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IMPORTANCE OF VULNERABLE MARINE ECOSYSTEMS (VMEs) AND ESSENTIAL FISH HABITATS (EFHs) IN THE CENTRAL MEDITERRANEAN SEA

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List of talk, poster, and papers

Palummo V., Milisenda G., Canese S., Salvati E., Pica D., Passarelli A., Spanò N., Romeo T., Greco S. (2022). Oral presentation: Effect of environmental and anthropogenic factors on the distribution of cold-water corals. In Proceedings of the 3rd Mediterranean Symposium on the conservation of Dark Habitats (Genoa, Italy, 21 September 2022). UNEP/MAP-SPA/RAC.

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General Abstract

Vulnerable marine ecosystems (VMEs) are dee-sea biodiversity and ecological functioning hotspots. The term VMEs is based on the presence of unique and diversified benthic assemblages that consist of rare or endemic species that are physically fragile and sensitive to threats from human activities. VMEs component species frequently exhibit ecological traits such as late age of maturity, slow growth rate, uniqueness, low recruitment, high longevity and slow recovery ability after mechanical impact, which make them more vulnerable to human activities (e.g. fishing activities). These benthic species are able to increase habitat heterogeneity by providing more associated fauna in the surrounding areas, serving as feeding, breeding, nursery and refuge areas for a wide range of fish and invertebrate species. For these reasons, they have also been recognized as Essential Fish Habitats (EFHs). Despite the ecological importance of VMEs, and the use of new technologies for the exploration of deep-sea environments the information about their distribution and ecological role still remains scarce.

Through the combination of non-invasive acoustic and visual methods such as Multibeam Echosounder (MBES) and Remotely Operated Vehicles (ROVs), this thesis aims to study the ecological role of VMEs in terms of associated fauna in neighbouring areas, and the preferences of the habitats, using two different multivariate approaches (Redundancy Analysis and Multiple Co-inertia Analysis) and in addition, to study their actual and potential distribution through Habitat Suitability Models (HSMs) using Maximum Entropy (MaxEnt) modelling. This approach allowed us to link presence data and environmental and anthropogenic predictors that characterised the study area, in order to obtain predictive distribution maps, determining the contribution of each predictor. The thesis includes case-studies both in the Ionian Sea and the Strait of Sicily. These results enhance our knowledge on the spatial distribution of the main VMEs observed during ROV expeditions, emphasizing the importance of environmental and anthropogenic parameters in determining their distribution and/or presence, as well as providing useful information on the important ecological role of these marine ecosystems, conferring them the definition of Essential Fish Habitats (EFHs).

Chapter 1. General introduction

1.1 The Mediterranean Sea

The Mediterranean Sea (Figure 1) is a semi-enclosed basin with an incredible ecological value, beauty, and a wide variety of marine ecosystems (Chimienti *et al*., 2020a). Its surface area is 2.5 million km^2, and its length and width are 3000 km and 1500 km, respectively (Criado-Aldeanueva *et al*., 2020). The Mediterranean Sea is the deepest enclosed sea on Earth, with a maximum depth of 5267 m and an average depth of 1460 m (Criado-Aldeanueva *et al*., 2020). The basin makes up about 0.3% of the world's oceans in terms of overall volume (IUCN, 2019) and hosts about 7.5 % of marine species (Coll *et al*., 2010; IUCN, 2019). It is connected to the Atlantic Ocean on the west through the Strait of Gibraltar and to the Sea of Marmara and the Black Sea on the northeast part through the Dardanelles (Coll *et al*., 2010; Hayes *et al*., 2019; Rebesco & Taviani, 2019). The Suez Canal connects the Mediterranean to the Red Sea, the Indian Ocean, and the Middle East in the southeast (Coll *et al*., 2010). Furthermore, the Mediterranean Sea is divided into the Western (0.85 million km^2) and Eastern (1.65 million km^2) sub-basins by the Strait of Sicily (Coll *et al*., 2010; Criado-Aldeanueva *et al*., 2020). The basin's distinctive features include high salinities, temperatures, seawater density, moderate energy continental boundaries, and microtidal conditions (Rebesco & Taviani, 2019). A detailed description of the general oceanographic conditions in the Mediterranean has already been provided in the past (Bethoux, 1979; Bas, 2009; Coll *et al*., 2010).

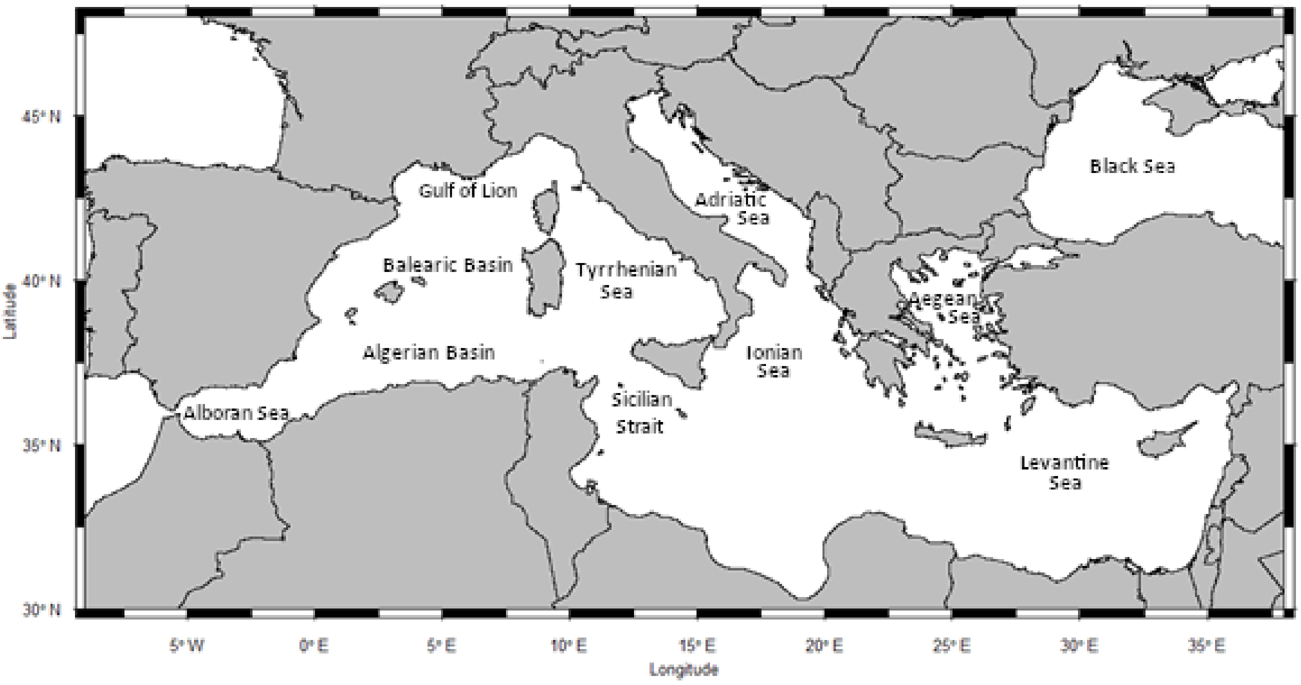

Figure 1: Map of the Mediterranean Sea and its sub-basins.

The circulation system of the Mediterranean Sea is one of its most notable features. The major process driving the Mediterranean circulation is atmospheric-ocean interactions (Figure 2). This force is influenced by heat and freshwater exchange, as well as meteorological and oceanic conditions (Tsimplis *et al*., 2006). The average evaporation (E) throughout the basin is insufficient to balance the average precipitation (P) and river runoff (R), necessitating a net influx of Atlantic waters (AW) through the Strait of Gibraltar. This compensatory mechanism generates a thermohaline circulation composed of many cells (Bethoux *et al*., 1999; Cusinato *et al*., 2018). As a result of evaporative losses, the largest one moves the AW entering the basin in the surface layer eastward, spanning the western and eastern basins. When this modified Atlantic water (MAW) enters the Levantine basin during the winter, the intense dry winds force it to intermediate depths (300-500 m), where it forms the Levantine Intermediate Water (LIW) (Robinson *et al*., 1991). LIW is the principal core of the Mediterranean waters that left the basin and recirculated westward before passing the Strait of Sicily and arriving at Gibraltar (Millot *et al*., 2006). During the winter, the water masses involved in the evaporation increase their salinity, and sink to the depth triggering deep convections, and creating secondary cells. The points in which these deep circulations occur are in the Eastern Mediterranean, in which the Eastern Mediterranean Deep Water (EMDW) is produced, specifically in the Adriatic and Aegean Seas. Regarding the Western Mediterranean deep water (WMDW), the convection occurs in the Gulf of Lions, and this water mass is then incorporated in the Mediterranean outflow through Gibraltar (Millot *et al.,* 2006; García-Lafuente *et al.,* 2009).

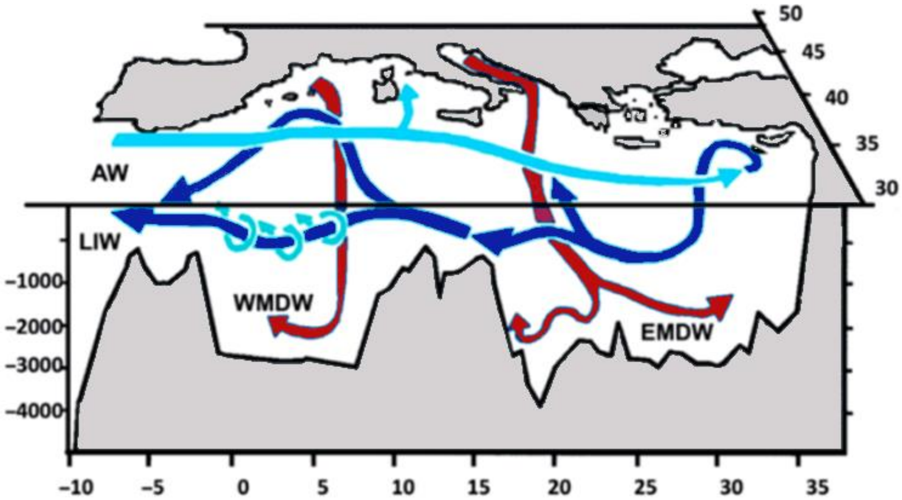

Figure 2: Graphic illustration of the thermohaline circulation in the Mediterranean Sea (modified by Criado-Aldeanueva and Javier Soto-Navarro, 2020).

1.2 Vulnerable Marine Ecosystems (VMEs) and Essential Fish Habitats (EFHs)

Despite covering only 0.7% of the ocean surface, the Mediterranean Sea hosts about 8% of the global biodiversity (Costello *et al*., 2010; Chimienti *et al*., 2020a). Though there are many anthropogenic impacts (e.g. pollutants, litter, fishing activities, etc.), it presents a high density of benthic habitats, from the coastal to the deep zone, which defines the area as a worldwide hot-spot of biodiversity (Danovaro *et al*., 2010; Chimienti *et al*., 2018; Chimienti *et al*., 2020a). In detail, the Mediterranean Sea presents several deep-sea ecosystems, including slope systems, submarine canyons, deep basins, seamounts, deep-water coral systems, carbonate mounds, cold seeps, hydrothermal vents, and permanent anoxic systems (Danovaro *et al*., 2010). These ecosystems were defined as Mediterranean Vulnerable Marine Ecosystems (VMEs) by the General Fisheries Commission for the Mediterranean (GFCM) (GFCM, 2017, 2018b). They can host several benthic ecosystems defined as VMEs indicator habitats (e.g. cold-water coral reefs, coral gardens, sea pens fields, deep-sea sponge grounds, tube-dwelling anemone patches, crinoids fields, oyster reefs and other giant bivalves, seep and vent communities, other dense emergent fauna), which in turn are inhabited by several species or group of species, called VMEs indicator species or taxa (Cnidaria, Porifera, Bryozoa, Echinodermata, Mollusca, Anellida, and Arthropoda) (Carpentieri *et al*., 2021). The classification of VMEs is based on several important characteristics, such as uniqueness, rarity, fragility, difficulty of recovery, and structural complexity (FAO, 2009). VMEs are primarily described based on their vulnerability, which is a substantial alteration of a population, habitat, or community induced by chronic or short-term disturbances. In other words, the vulnerability of these ecosystems is related to the likelihood of suffering substantial modifications due to chronic or short-term damage and the chances of recovery (Carpentieri *et al*., 2021). In such cases, the longer the recovery time, the greater the vulnerability of that specific community, habitat, or population. Conversely, the shorter the recovery time, the lower the vulnerability of a particular community, habitat, or population. The vulnerability of an ecosystem and its subsequent positive recovery outcome, if applicable, is related to the biological and structural aspects of the ecosystem itself. Therefore, easily disturbed environments with slow or non-existent recovery times are defined as vulnerable ecosystems (GFCM workshop, 2017). According to the Food and Agriculture Organization of the United Nations (FAO), the classification as VMEs is based on one or a combination of different criteria, such as:

- uniqueness or rarity: area, ecosystems or habitats that contain species whose loss cannot be replaced by ecosystems or similar locations. These zones include environments where indigenous species can be found, habitats of rare, endangered, or threatened species, or areas of feeding, breeding, nursery or spawning.

- functional significance of the habitat: areas or habitats essential for the reproduction, spawning, recovery or survival of different life-history stages, fish stocks or rare, endangered, or threatened species.
- fragility: habitats highly vulnerable to damage by anthropogenic activities.
- life-history traits of component species that make recovery difficult: habitats characterized by species with particular characteristics such as slow growth rates, late age of maturity, low recruitment or high longevity.
- structural complexity: habitats characterized by highly concentrated biotic and abiotic characteristics that produce complex physical structures. Frequently connected with a lot of diversity. The structuring of organisms is typically quite important for both diversity and ecological processes.

Thanks to their characteristics, VMEs, can be classified as Essential Fish Habitats (EFHs) (Oceana 2017). In particular, the term "Essential Fish Habitat" refers to a “habitat of fundamental importance for the life cycle of fish species subject to high exploitation based on biological and ecological requirements” (Rosenberg *et al*., 2000). They include waters and substrates that fish species depend on for survival, growth, reproduction, egg deposition, and feeding, playing a vital role in the life cycle of the species (FAO, 2018; D’Onghia *et al*., 2019a, D’Onghia *et al*., 2019b).

In the Mediterranean, a diverse range of habitats serves as EFHs, including seagrass meadows, rocky reefs, and soft-bottom areas. According to Oceana (2017) and GFCM, some examples of EFHs include:

- Spawning grounds: areas with a high concentration of mature females.
- Nursery grounds: areas with a high presence of juveniles.
- Migration corridors: areas where specific mobile species migrate during their life cycle.

- Feeding /Foraging grounds: areas where feeding activity is greater.

Defining these habitats is pivotal for preserving and enhancing stocks' status and long-term sustainability (Rosenberg *et al*., 2000). Conservation measures, both fishing-related and non-fishing-related, should be adopted to preserve and improve the quality and quantity of EFHs. Recognizing that marine resources hold immense environmental and social value, it has become necessary to develop a plan to identify and describe EFHs to ensure adequate habitats to conserve (Rosenberg *et al*., 2000)

1.3 Fishing impacts

VMEs are more impacted by human activities, in particular, fishing represents one of the main threats for these ecosystems. More specifically, trawling is among the most common and invasive fishing activities in the Mediterranean Sea (Consoli *et al*., 2017; IUCN, 2019). Together with bottom longlines, these fishing gears encounter the seabed to catch benthic or demersal species, causing deleterious damage to sessile species and, resulting in an ecosystem decline (Carpentieri *et al*., 2021), due to the life-history traits of the VME species and their co-existence with species of commercial value (Bo *et al*., 2014a; Bo *et al*., 2014b; Cau *et al*., 2017; D'Onghia, 2019b; Carpentieri *et al*., 2021). Despite several researchers have been highlighted the negative effect caused by fishing activities, with bottom-contact gears the relationship between this human activity and the health status of benthic communities is still poorly understood and depend also to other factors involved in this scenario, such as the own characteristics of species (size, shape, skeletal flexibility), of the environment (current circulation, topography of the seabed, movements of the fishing gears), fishing characteristics (type of fishing gears, technological equipment), and social factors (i.e. number of fishermen and vessels). Focusing on bottom trawling is important to highlight that can cause a decline of VMEs in two ways: directly by scraping the seafloor, resuspending sediment and removing habitat-forming species from the seabed, and indirectly by driving long-term alterations in benthic communities, lowering habitat heterogeneity and compromising ecosystem efficiency (Roberts, 2002; Colloca *et al*., 2004; Gray *et al*., 2006; Maynou & Cartes, 2012; Hinz, 2017; Carpentieri *et al*., 2021). More specifically, hard bottom VMEs, in particular those close to soft bottom, may be indirectly damaged by sediment resuspension and other unfavourable effects of trawling, such as bycatch and/or entanglement (Ragnarsson *et al*., 2017; Bilan *et al*., 2023). Soft-bottom VMEs are more sensitive to direct impacts of trawling, particularly in the case of fragile organisms (e.g. bamboo coral *Isidella elongata*, sea pens and the crinoid *Leptometra phalangium*), for which even low fishing effort can cause high mortality rates through displacement and crushing of sediment-anchored colonies (Murillo *et al*., 2010, 2018; Carpentieri *et al*., 2021).

1.4 Cold-Water Corals

Among Mediterranean VME indicator habitats, Cold-Water Corals (CWCs) habitat represents a key component in the Mediterranean Sea (Roberts *et al*., 2006; FAO, 2009; FAO, 2011), habitats able to greatly enhance the variability of the surrounding habitat, and providing an increase in heterogeneity (Ashford *et al*.,2019). CWCs are sessile azooxanthellate (i.e. lacking symbiotic dinoflagellates) cnidarians, which are generally referred as scleractinian, antipatharian, gorgonian and stylasterid corals, typically living beyond the photic zone (Roberts *et al*, 2009; Otero & Marin, 2019), usually below 200 m depth (Roberts *et al*., 2009; Chimienti *et al*.,2018; Otero & Marin, 2019). They create bio-constructions (Freiwald *et al*.,2009; Chimienti *et al*., 2020b) able to increase the heterogeneity of the surrounding habitat and its diversity (Chimienti *et al*., 2019a), that is why these habitats are defined as hotspots of biodiversity (Mastrototaro *et al*., 2010; Watling *et al*., 2011; Chimienti *et al*., 2020a; Carpentieri *et al*., 2021) with an important ecological and economic value (Capezzuto *et al*., 2018; Carpentieri *et al*., 2021). These complex three-dimensional structures provide microhabitats and niches that various commercial and non-commercial fish and invertebrate species rely on at different life stages, providing essential nurseries and offering protection and food sources (D'Onghia *et al*., 2010; Linley *et al*., 2017; Sion *et al*., 2019). These characteristics define these habitats as Essential Fish Habitats (EFHs) (D'Onghia *et al*., 2010; Sion *et al*., 2019).

The CWCs belong to two important classes, Anthozoa and Hydrozoa, but we will focus on the class Anthozoa because the other one is represented by one single species, *Errina aspera*, with a very limited geographical distribution in the Mediterranean Sea. The majority of the CWCs belong to the subclasses Hexacorallia and Octocorallia and precisely to the order Anthipatharia and Scleractinia for the Hexacorallia and to orders Sclerancyonacea and Malacalcyonacea for the Octocorallia.

The order Scleractinia includes many colonial and solitary corals like the white corals *Madrepora oculata* (Linnaeus, 1758), *Desmophyllum pertusum* (ex *Lophelia pertusa*) (Linnaeus, 1758), *Desmophyllum dianthus* (Esper, 1794), and the yellow coral *Dendrophyllia cornigera* (Lamarck, 1816). *Madrepora oculata* and *D. pertusum* can create deep-sea reefs like their shallow-water counterparts, and in the Mediterranean Sea six big reefs have been identified to date and these areas are classified as CWCs provinces (Taviani *et al*., 2017). These CWCs provinces are situated in the Strait of Sicily (South of Malta), in the Nora Canyon (South of Sardinia), in the south-western Adriatic Sea (Bari Canyon), in the northern part of the Ionian Sea (Santa Maria di Leuca), in the canyons of Gulf of Lions and the eastern part of the Alboran Sea.

Scleractinians include *Dendrophyllia cornigera*, a coral with colonies with sulphur-yellow coenenchyma and polyps on top of its typical branched structure (Chimienti *et al*., 2019a). They generally do not form complex carbonate structures but retain their individuality and can live both on hard substrates with a certain inclination (Chimienti *et al*., 2019a) and on flat muddy substrates (Enrichetti *et al*., 2023). The present geographic distribution of *D. cornigera* demonstrates that it is widespread from the east to the west of the Mediterranean Sea (Aegean Sea, Adriatic Sea, Ionian Sea, Strait of Sicily, Tyrrhenian Sea, Ligurian Sea, Balearic Sea, Alboran Sea) (Freiwald *et al*., 2009; Orejas *et al*., 2009; Bo *et al*., 2011, 2014b, 2015; Salomidi *et al*., 2010; Gori *et al*., 2013; Cau *et al*., 2015), and also in the six CWCs provinces and within a depth range of 80-733 m (Chimienti *et al*., 2019a).

Antipatharia, usually known as black corals, represent one of the main habitat-forming species in the Mediterranean Sea where four species have been described (Opresko and Försterra 2004): *Antipathes dichotoma* (Pallas, 1766), *Leiopathes glaberrima* (Esper, 1792), *Paranthipathes larix* (Esper, 1790) and *Antipathella subpinnata* (Ellis and Solander, 1786). Black corals, formerly thought to be uncommon species, have been extensively recorded thanks to ROV studies. Their Mediterranean distribution comprises the whole western basin, the Strait of Sicily, the Adriatic Sea, the Ligurian and the Tyrrhenian coast, the Aegean Sea, and the eastern part of the Alboran Sea (Bo *et al*., 2009, 2011, 2014c, 2015; Pardo *et al*., 2011; Angeletti *et al*., 2014; Deidun *et al*., 2015; Altuna and Poliseno., 2019). *Leiopathes Glaberrima* is generally distributed from 120 to 1000 m depth instead, *A. dichotoma* and *P. larix* have a depth range between 90-645 m and 90-460 m, respectively, with less occurrence in the deeper zones (Chimienti *et al*., 2019a). The last common black coral species, *A. subpinnata* is more distributed in shallower waters (60-150 m depth), with occasional occurrence in deep areas down to 600 m depth.

All these species may create mono or multi-specific gardens predominantly on hard substrates (Bo *et al*., 2011, 2012, 2015; Cau *et al*., 2017; Chimienti *et al*., 2019b, 2020b).

Sclerancyonacea order comprises species living on both hard and muddy substrates.

The most common Mediterranean hard bottom species of this order are the Primnoidae *Callogorgia verticillata*, and the Ellisallidae *Viminella flagellum.*

Callogorgia Verticillata has a wide distribution both in the Atlantic Ocean and the Mediterranean Sea (Braga-Henriques *et al*., 2013; Bullimore *et al*., 2013; Locke *et al*., 2013) and in the last one, it was recorded in the Alboran Sea, Gulf of Lions, Tyrrhenian Sea, Strait of Sicily, Ionian Sea, Adriatic Sea and Aegen Sea (Pardo *et al*., 2011; Fabri *et al*., 2014; Bo *et al*., 2011, 2012, 2014b, 2015; Angeletti *et al*., 2014; Cau *et al*., 2015, 2017; Knittweis *et al*., 2019). Its bathymetric distribution expands from 90 to 1000 m depth, its deeper observation was recorded by Mastrototaro *et al*., 2010 and Knittweis *et al*., 2019, in the Strait of Sicily. This species can create dense forests that are frequently combined with other CWCs species, resulting in a particularly heterogeneous and three-dimensional ecosystem (Angeletti *et al*., 2014).

Viminella flagellum is distributed only in the western part of the Mediterranean, more specifically in the Alboran Sea, Balearic Sea, Strait of Sicily, Tyrrhenian Sea and Ligurian Sea (Lo Iacono *et al.*, 2012; Aguilar *et al.*, 2013; Maldonato *et al.*, 2013; Bo *et al*., 2014b; Fourt & Goujard, 2012; Fabri *et al*., 2014). All arborescent species, such as *C. verticillata* and *V. flagellum* or the other gorgonian species belonging to the order Malacalcyonacea, such as *Acanthogorgia hirsuta, Swiftia pallida, Bebryce mollis, Paramuricea macrospina, Muriceides lepida, Villogorgia bebrycoides,* and *Placogorgia* spp., have an important ecological role in providing a three-dimensional habitat for a wide range of species (Chimienti *et al*., 2019a).

Among the Scleralcyonacea there are species able to live on muddy substrates and able to form wide forest like the bamboo coral *Isidella elongata* and several sea pens species like *Funiculina quandrangularis*.

Isidella elongata is more frequent on bathyal muddy sediments in a depth range between 500 and 1650 m (Maynou & Cartes 2012; Bo *et al*., 2015; Mastrototaro *et al*., 2017; Rueda *et al*., 2019), and it is more abundant in the Western part of the Mediterranean (Alboran Sea, Balearic Sea, Tyrrhenian Sea, Sardinian Channel, Strait of Sicily and Ionian Sea) (Hebblen *et al*., 2009; Mastrototaro *et al*., 2017; Maynou & Cartes 2012; Cartes *et al*., 2013, Fabri *et al*., 2014; Bo *et al*., 2015, Freiwald *et al*., 2009; Mytilineou *et al*., 2014). *Isidella elongata* is defined as habitat-forming species, due to its important ecological role, providing areas of spawning and refuge for several species (Mastrototaro *et al*., 2017; D’Onghia *et al*., 2019b). Living on soft sediments, commonly interested by trawling activities, it has been highly impacted and it is now a protected species listed in the IUCN Red List (Otero *et al*., 2017).

In the superfamily Pennatuloidea is possible to distinguish two groups based on their bathymetric distribution. *Pennatula rubra*, *Veretillum cynomorium* and *Pteroides spinosum* are more frequently recorded on continental shelf and for this reason are not rigorously considered as CWCs (Gori *et al*.,2017). The other group comprise two species: *Funiculina quadrangularis* and *Kophobelemnon stelliferum* for which the distribution includes the deepest part of the Mediterranean basin. Their distribution extends both in the western and eastern parts, including: Alboran Sea, Balearic Sea, Ligurian Sea, Tyrrhenian Sea, Strait of Sicily, Ionian Sea and Adriatic Sea. Sea pens are represented by other three species observed in the deepest part of the basin, such as *Protoptilum carpenterii*, *Pennatula phoshorea* and *Virgularia mirabilis* for which no consistent data of their aggregation are available (Chimienti *et al*.,2019a)

Due to their life-history traits (slow growth rate, low fecundity, late age of maturity, high longevity) and vulnerability, especially to human activities, CWCs have been incorporated into several management initiatives. At European level the EU Habitat Directive (92/43/EEC), and the OSPAR convention, present the list of threatened and/or declining species and habitats, and then the concept of Vulnerable Marine Ecosystems (VMEs) adopted both by United Nations General Assembly (UNGA) and Food and Agriculture Organization (FAO, 2009).

1.5 Deep-sea research

Over the past two decades, deep-sea research has advanced quickly, resulting in discoveries and an exponential rise in knowledge. This has been made possible by improvements in deep-sea visual (Remotely Operated Vehicles (ROVs)) and acoustic (Multibeam Echosounder (MBES), side-scan sonar, etc.) technology and the government’s willingness to support deep-sea research (Orejas & Jimenéz, 2019). Image technologies represent the most significant, non-destructive, recent advancements in studying deep-sea environments, especially for sessile organisms (Orejas & Jimenéz, 2019). In particular, ROVs aim to acquire georeferenced video capture and are equipped with scaling tools (such as laser pointers) that allow living beings to be seen, counted and measured, greatly increasing the likelihood of video footage being used as a key component of scientific study (Rossi & Orejas, 2019). The integration of these technologies (visual and acoustic) has made it possible to acquire high-resolution, continuous coverage of bathymetric and morphological data of the deep-sea environments and their exploration (Angeletti *et al*., 2019; Lo Iacono *et al*., 2019) and increase live CWCs occurrence registered (Chimienti *et al*., 2018). This is especially the case in areas that are difficult to explore, such as submarine canyons, seamounts, continental slopes and shelves (Buhl-Mortensen *et al*., 2017; Pierdomenico *et al*., 2016; Grinyo *et al*., 2018). This knowledge contributes to increasing our information on the CWC's biogeography, which is essential for creating and improve the maps for these habitats and improving fundamental knowledge of deep-sea biological resources and the ecosystem service they provide (Liang *et al*., 2021). According to the European Marine Strategy Framework Directive (.2008/56/EC), mapping VMEs, such as CWCs, represents the first step in the framework of environmental protection (Chimienti *et al*., 2018), and an important tool at the management level (Taranto *et al*., 2023), in implementing suitable management and protection plans (Otero & Marine, 2019; Sundahl *et al*., 2020; Lauria *et al*., 2021).

