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3rd MEDITERRANEAN SYMPOSIUM ON THE CONSERVATION OF THE DARK HABITATS

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Proceedings of the 3rd Mediterranean Symposium on the Conservation of the Dark Habitats

FORWARD

Dear Colleagues,

The United Nations Environmental Programme Mediterranean Action Plan – Specially Protected Areas Regional Activity Centre (UNEP/MAP – SPA/RAC) – Barcelona Convention has initiated since year 2000 a series of scientific symposia, dedicated to key habitats and NIS by organising the first Mediterranean Symposium on Marine vegetation. This initiative while aiming essentially to take stock of the recently available scientific data and to promote the cooperation between specialists and key actors working in the Mediterranean, has evolved since then to cover now Coralligenous, Dark Habibtats and Non-Indigenous species as well.

These symposia have been initiated as implementation of the UNEP/MAP regional action plans related to (i) **the Conservation of Marine Vegetation in the Mediterranean Sea** (adopted by the Contracting Parties to the Barcelona Convention in 1999 and updated in 2012), (ii) **the Conservation of Coralligenous and other calcareous bio-concretions of Mediterranean** (adopted by the Contracting Parties to Barcelona Convention in 20018 and updated in 2016), (iii) **the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea** (Action Plan for Dark Habitats adopted by the Contracting Parties to the Barcelona Convention in 2013), and (iv) the **Action Plan concerning Species Introduction and Invasive Species** (Adopted by the Contracting Parties to the Barcelona Convention in 2003 and updated in 2016).

The "Mediterranean Symposia on Marine Key Habitats and NIS" are an important output, not only of the UNEP/MAP Mid-Term Strategy for the period 2016-2021 (Decision IG.22/1), but also for NTZ/MPA project "Empowering the legacy: scaling up co-managed and financially sustainable No-Take Zones / Marine Protected Areas", financed by MAVA foundation under its Mediterranean Strategy and by the IMAP-MPA project "Towards achieving the Good Environmental Status of the Mediterranean Sea and Coast through an Ecologically Representative and Efficiently Managed and Monitored Network of Marine Protected Areas" .

For more than two decades, the symposia have provided, within the framework of the Barcelona Convention, a platform for dialogue between the scientific community, managers, and decision makers.

This year, SPA/RAC in collaboration with the Italian Institute for Environmental Protection and Research (ISPRA), the university of Genoa and its Department of Earth, the Environment and Life Sciences of the University of Genoa (DISTAV) and the association "Società Italiana di Biologia Marina" (SIBM), organized a new edition of the Mediterranean Symposia in Genoa, from 19 to 23 September 2022, as follows:

- 7th Mediterranean Symposium on Marine Vegetation (19-20 September 2022)
- 4th Mediterranean Symposium on the conservation of coralligenous and other calcareous bio-concretions (20-21 September 2022)
- 3rd Mediterranean Symposium on the conservation of dark habitats (21-22 September 2022)
- 2nd Mediterranean Symposium on the non-indigenous Species (22-23 September 2022)

This edition was also a good opportunity to discuss new topics such as monitoring and definition of Good Environmental Status (GES), monitoring and assessment scale in the Mediterranean and, in this way, contribute to enhancing Science-Policy interface and to strengthening links ang cooperation between SPA/RAC and Barcelona Convention system and scientists and scientific institutions in the Mediterranean.

Khalil ATTIA
SPA/RAC Director

Tatjana Hema
UNEP/MAP Coordinator

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14:30-14:45 "**Distributions of some poorly known atlanto-mediterranean deep-sea anthozoans in the western mediterranean**" by **Ricardo AGUILAR,** BLANCO J., PERRY A.

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10:45-11:00 **Closure of the Symposium**

13:00-14:00 ***Lunch***

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KEYNOTE CONFERENCE

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THE LIGURIAN WHITE CORAL PROVINCE: NEW SUPPORTING EVIDENCE

Abstract
Cold-Water Coral (CWC) provinces are defined as discrete areas with an important presence of scleractinian bioconstructions created by the so-called white corals. Their high ecosystemic value coupled to their high vulnerability to mechanical damages, supported their recognition as Vulnerable Marine Ecosystems (VMEs) with a high conservation priority. The mapping and characterization of these sites is therefore essential to implement appropriate management measures to guarantee their conservation. Until now, the quest for living reefs only marginally targeted the Italian Ligurian Sea (NW Mediterranean basin). A significant effort was made in the 60s-70s by Lieutenant Fusco, who mapped twelve coral areas from 500 to 700 m based on fishers' entanglements and bycatch coral rubble. Modern records of living colonies followed, but no systematic direct exploration of all the areas identified by Fusco and their proximities was ever attempted. Within the project framework Curiosity Driven (University of Genoa, 2021-2022), Side Scan Sonar profiles, multibeam maps and trawling routes were used to identify the sites that could potentially host the bioconstructions, including new sites from the Levante Canyon discovered in 2014. Remotely Operated Vehicle (ROV) technology was then employed to explore four areas and their proximities where reliefs were detected, namely Genoa Plateau, Portofino and Entella Canyon, Deiva Marina Canyon, and Levante Canyon. With the exception of the Genoa Plateau coral mound, destroyed by trawling activities, massive bioconstructions were found in the other three areas accounting for approximately 9 km^2 *of both bioherms and living reefs of* Madrepora oculata *and* Lophelia pertusa *supporting the creation of a distinct coral province.*

