sea ecosystems. Aquatic Conservation: Marine and Freshwater Ecosystems, DOI: 10.1002/aqc.2930.

Betti F., Bavestrello G., Bo M., Enrichetti F., Loi A., Wanderlingh A., Pérez-Santos I., Daneri G. (2017). Benthic biodiversity and ecological gradients in the Seno Magdalena (Puyuhuapi Fjord, Chile). Estuarine, Coastal and Shelf Science, 198: 269-278.

Betti F., Bavestrello G., Bo M., Coppari M., Enrichetti F., Manuele M., Cattaneo-Vietti R. (2017). Exceptional strandings of the purple snail *Janthina pallida* Thompson, 1840 and first record of an alien goose barnacle along the Ligurian coasts (western Mediterranean Sea). The European Zoological Journal, 84: 488-495.

SUBMITTED ARTICLES

Enrichetti F., Bava S., Bavestrello G., Betti F., Lanteri L., Bo M. Artisanal fishing impact on deep coralligenous animal forests: a Mediterranean casa study of marine vulnerability. Ocean & Coastal Management, review.

Betti F., Bavestrello G., Bo M., Coppari M., Enrichetti F., Fravega L., Cattaneo-Vietti R. On the effects of recreational SCUBA diving on fragile benthic species: the Portofino MPA (NW Mediterranean Sea) case study. Ocean & Coastal Management, under review.

van de Water J. A. J. M., Coppari M., Enrichetti F., Ferrier-Pagès C., Bo M. Local environmental conditions shape the microbiota of the mesophotic black coral *Antipathella subpinnata*. FEMS - Microbiology Ecology, under review.

Enrichetti F., Dominguez-Carrió C., Toma M., Bavestrello G., Betti F., Canese S., Bo M. Megabenthic assemblages of the Ligurian deep continental shelf (NW Mediterranean Sea). PLOS ONE, under review.

Enrichetti F., Dominguez-Carrió C., Toma M., Bavestrello G., Canese S., Bo M. Marine litter assessment and distribution on the deep Ligurian continental shelf (NW Mediterranean Sea). Marine Pollution Bulletin, under review.

NATIONAL AND INTERNATIONAL CONGRESS, WORKSHOP AND SEMINAR

3rd International Workshop on Taxonomy of Atlanto-Mediterranean Deep-Sea Sponges, 6-11 June 2016, Spanish Oceanographic Institute (IEO), Spain. Enrichetti F., Bavestrello G., Bertolino M., Bertolotto R.M., Pansini M., Pronzato R., Bo M. Horny Sponge Grounds of the Ligurian Sea. Contributed talk.

48th Congresso della Società Italiana di Biologia Marina (SIBM), 7-9 June 2017, Rome, Italy. Ravanetti G., Bavestrello G., Betti F., Bo M., Cappanera V., Enrichetti F., Nonnis-Marzano F., Venturini S., Cattaneo-Vietti R. Fishing impact on the red Mediterranean gorgonian *Paramuricea clavata* of the Portofino MPA. Contributed talk.

Società Italiana di Biologia Marina (SIBM) Workshop on the coralligenous of the Italian waters, 5-8 September 2017, Ustica Island, Palermo, Italy. Bo M., Enrichetti F., Bianchi C.N., Canese S., Montefalcone M., Morri C., Salvati E., Tunesi L., Bavestrello G. Deep coralligenous environmental status: Marine Strategy ROV working protocol and resulting index. Contributed talk.

Second Joint Meeting of Société Zoologique de France (SZF) and Unione Zoologica Italiana (UZI), 18-23 September 2017, Torino, Italy. Coppari M., Bavestrello G., Carpenteri P., Enrichetti F., Mancusi C., Serena F., Bo M. Trawling impact in the Italian Seas: biodiversity, trends and conservation of megabenthic communities. Contributed talk.

Second Joint Meeting of Société Zoologique de France (SZF) and Unione Zoologica Italiana (UZI), 18-23 September 2017, Torino, Italy. Betti F., Bavestrello G., Bo M., Cappanera V., Coppari M., Enrichetti F., Fravega F., Ravanetti G., Venturini S., Cattaneo-Vietti R. Anthropic pressure and environmental sustainability in the MPA of Portofino (Ligurian Sea). Poster.

Second Joint Meeting of Société Zoologique de France (SZF) and Unione Zoologica Italiana (UZI), 18-23 September 2017, Torino, Italy. Enrichetti F., Bava S., Bavestrello G., Betti F., Bo M. Deep coralligenous biodiversity and artisanal fishing effort: the case of the Maledetti Shoal (Savona, Ligurian Sea). Poster.