In addition, it is possible to investigate how geological, biological, and oceanic processes interact, enabling the extension of point data to full-coverage maps (Lo Iacono *et al*., 2019).

Data on species distribution, particularly for vulnerable species, is critical for informing spatial management strategies and better understanding processes of evolution and ecology (Hortal *et al*., 2015; Taranto *et al*., 2023). However, deep-sea field surveys are challenging and expensive (Stephenson *et al*., 2021), so the distribution and influence of environmental and anthropogenic factors on these ecosystems are not well known (Bargain al., 2017).

In particular, abiotic conditions represent the most important process controlling the development processes of biological communities (D'Amen *et al*., 2017; Taranto *et al*., 2023). In this regard, abiotic and biotic factors together influence individual organisms by determining the presence or absence of species in certain areas (D'Amen *et al*.,2017; Kraft *et al*.,2015; Taranto *et al*.,2023). In a specific area, only species that tolerate the same environmental circumstances may coexist. While distinguishing the function of environmental variables from other factors that may influence the spatial distribution of species is difficult (Cadotte and Tucker, 2017), it is appropriate to anticipate the role of the surrounding environment, both direct and indirect, in structuring biodiversity in a specific area (Taranto *et al*., 2023).

As mentioned before, due to their life-history traits and vulnerability to human impacts (Ragnarsson *et al*., 2017), have been included in several management measures. Furthermore, multiple studies have shown that CWCs can regulate nutrient fluxes (Middelburg *et al*., 2016; Rix *et al*., 2016), offer habitat for other benthic and demersal species (Buhl-Mortensen *et al*., 2017; Linley *et al*., 2017) and are most likely involved in regulating, sustaining, and providing ecosystem services (Thurber *et al*., 2014).

For all these reasons, studying and knowing of their distribution began to be a priority for marine space planners and benthic ecologists (Taranto *et al*., 2023). In this context, Habitat Suitability Models (HSMs) or Species Distribution Models (SDMs) play a key role and have been increasingly important in resource management and conservation biology in recent years. These models are presently being used to map the distribution of VMEs-suitable habitats in deep oceans (Stephenson *et al*., 2021), enhancing our understanding of their global distribution (Bargain *et al*., 2017). In particular, HSMs can correlate georeferenced occurrence data and environmental parameters (biotic and abiotic) (Elith & Leathwick, 2009; Taranto *et al*., 2023) comparing the particular conditions of the sites where the species were observed with the conditions of the entire study area, to highlight the distribution of suitable environments in space (Pearson, 2008; Bargain *et al*., 2018), also in areas where occurrence data are not available (Bargain *et al*., 2018) HSMs are widespread used to predict the spatial distribution of the species, especially in deep-sea environments (Davies & Guinotte, 2011; Savini *et al*., 2014; Fabri *et al*., 2017; Yesson *et al*., 2017; Bargain *et al*., 2018; Taranto *et al*., 2023) where this technique is still recent (Bargain *et al*., 2018).

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Aims of the Thesis

This thesis aims to investigate the deep-sea benthic communities by performing deep-sea acoustic (Multibeam Echosounder (MBES) and visual (Remotely Operated Vehicles (ROVs)) technologies in two study areas of the Mediterranean Sea: Amendolara Bank (Ionian Sea) and Strait of Sicily. The overarching objectives are to investigate the role of the environmental variables in determining the distribution of benthic communities, highlight the ecological role that these species can have in deep-sea environments and emphasise the vulnerability of these habitats to human fishing activities. More in detail, this thesis includes three case studies:

Chapter 2: **New contribution on the distribution of Vulnerable Marine Ecosystems in the Amendolara Bank (Ionian Sea)**

This study aims to: i) investigate the Amendolara Bank, ii) identify benthic species with ecological importance, iii) assess the associated fish fauna to VMEs and iv) assess anthropogenic influence by evaluating abandoned, lost or otherwise discarded fishing gear (ALDFG).

Chapter 3: **Effect of environmental and anthropogenic factors on the distribution and co-occurrence of cold-water corals**

This study aimed to i) provide new data on the spatial distribution of six CWCs species in the Strait of Sicily, ii) describe the main environmental and anthropogenic variables that shape their distribution, and iii) identify hotspots where individuals from different species can co-occur.

Chapter 4: **Ecological role of habitat-forming benthic species on muddy sediment**

The study was conducted in the Strait of Sicily and aimed at assessing the ecological importance of benthic species living mainly on soft bottoms. The objective is to assess how the distribution of associated fauna varies in relation to the habitat-forming species and the environmental variables that characterize the study area.

Chapter 2. New contribution to enlighten the distribution of Vulnerable Marine Ecosystems in the Amendolara Bank (Ionian Sea)

New contribution to enlighten the distribution of Vulnerable Marine Ecosystems in the Amendolara Bank (Ionian Sea)

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Abstract

The Amendolara Bank has recently been established as a Site of Community Importance however the knowledge about it is still poor and, particularly, the deeper zone (>50m) has received scant attention. Previous studies have discovered the existence of VMEs inside the bank, emphasizing the need for more information and its relevance in terms of conservation in the Ionian Sea. This study aims to investigate various aspects of the bank using Remotely Operated Vehicles (ROVs) in order to highlight the possible presence of VMEs, the relationship between VMEs and environmental variables, assess the relationship between the observed fish and VMEs, and evaluate the anthropogenic impact through the identification of fishing gear present in the study area. This research presents new information on the biodiversity of the Amendolara Bank, identifying the presence of four VMEs species including the first sightings of the black coral *Antipathella subpinnata* and the precious red coral *Corallium rubrum* along the Calabrian Ionian coast. Furthermore, evidence of the interaction between fish fauna and VMEs species, as well as evidence of anthropogenic influence produced by abandoned, lost or otherwise discarded fishing gear (ALDFG), highlights the urgent need for additional research to increase the knowledge about the biodiversity of this unique spot in the Ionian Sea.

Keywords: VMEs, Mediterranean Sea, anthropogenic impact, EFHs, fish assemblages

2.1 Introduction

Banks and Seamounts are deep-sea structures characterized by a vertical elevation of more than 100 m from the sea bottom influencing the direction and flow of ocean currents and therefore affecting the food availability for the suspension filter feeder species (Watling *et al*., 2017; Goode *et al*., 2020). Moreover, these structures provide a hard substrate for the settlement and growth of several habitat-forming species (Clark *et al*., 2010; Watling *et al*., 2017; Goode *et al*., 2020) and favourable conditions for the occurrence of a well-diversified fish fauna (Vassallo *et al*.,2018; Bo *et al*.,2020b). In fact, the seamounts (thereafter this term include also Banks) are considered good fishing areas for recreational and commercial fisheries (Würtz & Rovere, 2015) thanks to their characteristics, and are worldwide recognized as hot-spot of biodiversity (Bo *et al*.,2020b). Considering this, Mediterranean seamounts have received little attention, particularly compared to their oceanic counterparts. Only a few of the 250 seamounts present in the Mediterranean Sea have been thoroughly studied highlighting their biological and ecological role (Bo *et al*., 2011, 2020b; Danovaro *et al*., 2010; Morato *et al*., 2013; Würtz & Rovere, 2015). This is the case of the Amendolara Bank (Italy, Ionian Sea) which was studied in the past only in its shallow zone (upper portion of the euphotic zone), providing significant information on the coralligenous habitat (Rossi & Colantoni, 1976; Panetta *et al*., 1985; Perrone, 1985; Strusi *et al*., 1985; Cecere & Perrone, 1988). However, there is very scant information available about the deeper zone below 100 m depth, where the alternation of mobile and hard substrates generates suitable conditions for mesophotic and deep-sea suspension feeders (Bo *et al*.,2012). Among these species inhabiting the deep part of seamounts is possible to recognize Vulnerable Marine Ecosystems (VMEs) indicator species (e.g. corals and sponges) (Watling *et al*., 2017; Goode *et al*., 2020) that are considered to have an important role to enhance the biodiversity in the deep sea (Beazley *et al*., 2013, 2015; Ashford *et al*., 2019). The VMEs include groups of species characterized by peculiar life history traits (e.g. slow growth rate, late age of maturity, low fecundity, high longevity) that make them particularly vulnerable to different impacts, such as fishing activities (FAO,2009; FAO, 2016; Aguilar *et al*., 2017).

The great habitat complexity provided by VME species promotes greater richness and biodiversity and enhances ecosystem functioning (Cerrano *et al*., 2010; de la Torriente *et al*., 2020). Indeed, the environments created by these ecosystems provide shelter from predators and adverse hydrological conditions (Amsler *et al*., 2009; Ashford *et al*., 2019), as well as serve as nursery and aggregation areas for a wide range of taxa, including those of commercial interest (Baillon *et al*., 2012; Ashford *et al*., 2019).

The Amendolara Bank has been established as a Site of Community Importance (SCI) (SCI code IT9310053) and designed as a Special Conservation Area (DM 27/06/2017 - G.U. 166 del 18-07-2017). Moreover, recently the Calabria Region decided to insert it in the list of the Regional Marine Parks (Legge Regionale 16 Dicembre 2022, n°46) raising the importance of better studying its biodiversity and importance in the Ionian Sea.

The principal aims of the study are to investigate the main aspects of the mesophotic zone of the Amendolara Bank i) highlight the presence of VMEs, ii) assess the relation between VMEs species and environmental variables, iii) study the associated fish fauna to VMEs species, and iv) evaluate anthropogenic impact through the assessment of abandoned, lost or otherwise discarded fishing gear (ALDFG).

2.2 Materials and Methods

Study area

The survey was conducted from 9th to 19th March 2021 in the Amendolara Bank (39,86838°N – 16.72344°E) (Figure 1) on board the Research Vessel *Astrea* of ISPRA. The Amendolara Bank is located in the northern Ionian Sea, within the Gulf of Taranto (Ferranti *et al*., 2012; Würtz & Rovere, 2015) and it is characterized by irregular morphology due to the presence of peaks and valleys (Cecere & Perrone, 1988;). In detail, the top of the bank ranges between 21 and 50 meters deep and the sloping sides reach the bottom between 125 and 250 m deep. The lowest bathymetries are concentrated on the western side of the bank, while to the east the rock structure slopes more steeply towards the muddy bottom. To the southeast, there is a separate outcrop that rises to approximately 140 m.

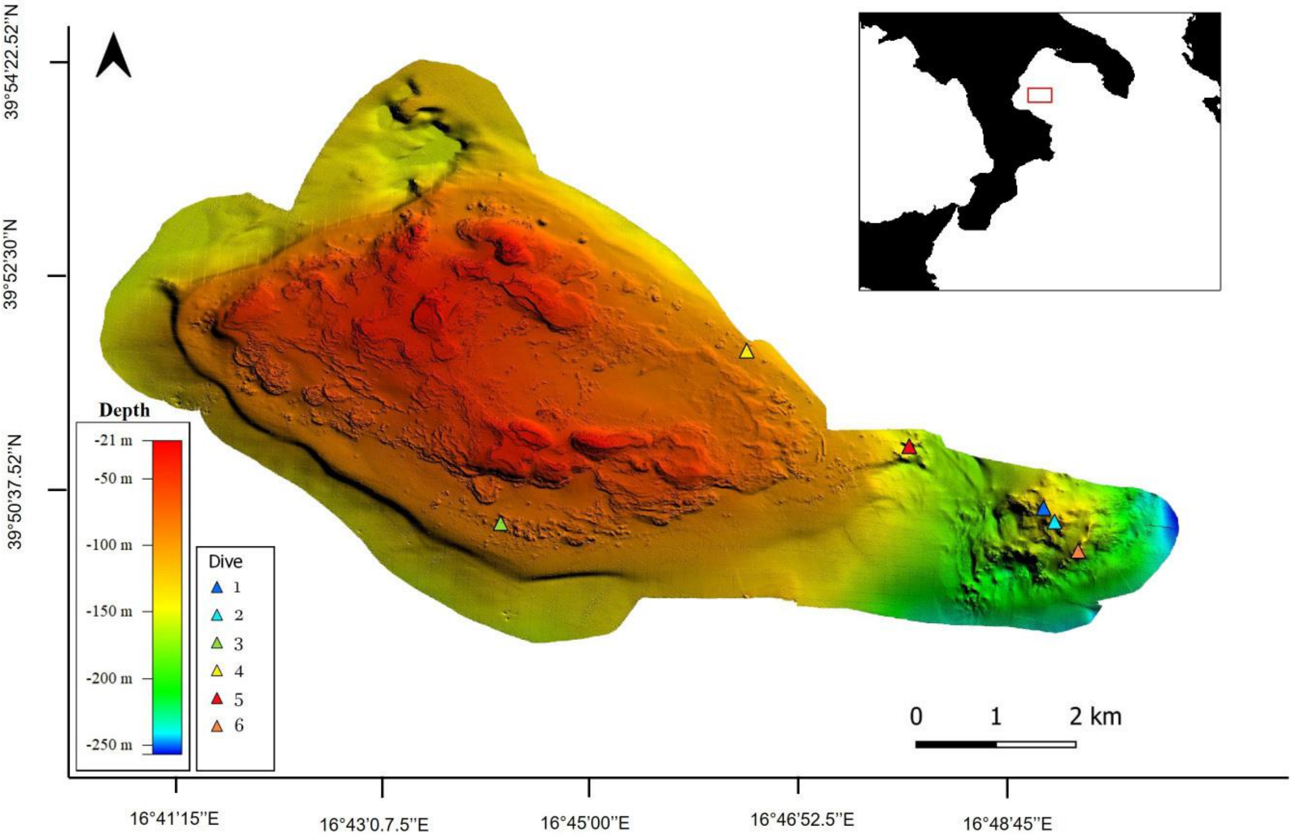

Figure 1: High-resolution bathymetry data map of Amendolara Bank. Tringles show the points of the 6 dives performed.

Data collection

The survey was conducted firstly using an MBES Kongsberg EM2040. This is a mainstream tool for mapping ocean, benthic habitats and for performing seafloor geotechnical surveys (Brown *et al*.,20019). MBES allows us to obtain a high-resolution morpho-bathymetric map, from which is possible to extract other data layers, including information about seafloor morphology (e.g. slope, aspect, roughness, etc.). The combination of these layers is useful for identifying and segmenting seabed typology propaedeutic to mapping benthic habitats (Brown *et al*., 2019). The data obtained were processed to remove spikes generated by navigation system problems and/or acquisition systems (Bo *et al*., 2015).

Transects were located on the basis of the bathy-morphological map of the seabed with a preference for those parts of the seabed with greater heterogeneity (in the meaning of frequent variations in depth and slope). The transects were performed using ROVs (Remotely Operated Vehicles) Pollux III equipped with a digital camera, strobe, and high-definition video camera (HD SONY HDR-CX405). The depth sensor and underwater acoustic tracking position system (Ultra Short Baseline Linquest Tracklinq 1500 MA) on the ROV provided geographic position every second during the dives. During this study on the Amendolara Bank, 6 dives were carried out in a depth range between 80 – 160 meters.

Data analysis

During the survey, video data logging was performed using OFOP (Ocean Floor Observation Protocol) software (van den Beld *et al*., 2007) and then processed using the software VLC. Three environmental variables (slope, aspect, roughness) were extracted from high-resolution Multibeam bathymetry (0.25 cm) data through the package “raster”, and function “terrain” in the R software (Hijmans, 2023). In each dive, the seabed substrate type, the VMEs, and fish species were recorded. In order to assess the relationship among VMEs, fish species, and environmental variables a Redundancy Analysis (RDA) analysis was performed. This multivariate analysis assumes that each species' reaction and the ordination axes follow a linear relationship (Gori *et al*., 2011). For the RDA, dives were divided into segments on the base of the substrate type removing the part without the presence of VMEs or fish species (Figure 2). Each segment was characterized by the abundance of the VME species, the abundance of the fishes, and the mean value of the environmental variables considered in the analysis (depth, slope, aspect, and roughness). The statistical contribution of each variable in the model was calculated using the Monte Carlo permutation test with 999 permutations. This process was performed using the "vegan" package in R software (Oksanen *et al*.,2019). The results of RDA allow to identify the environmental factors that explain the variation in the presence and abundance of the species (Gori *et al*.,2011).

For each dive, the number of ALDFG was recorded to assess anthropogenic impacts.

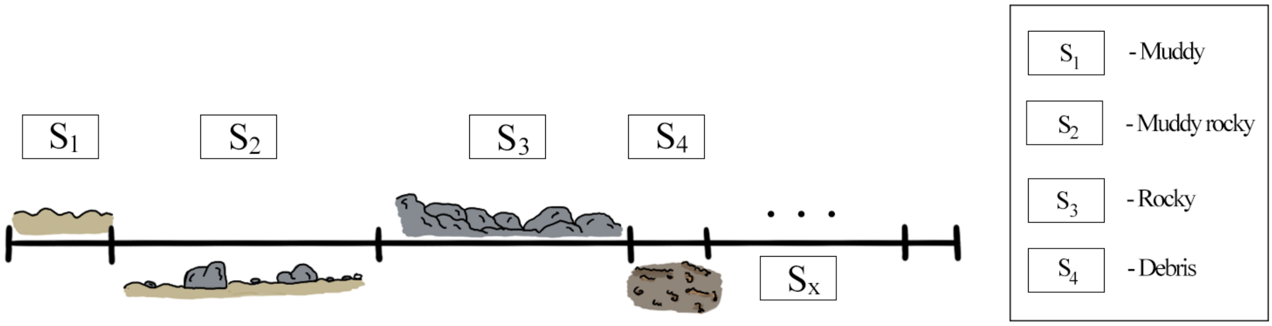

Figure 2: Graphical representation of the substrate type identified in the analyzed transect.

2.3 Results

Presence of VMEs in the Amendolara Bank

A total of 6 dives were performed in the Amendolara Bank in a depth range between 80 -166 m (Table 1). The dives were characterized by four types of substrates: muddy, muddy rocky, rocky, and debris, and in total we obtained 60 segments. Video analysis showed the presence of four VME species, discontinuously distributed or in patches: the black coral *Antipathella subpinnata* (Ellis & Solander, 1786) (Figure 3A), the yellow coral *Dendrophyllia cornigera* (Lamarck, 1816) (Figure 3B), the red coral *Corallium rubrum* (Linnaeus, 1758) (Figure 3C) and the sea fans sponge *Poecillastra compressa* (Bowerbank, 1866) (Figure 3D). These species were observed in a depth range of about 147 – 164 m, 82 – 96 m, 122 – 150 m, and 125 – 150 m respectively.

The black coral *A. subpinnata* was observed in the deeper investigated area in the south-eastern area of the Bank (Dive 6). This species was recorded exclusively on muddy rock substrates where it formed one dense forest with a density that can reach 2.8 ind/m.

Corallium rubrum has been observed only on rocky substrates, mainly on vertical walls in two dives in the western and eastern parts of the Amendolara Bank. The distribution of this species was patchy with a density of 0.2 ind/m in the western side (Dive 3) and a density of 0.11 n° ind/m in the eastern side (Dive 4) (Table 1).

Dendrophyllia cornigera, was observed only on muddy rock substrate in four different dives located in the south-eastern part of the Amendolara Bank. This species showed a scattered distribution and is mostly present in Dive 5 (Table 1).

The orange fan-shaped sponge *P. compressa* was observed scattered on muddy sediment in two different sites in the south-eastern part. In Dive 6 this sponge was recorded associated with the *A. subpinnata* forest.

In the Dive 6 at a depth of 148 m the occurrence of the echinoderm *Coronaster briareus* was recorded (Figure 4A, B) inside a crevice of small rocky outcrop.

Table 1: ROV transects carried out in the Amendolara Bank (Ionian Sea). For each VMEs species number of colonies observed is reported.

Dive	Lat	Long	Mean Depth (min-max)	Lenght (m)	*A. subpinnata*	*C. rubrum*	*D. cornigera*	*P. compressa*
1	39.84131°	16.81221°	97 (20 - 128)	268			3	
2	39.83987°	16.81342°	112 (20 - 133)	948			6	2
3	39.83918°	16.73576°	83 (31 - 90)	1582		315		
4	39.86349°	16.77031°	90 (22 - 98)	658		73		
5	39.85002°	16.79394°	123 (25 - 152)	536			37	
6	39.83526°	16.81709°	133 (21 - 166)	730	113		23	46

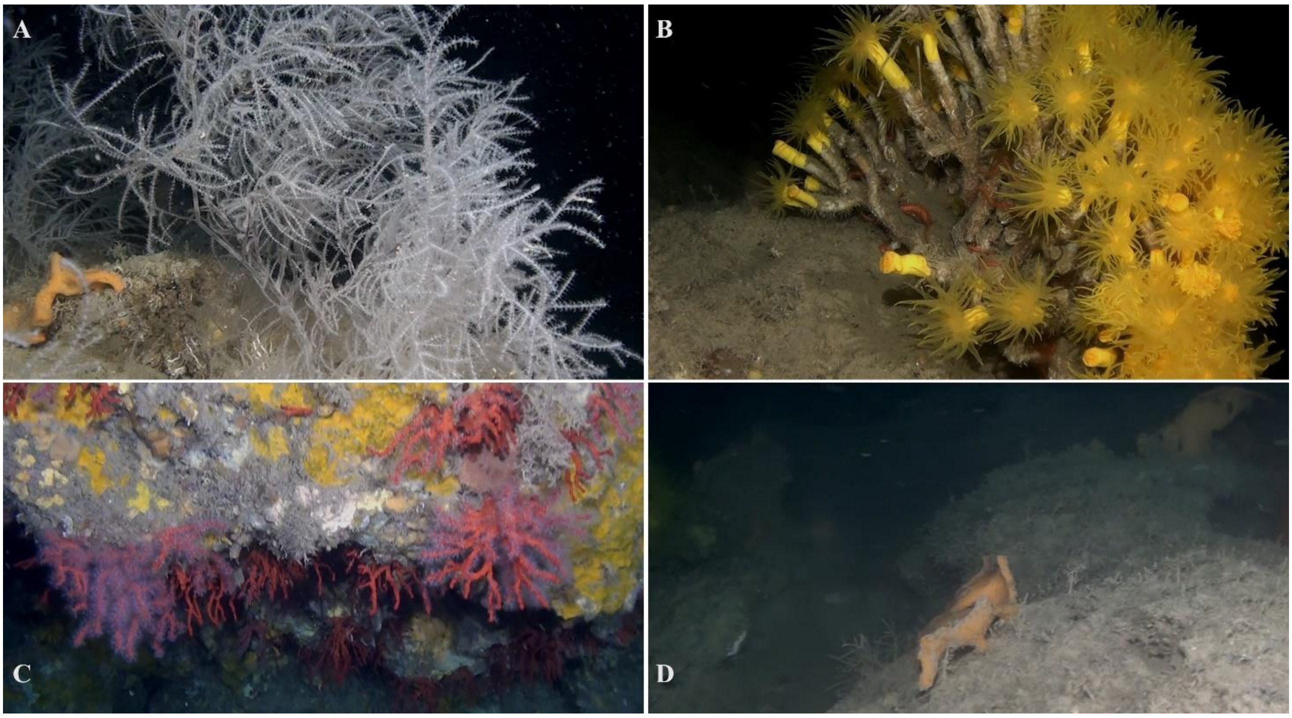

Figure. 3: VMEs species observed in the Amendolara Bank: *A. subpinnata* (**A**), *D. cornigera* (**B**), *C. rubrum* (**C**) and *P. compressa* (**D**).

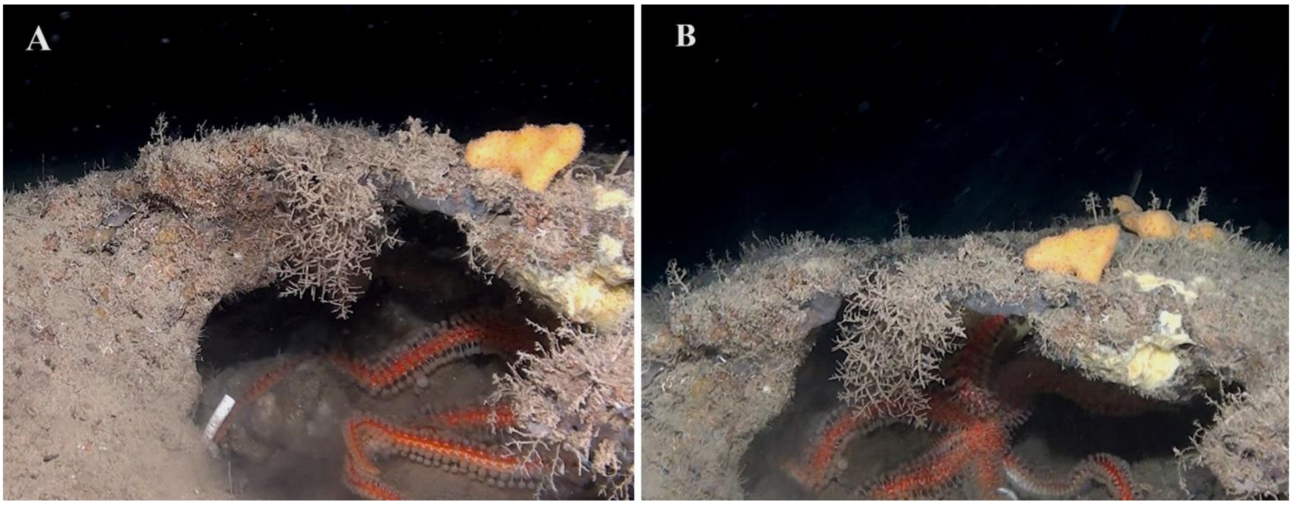

Figure. 4A, B: ROV image of *Coronaster briareus* observed in the study area. On the top of the outcrop is possible observe specimens of *P. compressa* and encrusting sponges.

Redundancy analysis (RDA)

The first two axes of the RDA model ($P < 0.05$) explained 44% of the species data variance; with the first and second axes explaining 29% and 15%, respectively (Figure 5).

The RDA analysis (Figure 5) shows that seabed substrate type and morphology have a significant influence on VMEs species distribution. More specifically, *A. subpinnata*, *D. cornigera* and *P. compressa* are associated to muddy rocky sediment. On the other hand, the presence of red coral *C. rubrum* is more probable in areas with rocky substrates and high values of slope and roughness.

A total of 22 fish species were observed in the explored sites with the most common species being *Anthias anthias*. Only 6 species are of commercial interest, in detail *Helicolenus dactylopterus*, (Delaroche, 1809), *Conger conger* (Linnaeus, 1758), *Zeus faber* (Linnaeus, 1758), *Mullus barbatus* (Linnaeus, 1758), *Scorpaena scrofa* (Linnaeus, 1758), and *Lepidorhombus boscii* (Risso, 1810). Only *H. dactylopterus* was associated with VMEs species (*D. cornigera*). Other fish species are associated with VMEs. In particular, *Lappanella fasciata* (Cocco, 1833), *Labrus mixtus* (Linnaeus,1758), *Boops boops* (Linnaeus,1758), *Macroramphosus scolopax* (Linnaeus,1758) and *Aulopus filamentosus* (Bloch,1792) are associated to *A. subpinnata*, *D. cornigera* and *P. compressa*. While *Phycis phycis* (Linnaeus,1766), *Gadella maraldi* (Risso,1810), *Callanthias ruber* (Rafinesque, 1810), *Scorpaenodes arenai* (Torchio, 1962), *Serranus cabrilla* (Linnaeus, 1758), and *Anthias anthias* (Linnaeus, 1758) are preferentially associated to *C. rubrum*.