Key-words: ROV-imaging, white coral reefs, province, vulnerability, Ligurian Sea

Introduction
Bioconstructions dominated by scleractinians, usually referred to as white corals reefs, are considered one of the most important and characteristic habitats of the deep-sea realm and the world-wide climax biocoenosis of the bathyal zone, including the Mediterranean Sea (SPA/RAC–UN Environment/MAP, 2019). Thanks to their structural complexity, these bioconstructions hold a paramount role in bathyal environments representing poles of attraction of deep-sea fauna (Rueda *et al*., 2019), influencing biogeochemical cycles, enhancing pelagic-benthic coupling processes (Rossi *et al*., 2017), and supporting highly profitable fishing grounds both for professional and recreational fishers (D'Onghia *et al*., 2017). Their high sensitivity to mechanical impacts and slow recovery ability, however, make them among the most vulnerable deep-sea ecosystems (VMEs), supporting high levels of protection (Otero & Marin, 2019).
In the Mediterranean Sea, white corals are considered a relic of the cold fauna of the Quaternary period (Plio-Pleistocene) and the majority of sites host subfossil coral rubble or frameworks (bioherms) (Vertino *et al*., 2014). Living bioconstructions are mainly found on hard substrata where slope and seafloor heterogeneity sustain a significant hydrodynamic flux, in particular that of the cold, oxygen- and aragonite-rich Intermediate Levantine Water

(Chimienti *et al*., 2019). In the Mediterranean basin, eight flourishing sites of occurrence of white coral reefs and mounds, usually referred to as provinces, have been identified in the last 20 years of investigations (Angeletti *et al*., 2020). In particular, the North-western Mediterranean Sea hosts the Gulf of Lion canyon system provinces (Fabri *et al*., 2014; Orejas *et al*., 2009), and, eastward, the newly discovered Corsica Channel province (Angeletti *et al*., 2020). Along the continental slope of the Ligurian Sea, however, the quest for living reefs has always been fragmentary.

Scattered coral bycatch has been reported off Portofino, Entella Canyon (Sestri Levante), Deiva Marina Canyon (Moneglia), and in the easternmost part of the Levante Canyon (Monterosso, La Spezia) since the 1920s (Brunelli & Bini, 1934; Rossi, 1958; Fanelli *et al*., 2017) (Fig.1). In 1957, topographic and sedimentologic surveys were conducted by the vessel *Calypso* in the Portofino area detecting a massive coral framework between 700 and 800 m (Blanc 1959), with living colonies found around 200-500 m in the easternmost sector (Rossi, 1958). In 1967, 1968 and 1972, the First Lieutenant Nicola Fusco summarized the available information in three charts (n°6-8) identifying the known coastal fishing grounds of Liguria and the possible obstacles, including coral areas. Twelve coral areas were reported from the Ligurian Sea (500-700 m), of which ten distributed in the eastern sector, from Genoa to Monterosso (Fig. 1).