Università degli Studi di Genova. Invited speaker within the course of Marine Landscape Ecology, Prof. Carlo Nike Bianchi (Specialist Bachelor's Degree in Marine Science), 30 November 2017. Title of the seminar: "Mesophotic animal forests and ecological indices".

15th Deep-Sea Biology Symposium (DSBS), 9-14 September 2018, *Monterey, CA, USA.* Enrichetti F., Toma M., Dominguez-Carrió C., Bavestrello G., Betti F., Canese S., Cattaneo-Vietti R., Bo M. Biodiversity and vulnerability of the Ligurian deep circalittoral megabenthic communities. Contributed talk.

75th Congresso Nazionale dell'Unione Zoologica Italiana (UZI), 25-28 September 2018, Lecce, Italy. Enrichetti F., Toma M., Dominguez-Carrió C., Bavestrello G., Betti F., Bo M. Megabenthic biodiversity and anthropogenic disturbance of the Ligurian deep continental shelf. Contributed talk.

Forum on Fisheries Science in the Mediterranean and Black Sea. General Fisheries Commission for the Mediterranean (GFCM), 10-14 December 2018, FAO Headquarters, Rome, Italy. Enrichetti F., Bavestrello G., Betti F., Bava S., Bo M. Deep-sea sensitive habitats identification: two examples from the Ligurian Sea (NW Mediterranean Sea). Contributed talk.

Working Group on Marine Protected Areas (WGMPA), including a session on Essential Fish Habitats (EFH). General Fisheries Commission for the Mediterranean (GFCM), Scientific Advisory Committee on Fisheries (SAC). FAO headquarters, Rome, Italy, 18 – 21 February 2019. New FRA Proposal: Deep-sea sensitive habitats from the Ligurian Sea (NW Mediterranean Sea). Contributed talk.

Subregional Committee for the Western Mediterranean (SRC-WM). General Fisheries Commission for the Mediterranean (GFCM), Scientific Advisory Committee on Fisheries (SAC). Sète, France, 5 April 2019. New FRA Proposal: Deep-sea sensitive habitats from the Ligurian Sea (NW Mediterranean Sea). Contributed talk.

UNITE – Università della terza età. Genova, 8 April 2019. Comunità bentoniche del circalitorale profondo ligure: biodiversità ed impatto antropico. Invited speaker.

50° Congresso della Società Italiana di Biologia Marina, Livorno, 10-14 giugno 2019. Enrichetti F., Dominguez-Carrió C., Toma M., Bavestrello G., Betti F., Bo M.. Foreste animali mesofotiche del Mar Ligure. Contributed talk.

50° Congresso della Società Italiana di Biologia Marina, Livorno, 10-14 giugno 2019. Betti F., Bo M., Enrichetti F., Bavestrello G. Idrozoi, sentinelle dei cambiamenti climatici sulle scogliere mediterranee. Contributed talk.

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Knowledge about deep-sea megabenthic communities has greatly increased during the last two decades thanks to the improvement of technical diving equipment, submersibles, remotely operated and autonomous vehicles. In the last 10 years, several studies, targeting the Mediterranean deep continental shelf or mesophotic zone (40-200 m), have reported the occurrence of rich hotspots of biodiversity, namely marine animal forests, dominated by habitat-forming species such as corals and sponges thriving in dim light conditions. This bathymetric range hosts the deepest extension of shallow-water animal forests as well as proper mesophotic assemblages thriving on the so-called *roche du large* ecosystems. These complex aggregations play a fundamental ecological role in the marine ecosystems (refuge effect, nursery area, benthic-pelagic coupling, biogeochemical cycles) and the characterization of their diversity and distribution is considered of primary interest worldwide. Explorations have depicted not only the diversity of these forests but also their vulnerability towards mechanical impacts inflicted by demersal fishing activities, especially in the overexploited Mediterranean basin. This leads to an urgent need to quantify the damages burdening on these sensitive ecosystems and to develop easy-to-apply tools to evaluate and monitor their environmental status, in order to provide effective conservation measures. This thesis aims to address these topics focusing on the marine animal forests of the Ligurian deep continental shelf. The impact on the megabenthic communities of two fishing techniques highly employed in the Ligurian Sea has been quantified. Than, a georeferenced database of the location of diversity hotspots and sites of litter accumulation has been created, providing managers and stakeholders the scientific information and the tools for the creation of an extensive network of deep-sea marine protected areas in the Ligurian basin.

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