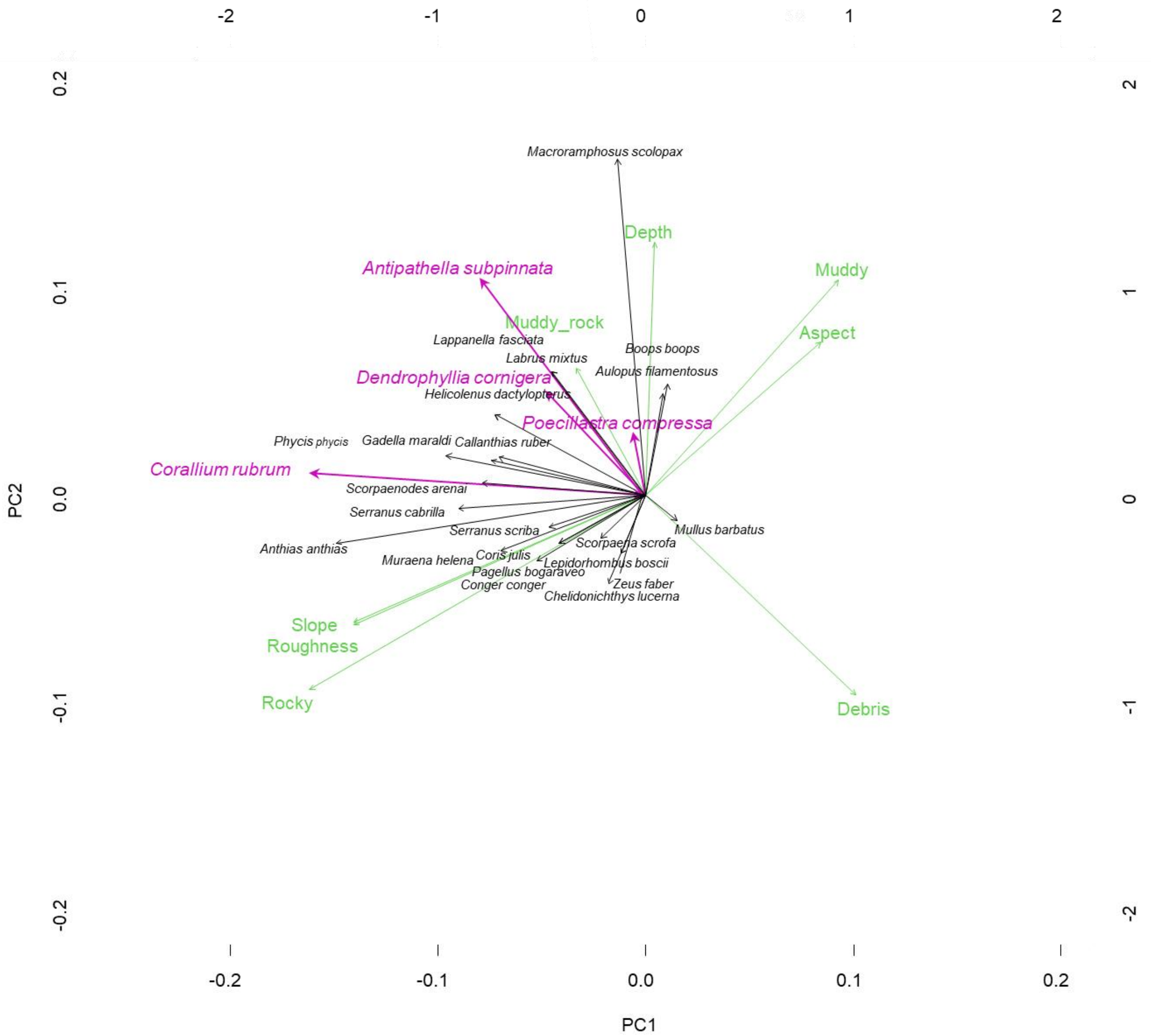

Figure 5: RDA analysis shows the relationship of VME species with environmental variables and associated fish fauna.

Anthropogenic impacts

Along the dives, 31 longlines were counted, most of them entangled on muddy rock and/or rocky substrates (Figure 6A). The most impacted species was *A. subpinnata* (Figure 6B), in fact, 18 of the total longlines were observed entangled on black coral colonies in the deepest part of the Amendolara Bank (Figure 7).

The other species impacted by fishing activities, even if with lower intensity, were *D. cornigera* and *P. compressa*. No ALDFG were observed around the colonies of *C. rubrum*.

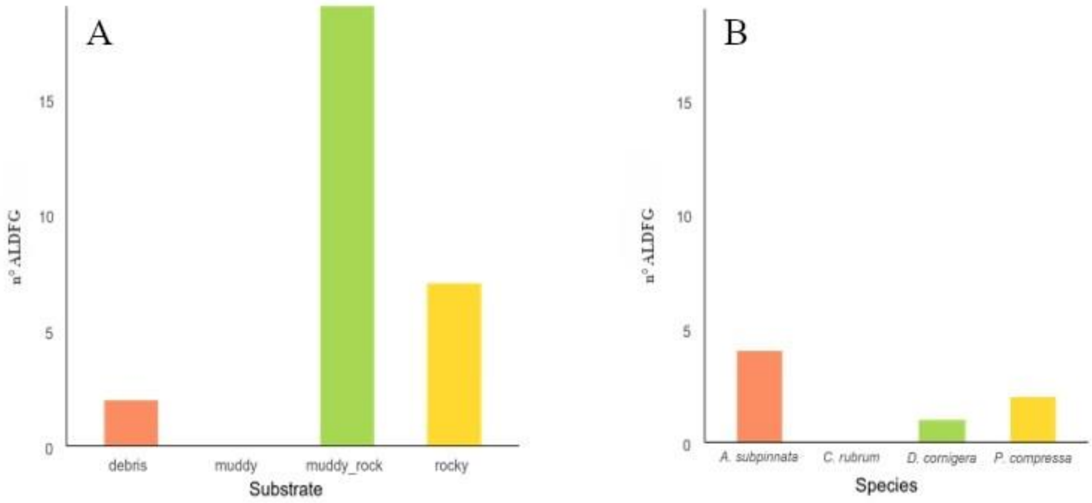

Figure 6: Number of ALDFG in relation to the typology of substrate (**A**) and VMEs species (**B**).

Figure 7: Longline entangled around *A. subpinnata* colonies.

2.4 Discussion and Conclusions

Mesophotic and deep-sea VMEs represents the most significant Mediterranean ecosystems below 50 m depth, which are also extremely sensitive to fishing activity (Chimienti *et al*., 2020b). In the present study, ROVs survey documented the presence of 4 different species classified as VMEs belonging to 4 different orders. The species are *A. subpinnata* (Antipatharia), *C. rubrum* (Scleralcyonacea), *D. cornigera* (Scleractinia) and *P. compressa* (Astrophorida).

Records of the black coral A. *subpinnata* and the red coral *C. rubrum* in the Ionian Sea are limited to the Apulian coast (Bo *et al*., 2008; Toma *et al*., 2022), the Amendolara Bank is the westernmost Ionian site that hosts these two protected species. Whereas *D. cornigera* and *P. compressa* have been previously observed in the area (Bo *et al*., 2012a; Castellan *et al*., 2019).

The main habitat-forming species on the bank are Cnidaria, and Porifera which are known to play an important role in marine ecosystems contributing to the restocking of fish populations by serving as Essential Fish Habitats (EFHs). Providing shelter from predators, spawning and nursery grounds, and greater availability of prey are only a few of the hypotheses that have been formulated to explain the association between fish and VMEs (D'Onghia *et al*., 2019a). The orange sponge *P. compressa*, due to its fan morphology, can create three-dimensional habitats, acting as appropriate refuges for other benthic species and vagile fauna (Bo *et al*., 2012a), and perform the same ecological role as deep water corals (Hogg *et al*., 2010). In fact, as shown by the RDA results, it seems that several fish species are associated to *P. compressa* habitat. Despite their important ecological role, sponge grounds are less studied with respect to coral habitats, and this could be explained by the difficulty of classifying sponge species by video survey (Bo *et al*., 2012a). RDA analysis confirm a relation between VME species and the fish community. As reported by D'Onghia *et al*. (2019a, 2019b) VME species can play an important ecological role for fish fauna. In particular, both commercially (*Helicolenus dactylopterus*, *Phycis phycis*) and non-commercially fish species (*Lappanella fasciata*, *Gadella maraldi*, *Scorpaenodes arenai*, *Serranus cabrilla*, *Anthias anthias, Callanthias ruber*) seems to be associated to the 4 VME species recorded. *Helicolenus dactylopterus* is known to be associated to Cold-Water Corals, including black corals, gorgonians, and sponges (Gomes-Pereira *et al*., 2014; Capezzuto *et al*., 2018; Kapiris *et al*., 2022). Also, *L. fasciata, A. anthias,* and *C. ruber* have been observed around coral habitats (Gomes-Pereira *et al*., 2014; Gomes-Pereira *et al*., 2017).

Concerning the substrate type preference, in the area *A. subpinnata*, *D. cornigera,* and *P. compressa* were associated to muddy rocky substrates while *C. rubrum* is associated to rocky substrate, and to the high value of slope and rugosity. As reported in previous studies *D. cornigera* can form aggregations on rocky substrate but also on soft sediments (Enrichetti *et al*., 2023) and *P. compressa* has been observed on both rocky and muddy rock substrates (Bo *et al*., 2012a; Bo *et al*., 2012b). *Corallium rubrum* is typically associated with a rocky substrate characterized by steep walls (> 60), as already reported in several papers which define the slope as a key factor for the distribution of this species (Carugati *et al*., 2022; Toma *et al*., 2022).

Another interesting finding of this study was the record of the echinoderms *C. briareus.* This species is mostly distributed in the Atlantic Ocean and its first observation in the Mediterranean Sea were reported from the Alboran Sea (Hebbeln *et al*., 2009) and Maltese waters during an ROV survey in which 26 individuals were observed (Evans *et al*.,2018). The first occurrence of *C. briareus* along the Italian coasts (Amendolara bank) was reported during an ROV survey in 2009 (Bo *et al*., 2020a). To date, with this new observation, the number of *C. briareus* observations rises to 29 specimens in the entire Mediterranean Sea and the second along the Italian coasts.

Banks and seamounts are ecologically and biologically significant marine regions that play an essential role in operating deep-sea ecosystems, principally contributing to offshore benthic and pelagic biodiversity (Bo *et al*., 2020b). Several studies have been carried out focused on Mediterranean seamounts, highlighting the presence of VMEs (like corals and sponges) and their vulnerability to anthropogenic pressure, mainly to fishing activities (trawling and longline fisheries)

(Bo *et al*., 2014a; Bo *et al*., 2020b; Goode *et al*., 2020). The Amendolara Bank appears to be highly impacted by ALDFG from artisanal fishing (e.g. longlines) which are known to cause damage (Bo *et al*., 2014b; Lauria *et al*., 2020). Although the impact of trawling hits larger surfaces, it is generally limited to muddy bottoms. The damage caused by longlines can affect rare or structuring benthic species since it can remove, broke or abrase the tissue of the organism due to strong currents or during recovery operations. Fishing gears is estimated to represents 98% of the total litter in certain areas (Angiolillo *et al*., 2015; Cau *et al*., 2017; Consoli *et al*., 2019) that cause degradation of marine habitats, sediment re-suspension, reduction of habitat-forming species, decrease of fish abundance, and diversity (Valderrama Ballesteros *et al*., 2018; Consoli *et al*., 2019).

The geomorphology of Amendolara Bank favours the presence of arborescent and branched benthic species with a major risk of entanglement (Angiolillo & Canese., 2018; Gori *et al*., 2017; Consoli *et al*., 2019; Moccia *et al*., 2020). Many of the VME indicator taxa , such as corals and sponges, have three-dimensional growth so are more sensitive; the use of longlines on VMEs should be prohibited (Chimienti et al., 2020a). In this study, the black coral *A. subpinnata* results to be more sensitive and impacted by anthropogenic pressure as more than half of the longlines were observed on this species. Other studies reported similar results with *A. subpinnata* as the most impacted species, easily getting entangled due to their arborescent morphology, colony size, and flexible skeleton (Angiolillo & Fortibuoni, 2020; Terzin *et al*., 2021).

The present study reports new information about the biodiversity in the Ionian Sea with the first records of the black coral *A. subpinnata*, and the red coral *C. rubrum*. Moreover, evidence of the relation between fish and VME species together with records of anthropogenic impact caused by fishing activities emphasizes the urgent need for further studies in the area aimed at increasing biodiversity knowledge. All this information will be essential in order to make Amendolara Bank's protection measures effective as well as to plan the extension of the actual perimeter of the Site of Community Interest (SCI) for the inclusion of the recorded VMEs habitats.

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Chapter 3. Effect of environmental and anthropogenic factors on the distribution and co-occurrence of cold-water corals

EFFECT OF ENVIRONMENTAL AND ANTHROPOGENIC FACTORS ON THE DISTRIBUTION AND CO-OCCURRENCE OF COLD-WATER CORALS

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Abstract

Cold-water corals (CWCs) are bioengineering species that can increase habitat heterogeneity and improve the deep sea's biological diversity and ecosystem functioning. Knowledge of their distribution provides a critical baseline for assessing the effect of natural and anthropogenic impacts on these important deep-sea habitats. The aims of this study are: i) provide new data on the spatial distribution of six CWCs species in the Strait of Sicily, ii) describe the principal environmental and anthropogenic variables that play a role in shaping their distribution, iii) identify hotspots in which individuals belonging to the various species co-occur. Presence-only data of six CWCs species, ten environmental variables (depth, slope, rugosity, aspect, flowdir, temperature, salinity, current North, current East, chlorophyll-a), and one variable relating to fishing effort (Automatic Information System – AIS) were used to predict the suitable habitats. We used Maximum Entropy modelling (MaxEnt) approach and used the AUC (area under the receiver operating characteristic curve) and TSS (true skill statistics) to evaluate the model performance. The results showed excellent AUC, TSS and standard deviation mean values for all six species. The validation, show excellent high predictive performance. MaxEnt identified slope, depth, and rugosity as the most important predictors, showing the highest percentage contribution for all six species considered. Throughout the study area, highly persistent density hotspots of CWC co-occurrence were discovered, with a total extension of 4.05 km^2 where all species co-occur. Although studies on the effect of environmental and anthropogenic factors that impact the distribution of these species of conservation interest remain scarce, the results of this study offer useful guidance for decision-makers to develop necessary conservation measures.

Keywords: CWCs, MaxEnt, SDMs, habitat suitability, deepsea, co-occurrence

3.1 Introduction

Cold Water Corals (CWCs) are among the most important habitat-forming species in the Mediterranean Sea (Chimienti *et al*., 2020). These ecosystems are made by cold affinity azooxanthellate cnidarian species from Scleractinia, Octocorallia, Anthipatharia and Stylasteridae (Otero & Marine, 2019; Chimienti *et al*., 2018, 2019; Barbosa *et al*., 2020). Due to their three-dimensional shape, they are considered bioengineering species that can increase habitat heterogeneity improving biological diversity and ecosystem functioning (Buhl-Mortensen *et al*., 2010; Chimienti *et al*., 2019; Barbosa *et al*., 2020). Due to their life-history characteristics, such as slow growth rate, high fragility, low recovery, low fecundity, and high longevity (Huvenne *et al*., 2016; Bargain *et al*., 2017; Barbosa *et al*., 2020), CWCs are very sensitive to environmental modification and anthropogenic pressure (Chu *et al*., 2019; Sundahl *et al*., 2020), such as bottom trawling, ocean acidification, and pollution (Bargain *et al*., 2017). Considering these characteristics, CWCs are categorised as Vulnerable Marine Ecosystems (VMEs) (FAO, 2009; Ashford *et al*., 2019; Barbosa *et al*., 2020) and many of these species are also listed in the International Union for Conservation of Nature (IUCN) Red List as "threatened" or "endangered" (Otero *et al*., 2017). In addition, many commercial and non-commercial fish and invertebrate species use these habitats as nursery, feeding, and refuge areas highlighting their key ecological role as Essential Fish Habitats (EFHs) (D'Onghia *et al*., 2017; D'Onghia, 2019; Lo Iacono *et al*., 2018).

Due to its semi-enclosed configuration, the Mediterranean Sea represents one of the most vulnerable regions to climate change and anthropogenic stressors (Otero & Marin, 2019; Chimienti *et al*., 2020; Fanelli *et al*., 2021). Despite its small dimensions, the basin hosts an elevated percentage of the planet's biodiversity (more than 7.5%) (Danovaro *et al*., 2010). It supports a wide variety of benthic habitats from the shallows to the deepest bottoms, making it a significant hotspot for biodiversity (Chimienti *et al*., 2020). In the last decade, new technology (e.g. Remotely Operated Vehicles (ROVs)) has allowed observation of the presence of CWC habitats in several parts of the Mediterranean Sea. However, very few of these species have been mapped and, as a result, the information on these habitats in the basin remains scarce (Savini *et al*., 2014). It is essential to increase the knowledge of deep-sea habitats (mesophotic and deep zone) to ensure adequate protection and that management measures are implemented (Chimienti *et al*., 2020).

In this framework, understanding the spatial distribution of CWC ecosystems and the primary factors determining their occurrence represent the first step in applying the appropriate management and protection plans (Otero & Marin, 2019; Sundhal *et al*., 2020; Lauria *et al*., 2021). However, field surveys in deep-sea areas are challenging and expensive (Stephenson *et al*., 2021), so the distribution and the effect of environmental and anthropogenic factors on these ecosystems are poorly understood (Bargain *et al*., 2017).

Nowadays, modern technology, such as multi-beam echosounders and ROVs, which can obtain complete coverage of species distribution, can be used for much more accurate prediction modeling (Yesson *et al*., 2012; Buhl-Mortensen *et al*., 2015; Sundahl *et al*., 2020; Abad-Uribarren *et al*., 2022). Habitat Suitability Models (HSMs) have grown significantly in resource management and conservation biology in the last few years. These models are currently used for determining the distribution of suitable habitats for VMEs in deep oceans (Stephenson *et al*., 2021), expanding the knowledge of their distribution at a global scale (Bargain *et al*., 2017).

Within this study, habitat modelling technique were used to create predictive distribution maps of six CWC species of conservation concern in the Mediterranean Sea: the scleractinian *Madrepora oculata, Desmophyllum pertusum, Dendrophyllia cornigera*, the antipatharian *Leiopathes glaberrima* and the scleralcyonacean *Callogorgia verticillata* and *Isidella elongata*, and to find areas where the greatest number of these species co-occur. The species were chosen because they were among the main arborescent CWCs found in the study area, taking into account both hard and soft substrate. The study aimed to: i) provide new data on the spatial distribution of six CWC species in the Strait of Sicily, ii) describe the principal environmental and anthropogenic variables that play a role in shaping their distribution, iii) identify hotspots in which individuals belonging to the various species co-occur.

3.2 Materials & Methods

Study area

The study area is located in the Northern part of the Strait of Sicily (38°0.587'N; 11°19.329'E, study area's centroid) (Figure 1) and cover an extention of about 1651 km^2. This area presents a pronounced bathy-morphological heterogeneity between 130 and 1000 m of depth, peculiar circulation patterns and diverse seabed typology, including rocky, muddy, coral rubble, muddy rock. In this area, the Atlantic waters divide into two branches, one flowing into the Tyrrhenian Sea and the other into the Sicilian Channel (Di Lorenzo *et al*., 2018). The latter turns into two branches: Atlantic Ionic water and Atlantic Tunisian water. The first one favours the formation of two vortices (one over the Adventure bank and the second off Cape Passero), with a complex circulation system that transports water between the eastern and the western sub-basins and generates upwelling (Manzella *et al*., 1990; Di Lorenzo *et al*., 2018) making the Strait of Sicily a highly productive area (Agostini *et al*., 2002). Currents are considered one of the most critical variables governing CWCs dispersion since they supply food for the corals and preserve their burying by sediments (Thiem *et al*., 2006; Taviani *et al*., 2011; Chimienti *et al*., 2019).

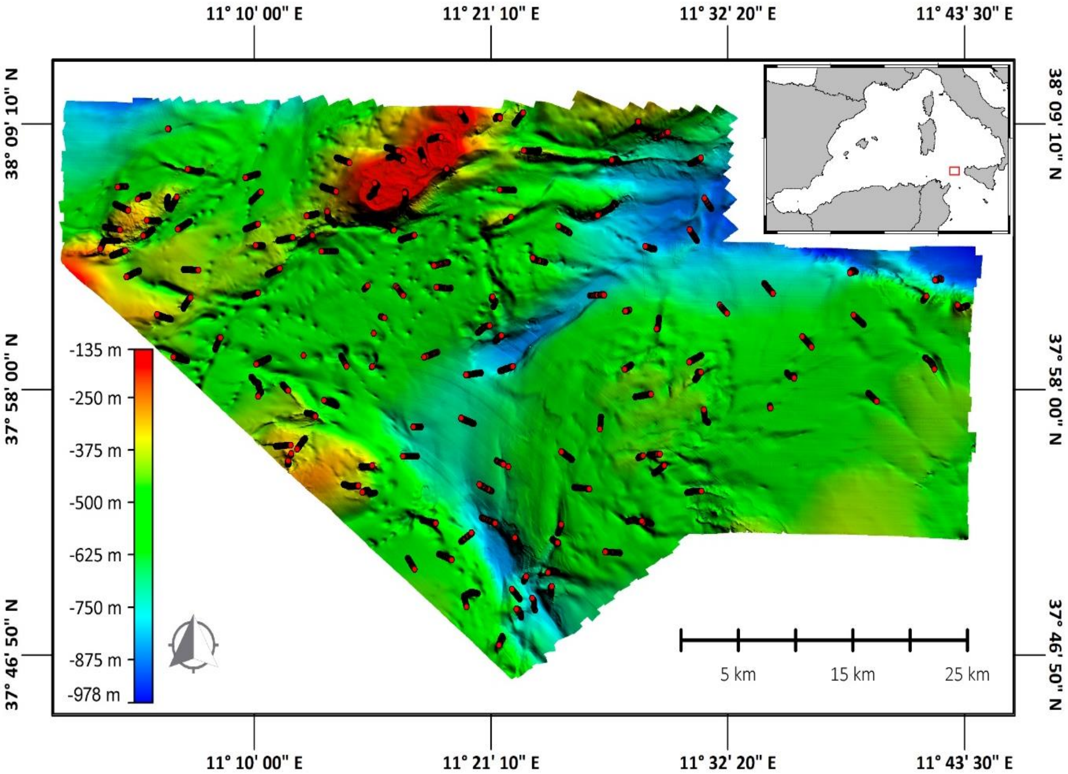

Figure. 1: 5 m resolution Multi-beam (MB) bathymetric model of the study area. The lines (in red) indicate the 140 transects performed in the area.

Data collection

Acoustic surveys were conducted from August 12th to September 6th, 2021. Seafloor morphology was acquired using a Multi-beam echosounder Kongsberg EM2040 in shallower waters (150 to 300 meters) and a Kongsberg EM712-MK2 in deeper waters (300 to 1000 meters). Raw acoustic data were processed to produce a 5 m cell Digital Terrain Model (DTM) of the entire area. ROV surveys were carried out from September 11th to November 17th, 2021 (67 days in total) under the MedWind project. The survey was carried out on board the vessel MainportGeo, which was equipped with a Tomahawk ROV Light Work Class with two manipulators five functions, four cameras (full HD, standard colour, standard black and white and high definition 6K camera), two laser beams, sampling box, beacon, DVL system and Seabird Microcat SBE 37. A total of 140 transects were carried out, in a depth range from 135 to 985 m. The length of each transect was variable, for a total of 129.5 km explored (mean 929 ± 257 dev.st). During video surveys, OFOP (Ocean Floor Observation Protocol) (van den Beld *et al.*, 2007) data logging software was used to record each observation with its corresponding information (date, time, the ROV's and ship's positions, depth).

Georeferenced presence data for the six species was extrapolated from the data set. The number of organisms of each species was standardised according to the length of each transect. As a result, the final unit of measurement is n°organisms x linear km. In the same period of the ROV survey, Oceanographic parameters (Temperature and Salinity) were collected in 97 random stations using a Rosette and CTD (Sea-Bird Scientific SBE 911 Plus V2).

Predictor variables

Eleven predictor variables were considered in the models, subdivided into terrain (depth, slope, rugosity, aspect, flowdir), oceanographic variables (chlorophyll-*a*, temperature, salinity, current North, current East) and fishing effort of bottom trawling activities (Automatic Identification Systems - AIS data) (Table 1; Figure 2). Terrain variables (slope, rugosity, aspect and flowdir) were extracted from high-resolution Multi-beam depth data (resolution 5 m) through the package "raster", function "terrain" in the *R software* (Hijmans, 2023).

The slope is an important variable for determining benthic habitat distribution, contributing to an increase in current flow, which benefits food supply, crucial for filter-feeding organisms (Mohn & Beckmann, 2002). Furthermore, because it restricts the use of some fishing gears, the slope can significantly lessen anthropogenic influence in some regions (Wilson *et al*., 2007). Its values range from 0 to (π)/(2) (1.57 radians), with low values often corresponding to gentle slopes and high values typically corresponding to steep slopes (Wilson *et al*., 2007), and are not always connected to rocky substrates. Increasing slope value is related to increased terrain complexity (Bargain *et al*., 2018).

"Rugosity" indicates the seafloor's complexity; in this case, low values indicate a soft seabed, while high values indicate a rocky seabed. The values were calculated as the difference in elevation between two adjacent pixels, with low values meaning no terrain variation and high values meaning terrain variation. Aspect indicates the orientation of the seabed and provides data on a certain area's exposure to local and regional currents (Wilson *et al*., 2007). Flowdir indicates the direction of the substrate slope. Along with rugosity, aspect and slope provide a wider and clearer view of the substrate (Qin *et al*.,2007).

Regarding temperature and salinity, a continuous raster was created for both variables from the CTD sampling points within the entire study area. To do this, a co-kriging analysis was carried out using temperature or salinity as respondent variables and bathymetry as a co-variable, which is important to predict the trend of the two respondent variables.

The co-kriging analysis, performed in R using the 'gstat' package (Pebesma, 2004), provided the prediction of the target variable at unsampled points from the co-variables. Current North, current East and chlorophyll-*a* (chl-*a*) were collected from the Copernicus Marine Service. The amount of fishing effort deployed by the trawlers operating in the selected area was estimated using all the available data collected by the AIS for the year 2021. The temporal frequency of vessel positions was standardised and interpolated at 10'; fishing trips by vessels were identified and fishing set positions by trip (hauls) were identified using speed and depth filters (Russo *et al*., 2016). The yearly amount of effective trawling effort (ETE) was finally estimated concerning the cells of the same 1×1 km grid and expressed as the number of fishing hours.

Table 1*:* Principal predictor variables used to produce the habitat suitability model for CWC species in the Northern part of the of Strait of Sicily.

	Variables	Units	Source	Method
Terrain	Depth	m		Multibeam echosounder Kongsbers EM2040 (shollow water) Multibeam echosounder Kongsbers EM2040 (shollow water)
	Slope	radians	Derived from Bathymetry	Terrain function (R software)
	Aspect	0-360°	Derived from Bathymetry	Terrain function (R software)
	Rugosity	no unit	Derived from Bathymetry	Terrain function (R software)
	Flowdir	no unit	Derived from Bathymetry	Terrain function (R software)
Oceanographic	Temperature	°C		Sea Bird Scientific SBE 911 Plus V2
	Salinity	psu		Sea Bird Scientific SBE 911 Plus V2
	Current East	m/s	Copernicus Marine Service	Satellite
	Current North	m/s	Copernicus Marine Service	Satellite
	Chlorophyll-a	mg m^{-3}	Copernicus Marine Service	Satellite
Fishing effort	AIS data	hours	Astra paging Ltd	Satellite

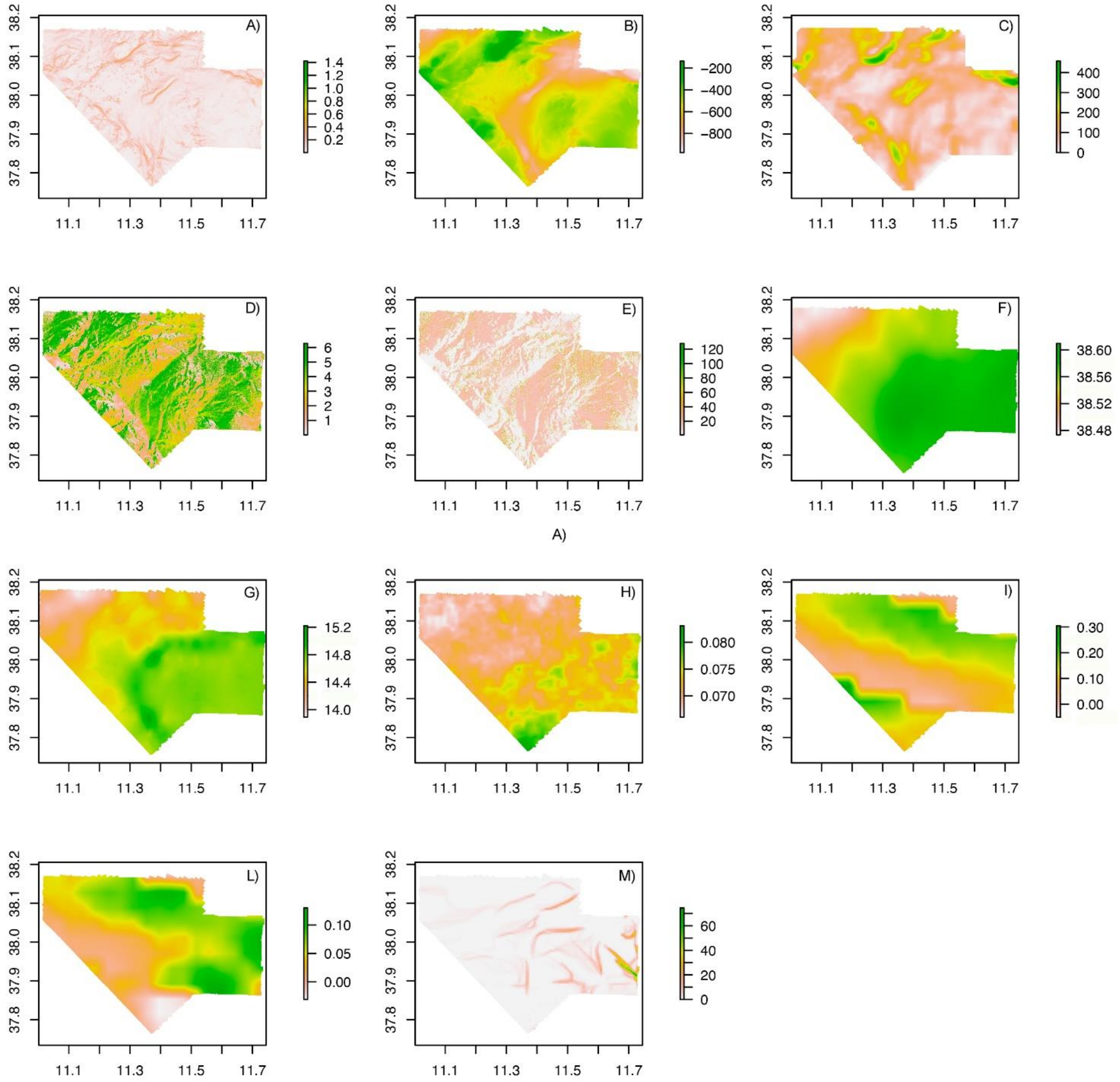

Figure. 2: Spatial patterns of the environmental predictors used in the MaxEnt model. These consist of (**A**) Slope; (**B**) Depth; (**C**) Rugosity (**D**) Aspect; (**E**) Flowdir; (**F**) Salinity; (**G**) Temperature; (**H**) Chlorophyll-*a*.; (**I**) Current East; (**L**) Current North; (**M**) Fishing effort (AIS data). These maps were performed in R environment.

Modelling approach

Habitat suitability maps for the six species of CWCs were performed using Maximum Entropy method (MaxEnt), a modelling approach used to identify probability distribution with the highest level of entropy when only species presence data are provided (Etnoyer *et al*., 2017). MaxEnt was used to create a model that connected every georeferenced observation to a set of predictor variables to predict habitat distribution in terms of the probability of suitability for species distribution (Fabri *et al*.,2017; Elith *et al*., 2011; Phillips *et al*., 2006). A correlation matrix using Pearson's coefficient was produced to identify the variables to be included in MaxEnt models and eliminate those with high collinearity.

The MaxEnt model was trained using batch files that allowed us to generate multiple models. Several default parameters were left unchanged, such as: 10^{-5} and 500 for convergent threshold and maximum interaction value, respectively, and maximum background points of 10,000 as suggested by other authors (Phillips & Dudík, 2008; Anderson *et al*., 2016; Bargain *et al*., 2017). For each species, we made four models (with ten replicates each) in which the "Regularization Multiplier" (RM) parameter varied, with values of 1, 1.5, 2 and 2.5, respectively. RM is a key parameter that, at higher values, represents if the forecast will be more widespread and less localised (Phillips, 2005), reducing the 'overfitting' of the data (Bargain *et al*., 2018). Moreover, the logistic outputs were chosen since they assess the probability of existence conditional on environmental variables and are simpler to understand. A k-fold cross-validation approach was employed with the MaxEnt model to evaluate the degree of uncertainty in the model's predictions. To compare the training and test datasets to validate the model, presence data were divided into 10 randomly generated partitions. This made it possible to acquire estimates of the predictive performance and uncertainty surrounding the fitted functions outside of the sample. The accuracy of the prediction of the models was calculated using Receiver Operating Characteristics (ROC) analysis through a comparison of the Area Under the ROC Curves (AUC) and the True Skill Statistic (TSS) value (Phillips *et al*., 2006; Liu *et al*.,2016). The AUC value is related to the model's reliability concerning the data. AUC value ranges from 0 to 1: less than 0.5 indicates that the model does not fit the data well, more than 0.7 is considered acceptable, and a value of 1 indicates an ideal model result (Bargain *et al*., 2017). Considering TSS value range, 0.2-0.5 indicate a poor model performance, 0.6-0.8 is useful and greater than 0.8 indicate an excellent model performance (Liu *et al*.,2023). According to the study of Liu *et al*., 2016, for each model, we selected the maxSSS values (maximum sum of sensitivity and specificity) as the threshold and calculated the average TSS of the results of the 10 replicates of the MaxEnt model to evaluate model performance. Test gain (which is a measure of goodness of fit) was also applied to evaluate how close the model is to the test presence samples (if the gain is 2, it means that the average likelihood of the presence samples is exp (2) =7.4 times higher than that of a random background pixel (Phillips *et al*.,2017). Moreover, each variable's contribution to the predictive model was also examined. A Jackknife test highlighted the percentage that each variable contributed to the final MaxEnt habitat suitability model. Jackknife tests examine how well the model predicts when only one of each variable is present, followed by all the variables save the one tested first. Then, habitat suitability mapping was done using the mean model of 10 replicates. The obtained probability maps of CWC distribution have been processed using the R environment to visualise the probability of presence in the study area. From the predictive maps obtained for each species, the coverage of the species in the study area, in terms of km^2, was determined. Four different ranges of occurrence probability were used.

Co-occurrence

The R package "geostats" (Vermeesch, 2022) was used to identify density hotspots on species distribution maps. Using the local Getis-Ord Gi statistic index (Getis & Ord, 1992; Ord & Getis, 1995), it was possible to identify statistically significant spatial groupings of high value (hot spot) and low value (cold spot). Z-core, p-value, and confidence level bin (Gi Bin) are the results of the analysis. The Gi Bin variable categorises the data into one of the three categories, ranging from -3 (cold spot – 99% confidence) to 3 (hot spot – 99% confidence). Features in the ± 3 bins have statistical significance with a confidence level of 99%; features in the ± 2 bins have a confidence level of 95%; features in the ± 1 bins have a confidence level of 90%; and clustering for features in bin 0 are not statistically significant (Milisenda *et al*., 2021).

An area of species co-occurrence was defined as that with the largest probability of occurrence of individuals belonging to various species. This area was identified using R software, which extracted the area where the hot spots overlapped. The overlap rate for each grid cell was calculated using the Index of Co-occurrence (CI) (Fiorentino *et al*., 2003; Colloca *et al*., 2009; Milisenda *et al*., 2021) to determine the relative persistence of a cell as a potential zone for species aggregation. This index was calculated as the sum of the number of species categorised as a hot spot in a specific area using the formula:

$$CI_i = \sum_{j=1}^{n} ij$$

When grid cell ("i") is included in a hot spot of species "j", ij = 1; otherwise, ij = 0; n is the total number of species. When density hotspots were not observed, the CI is reduced to 0, but when density hotspots exist for all species considered, CI increases to 6 for the cell "i". Results were plotted in a single co-occurrence map showing a scale of different co-occurrence classes (from 0 to 6).

In addition, Spearman analysis was used to evaluate correlations between species and the R function "ggcorrplot" was used to calculate and visualise positive or negative correlations among species distribution (Kassambara, 2022).

3.3 Results

Modelling evaluations

ROV surveys allowed observation and mapping of the six target CWC species (Figure 3): *M. oculata* n=4342, *D. pertusum* n=582, *D. cornigera* n=192, *L. glaberrima* n=375, *C. verticillata* n=3221, *I. elongata* n=10378.

Considering all species, MaxEnt model performed well using cross-validation, with a mean AUC value greater than 0.8 and a standard deviation between 0.008 and 0.02, showing that our models were significantly better than random. Therefore, a maximum and minimum test gain value of 2.87 (for *D. cornigera*) and 0.69 (for *I. elongata*), meaning that the average likelihood of occurrence samples for these two species is 17.63 and 1.94 respectively times higher than that of a random background pixel. Only for *I. elongata*, the TSS value is 0.6; for the other five species is more than 0.8. Table 2 shows the value of AUC, standard deviation, test gain with all variables, the average likelihood of the presence of samples and mean TSS for all six species. These data allow us to measure the goodness of fit. These results suggest the effectiveness of the models and the accuracy of their predictions of habitat distribution of the six CWCs species in the Strait of Sicily.

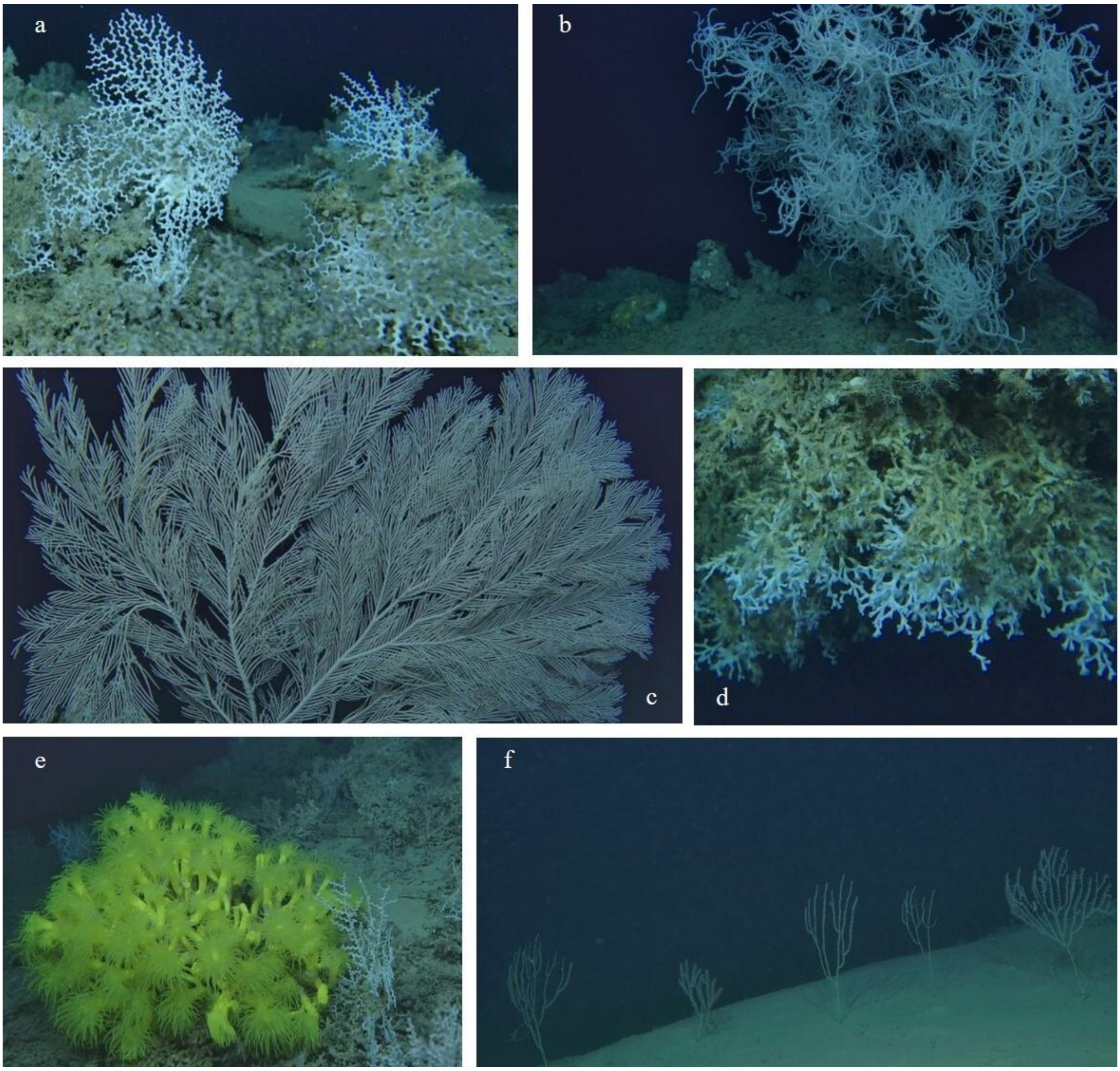

Figure. 3: Representative images of the six target CWCs species whose distribution and co-occurrence were modelled in this study: *Madrepora oculata* (**a**), *Leiopathes glaberrima* (**b**), *Callogorgia verticillata* (**c**), *Desmophyllum pertusum* (**d**), *Dendrophyllia cornigera* (**e**) and *Isidella elongata* (**f**).

Table 2: MaxEnt model validation for CWCs species. Average True Skill Statistics (TSS), Area Under the receiver operating characteristic Curve (AUC) and Standard Deviation (dev/st) of the results of 10 interactions of the MaxEnt models for each species are shown.

Species	Threshold	TSS	AUC	dev/st
Madrepora oculata	0.24	0.81	0.935	0.015
Desmophyllum pertusum	0.206	0.89	0.971	0.023
Dendrophyllia cornigera	0.071	0.86	0.973	0.016
Leiopathes glaberrima	0.129	0.83	0.956	0.02
Callogorgia verticillata	0.164	0.83	0.95	0.008
Isidella elongata	0.348	0.59	0.819	0.013

Evaluation of the importance of variables within the model

Salinity was deleted from all models after the Pearson correlation index. Table 3 provides the percentage variable contribution retained in the final model for each species, using the jackknife contribution test. MaxEnt identified slope, depth, rugosity, temperature and current north as the four variables contributing to all six CWCs species (all predictors outputs for all species are present in Supplementary material). The slope represents the key environmental predictor explaining most of the variance. The maximum and minimum values are expressed for *M. oculata* (70.2%) and *I. elongata* (46%), respectively.

Table 3: Percentage of contribution for each predictor on the distribution of the six CWC species included in the final MaxEnt model.

Species \ Variables	Slope	Depth	Chl-*a*	Current East	Current North	Temperature	Rugosity	AIS	Aspect	Flowdir
Madrepora oculata	70.2	4.8	3.2		11.7	7.4	0.9		0.6	1.3
Desmophyllum pertusum	69.4	6.4			3.8	3.6	5.3	8.5	1.9	1.1
Dendrophyllia cornigera	59.6	15	12.2		5.1	1.7	2.4		0.7	3.3
Leiopathes glaberrima	59.4	27.1		1.4	0.8	3.1	4.3	3.9		
Callogorgia verticillata	49	22.5	3.2	1.8	14.6	1.5	3.8		1.9	1.8
Isidella elongata	46	12.1	13.6	6.6	6.1	4.1	6.8	4.8		

All species exhibit a curvilinear positive connection with slope, with a greater probability of occurrence in areas with high slope values. More specifically, *M. oculata* (Supplementary Figure 1A), *D. pertusum* (Supplementary Figure 2A), *D. cornigera* (Supplementary Figure 3A) and *L. glaberrima* (Supplementary Figure 4A) showed a constant positive correlation. The sea fans *C. verticillata* (Supplementary Figure 5A), show an optimum range of 0.49 radians. Furthermore, the bamboo coral *I. elongata* (Supplementary Figure 6A), also shows a positive correlation and plateau at low slope values can be observed (around 0.3 radians).

Depth also had a significant impact on the habitat preferences of CWCs species. It showed a high probability of occurrence associated to the depth range of 430/750 m for *M. oculata*, (Supplementary Figure 1B) and *D. pertusum* (Supplementary Figure 2B). The graphs show a continuous positive correlation from 900 to 200, 700 to 200 and 900 to 150 m for *D. cornigera* (Supplementary Figure 3B), *L. glaberrima* (Supplementary Figure 4B) and *C. verticillata* (Supplementary Figure 5B), respectively. Instead, *I. elongata* (Supplementary Figure 6B) shows an optimum depth range between 800 and 500 m.

Rugosity is an important terrain variable that explains the complexity of the substrate. The highest values were associated with *M. oculata* (Supplementary Figure 1C) and *L. glaberrima* (Supplementary Figure 4C) (300 and 250, respectively), and the lowest value was associated with *I.*

elongata (Supplementary Figure 6C) (< 20). *D. cornigera* (Supplementary Figure 3C) showed an optimal value between 150 and 200. *C. verticillata* (Supplementary Figure 5C) showed values of around 50, while *D. pertusum* (Supplementary Figure 2C) showed an optimum of around 250 and 350.

The average response curve for Temperature shows higher probabilities of occurrence between 14 °C and 15 °C for *M. oculata* (Supplementary Figure 1G), *D. pertusum* (Supplementary Figure 2F), *D. cornigera* (Supplementary Figure 3G) and *L. glaberrima* (Supplementary Figure 4D). *Callogorgia verticillata* (Supplementary Figure 5F) show two peaks, of 14.3 °C and 15 °C. For *I. elongata* (Supplementary Figure 6E), the higher probability of occurrence is between 14.2 °C and 14.7 °C.

Current North appeared to be positively correlated with *M. oculata* (Supplementary Figure. 1H), *D. pertusum* (Supplementary Figure 2G) and *I. elongata* (Supplementary Figure 6G) with an optimal of 0.9 and 0.10 m/s for the first two species and 0.7m/s for *I. elongata,* while was negatively correlated with *D. cornigera* (Supplementary Figure 3H), *L. glaberrima* (Supplementary Figure 4F) and *C. verticillata* (Supplementary Figure 1H) the probability of presence decreased at high current values.

Current East was only relevant for three species: *L. glaberrima* (Supplementary Figure 4E) *C. verticillata* (Supplementary Figure 5G) and *I. elongata* (Supplementary Figure 6F). The first one shows a linear positive correlation starting from low current values. For the second, two optimality ranges can be observed, one between 0.05 – 0.012 m/s and one at 0.25 m/s. Instead, *I. elongata* showed a negative correlation.

Other main predictors with a significant contribution rate for the considered species, were chl-*a* for *M. oculata* (Supplementary Figure 1F) *D. cornigera* (Supplementary Figure 3F) *C. verticillata* (Supplementary Figure 5E) and *I. elongata* (Supplementary Figure 6D). The highest suitability was found for values between 0.077 – 0.082 mg*m^3 for *M. oculata*, and an optimal peak at the value of 0.82 mg*m^3 for *D. cornigera*, and 0.077 mg*m^3 for both *C. verticillata* and *I. elongata.*

Fishing effort (AIS) was found to be relevant for *D. pertusum* (Supplementary Figure 2H), *L. glaberrima* (Supplementary Figure 4G) and *I. elongata* (Supplementary Figure 6H). Fishing effort significantly and negatively affects the probability of occurrence of *D. pertusum* and *I. elongata.* As fishing efforts increased, the probability of occurrence of the species decreased. In the case of *L. glaberrima*, however, we can observe a positive correlation. Aspect and flowdir, on the other hand, provide a low contribution for four species considered: *M. oculata* (Supplementary Figure 1D-1E), *D. pertusum* (Supplementary Figure 2D - 2E), *D. cornigera* (Supplementary Figure 3D - 3E), and *C. verticillata* (Supplementary Figure 5D).

Predicted distribution

Areas with suitable conditions for the occurrence of CWCs species are shown in Figure 4. *Madrepora oculata* shows a higher probability of occurrence in the South and South-West area, *L. glaberrima* in the North, West and South-West area, and *C. verticillata* in the North and South-West area. These three species show the presence of small patches in delimitated geographical areas. *Desmoplyllum pertusum* and *D. cornigera*, were more randomly distributed, while for *I. elongata,* suitable habitat extended throughout the entire study area, with a higher probability in the south-east area.

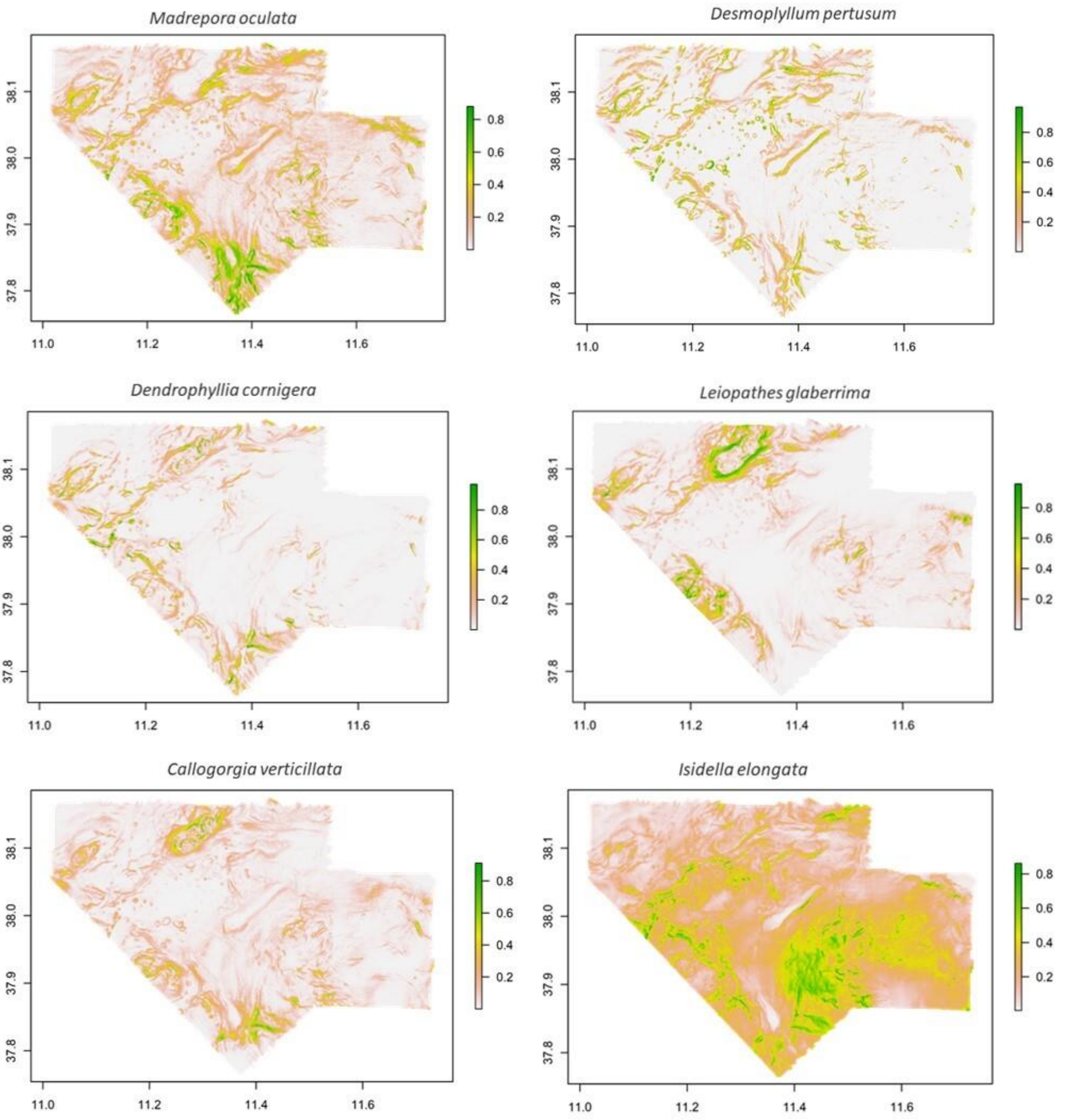

Figure. 4: Habitat suitability maps for *Madrepora oculata* (**A**), *Desmophyllum pertusum* (**B**), *Dendrophyllia cornigera* (**C**), *Leiopathes glaberrima* (**D**), *Callogorgia verticillata* € and *Isidella elongata* (**F**) in the Northern part of the Strait of Sicily using MaxEnt model. The legend shows the probability of occurrence values (high values in green and low values in white).

Co-occurrence and correlations

The probability of highly persistent density hotspots of CWCs occurrence were found scattered throughout the entire study area, with relevance in the northern, southern, south-eastern, and southwestern parts (Figure. 5). Areas identified as suitable (hotspots) for the six CWC species covered an extension of 206.8 Km^2, 172.4 km^2 and 105 km^2 for the Scleractinia *M. oculata, D. pertusum* and *D. cornigera* respectively, 119.3 km^2 for the Antipatharia *L. glaberrima* and 168.3 km^2 and 279.81 km^2 for the Alcyonacea *C. verticillata* and *I. elongata*, respectively (Table 4). The extension of the area in which the hotspots of all six species are present covers an area of 4.05 km^2 (CI 6) (CI 1 = 299.30 $km^{2,}$ CI 2 = 114,79 km^2, CI 3 = 70.20 km^2, CI 4 = 41.86 km^2, CI 5 = 24 km^2, CI 6 = 4.05 km^2).

All species exhibited a positive correlation, according to Spearman's correlation matrix (Figure 6). From the graph the highest degree of correlation was given by the pairs *M. oculata* - *D. pertusum* and *D. cornigera* - *C. verticillata*, while the lowest correlation was found between *I. elongata* - *L. glaberrima.*

Table 4: Hotspots of CWCs extension measured as km^2 in the study area.

Species	Hotspot extension (km^2)	% Coverage in the study area
Madrepora oculata	206.8	0.12
Desmophyllum pertusum	172.4	0.10
Dendrophyllia cornigera	105	0.06
Leiopathes glaberrima	119.3	0.07
Callogorgia verticillata	168.3	0.10
Isidella elongata	279.81	0.17

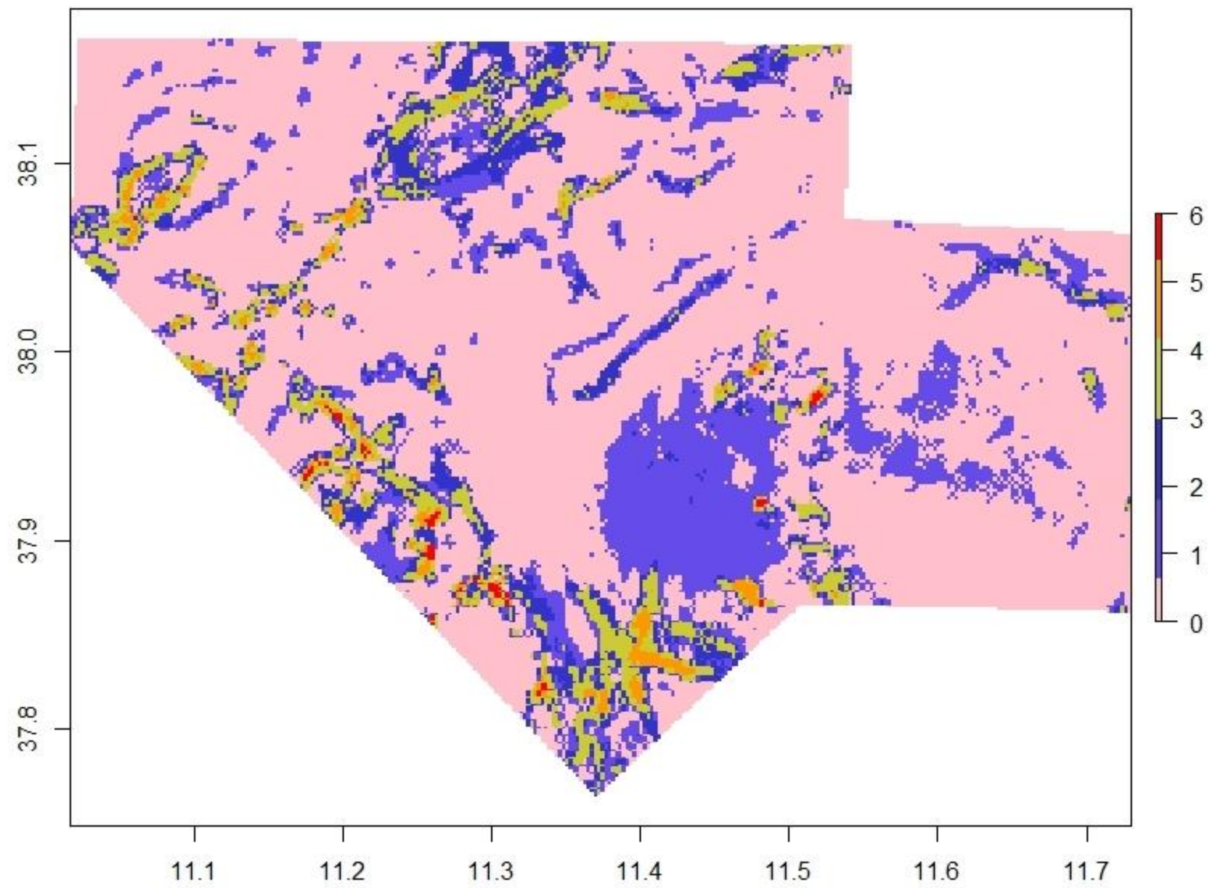

Figure. 5: Co-occurrence Index of CWCs. The different colours express the number of species co-occurring in the same area (e.g. pink indicates 0 species, red indicates six species co-occur in the same area).

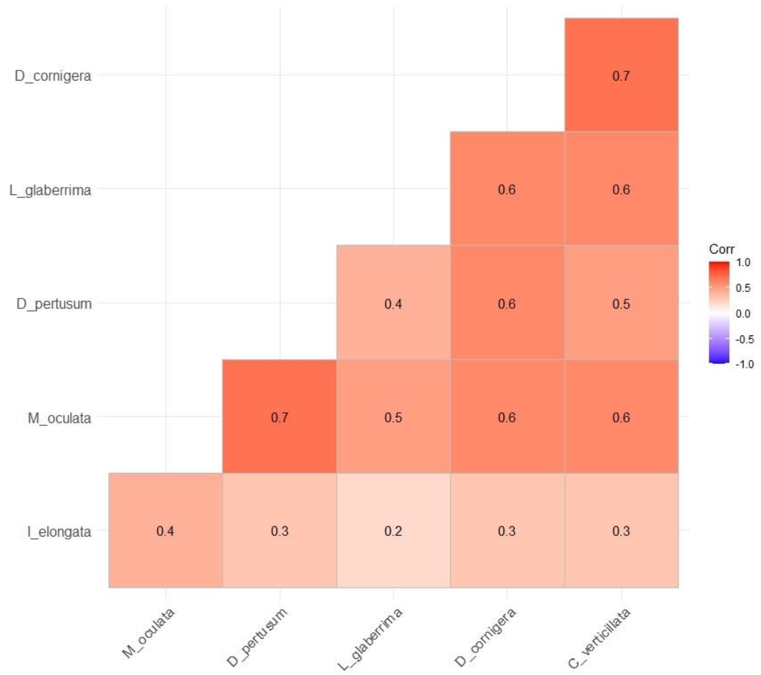

Figure. 6: Spearman correlation matrix of all CWC species.

3.4 Discussion

The present study modelled the distribution of six CWC species based on 10 potential drivers using a MaxEnt approach in the Northern part of the Strait of Sicily. For the first time, several CWC species were studied together through high-resolution sampling, and their probability of occurrence was used to produce co-occurrence maps, which will be a powerful tool in supporting the implementation of conservation actions in the area.

Habitat suitability models generated showed an AUC and TSS value of more than 0.8 for all species except for *I. elongata*, for which the TSS value is 0.59 (useful), suggesting that the MaxEnt models predicting successfully the six CWC species' distribution in the study area.

Among all predictors, slope, depth, rugosity, temperature and Current North were the main variables influencing the likelihood of distribution and habitat suitability of CWCs species.

As reported by several other authors, slope, depth, and rugosity are among the factors that most influence the habitat preferences of CWCs (García-Alegre *et al*., 2014; Lauria *et al*., 2021; Abad-Uribarren *et al*., 2022).

The species' habitat preferences are strongly influenced by slope, showing that the probability of occurrence appears to be positively correlated with this variable. This factor influences current flow, and consequently influences food availability for benthic species (Mohn and Beckmann, 2002; Wilson *et al*., 2007; García-Alegre *et al*., 2014). Considering that CWCs are suspension feeders, the previously mentioned conditions (exposure to currents and increased food supply) are of fundamental importance in modelling their distribution (Portilho-Ramos *et al*., 2022). In addition, the slope may also be a limiting factor for fisheries (Grehan *et al*., 2005; García-Alegre *et al*., 2014) and indirectly protects the species from fishing activities providing refuge for benthic fauna (Huvenne *et al*., 2011; Pierdomenico *et al*., 2016; Pearman *et al*., 2020).

Slope can also influence the seabed's exposure to currents (Bargain *et al*., 2018), representing one of the key environmental variables influencing deep-sea ecosystems (Hebbeln *et al*.,2016; Rebesco

& Taviani, 2019). Hydrodynamics was among the factors affecting the distribution of some CWCs under consideration. The currents, in addition to increasing the food supply, promote the spread of coral propagules and, as a result, the species' success, while also preventing burial (White *et al*., 2005; Henry *et al*., 2014; Bargain *et al*., 2018; Chimienti *et al*., 2019; Rebesco & Taviani, 2019; Pearman *et al*., 2020; Lim *et al*., 2020). In agreement with this, many observations of CWCs have been made in association with steep walls (Davies *et al*., 2014; Robert *et al*., 2015; Pearman *et al*., 2020).

Depth also significantly influenced the habitat preferences of CWC species. It showed a high probability of occurrence associated with different depth ranges depending on the species considered. The ranges recorded support previous findings for these CWC species (Hebbeln *et al*., 2009; Mastrototaro *et al*., 2010; Lo Iacono *et al*., 2012; Gori *et al*., 2013; García-Alegre *et al*., 2014; Carbonara *et al*., 2022) confirming the extrapolation potential of our study and the selected model applied.

Rugosity of the seafloor is another main factor driving the distribution of CWC, confirming that corals promote substrate complexity (Bargain *et al*., 2018), except for some species that prefer muddy/sandy bottoms. This is the case of the bamboo coral *I. elongata* which presented the lowest rugosity value. This species is the only one among those analysed that is suggested to prefer muddy sediments (Mastrototaro *et al*., 2017; Pierdomenico *et al*., 2018; Chimienti *et al*., 2019; Carbonara *et al*., 2020), which was also confirmed in this present study.

Other relevant variables are temperature and fishing effort. Regarding the first one, it is widely acknowledged that the distribution of CWCs is restricted by temperature instead of depth, and they are most typically found at temperatures ranging from 4 to 12 °C (Roberts *et al*.,2009; Maier *et al*.,2012). They exist in the Mediterranean Sea at temperatures ranging from 12.5 °C to almost 14 °C, with occasional higher temperatures (Freiwald *et al*., 2009; Castellan *et al*., 2019; Reynaud *et al*.,2021).

Fishing activities, especially bottom trawling, negatively impact CWC communities. This activity removes living benthic fauna from the substrate and secondarily, it harms the substrate that has a crucial role in the settlement of new coral colonies and, therefore, the possibility of natural restoration of the original habitat (Hall-Spencer *et al*., 2002; Fanelli *et al*., 2017). The species can be damaged even if not removed by trawling activity, resulting in more susceptibility to epibiosis, parasitism, and predation (D'Onghia, 2019). CWCs habitats are classified as EFHs as they have a key ecological role for many commercial and non-commercial fish and invertebrate species that use these habitat as nursery, feeding, and refuge areas. Regarding *I. elongata*, it is assumed that 5% of the entire income of professional fishing in the Mediterranean comes from species associated with bamboo coral forests, for example, the red shrimps *Aristeus antennatus* and *Aristeomorpha foliacea* (Cartes *et al*., 2013; Mastrototaro *et al*., 2017; Pierdomenico *et al*., 2018; STECF, 2019; Carbonara *et al*., 2020). Fishing these species of commercial interest using trawl nets has a significant impact on *I. elongata* populations, also in the Strait of Sicily (Lauria *et al*., 2017; Mastrototaro *et al*., 2017; Carbonara *et al*., 2020).

The analysis of Spearman's matrix suggests that all the species showed a positive correlation in terms of co-occurrence. Species that prefer more complex substrate types, mainly rocky bottoms, such as *M. oculata, D. pertusum, D. cornigera, L. glaberrima*, and *C. verticillata* are more associated and presented a high value of correlation (Spearman correlation: 0.4 - 0.7). On the other hand, the bamboo coral *I. elongata*, showed low correlation values with other CWC species (Spearman correlation value: 0.2 – 0.4). Regarding the correlation between *I. elongata* and *M. oculata* (0.4), this could be due to the presence of many living colonies of *M. oculata* being able to colonise other types of substrates (e.g. small rocky, thanatocoenosis, wrecks) observed during ROV surveys in areas characterised by muddy bottoms. In addition, many colonies of *M. oculata* and *D. cornigera* were observed on stretches of thanatocoenosis. As reported by other authors, CWCs can grow and live over dead coral structures (Hebbeln *et al*., 2016).

This study provides a co-occurrence analysis among CWC species, which allowed, for the first time, the identification of suitable habitat in km2 for all six CWC species within the study area. This new approach is of fundamental importance for conserving these vulnerable habitats. Based on these results, adopting new management measures could enable the protection of more species and, indirectly, the associated fauna. Co-occurrence analysis, and HSMs, provide information of great importance from a management perspective. Nevertheless, the high-resolution data used in this study will be increasingly needed to study the distribution and importance of environmental and anthropogenic factors that reduce the presence of CWCs. However, these data may be difficult to obtain or collect in some challenging and large areas; therefore, predicting their distribution using appropriate habitat models provides a useful tool for decision-makers (Kinlan *et al*., 2020).

3.5 Conclusions

The present study enhances our knowledge of the spatial distribution of some important CWC species (*M. oculata, D. pertusum, D. cornigera, L. glaberrima, C. verticillata* and *I. elongata*) using a fine spatial scale (5m). Our results support the idea that anthropogenic and environmental variables play a significant role in controlling species distribution. Considering this and that these ecosystems are crucial for many other species (with commercial and non-commercial value), it is critical to implement effective management strategies to ensure the protection and conservation of these populations and their associated biodiversity.

Despite the advancement of technologies (e.g. ROVs, Multi-beam echosounder, high-resolution data), data on the distribution of deep-sea species are often difficult to obtain. Predicting their distribution using habitat suitable models is crucial because mapping vulnerable environments of conservation concern is considered the first step in the environmental protection framework. It is important to emphasise that environmental factors driving species distribution can vary even at small scales, altering the probability of species presence. For this reason, this study highlights the importance of continuing to use high-resolution (5 m) spatial scales to accurately estimate the habitat suitability for benthic communities.

This paper also reports, for the first time, the co-occurrence analysis between CWC species, showing that in some zones within the study area, there is a partially overlapping distribution among some or all the species considered.

The valuable and new information obtained from this study can be useful for fisheries management and used to guide the establishment of new Fisheries Restricted Areas (FRAs) to preserve and increase the conservation of its area and natural restoration. In addition, the modelling approach used in this study may be extended to the entire Mediterranean, providing a large-scale view of these species' distribution.

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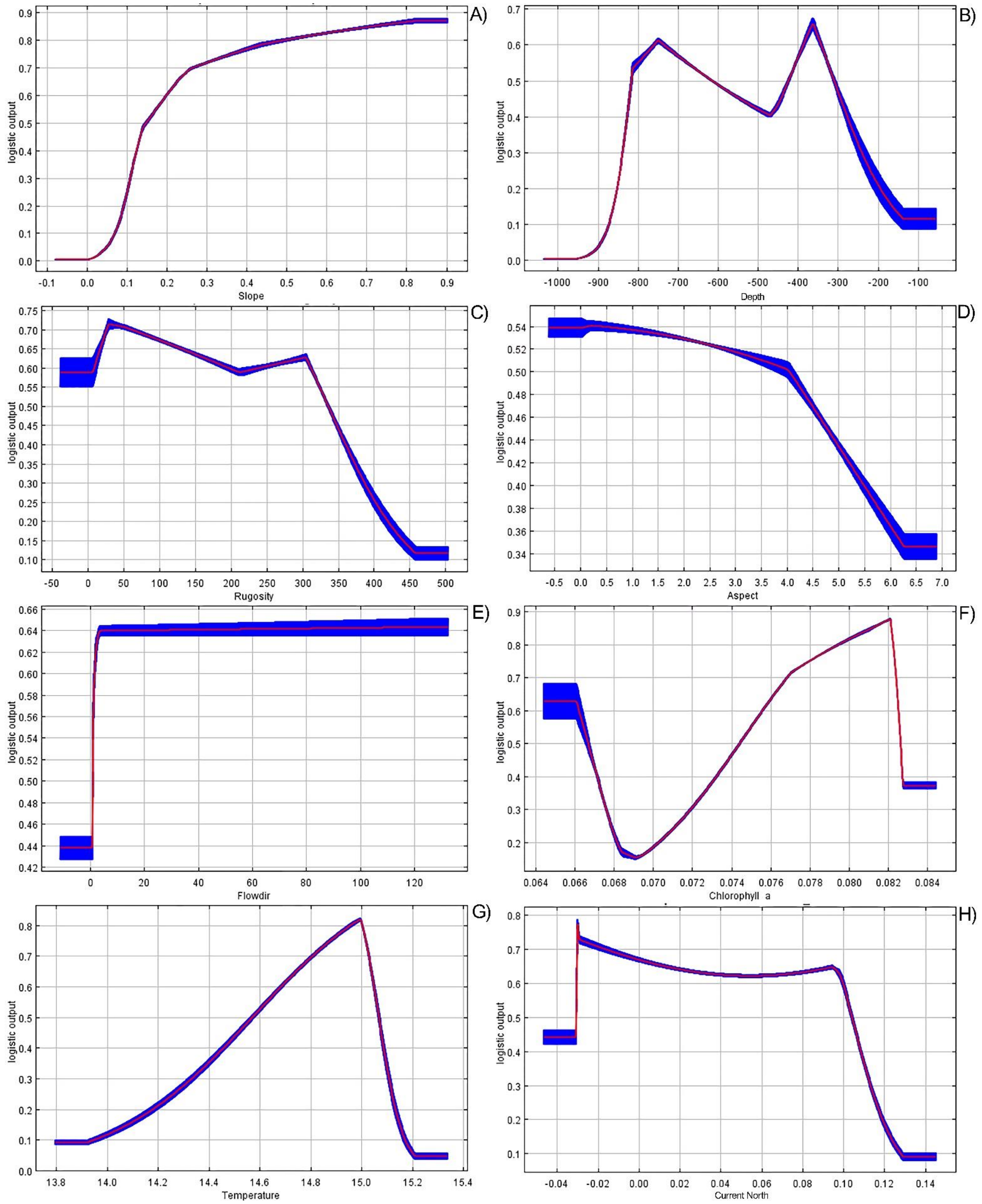
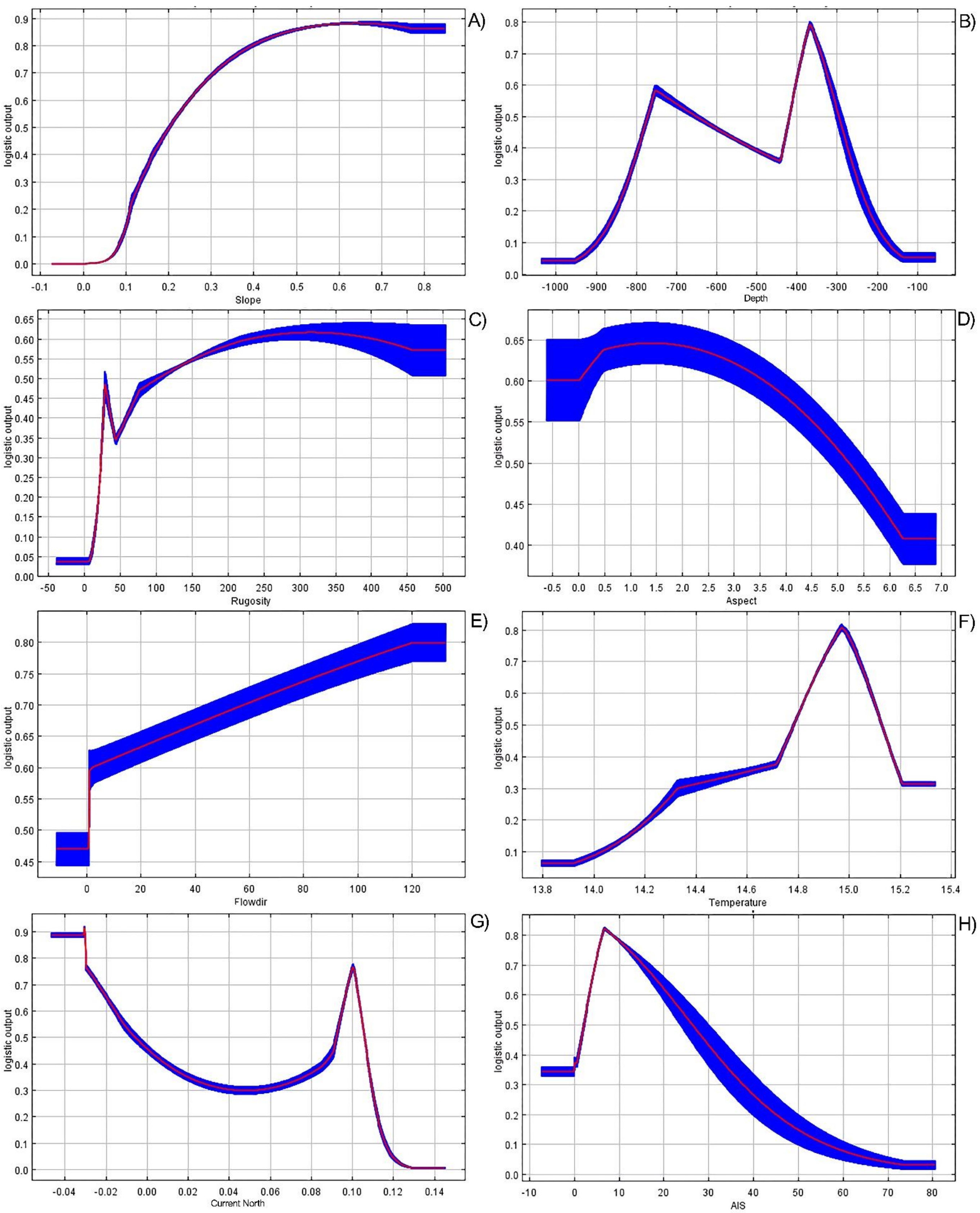
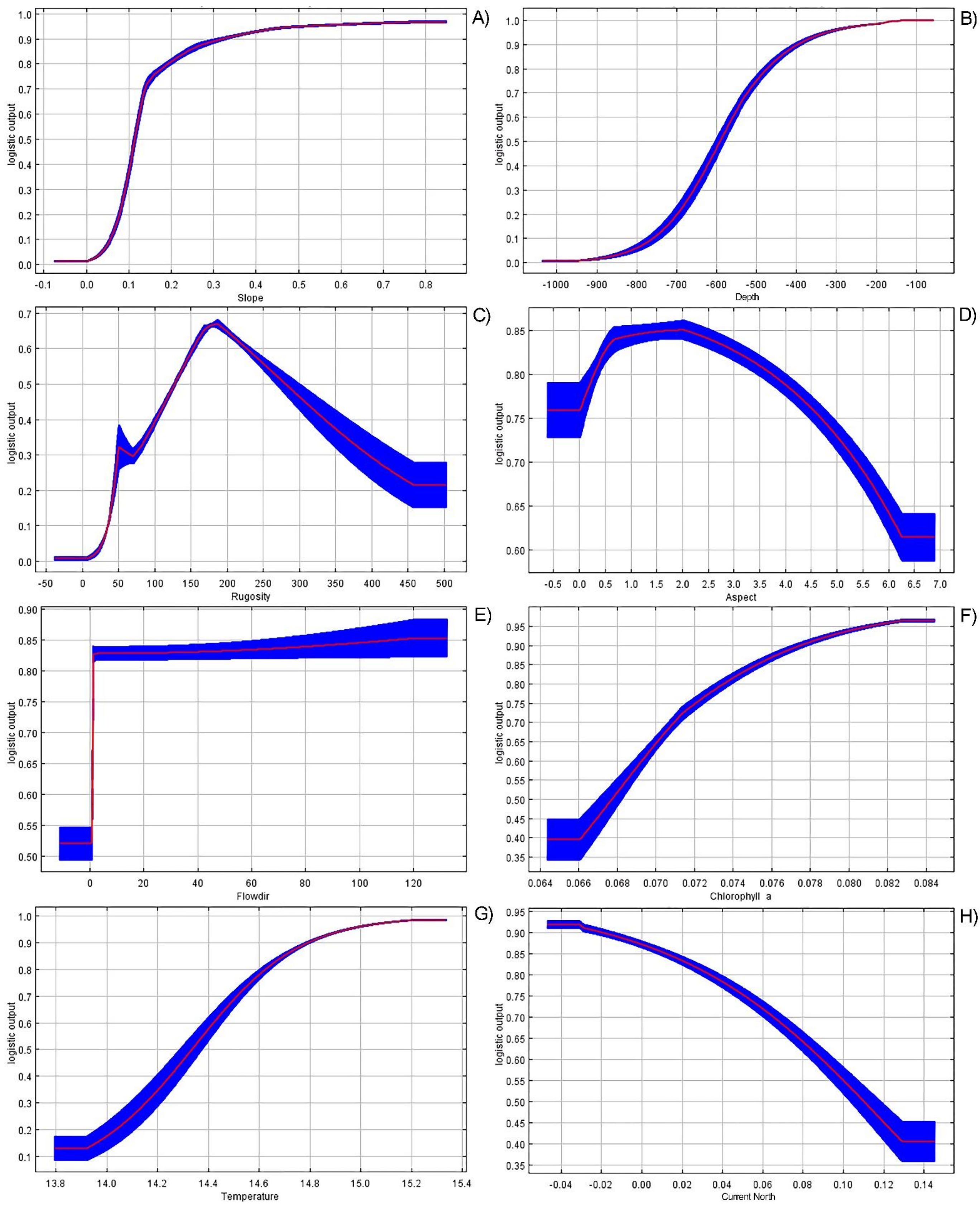
Supplementary Material



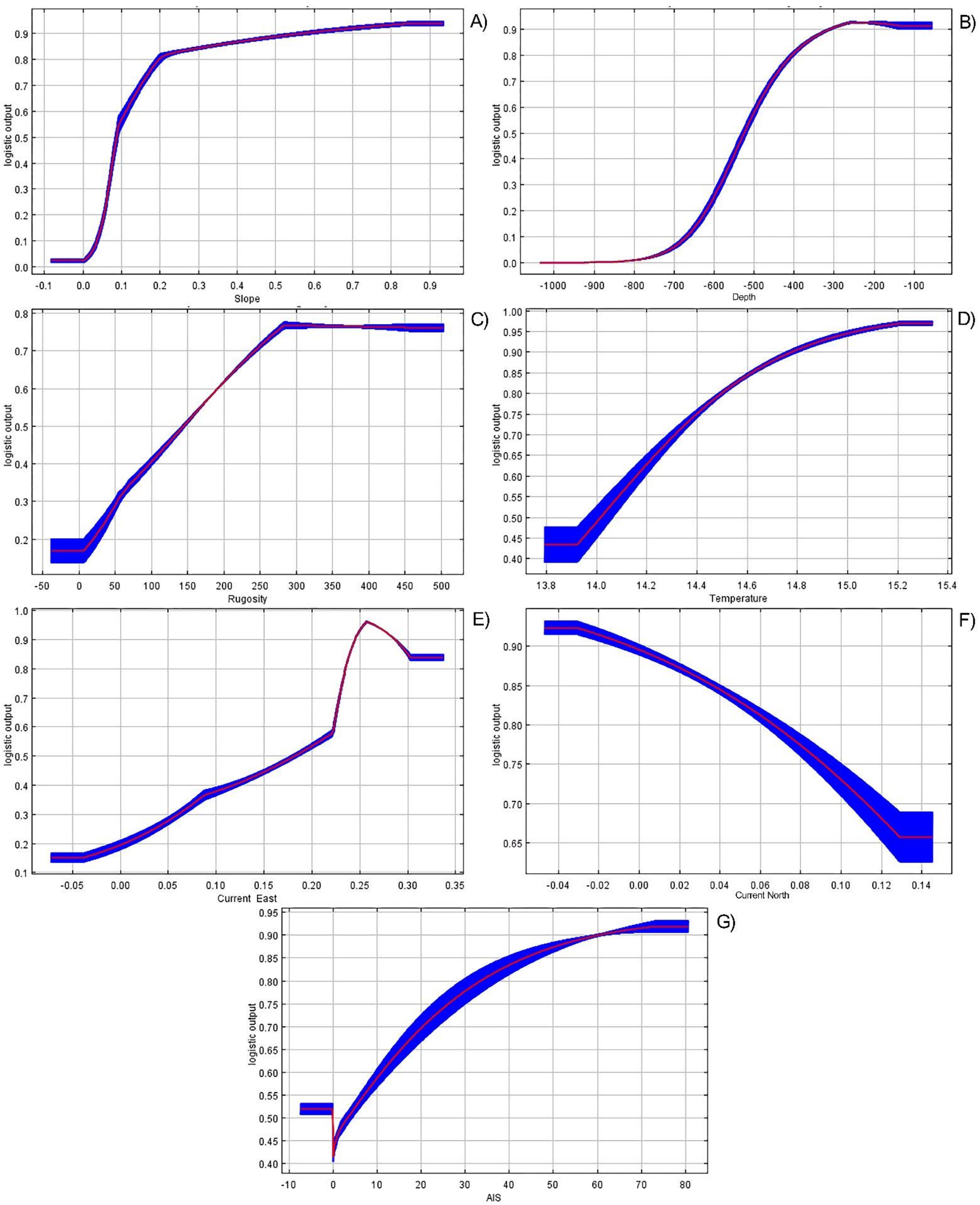
Supplementary Figure 1. Response curves representing the expected likelihood of *Madrepora oculata* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Aspect, **E**) Flowdir, **F**) Chlorophyll-*a*, **G**) Temperature and **H**) Current North.



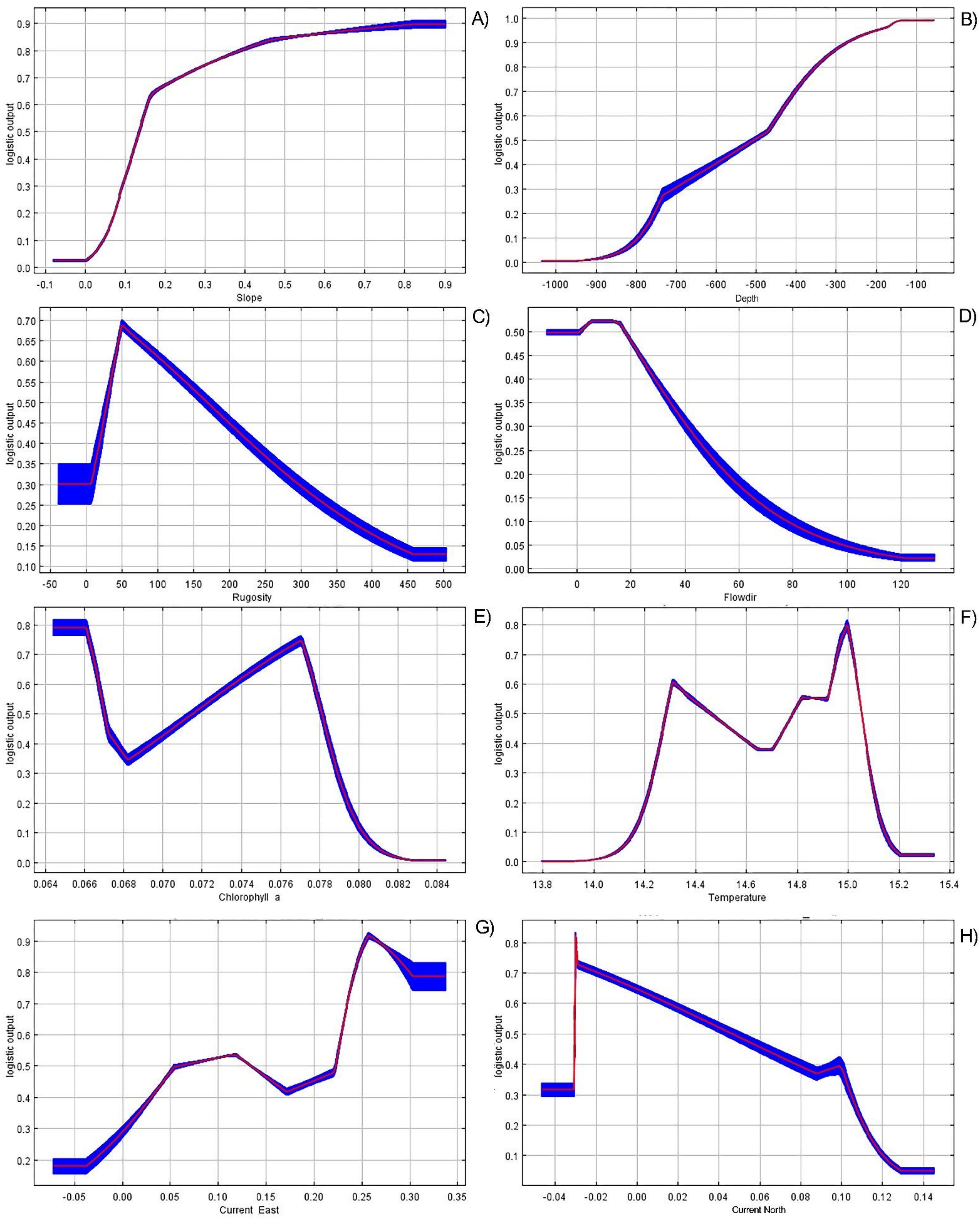
Supplementary Figure 2: Response curves representing the expected likelihood of *Desmophyllum pertusum* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Aspect, **E**) Flowdir, **F**) Temperature, **G**) Current North and **H**) AIS (fishing effort).



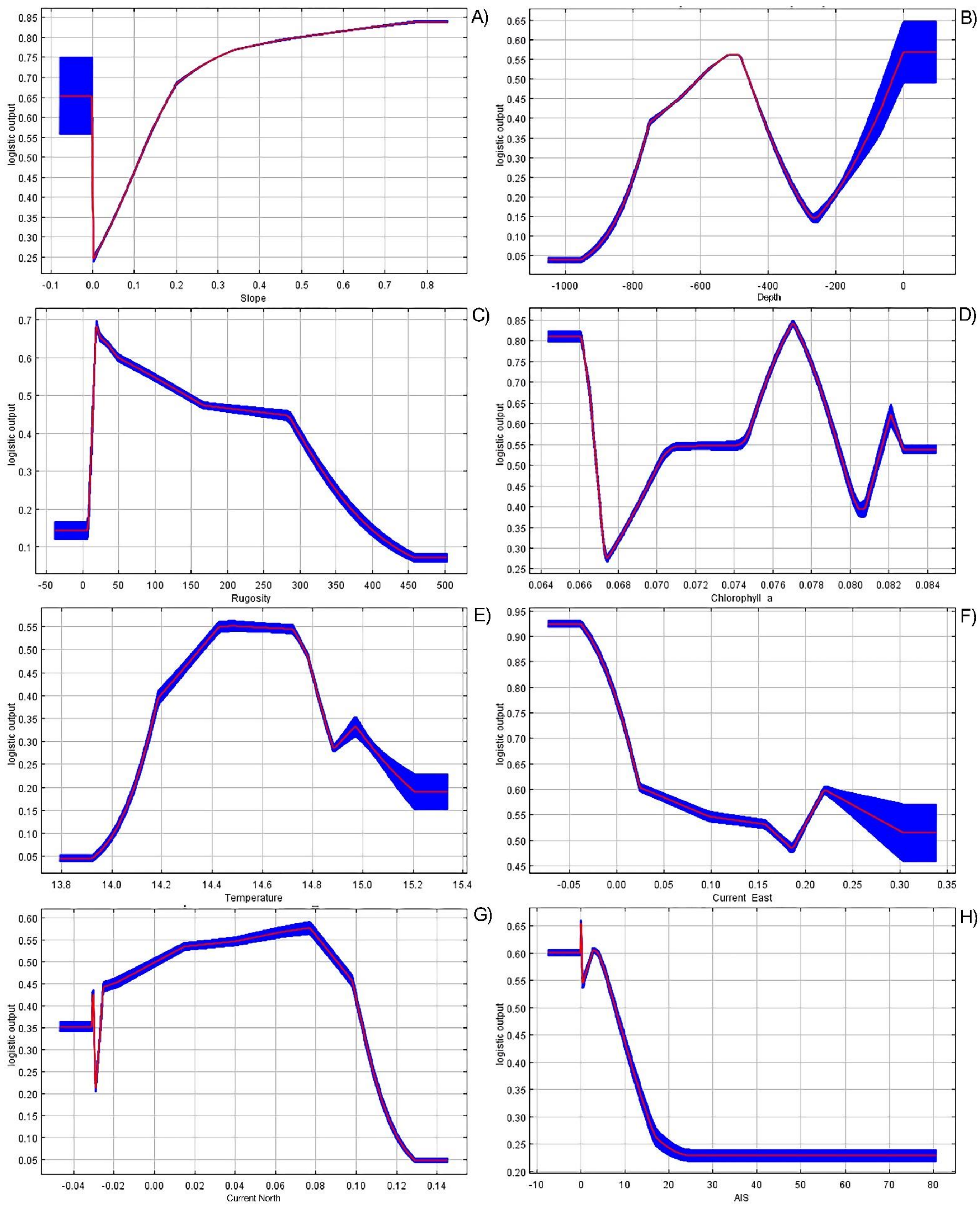
Supplementary Figure 3: Response curves representing the expected likelihood of *Dendrophyllia cornigera* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Aspect, **E**) Flowdir, **F**) Chlorophyll-*a*, **G**) Temperature and **H)** Current North.



Supplementary Figure 4: Response curves representing the expected likelihood of *Leiopathes glaberrima* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Temperature, **E**) Current East, **F**) Current North, and **G**) AIS (fishing effort).



Supplementary Figure 4: Response curves representing the expected likelihood of *Callogorgia verticillata* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Flowdir, **E**) Chlorophyll-*a*, **F**) Temperature, **G**) Current East and **H**) Current North.



Supplementary Figure 6: Response curves representing the expected likelihood of *Isidella elongata* presence with respect to the continuous environmental variables in our study area. The curves display the mean response of the ten replicate MaxEnt runs (red) +/- the standard deviation (blue) for: **A**) Slope, **B**) Depth, **C**) Rugosity, **D**) Chlorophyll-*a*, **E**) Temperature, **F**) Current East, **G**) Current North and **H**) AIS (fishing effort).

Chapter 4. Ecological role of benthic habitat-forming species on muddy sediment

ECOLOGICAL ROLE OF BENTHIC HABITAT-FORMING SPECIES ON MUDDY SEDIMENTS

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Abstract
Habitat-forming species play a crucial ecological role, also on soft muddy sediment, by providing three-dimensional structures, enhancing physical conditions, and offering shelter and food for a variety of linked species. Due to their important ecological role and vulnerability to human activities have been classified as Essential Fish Habitats (EFHs) and Vulnerable Marine Ecosystems (VMEs). Several of these habitats still require information regarding their distribution, biological importance, protection, and management. In this study, we focused on several VMEs species that characterize predominantly muddy sediment, the species are: bamboo coral *Isidella elongata*, sea pens (*Funiculna quadrangularis*, *Pennatula* spp., *Virgularia mirabilis*, *Protoptilum carpenterii*, *Kophobelemnon stelliferum*), and the crinoid *Leptometra phalangium*, also classified as deep-sea habitat-forming vulnerable taxa. The aim of the study is to investigate the ecological role that VMEs have in habitats with muddy sediment, in terms of associated fauna. Multiple co-inertia analysis was performed in order to highlight the relationship between the associated fauna (fish and crustaceans), VMEs, environmental (Temperature, Chlorophyll-a (Chl-*a*)), morphological (Depth, Slope, Aspect, Rugosity), sediment type and anthropogenic variables (Fishing effort) that characterized the study area. The results confirm that habitat-forming species considered in this study, play an important ecological role in deep-water soft-bottom habitats for a wide range of species, including also those of economic value.

Keywords: EFHs, Habitat-forming species, associated fauna, Co-inertia analysis, soft-sediment environment

4.1 Introduction

The preservation of biodiversity and the control of ecological and biochemical processes, many of which are directly related to the provision of ecosystem services, are greatly aided by habitat-forming species (Mayer-Pinto *et al*., 2020). Deep sea is one of the most remote and least explored environments on Earth, characterized by extreme conditions such as high pressure, low temperature, and low food availability (Feng *et al*., 2022). Despite these harsh conditions, is home to a remarkable diversity of life, including a wide range of habitat-forming species that create complex structures and provide essential ecological services for the functioning of benthic ecosystems. These species are known to play a critical role in shaping the structure and function of marine ecosystems (Murillo *et al*., 2011; Bastari *et al*., 2018; Lefkaditou *et al*., 2022). In the deep sea, habitat-forming species are equally important, creating complex three-dimensional structures that provide shelter, food, and attachment sites for a diverse assemblage of fauna, including fish and crustaceans. However, unlike their shallow-water counterparts, deep-sea habitat-forming species are poorly known and understood, and their ecological significance remains largely unexplored (Feng *et al*., 2022). Due to their important ecological role, vulnerability to human activities and their life-history traits have been classified as Essential Fish Habitats (EFHs) and Vulnerable Marine Ecosystems (VMEs) (FAO 2009; FAO, 2011, Rogers & Gianni, 2010). Among vulnerable habitat-forming species, we identify coral gardens, sea pens, sponges, and crinoids, which play a crucial ecological role in soft muddy sediment by providing three-dimensional structures, enhancing physical conditions, and offering shelter and food for a variety of linked species (Cerrano *et al*., 2006; Bastari *et al*., 2018; Lefkaditou *et al*., 2022). Several of these habitats still require information regarding their distribution, biological importance, protection, and management. To better understand how marine ecosystems function and what services they provide, as well as to conserve or restore them in the face of exploitation and human influences, this knowledge gap must be filled.

In this context, we will focus on several groups of VMEs which live predominantly on muddy bottoms. They are represented by the bamboo coral *Isidella elongata*, sea pens (*Funiculina quadrangularis, Pennatula* spp., *Virgularia mirabilis, Protoptilum carpenterii, kophobelemnon stelliferum*), and the crinoid *Leptometra phalangium*.

The bamboo coral *I. elongata*, belonging to the Scleralcyonacea order, is an important ecosystem engineering found in various parts of the world, including the Mediterranean Sea. Additionally, as filter feeders, it plays a crucial role in improving water quality by removing particles and pollutants from the water column (Buhl-Mortensen *et al*., 2010). Unfortunately, bamboo corals are vulnerable to various threats, including destructive fishing practices, ocean acidification, and climate change (Mastrototaro *et al*., 2017). Nevertheless, the species provide essential habitats for a diverse range of marine organisms, including many commercially important species such as the red shrimps *Aristeus antennatus* and *Aristeomorpha foliacea*, and the Norway lobster *Nephrops norvegicus* (Matrototaro *et al*., 2017). These commercial species use the complex structures formed by bamboo corals as shelter and feeding grounds, and removing these structures can significantly impact populations and productivity of local fisheries. The loss of these habitats can have severe consequences for the reproductive success and survival of these vulnerable species, further highlighting the importance of protecting bamboo corals (Buhl-Mortensen *et al*., 2010). In the Mediterranean, little is known about the distribution and abundance of bamboo corals and the associated fauna, despite their ecological importance (D'Onghia *et al*., 2007). Another group living on soft bottoms sediment, primarily muddy or sandy (Bastari *et al*., 2018), are the Pennatuloidea, (Cnidaria, Octocorallia), also known as sea pens, which are a subclass of Anthozoans. These species are able to change the direction of current flow, keeping nutrients in place and drawing plankton close to the sediment (Tissot *et al*., 2006). In addition, they increase food delivery and lower predatory activity for the associated species, as reported for the rockfish (*Sebastes alutus*) and the red fish (*Sebastes* spp.) (Brodeur, 2001; Baillon *et al*., 2012; Bastari *et al*., 2018). Sea pens are also able to provide refuge and substrate for eggs, larvae and juvenile, including vertebrate and invertebrate species (Baillon *et al*., 2012; Bastari *et al*., 2018).

Crinoids are delicate species that, in certain cases, live raised off the sea bottom by a stalk that is linked to them, making them vulnerable to bottom-contact fisheries (Murillo *et al*., 2011). Also in this case, a variety of associated fauna can find shelter and substrate to live in them (Tissot *et al*.,2006; Murillo *et al*., 2011). This is the case of *Leptometra phalangium* in the Mediterranean Sea, which represent an important habitat for bentho-pelagic fish that are still growing and spawning (Colloca *et al*., 2004;). Like deep-sea corals, *L. phalangium* is also able to improve habitat heterogeneity (Colloca *et al*., 2004; Leonard *et al*., 2020), having a significant impact on the structure of soft bottoms (Carpentieri *et al*., 2021). Although it is a vagile species, *L. phalangium* can create habitats that support a diverse associated fauna and high densities of recruits and juveniles that include species of commercial value like the European hake (*Merluccius merluccius*), the greater forkbeard (*Phycis blennoides*), and the deep-water rose shrimp (*Parapenaeus longirostris*) (Ordines & Massutí, 2009; Colloca *et al*., 2004; Carpentieri *et al*., 2021).

However, despite the growing recognition of the ecological significance of deep-sea habitat-forming species, there are still major gaps in our understanding of their ecological roles and the associated fauna they support.

One of the main challenges in studying deep-sea environments is their inaccessibility and the difficulty of sampling and studying species in their natural environment. The functional traits of species inhabiting deep-sea (e.g. slow growth rate, late age of maturity, high longevity, slow recovery) and the fact that they provide refuge, nursery and foraging areas for several commercial species, makes this habitat-formers more vulnerable to fisheries, especially for those activities conducted on muddy sediments (e.g. bottom trawling) (Ramirez-Llodra *et al*., 2010). Bottom trawling, in particular, has been shown to have a devastating impact on soft bottom benthic communities, with recovery taking decades or even centuries (Bastari *et al*., 2018; Carbonara *et al*., 2022). A study in the Strait of Sicily found that bamboo coral assemblages were more impacted by trawling, with a significant decline in biomass and diversity in heavily fished areas (Chimienti *et al*., 2019). More studies have been carried out regarding relationship between fish and hard-bottom VMEs (Ross *et al*., 2015; Arnaud-Haond *et al*., 2017; D'Onghia *et al*., 2019; Devine *et al*., 2020), but very few studies have been investigated this relationship taking into account soft-sediment environment (D'Onghia *et al*., 2011; Baillon *et al*., 2012; D'Onghia *et al*., 2012). The widespread belief that muddy bottoms are homogeneous and stable is one theory put up as to why this discrepancy exists (Danovaro *et al*., 2014). Therefore, the aim of this study is to assess the ecological role of the most important habitat-formers living predominantly on muddy sediment, focusing on their contribution in terms of associated biodiversity. Through this study, we aim to provide a better understanding of the ecological roles of deep-sea habitat-forming species and their importance for the conservation and management of deep-sea ecosystems.

4.2 Materials and Methods

Study area

The study area which covered 1650 km^2 was in the central Mediterranean Sea and included the Northern part of the Strait of Sicily (38°0,587'N; 11°19,329'E, study area's centroid) (Figure 1). This area presents pronounced bathy-morphological heterogeneity between 130 and 1000 m of depth, and peculiar circulation patterns. In this area, the Atlantic waters divide into two branches, one flowing into the Tyrrhenian Sea and the other into the Sicilian Channel (Di Lorenzo *et al*., 2018). The latter turns branches into two 'arms': Atlantic Ionic water and Atlantic Tunisian water. The first one favours the formation of two vortices (one over the Adventure bank and the second off Cape Passero), with a complex circulation system that transports water between eastern and western sub-basins and generates upwelling (Manzella *et al*., 1990; Di Lorenzo *et al*., 2018) making the Strait of Sicily a highly productive area (Agostini *et al*., 2002). Currents are considered one of the most critical variables governing CWCs dispersion since they supply food for the corals and keep them from getting buried by sediments (Thiem *et al*., 2006; Taviani *et al*., 2011; Chimienti *et al*., 2019).

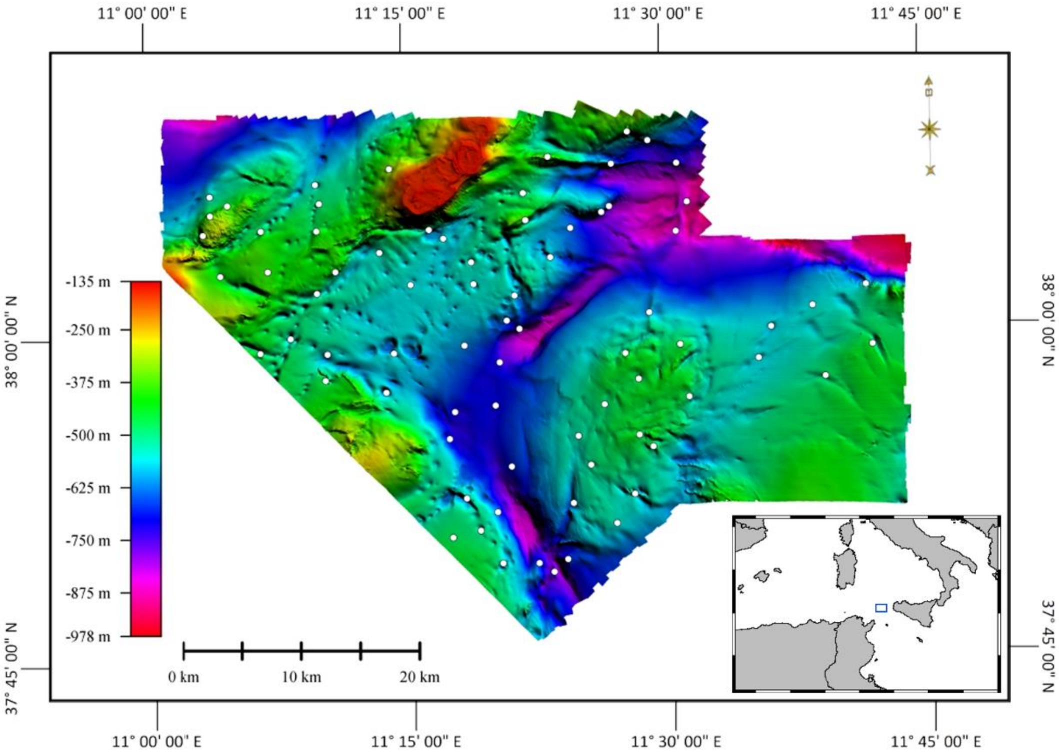

Figure 1: 5 m resolution Multi-beam (MB) bathymetry model of the study area. White dots indicate centroids of the 74 transects analysed.

Data collection

The morpho-bathymetric data were collected using a Multibeam echosounder (MBES) Kongsberg EM2040 in shallower waters (from 150 to 300 meters) and a Kongsberg EM710-MK2 in deeper waters (from 300 to 1000 meters). Raw acoustic data were processed to produce a 5 m cell Digital Terrain Model (DTM) of the entire area. A total of 140 transects were performed through Remotely Operated Veichles (ROVs) survey from September 11th to November 17th 2021 (67 days in total), under the MedWind project, using Tomahawk ROV Light Work Class with two manipulators five functions, four cameras (full HD, standard color, standard black and white and high definition 6K camera), two laser beams, sampling box, beacon, DVL system and Microcat SBE 37. To cover the entire study area, transects were randomly placed. Overall, total of 129,509 km in length were investigated. Video analysis was performed on board using Ocean Floor Observation Protocol (OFOP) software (van den Beld *et al*., 2007) and then using VLC media player after the survey.

Set of Variables used for modelling

Several variables were used for the analysis, and we decided to classify them into different groups; environmental variables (Temperature and Chlorophyll-*a*), morphological variables (Slope, Depth, Rugosity, Aspect), sediment type (observed during ROV survey) and Fishing effort (AIS data). Slope, rugosity and aspect were extracted from the high-resolution Multibeam bathymetry data (5m) using “terrain” function in R environment (Hijmans, 2023). For Temperature, a continuous raster was constructed from CTD sample sites over the whole study area. In order to achieve this, a co-kriging analysis was performed with temperature as a response variable and depth as the co-variable. The co-kriging analysis, carried out in R using the “gstat” package (Pebesma, 2004), predicted the target variable from the co-variables at un-sampled locations. Chlorophyll-*a* was obtained from in Copernicus Marine Services.

The Fishing effort employed by trawling activities in the study area, was assessed data acquired by the Automatic Identification System (AIS). The temporal frequency of vessel locations was standardized and interpolated at 0.01 degree; fishing trips and fishing set positions by trips (hauls) were detected using speed and depth filters (Russo *et al*., 2016). Finally, the yearly amount of effective trawling effort (ETE) was estimated, with respect to the cells of the same 1 km × 1 km grid described above, as the cumulated sum of the number of fishing hours.

Statistical analysis procedure

From all 140 transects, only those characterized by, at least, 40% of muddy sediment, were selected for the analysis. In total were considered 74 transects (Figure 1 and Table 1). For all 74 transects the principal VMEs species were counted and standardized to one linear kilometre. The variables considered in this study, were grouped in six different data-frames (DFs): 1) DF containing sediment type; 2) DF containing environmental variables measured in each transect (Temperature and Chlorophyll-a); 3) DF containing fishing effort (AIS data); 4) DF containing the frequency of VMEs species; 5) DF containing the frequency of associated fauna and 6) DF containing morphological variables (Slope, Depth, Rugosity, Aspect).

For both fish and crustaceans, the sighting frequency distribution of each species was calculated (n° of individuals on linear transects of 1 km). Next, the quartiles of these two distributions were calculated, and species falling in the first quartile (both fish and crustaceans) were removed from the analysis.

In order to compare different DFs containing different types of data and measures, all variables were standardised using the "decostand" formula of "vegan" package in R environment. With the aim of characterising each data frame and then making useful associations between them, we first carried out a Principal Component Analysis (PCA) for each data frame. The correlation between each pair of DFs was measured through random variable "RV" coefficients. RV coefficient is a multivariate generalization of Pearson's quadratic correlation coefficient. It can have values ranging from 0 to 1, with 0 indicating no correlation and 1 indicating complete association. The RV coefficient's significance was determined using the permutation test (Heo, 1998).

Following that, we performed a Multiple Co-inertia Analysis (MCOA) (Chessel, 1996), applied to all PCA results. This is a multivariate method for linking k data frames ($k > 2$), that share the same rows (individuals or variables). All analyses were performed in R environment, and the "ade4" package (Dray, 2003) was used to perform MCOA.

Transect	Mean Long	Mean Lat	Mean Depth	Lenght	Transect	Mean Long	Mean Lat	Mean Depth	Lenght
1	11.69916	37.98464	-469.51	1502.45	38	11.07913	38.10153	-411.23	1017.18
2	11.65287	37.96111	-480.49	1165.54	39	11.06238	38.10865	-536.06	616.99
3	11.64202	38.01529	-557.68	1260.02	40	11.06237	38.09418	-381.65	1091.97
4	11.60156	37.99986	-573.79	1174.20	41	11.07114	38.04755	-427.05	1047.40
5	11.58883	37.97616	-564.02	958.80	42	11.10819	37.98798	-478.13	1112.24
6	11.52115	37.94770	-491.76	1220.32	43	11.13782	37.99888	-543.12	1001.46
7	11.48449	38.01285	-576.35	1323.99	44	11.16425	38.03295	-516.56	1048.70
8	11.47260	37.96228	-461.94	1197.84	45	11.18230	38.04931	-449.66	1128.34
9	11.46038	37.98203	-475.37	677.17	46	11.16488	38.08082	-442.87	1009.53
10	11.43903	37.94334	-486.45	4679.49	47	11.16808	38.10178	-493.03	1023.62
11	11.47209	37.91958	-441.62	2052.10	48	11.11118	38.08150	-478.00	1030.72
12	11.42447	37.89728	-509.18	1203.58	49	11.11708	38.05036	-395.31	1045.54
13	11.48511	37.91030	-480.83	1095.98	50	11.22504	38.06339	-536.52	1033.08
14	11.44818	37.85232	-541.10	1058.01	51	11.17313	37.98629	-558.77	994.26
15	11.35885	38.00262	-754.07	875.77	52	11.17065	37.96617	-464.08	1192.55
16	11.31536	38.03771	-555.76	1051.98	53	11.30408	37.87384	-549.06	994.10
17	11.35488	38.02820	-574.80	1055.77	54	11.29013	37.84423	-471.10	1031.78
18	11.39000	38.05688	-592.90	1013.01	55	11.31703	37.84909	-511.12	1018.93
19	11.41001	38.07886	-633.35	967.04	56	11.33761	37.82370	-535.24	1052.36
20	11.36685	38.08568	-434.63	653.47	57	11.38653	37.81625	-756.99	1081.69
21	11.36525	38.10630	-491.05	1035.89	58	11.37227	37.82313	-683.50	1019.47
22	11.27359	38.08003	-543.77	867.15	59	11.40009	37.82567	-672.12	954.15
23	11.28720	38.07301	-562.25	1114.05	60	11.40676	37.86846	-592.68	1023.43
24	11.31371	38.05443	-546.71	1048.27	61	11.33370	37.86293	-633.22	1087.50
25	11.23647	38.12721	-336.65	1033.95	62	11.34831	37.89749	-686.28	994.32
26	11.46740	38.15153	-431.15	1016.49	63	11.41306	37.91960	-518.31	999.39
27	11.48692	38.14447	-629.34	1036.58	64	11.33891	37.97738	-729.34	1062.94
28	11.45110	38.12728	-637.10	785.21	65	11.33411	37.94443	-702.29	1027.75
29	11.38979	38.13373	-369.90	670.71	66	11.29475	37.94023	-706.87	566.63
30	11.44813	38.09484	-709.32	890.79	67	11.22891	37.95609	-591.69	2431.28
31	11.44040	38.09044	-676.30	817.70	68	11.25453	38.03813	-557.52	381.04
32	11.51418	38.12669	-735.27	1002.22	69	11.30531	37.99088	-612.80	1000.35
33	11.52337	38.09686	-859.60	1014.41	70	11.28909	37.91957	-699.76	993.70
34	11.51199	38.07461	-842.21	1093.69	71	11.23714	37.98609	-583.74	951.41
35	11.34679	38.00926	-651.20	1040.54	72	11.46624	37.87440	-536.11	1453.02
36	11.16468	38.11630	-545.38	935.66	73	11.69412	38.03024	-541.17	584.70
37	11.05511	38.07941	-363.38	1119.69	74	11.51355	37.98806	-465.67	1060.28

Table 1: Numbers, geographic positions and total length for the 85 ROV transects selected for the analysis.

4.3 Results

Six different types of sediment were identified in the chosen transects (Debris, Muddy, Muddy rock, Rocky, Sand and Tanatocenosis). Among the principal VMEs species which live predominantly on muddy sediment we focused on bamboo coral *Isidella elongata*, sea pens (*Funiculina quadrangularis*, *Pennatula* spp., *Virgularia mirabilis*, *Protoptilum carpenterii*, *Kophobelemnon stelliferum*), and the crinoid *Leptometra phalangium* (Figure 2).

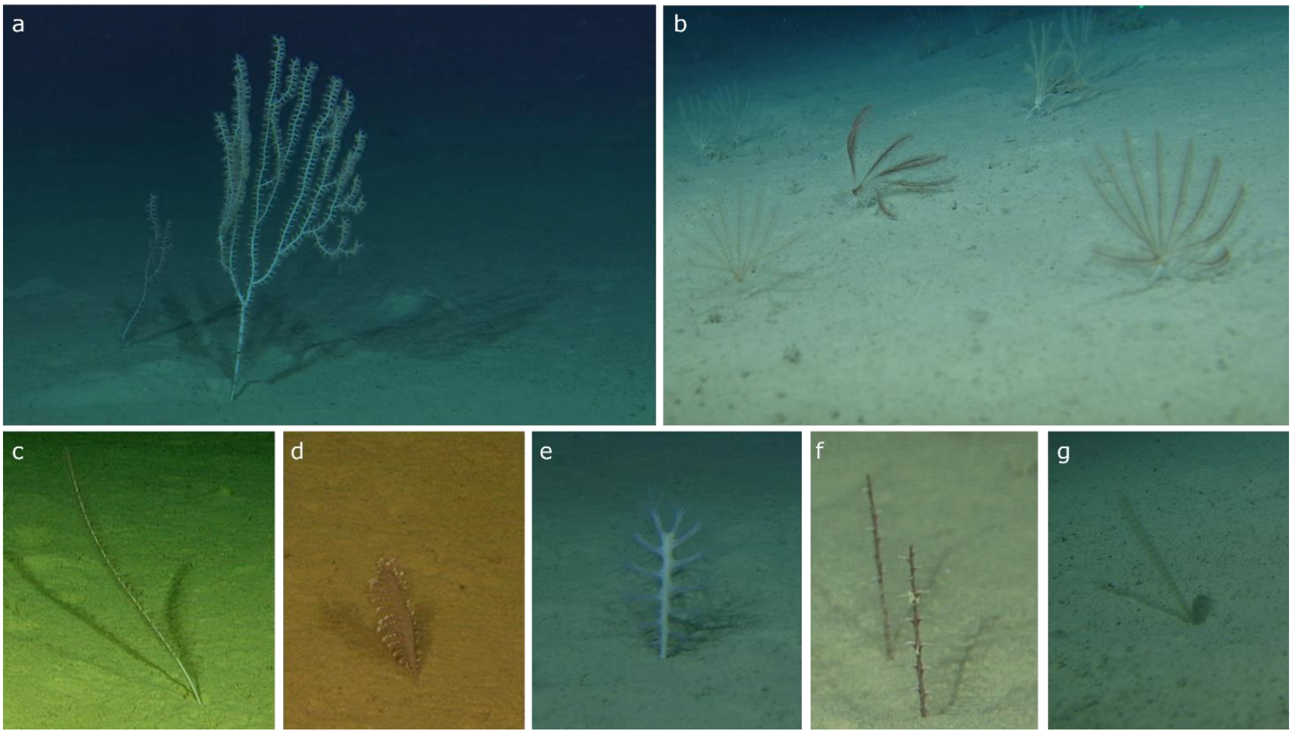

Figure 2: Figure shows VMEs of muddy bottom observed during the ROV survey, we have: a) *Isidella elongata*, b) *Leptometra phalangium*, c) *Funiculina quadrangularis*, d) *Pennatula* spp., e) *Kophobelemnon stelliferum*, f) *Protoptilum carpenterii* and g) *Virgularia mirabilis*.

Table 2 shows the transects used for this study specifying the number of occurrence as well as the percentages of the various types of sediment present in each transects.

In total, 11 crustaceans and 29 fish species were maintained in the analysis. For the latest, we had 3 Chondrichthyes and 26 Osteichthyes (Table 3). The most representative species are shown in Fig.3.

In Table 4, we reported *p*-values of correlations and RV coefficient for fish (a) and crustaceans (b). The interpretation of these values could be more easily explained by considering a correlation plot obtained through a MCOA and shown in Figure 4 and 5 for fish and crustaceans, respectively. Multiple co-inertia spaces comprised two axes, accounting for 15.46% and 10.76% for fish and 16.4% and 12.9% for crustaceans of the variance, respectively.

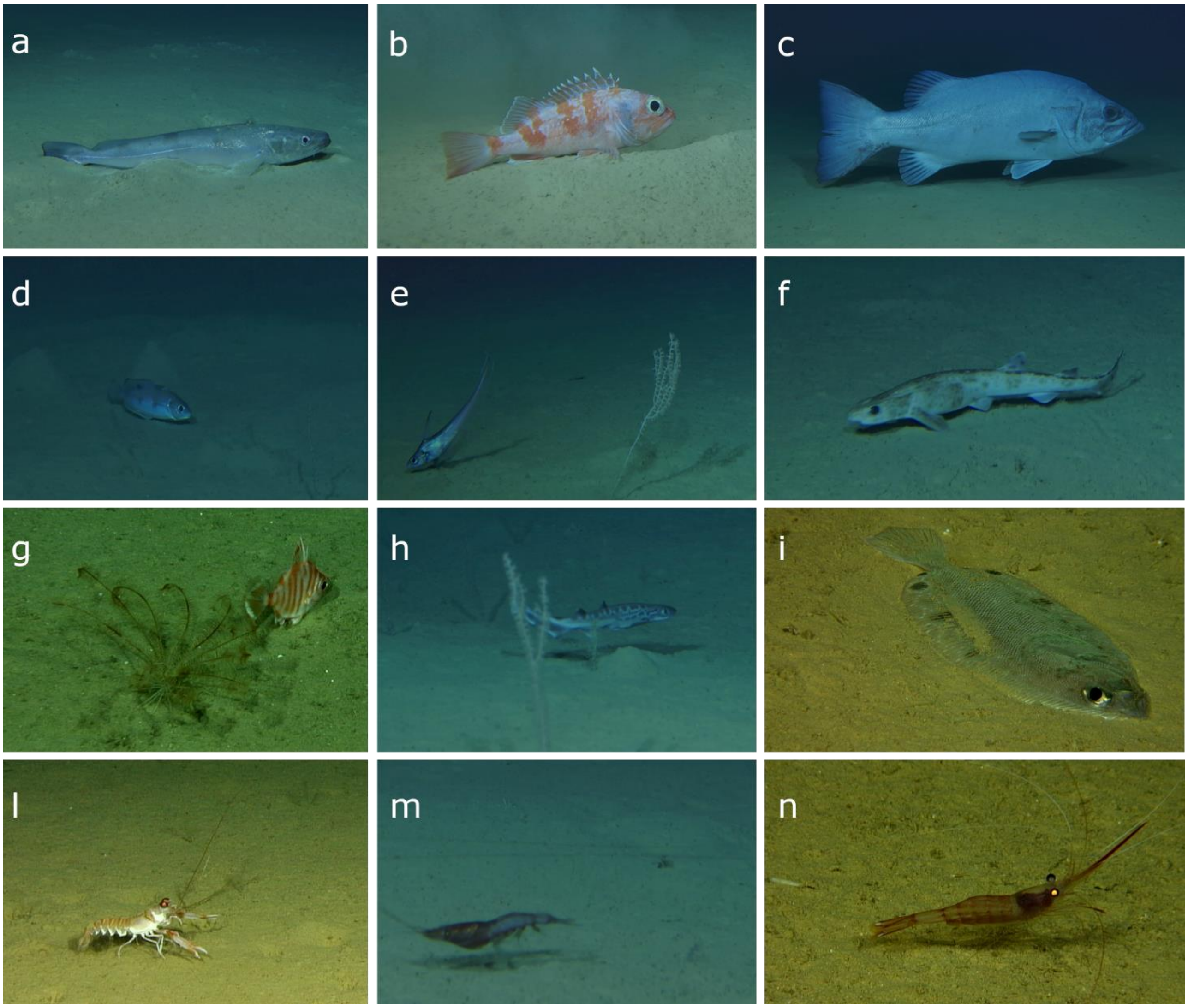

Figure 3: Underwater video frame of the dominant associated fauna taxa observed in the selected transects. a) *Merluccius merluccius*, b) *Helycolenus dactylopterus*, c) *Polyprion americanus*, d) *Phycis blennoides*, e) *Nezumia* spp., f) *Scyliorhinus canicula*, g) *Capros aper*, e) *Galeus melastomus*, i) *Lepidorhombus boscii*, l) *Nephrops norvegicus*, m) *Aristeus antennatus*, n) *Plesionika* spp..

Table 2: the table shows the transects within which the VMEs species were observed, as well as the number of observations made. The table also indicates the percentage of substrate that typified the transect.

	VMEs			Sediment type %					
Transect	***I. elongata***	**Pennatuloida**	***L. phalangium***	**Debris**	**Muddy**	**Muddy rock**	**Rocky**	**Sand**	**Tanatocenosis**
1		6	73		0.4106	0.5894			
2	306	5			1				
3	22	3			1				
4	681	3	10		1				
5	1749	2	2		1				
6	167	5	2		1				
7	198	1			1				
8	193	34	12		1				
9	6	25	1583		1				
10	340	54			1				
11	64	4	907		0.6226	0.2097	0.1197		0.0479
12	1033	22			1				
13	1291	20			1				
14	913	4			1				
15	73	1			1				
16	6	4			1				
17	26		1		1				
18	24	3			1				
19	17	4			1				
20	50				0.6774	0.3226			
21	46		1		1				
22	26	13			0.9924		0.0076		
23	151	7			1				
24	9	2			1				
25			648	0.0536	0.9464				
26	68	1	121		0.7456		0.2515		0.0029
27	3				1				
28	76	2			0.9180	0.0820			
29			1		0.4054	0.5946			
30	28	1			1				
31	61	2			1				
32	150	8	1		1				
33	20	2			1				
34	1	4			1				
35					1				
36	22	4	1		1				
37		2	418		0.4475	0.3957	0.1568		

	VMEs species			Sediment type %					
Transect	***I. elongata***	**Pennatuloida**	***L. phalangium***	**Debris**	**Muddy**	**Muddy rock**	**Rocky**	**Sand**	**Tanatocenosis**
38	31	56	255		0.4211	0.4089	0.1066	0.0148	0.0486
39	6	4			0.9804	0.0196			
40	25	12	284		0.5033	0.3067		0.1900	
41			135		1				
42	98	20	126		0.8713	0.1287			
43	19	34			1				
44	20	53			1				
45	18	74	111		0.5707	0.4293			
46	75	64	164		0.9821	0.0179			
47	8	21			1				
48	3	6	2		1				
49			214		1				
50	6	26	1		1				
51	10	6			1				
52	11	87	31		0.9501				0.0499
53	454	4			1				
54	80	23			1				
55	58	10			1				
56	151				0.5159				0.4841
57		1			0.5753				0.4247
58	1	1			0.6218				0.3782
59	33		2	0.0105	0.5461	0.0834			0.3600
60	219	2			0.4682	0.1689			0.3629
61			1		1				
62					1				
63	965	13	1		1				
64	19	1			1				
65	2				1				
66	3	1			1				
67	84		1		1				
68	7				1				
69	13				1				
70	11				1				
71	81	3			1				
72	1047	1	1	0.2188	0.6493	0.0819			0.0500
73		5			1				
74	23	106	346		1				

Table 3: Fish and crustacean species maintained in the final analysis. The table also shows the major fishing methodology for each commercial species and the status within the IUCN for all observed species (both of commercial and conservation interest).

	Commercial species					
	Small-scale fisheries	**Bottom Trawling**	**Bottom Longline**	**IUCN Global**	**IUCN Med**	**IUCN Italy 2017**
Fish						
Hymenocephalus italicus				LC	LC	LC
Coelorinchus caelorhincus				LC	LC	LC
Lepidopus caudatus			X	DD	LC	LC
Trachurus sp.	X			LC	LC	LC
Hoplostethus mediterraneus				LC	LC	LC
Lepidorhombus boscii		X		LC	LC	LC
Chlorophthalmus agassizi		X		LC	LC	LC
Merluccius merluccius	X	X		LC	VU	NT
Helicolenus dactylopterus	X	X		LC	LC	LC
Chauliodus sloani				LC	LC	LC
Arctozenus risso				LC	LC	LC
Chlopsis bicolor				LC	LC	LC
Polyprion americanus	X		X	DD	DD	VU
Scyliorhinus canicula		X	X	LC	LC	LC
Synchiropus phaeton				LC	LC	LC
Capros aper				LC	LC	LC
Gadella maraldi				LC	LC	LC
Gnathophis mystax				LC	LC	LC
Benthocometes robustus				LC	DD	LC
Facciolella oxyrhyncha					DD	LC
Stomias boa				LC	LC	LC
Conger conger	X	X	X	LC	LC	LC
Gadiculus argenteus					LC	LC
Notacanthus bonaparte					LC	LC
Nettastoma melanurum				LC	LC	LC
Nezumia spp.				LC	LC	LC
Galeus melastomus				LC	LC	LC
Etmopterus spinax				VU	LC	LC
Phycis blennoides		X	X		LC	LC
Crustaceans						
Aristaeomorpha foliacea		X				
Nephrops norvegicus		X				
Penaeus kerathurus	X	X				
Aristeus antennatus		X				
Plesionika spp.	X	X				
Parapenaeus longirostris		X				
Geryon longipes						
Paromola cuvieri						
Munida sp.						
Bathynectes maravigna						
Anamathia rissoana						

Both graphs show that different variables influence the distribution of associated fauna. Regarding fish (Figure 4), MCOA evidences a spatial trend between the variables, allowing us to make a separation between fauna associated with the three habitat-forming species category. More specifically, *Phycis blennoides, Galeus melastomus, Nezumia* spp., *Etmopterus spinax,* and *Himenocephalus italicus* showed a positive correlation with *Isidella elongata* in areas characterised by soft muddy sediment and high values of Temperature and Chl-*a.* Moreover, *Coelorinchus caelorhincus, Lepidopus caudatus, Lepidorhombus boscii*, *Trachurus* sp., *Hoplostethus mediterraneus, Chlorophthalmus agassizii, Merluccius merluccius, Helycolenus dactylopterus, Gadiculus argenteus, Clopsis bicolor, Scyliorhinus canicula*, and *Synchirophus phaeton* showed a positive correlation with Pennatuloidea and *Leptometra phalangium*, predominantly on debris sediment. However, MCOA analysis has shown no relevant correlation between other fish and habitat-forming species, but a correlation with the type of substrates and morphological variables of the sediment. In particular, *Nettastoma melanura, Notacanthus bonapartei* and *Conger conger* are correlate with high bathymetry values and substrate characterised by tanatocenosis. On the other hand, *Polipryon americanus, Capros aper, Gadella maraldi, Gnathophis mystax, Benthocometes robustus*, *Facciolella oxyrhyncha, Stomias boa* are predominantly correlated with sand, rocky, and muddy rock sediment in areas with high values of slope and rugosity.

Considering the circulation circle plot for crustacean's data frame (Figure 5) is possible to observe a correlation with habitat-forming species, particularly for crustaceans of commercial value. More specifically, *Parapenaeus longirostris, Aristeomorpha foliacea, Nephrops norvegicus* are positively correlate with *I. elongata* in areas characterized by muddy sediment and high values of Temperature and Chl-*a.* The red shrimp *Aristeus antennatus, Plesionika* spp. and *Penaeus keraturus* shown a positive correlation with *L. phalangium* and Pennatuloidea in areas characterized predominantly by debris sediment type and high aspect values. Species with no commercial value are not correlated with habitat-forming species but present a positive correlation with morphology and sediment types.

In particular, *Geryon longipes* seem correlated with high values of bathymetry. On the other hand, *Paromola cuvieri, Bathynectes maravigna, Munida* sp, *Anamathia rissoana* are correlated with different types of sediment; tanatocenosis, rocky, muddy rock and sand, in areas with high values of rugosity and slope.

Table 4: *p*-value of correlation (upper part of the table) and RV coefficient (lower part of the table) for Fish (a) and Crustaceans (b)

(a)	Fish	VME	Env	Morfo	Substrate	AIS	MFA
Fish	1	< 0.001	< 0.001	< 0.001	< 0.001	0.36	< 0.001
VME	0.18	1	0.015	< 0.001	0.571	0.214	< 0.001
Env	0.148	0.07	1	< 0.001	0.075	0.29	< 0.001
Morfo	0.228	0.13	0.091	1	< 0.001	0.408	< 0.001
Substrate	0.099	0.015	0.042	0.128	1	0.043	< 0.001
AIS	0.045	0.026	0.015	0.021	0.053	1	0.309
MFA	0.699	0.517	0.399	0.568	0.38	0.31	1

(b)	Crustaceans	VME	Env	Morfo	Substrate	AIS	MFA
Crustaceans	1	0.055	0.58	< 0.001	< 0.001	0.05	0.015
VME	0.077	1	0.015	< 0.001	0.56	0.2	< 0.001
Env	0.026	0.07	1	< 0.001	0.07	0.29	0.001
Morfo	0.102	0.13	0.09	1	< 0.001	0.4	< 0.001
Substrate	0.14	0.015	0.04	0.128	1	0.043	0.001
AIS	0.059	0.026	0.015	0.02	0.05	1	0.28
MFA	0.52	0.525	0.38	0.56	0.44	0.35	1

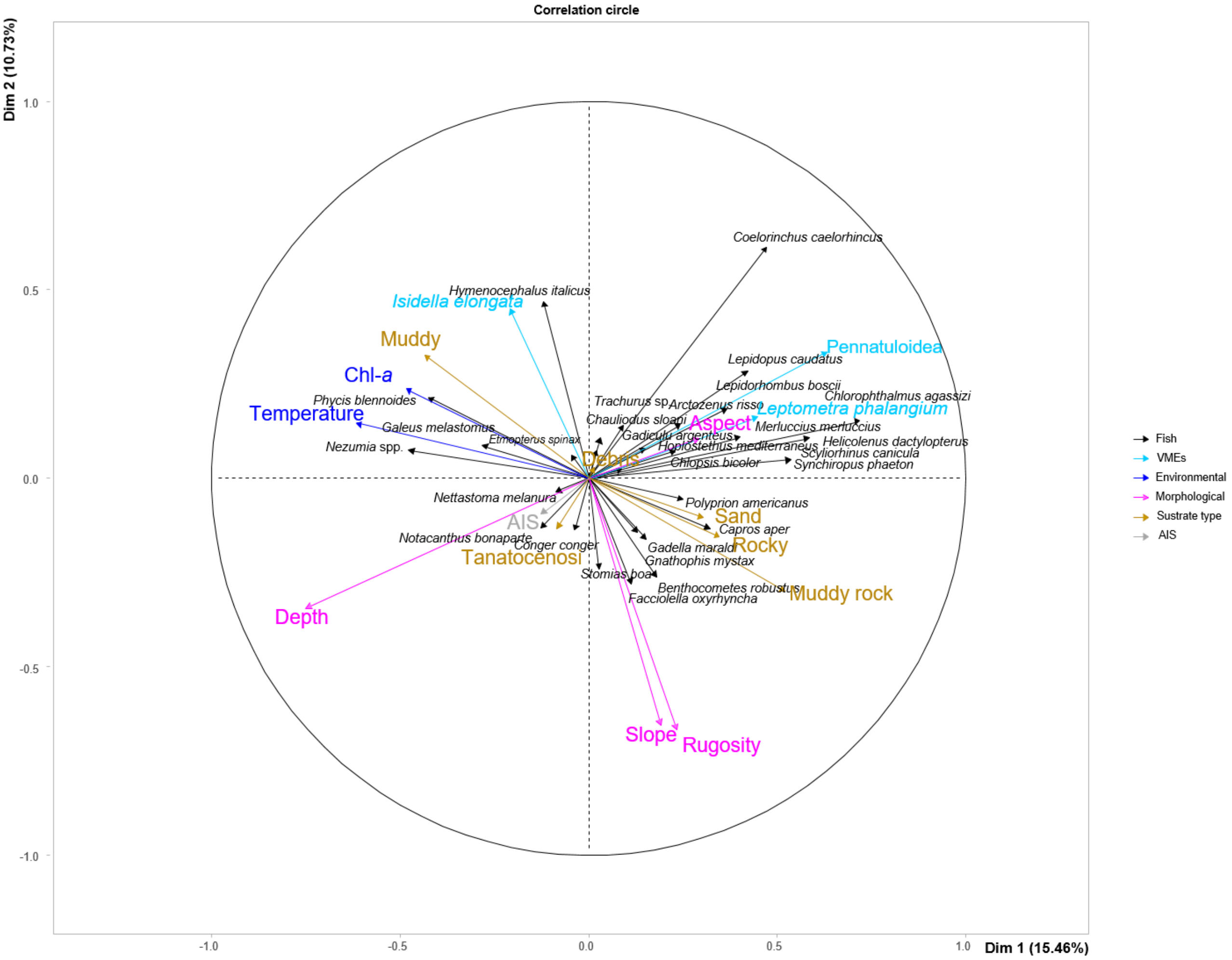

Figure 4: Correlation circle plot of Multi Factor Analysis for Fish.

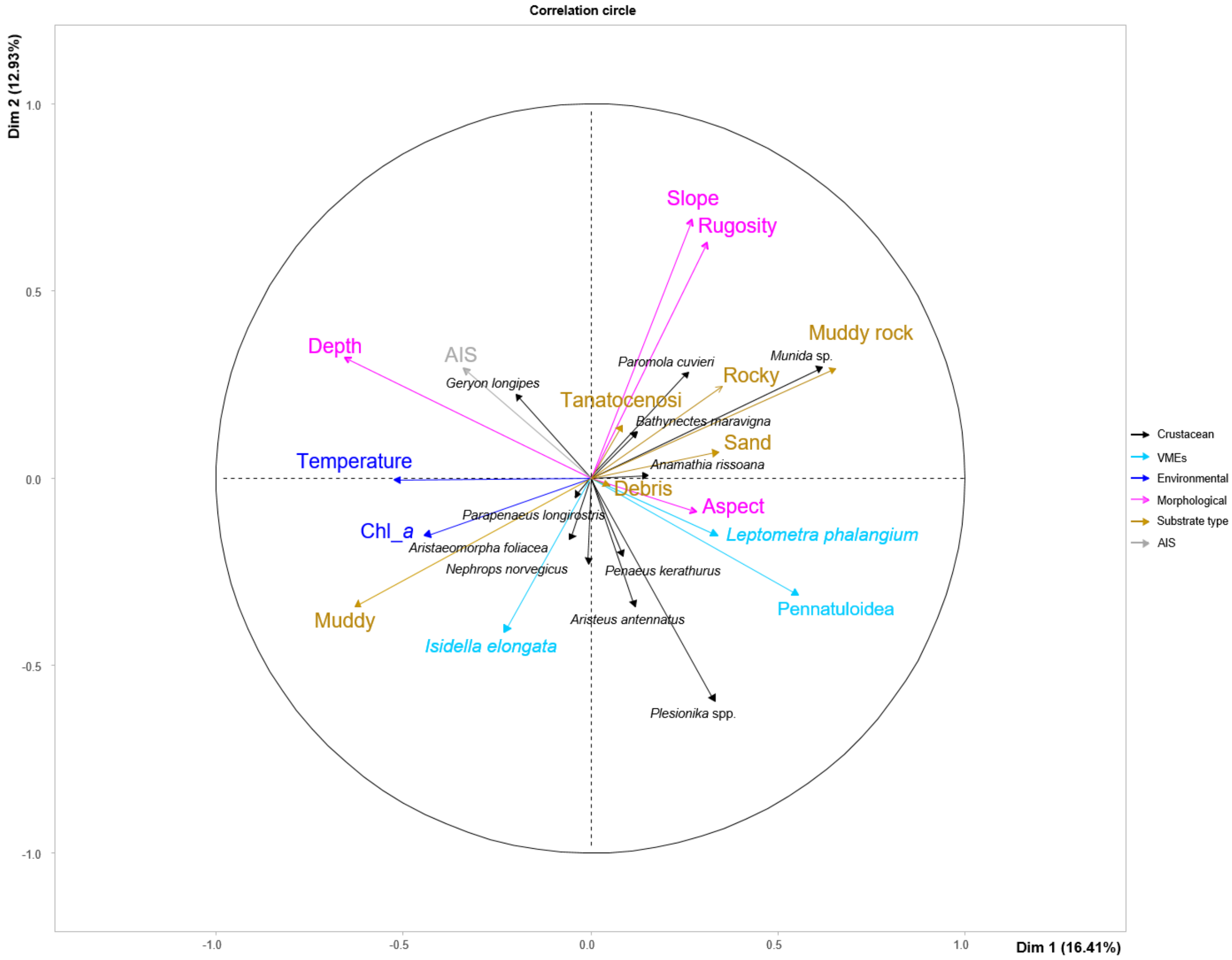

Figure 5: Correlation circle plot of Multi Factor Analysis for Crustaceans.

Anthropogenic impact of trawling activities: focus on *Isidella elongata*

Although AIS was not significant in the MCOA analysis, during ROV survey, the study area characterized by muddy sediment was found to be severely impacted by trawling activity. Among the VMEs considered in this study, the most affected species turned out to be *I. elongata*, as shown in Figure 6. Damage to tissues and exposed skeleton are prevalent on corals as a result of fishing gear, and this was observed at numerous ROV locations, where several colonies of *I. elongata* have been observed uprooted from the sediment, affected by epibiosis or entangled by anthropogenic litter.

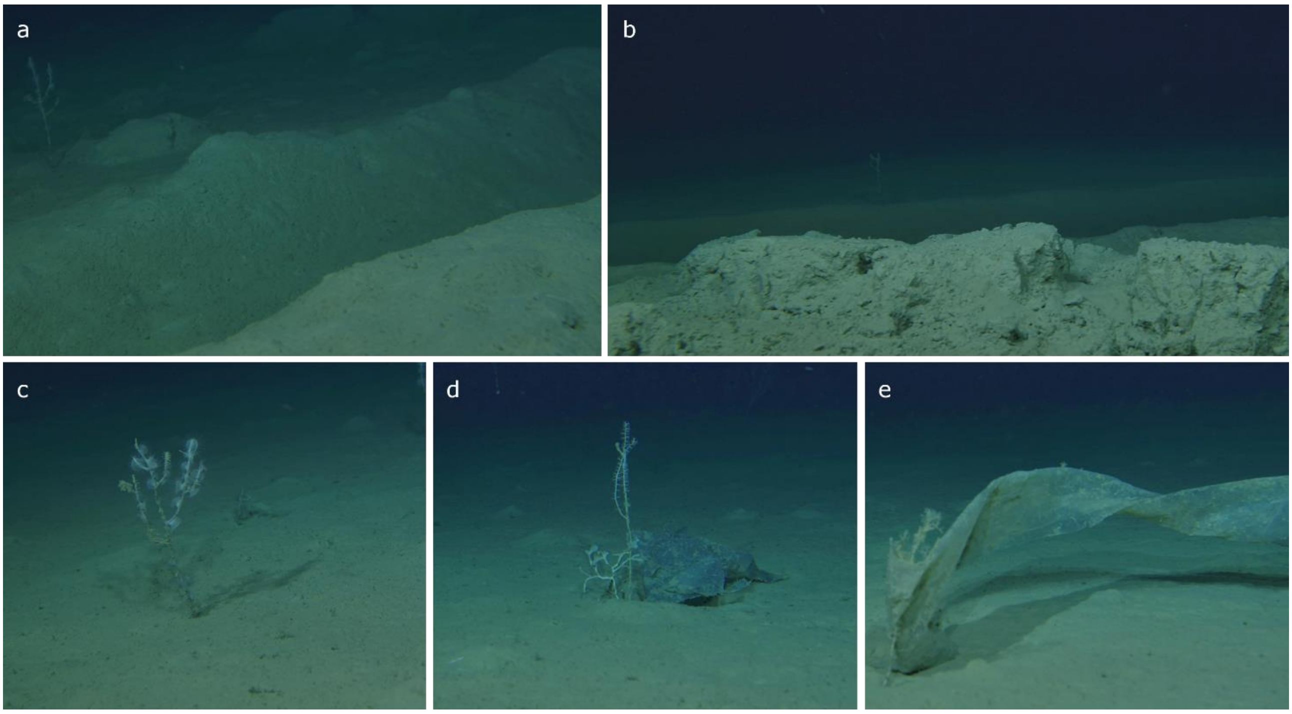

Figure 6: Evidence of fishery impact; a), b): trawl marks, c) colony of *I. elongata* affected by epibiosis (anemone *Amphianthus dohrnii*), d) colony of *I. elongata* uprooted from sediment, e) litter (plastic) entangled around colony of *I. elongata.*

4.4 Discussion and Conclusions

Studying the relationship between vulnerable habitat-forming species and their associated fauna, like fish and crustaceans, isn't straightforward. There are different opinions on whether the complexity of the habitat, rather than the presence of VMEs determines the distribution of other species (Harter *et al*., 2009; Söffker *et al*., 2011). Some believe that there is a connection between these species, but it may not be an essential condition for the survival of associated species (Auster, 2007). However, it's well known that, especially in habitats with muddy sediments, the presence of habitat-forming species adds complexity to the habitat, ensuring the presence of both conservationally and commercially important species (Boulard *et al*.,2023). Several hypotheses attempt to explain this association, including the idea that habitat-forming species provide refuge against predators and/or serve as nursery (Costello *et al*., 2005; D'Onghia *et al*., 2012; D'Onghia *et al*., 2019) and spawning areas (Costello *et al*., 2005; Marliave *et al*., 2009; D'Onghia *et al*., 2010; Baillon *et al*., 2012). In other cases, it's been suggested that they increase predation rates (Husebø *et* *al*., 2002; Costello *et al*., 2005). There's also evidence of direct use of these species, such as the presence of larvae on sea pens colonies (Baillon *et al*., 2012) and shark eggs (Henry *et al*., 2013; Cau *et al*., 2017). Although the nature of this relationship is not well-studied, it's important to emphasize that the presence of habitat-forming species, both on rocky and muddy substrates, influence the distribution of associated fauna.

Few studies have been conducted to investigate the fauna associated with VMEs on muddy sediment, as expected. For this reason, we have opted to concentrate on muddy bottom VMEs and the fauna that inhabits them.

More specifically, our study revealed that differences in the distribution of associated fauna are primarily correlated with sediment type, morphology, presence of VMEs and environmental variables (Tempertaure and Chl-*a*), for which the role in the distribution of fauna, mainly fish, is known (Shaari & Mustapha, 2018). Depth did not appear to significantly influence the distribution of associated fauna, possibly because the depth ranges of the selected transects were similar. Furthermore, our research indicates that *I. elongata* is most closely associated with muddy and monotonous substrates, which are more influenced by fishing activities. While *L. phalangium* and sea pens were more typically found in more heterogeneous transects characterised by sand, muddy rock or rocky. Despite this diversification, the VMEs groups considered in this study, are truly habitat-forming species, due to their morphology and their ability to create intricate habitats even in areas with flat, predominantly characterized by muddy sediment. These characteristics can contribute to the three-dimensionally structure of soft-bottom, enhancing habitat heterogeneity and providing, especially, refuge, spawning and nursery areas for a wide range of species, also of commercial values (Colloca *et al*., 2004; Pham *et al*., 2015; Pierdomenico *et al*., 2018; Mastrototaro *et al*., 2017; Carbonara *et al*., 2020). In particular, our results show an evident correlation between VMEs species and associated fauna, including both species of commercial and conservation interest, such as the principal commercial Osteichthyes *M. merluccius, P. blennoides, C. agassizii,* *H. dactylopterus*, *L. boscii*, and the Chrondrichthyes blackmouth shark *G. melastomus*, the Velvet belly lantern shark *E. spinax*. Other studies report the possible correlation among these fish species and VMEs considered in these study (Cartes *et al*., 2013; Mytilineou *et al*., 2014; Matrototaro *et al*., 2017; D'Onghia *et al*., 2019) More specifically, among fish we recognize the velvet belly lantern shark *E. spinax*, and the European Hake *Merluccius merluccius*, which present a positive correlation with the presence of VMEs. In particular, the first show a correlation with *I. elongata* and the second with the crinoid *L. phalangium* and sea pens.

The first one, *E. spinax*, was recently assesses by the IUCN rest list of Threatened species in 2016, as Least Concern in the Mediterranean (Guallart *et al*., 2016) and more recently has been assessed as "Vulnerable" to global level (Finucci *et al*., 2021). The species represent one of the most frequent by-catches of bottom trawling aimed to catching shrimps and lobsters, because it has no commercial value, once caught, this species is discarded. Although mortality is unknown, however it is anticipated to be substantial as to the severe injuries sustained when species are caught (Coelho *et al*., 2009). The second one, *M. merluccius,* was assessed for the IUCN in 2007 and listed as "Vulnerable" in the Mediterranean Sea (Di Natale *et al*., 2011). The species is mainly caught with trawling and other fishing gears (e.g. fixed nets, bottom longline). *M. merluccius* has a high commercial value in the Mediterranean Sea, because of this the species is over-exploited and a decline in the population has been observed (Abdul Malak, 2011). Regarding crustacean, as reported from our results and from other studies (Carbonara *et al*., 2020; Pierdomenico *et al*., 2018; Cartes *et al*., 2013; Mastrototaro *et al*., 2017), species of commercial values as the two red shrimps *A. foliacea* and *A. antennatus*, the deep-water rose shrimp *P. longirostris, Plesionika* spp. and the Norway lobster *N. norvegicus* are associated with VMEs. All these crustacean species are caught with bottom trawling and represents one of the main demersal sources of Mediterranean deep-water bottom trawl fishers (Kapiris *et al*., 2022), especially in the Strait of Sicily. Regarding the red shrimps, according to the latest stock assessment these resources appear to be over-exploited and the management advise was to reduce mortality from fishing and adopt sustainable trawl fishing operations (Kapiris *et al.*, 2022). The adoption of improved management measures, will allow both the protection of stocks and all associated fauna, and the protection of benthic ecosystems (Carbonara *et al*., 2020). This is the case of the bamboo coral *I. elongata* and the sea pens *F. quadrangularis,* which, as mentioned, are true EFHs for numerous species especially those of commercial value. Both of them present a particular morphological structure. On one hand *I. elongata* presents a rigid carbonaceous skeleton that breaks when it comes in contact with a fishing gear. On the other hand, *F. quadrangularis* colonies are characterized by a delicate axial rod and the inability to recede into the sediment as other sea pens species (e.g. *V. mirabilis* and *P. carpenterii*). These conditions; co-existence with commercial species and the presence of specific morphological structures, as well as their particular life history traits makes these two species extremely vulnerable and have been listed by IUCN Red List of Threatened species as "Critically Endangered" (Salvati *et al*., 2014).

Considering the bamboo coral, during this ROV survey *I. elongata* was found to be the species most impacted by trawling. This can be explained by the fact that, in addition to the conditions mentioned above, *I. elongata* was observed in monotonous transects characterised by muddy sediment where trawling activities are highly because the risks of entanglement of nets are lower. The unhealthy state of the colonies, is depending not only on direct trawling impact but also on indirect impact, such as high rates of sediment resuspension, which in turn damages the colonies, increasing rates of necrosis and subsequent epibiosis (Pierdomenico *et al*., 2018).

Efficiently managing species with high economic or conservation value requires understanding the processes governing the distribution of organisms and the scales at which these processes operate. Many studies have focused on the value of VMEs to deep-sea fish, though their precise function remains unclear (Milligan *et al*., 2016). What is obvious, is that the co-existence of vulnerable benthic species with species of high commercial and conservation value, and the intensive fishing not only causes over-exploitation of resources, but also degrades the entire ecosystem, resulting in a loss of the entire biodiversity supported by the ecosystems themselves (Kapiris *et al*., 2022).

Therefore, knowledge about the distribution of these vulnerable habitats and, concomitantly, the study of the fauna they support, is an essential step towards the application of appropriate management measures aimed at safeguarding ecosystems and the proper management of fishery resources.

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Chapter 5. General Discussion & Conclusions

5.1 Synthesis

The conservation of VMEs composed of fragile, sessile, slow-growing, and long-lived creatures vulnerable to fishing disturbance (FAO, 2009) is internationally acknowledged. Considering international rules, countries are now moving forward with attempts to safeguard them. The complexity supplied by habitat-forming species (HFS) in these benthic habitats has been associated to higher richness and biodiversity (Victorero *et al.*, 2018), as well as improved ecosystem functioning.

The long-term management and protection of these ecosystems has been a key focus of research. In recent years, the primary research goals have been, on one hand, to better understand their global distribution, which is critical for the implementation of effective marine spatial planning (Bargain *et al.*,2018). The study of the distribution of VMEs (which include a wide range of taxa) is considered an essential background for the purpose of assessing the biogeography of species of conservation interest, through an investigation of the possible and main factors, including both environmental and anthropogenic (e.g. fishing effort) factors capable of determining their presence or disappearance (Chimienti *et al.*,2018). On the other hand, to assess their ecological role through characterization of associated fauna (primarily fish) in a variety of benthic habitats (Milligan *et al.*,2016).

Taking this into account, throughout this thesis we attempt to provide some important results.

In chapter 2, investigation through ROVs survey, conducted in Amendolara Bank, report new information about the biodiversity in Ionian Sea. More specifically, the study showed the presence of 4 VMEs species belonging to 4 different orders. The VMEs species are the black coral *Antipathella subpinnata* (Antipatharia), the red coral *Corallium rubrum* (Scleralcyonacea), the yellow coral *Dendrophyllia cornigera* (Scleractinia) and the sea fans *Poecillastra compressa* (Astrophorida). For both *A. subpinnata* and *C. rubrum*, this was the first observation along the Ionian coast of Calabria. The other VMEs, *D. cornigera* and *P. compressa*, have been previously observed in the study area (Bo *et al*.,2012; Castellan *et al*.,2019). Another important observation was the echinoderms *Coronaster briareus* updating to 29 specimens observed in the Mediterranean Sea (2nd in the Ionian Sea). Concerning Redundancy Analysis (RDA) analysis, the results shown a preference of sediment type for VMEs species and a relationship between VMEs and fish species, emphasizing the important ecological role of these benthic species for both commercial and non-commercial fish species (D'Onghia *et al*.,2019a; D'Onghia *et al*.,2019b). In addiction during the exploration, *A. subpinnata* was the most impacted species from fishing activities, and this is due to their morphology and flexible skeleton (Angiolillo & Fortibuoni,2020; Terzin *et al*.,2021). The information carried out from this study can enhance the protection and management of the Amendolara Bank which was recently added in the list of the Regional Marine Park (Legge Regionale 16 Dicembre 2022, n°46).

In chapter 3, we aimed to provide new habitat suitability models (HSMs) (through Maximum Entropy Modelling approach (MaxEnt)) about six Cold Water Corals (CWCs) species in the Strait of Sicily, using 10 predictors variables (9 environmental and 1 anthropogenic) and presence only data, identify the principal factors, for each species, relevant for shaping their distribution and, identify the hotspots where specimens belonging to the different six CWCs species under-consideration can co-occur. MaxEnt results suggest that the models predict successfully the distribution about the six CWCs specie in the study area. Regarding the predictors, the models identify five (slope, depth, rugosity, temperature, current north) of the 10 variables influencing the distribution of all CWCs considered. In addition, the study provides a Co-occurrence analysis from all habitat suitability maps, allowing us the identification of the areas where hot spots of the various species may overlap, circumscribing a total of 4.05 km^2 (in which all six species can co-exist). This chapter contains new and relevant information about the distribution and ecology of CWCs species under examination, and also provide, for the first time, a co-occurrence analysis amongst CWCs species, demonstrating that there is a somewhat overlapping distribution among some, or all of the species investigated. All these information may be useful for modifying or improving existing conservation guidelines.

Chapter 4 documented another example the ecological importance of VMEs highlighting the relationship between habitat-forming species (living predominantly on muddy sediment) and their associated fauna in terms of fish and crustacean species. We analysed video ROV and recorded any occurrence of associated fauna, habitat-forming species, and substratum type along each transects. Then using a multivariate approach represented by the Multiple Co-inertia Analysis (MCOA), we tested the relationship between associated fauna, VMEs and environmental variables that characterized the study area. Our results highlight an association between VMEs and associated fauna, which includes both species of conservation interest and commercial value. Although various hypotheses on the coexistence of VMEs, in this case bottom trawlers, and their associated fauna have been proposed (e.g. they provide refuge and spawning areas for many species of conservation and commercial interest), what is certain is that they live in environments that are heavily exploited by bottom trawling. So, in order to protect these endangered environments with high ecological value and accomplish effective resource management, more and ongoing study on the distribution and fauna that these ecosystems host is required.

Now, it is reasonable to assume that, the thesis aims to advance research on the deep-sea at the Mediterranean level through an integrative approach that has allowed us to improve our knowledge at different levels. In particular it provides new information on biodiversity, highlighting the ecological role that VMEs play within marine ecosystems, the anthropogenic impact caused by fishing activities, and their spatial distribution in response to important environmental and anthropogenic variables. The valuable and new information obtained from these studies, as well as highlighting the effectiveness and importance of using both visual (ROVs) and acoustic (MBES) technologies, will be crucial in making effective protection measures aimed at preserving and increasing the conservation of the explored areas.

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Attachments

Talk abstract

3rd Mediterranean Symposium on the conservation of Dark Habitats (Genoa, Italy, 21-22 September 2022).

Abstract accepted.

EFFECT OF ENVIRONMENTAL AND ANTHROPOGENIC FACTORS ON THE DISTRIBUTION OF COLD WATER CORALS

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Abstract

Predictive habitat mapping is a useful tool for highlighting species-environment connections and for understanding the spatial distribution and complexity of benthic habitats. Most Cold-Water Coral (CWC) species have been designated as threatened by the IUCN, because they are directly impacted by human activities as well as fishery and climate change. The aim of the present research is to obtain habitat suitability map for several CWC species and understand the role of environmental factors on their distribution in the Northern part of Strait of Sicily. Multibeam high-resolution data, oceanographic data and ROV images were collected from August to November 2021. Presence-only data of six CWCs species, ten environmental variables (depth, slope, rugosity, aspect, flowdir, temperature, salinity, current North, current East, chlorophyll-*a*) and one variable relating to the fishing effort (Automatic Information System – AIS), were used to predict the suitable habitats through the Maximum Entropy modelling (MaxEnt). The predictive habitat of these species will be useful tool for decisionmakers in order to develop all necessary conservation measures for this area.

Keywords: Cold Water Corals, MaxEnt, species distribution modelling, habitat suitability

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EFFECT OF ENVIRONMENTAL AND ANTHROPOGENIC FACTORS ON THE DISTRIBUTION AND CO-OCCURRENCE OF COLD-WATER CORALS

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1. Introduction

Cold Water Corals (CWCs) are among the most important habitat-forming species in the Mediterranean Sea [1].Their three-dimensional structure makes them bioengineering species that enhance biodiversity and ecosystem functioning by increasing habitat variability [2]. However, limited mapping of CWCs has resulted in a lack of information about these habitats. In this study, habitat modeling techniques were used to create predictive maps of six CWCs species of conservation concern (Fig.1) in the Mediterranean Sea and to find areas where the greatest number of these species co-occur.

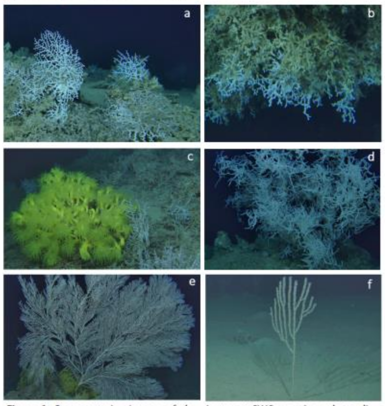


Figure 1: Representative images of the six target CWCs species, whose distribution and co-occurrence were modelled in this study: *Madrepora oculata* (a),*Desmophyllum pertusum* (b),*Dendrophyllia cornigera* (c),*Leiopathes glaberrima* (d),*Callogorgia verticillata* (c), and *Isidella elongata* (f)

2. Materials & Methods

The study area, which covered 1650 km², is located in the northern part of the Strait of Sicily (Fig. 2). Acoustic surveys were conducted from August 12th to September 6th and ROV surveys were carried out from September 11th to November 17th (67 days in total) using Tomawok ROV Light Work Class. Georeferenced data deriving from ROV survey and 11 predictors (environmental and anthropogenic), were used in order to perform Habitat Suitability models (HSMs) using Maximum Entropy modeling (MaxEnt) approach. The local Getis-Ord Gi statistic index was calculated to identify density hotspots on species distribution maps. The co-occurrence index was calculated to define the areas occupied by the highest number of hotspots belonging to different species. All analyses were performed in R environment.

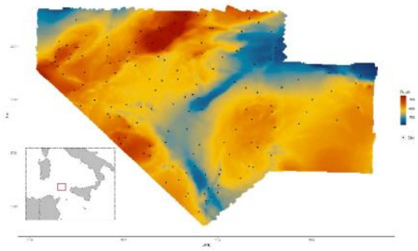


Figure 2: 5 m resolution Multibeam (MB) bathymetric model of the study area located in the Northern part of the Strait of Sicily. Black dots indicate the 140 dive.

3. Results

With an average AUC greater than 0.7, the MaxEnt model was successful in predicting the distribution of the six CWCs species in the study area. MaxEnt identified slope, depth, and rugosity as important predictors, showing percentage contribution for all six species considered (Fig. 3). These three variables are among the factors that most influence the habitat preferences of CWCs.

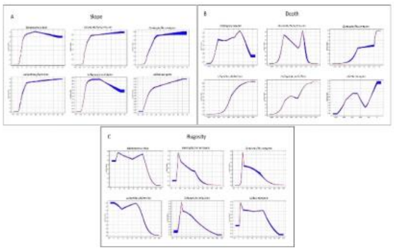
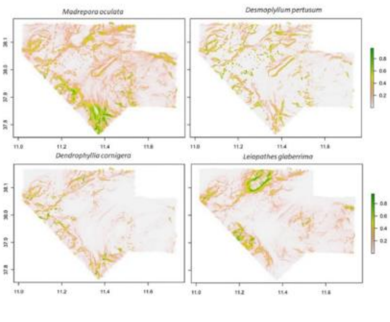


Figure 3: Response curves of three important predictors for all six CWCs species; Slope (A), Depth (B), Rugosity.

Concerning habitat suitability maps (Fig. 4) *M. oculata* (South and South-West), *L. glaberrima* (North, West and South-West) and *C. verticillata* (North, South-West) showed the presence of small patches in delimitated geographical areas.



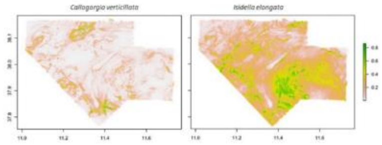


Figure 4: Habitat suitability maps for *Madrepora oculata* (A), *Desmophyllum pertusum* (B), *Dendrophyllia cornigera* (C), *Leiopathes glaberrima* (D), *Callogorgia verticillata* (E) and *Isidella elongata* (F) in the Northern part of the Strait of Sicily using MaxEnt model. The legend shows the probability of occurrence values.

D. pertusum and *D. cornigera*, were more randomly distributed, while for *I. elongata* suitable habitat ex- tended throughout the entire study area, with a higher probability in the South-East zone. The probability of highly persistent density hotspots of CWCs occurrence were found scattered throughout the entire study area, with relevance in the northern, southern, south-eastern and southwestern parts (Fig. 5). The extension of the area in which the hotspots of all six species are present cover an area of 4.05 km²

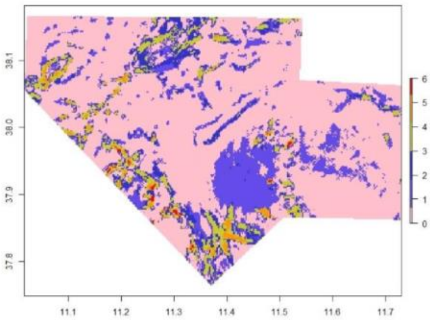


Figure 5: Co-occurrence Index of CWCs. The different colors express the number of species co-occurring in the same area (e.g., pink indicates 0 species, red indicates 6 species co-occur in the same area).

4. Discussions & Conclusions

The present study enhances our knowledge of the spatial distribution of CWCs species using a MaxEnt approach in the Northern part of the Strait of Sicily. Our results support the idea that anthropogenic and environmental variables play a significant role in deciding the distribution of species, in particular slope, depth, and rugosity are also considered by other authors as factors that most influence the habitat preferences of CWCs [3]. For the first time, several CWCs species were studied together through high-resolution sampling, and their probability of occurrence was used to produce co-occurrence maps, which will be a powerful tool in supporting the implementation of conservation actions in the area.

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Bamboo coral *Isidella elongata* (Cnidaria: Keratoisididae): New evidence on their ecological role and vulnerability in the Mediterranean Sea

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Introduction

Bamboo coral, sea pens, and crinoids are habitat-forming species that play a crucial ecological role in soft muddy sediment by providing three-dimensional structures, enhancing physical conditions, and offering shelter and food for a variety of linked species [1]. Due to their important ecological role and vulnerability to human activities have been classified as Vulnerable Marine Ecosystems (VMEs) and Essential Fish Habitats (EFHs). Several of these habitats still require information regarding their distribution, biological importance, protection, and management. In this study, we focused on several VME species that characterize predominantly muddy sediment, the species are: bamboo corals *Isidella elongata*, sea pens (*Funiculna quadrangularis, Pennatula spp., Virgularia mirabilis, Protoptilum carpenterii, kophobelemnon stelliferum*), and the crinoid *Leptometra phalangium* (Fig.1). The aim of the study is to investigate the ecological role that VMEs have in habitats with muddy sediment, in terms of the associated fauna.

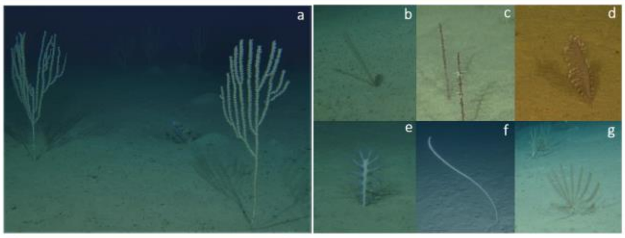


Fig.1: Main VME indicator taxa that characterize the muddy sediment in the study area: *Isidella elongata* (a), *Virgularia mirabilis* (b), *Protoptilum carpenterii* (c), *Pennatula* spp. (d),*Kophobelemnon stelliferum* (e), *Funiculina quadrangularis* (f)) and *Leptometra phalangium* (g)

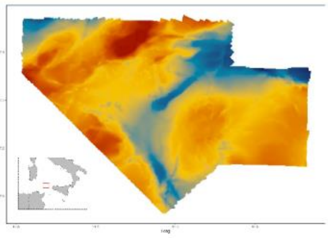


Fig.2: 5 m resolution Multibeam (MB) bathymetric model of the study area located in the Northern part of the Strait of Sicily

Materials and methods

ROV (Remotely Operated Vehicle) surveys were performed in the Northern part of the Strait of Sicily (Fig.2) from September 11th to November 17th (67 days). Video analysis was performed using OFOP (Ocean Floor Observation Protocol) software in order to acquire georeferenced presence data for all species observed and the typology of the sediment. Transects characterized by at least 40% muddy sediment were selected for this study, for a total of 85 dives in a depth range from 415 to 635 m. The main VME species and their associated fauna were considered for each transect. Multiple co-inertia analysis was conducted to study the relationship between the associated fauna (fish and crustaceans), VMEs, environmental (Temperature (T), Salinity (S), Chlorophyll-*a* (Chl-*a*), morphological (Depth, Slope, Aspect, Rugosity), type of sediment (Muddy, Muddy rock, Debris, Sand, Tanatocenosis) and anthropogenic variables (Fishing effort) that characterized the selected transects. The analysis were performed using "ade4" package in R environment.

Results

11 crustaceans and 31 fish species were maintained in the analysis, for the latest we had 3 Chondrichthyes and 28 Osteichthyes. The most representative species are show in Fig.3. Table 1 show the p-values of correlation for fish (a) and crustaceans (b). This values could be more easily explained by considering a correlation plot, obtained through a Multiple Factor Analysis (MFA) (Fig. 4a and 4b for fish and crustaceans respectively). Considering the correlation circle plots for fish (a), some species such as *Galeus melastomus, Etmopterus spinax, Phycic blennoides* are more associated with *I. elongata* in areas with muddy sediment and highest value of T, S and Chl-*a*. Other species (*Merluccius merluccius, Chlorophtalmus agassizi, Scyliorhunus canicula, Helicolenus dactylopterus*) seem to prefer *Leprometra phalangium* and Pennatuloidea habitats characterized by rocky/muddy rock sediment. For crustaceans (b), all species of commercial values prefer habitats with *I. elongata* that present muddy sediment and highest values of T, S and Chl-*a*.

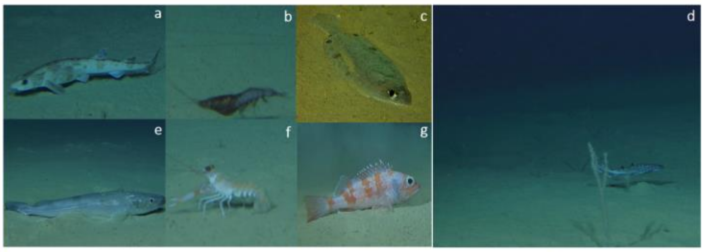


Fig.3: Representative associated fauna that mainly characterize the 85 dives selected: *Scyliorhinus canicula* (a), *Aristeus antennatus* (b), *Lepidorhombus boscii* (c), *Galeus melastomus* (d), *Merluccius merluccius* (e), *Nephrops norvegicus* (f), *Helicolenus dactylopterus* (g).

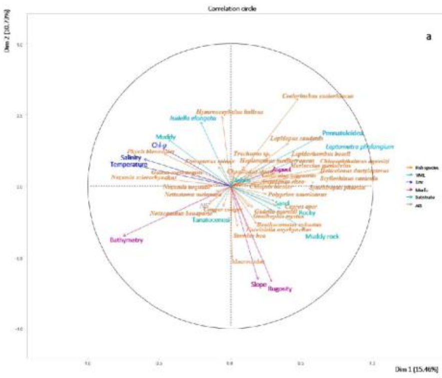
Table 1: p-value of correlation for Fish (a) and Crustaceans (b).

(a)

	Fish	VME	Env	Morfo	Substrate	AIS	MFA
Fish	1	<0.001	<0.001	<0.001	<0.001	0.36	<0.001
VME	0.18	1	0.015	<0.001	0.571	0.214	<0.001
Env	0.148	0.07	1	<0.001	0.075	0.29	<0.001
Morfo	0.228	0.13	0.091	1	<0.001	0.408	<0.001
Substrate	0.099	0.015	0.042	0.128	1	0.043	<0.001
AIS	0.045	0.026	0.015	0.021	0.053	1	0.309
MFA	0.699	0.517	0.399	0.568	0.38	0.31	1

(b)

	Crustacean	VME	Env	Morfo	Substrate	AIS	MFA
Crustaceans	1	0.055	0.58	<0.001	<0.001	0.05	0.015
VME	0.077	1	0.015	<0.001	0.56	0.2	<0.001
Env	0.026	0.07	1	<0.001	0.07	0.29	0.001
Morfo	0.102	0.13	0.09	1	<0.001	0.4	<0.001
Substrate	0.14	0.015	0.04	0.128	1	0.043	0.001
AIS	0.059	0.026	0.015	0.02	0.05	1	0.28
MFA	0.52	0.525	0.38	0.56	0.44	0.35	1



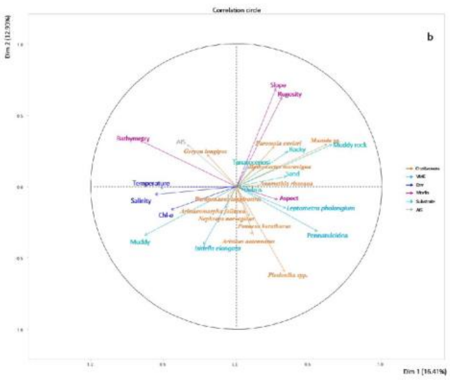
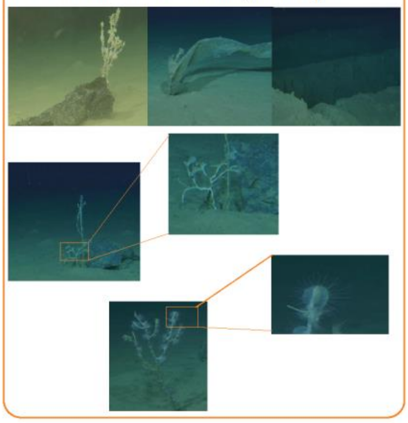


Fig.4: Correlation circle plot of Multi Factor Analysis for fish (a) and crustaceans (b).

Focus on anthropogenic impacts

Due to the co-existence, in particular of *I. elongata*, with species of commercial value make the latter more impacted by fishing activities, such as bottom trawling. A visual analysis indicates a general unhealthy status of the colonies. Further observations would be therefore useful to constrain the effects of trawling on this species.



Discussion and conclusion

All the species considered in this study (bamboo coral, sea pens, and crinoids bed) play an important ecological role in deep-water habitats for a wide range of species [2;3], including those of economic value as well as those most commonly caught in trawl bycatch (e.g., *G. melastomus, E. spinax, Scyliorhinus spp.*). The multiple co-inertia technique proved to be a useful tool for interpreting the correlations between all of the variables evaluated in this study. Furthermore, this study confirmed the possibility of using the associated fauna as a biomonitoring probe to assess the role of VME species.

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