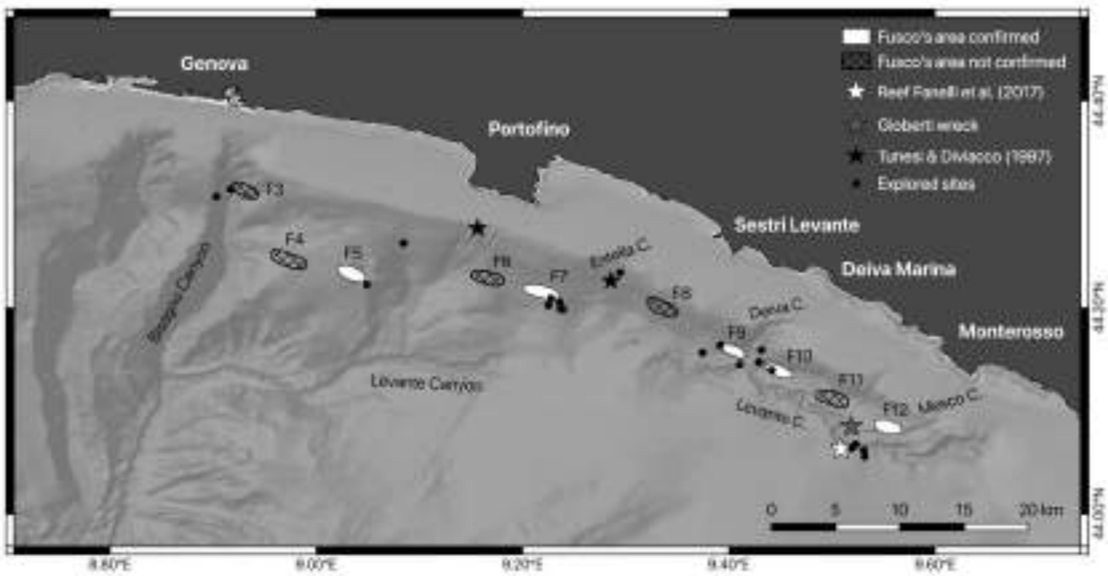

Fig.1. Localization of the Fusco's coral areas along the eastern Ligurian Canyon system with indication of the white coral findings and the Curiosity Driven Project ROV dives

In 1994, three dives were performed with the bathyscaphe *Cyana* in front of the Portofino Promontory and in the Entella Canyon reporting few living colonies at 210 m and 520 m (Morri *et al*., 1986; Tunesi & Diviacco, 1997; Tunesi *et al*., 2001). In 2011 and 2015, the ROV explorer Guido Gay reported *Madrepora oculata* Linnaeus, 1758 on the wrecks Transylvania and Gioberti, respectively off Savona (630 m) and Punta Mesco (530 m) (Fig. 1). In 2013 and 2014, ROV investigations of the eastern Ligurian Sea found scattered dead coral in the easternmost Fusco's area (F12) between 378 and 510 m, nearby Punta Mesco Canyon, and unveiled a living coral mound of *M. oculata* along the southern wall of the Levante Canyon at 510-580 m (Fanelli *et al*., 2017) (Fig. 1). Finally, in 2018, a ROV survey pictured for the first time the base of the massive bioherm of Portofino

(737- 773 m), occasionally impacted by the red shrimp trawling activities (Enrichetti *et al*., 2018).
These findings support an undisclosed potential for important discoveries, however, so far, nobody ever attempted to investigate the entire coral belt identified by Fusco. The possibility to investigate these areas is extremely valuable given that the deep Ligurian Sea is unique under many aspects: it is one of the most ancient sectors of the western Mediterranean Sea, it is located in the coldest part of the basin, and it is subjected to a high fishing pressure (Cattaneo *et al*., 2010). Geographical as well as depth distributional data are essential to know the real extent of this habitat and to implement appropriate management measures to guarantee its conservation. This paper presents new large-scale data on the occurrence of white coral bioconstructions in the eastern Ligurian Sea, supporting the creation of a distinct Ligurian coral province.

Materials and methods
The study area encompasses about 60 km of continental slope localized in the eastern part of the Ligurian Sea, from the Bisagno Canyon (Genoa) to Punta Mesco Canyon (Monterosso) (Fig. 1). In order to identify the sites that could potentially host the bioconstructions, Side Scan Sonar (SSS) profiles, multibeam maps and trawling routes of the entire area were used to detect reliefs within or in the proximities of the Fusco's coral areas. Previous literature data (e.g., Tunesi & Diviacco, 1997; Fanelli *et al*., 2017; Enrichetti *et al*., 2018) and indications of fishers have been also taken into consideration. Remotely Operated Vehicle (ROV) technology was then employed to explore four areas and their proximities where reliefs were detected, namely Genoa Plateau, Portofino and Entella Canyon, Deiva Marina Canyon, and Levante Canyon (Fig. 1). The ROV survey took place in the summer 2021 on board of the vessel *Daedalus* (Fondazione AzioneMare) with the ROV *Multipluto*. A total of 20 dives were carried out in the region from 350 to 800 m accounting for more than five hours of video footage and more than 8 km of explored seafloor.
To evaluate the occurrence and complexity of the bioconstructions in each site, 40 frames were randomly extrapolated from each video using the free software VLC and the substrate was characterized in terms of percentage composition in mud, rock, coral rubble and coral framework. When a coral matrix was detected, a surface percentage measure of the dead and living portions was made to evaluate the status of the habitat-forming species. In addition, an estimation of the height of the bioconstructions was given and, whenever possible, the dominant scleractinian species was also identified. Finally, the potential extension of the bioconstructions was calculated based on the bathy-morphological map of the area.

Results
Coral matrix was reported to a different extent in the four investigated regions (Fig. 2).

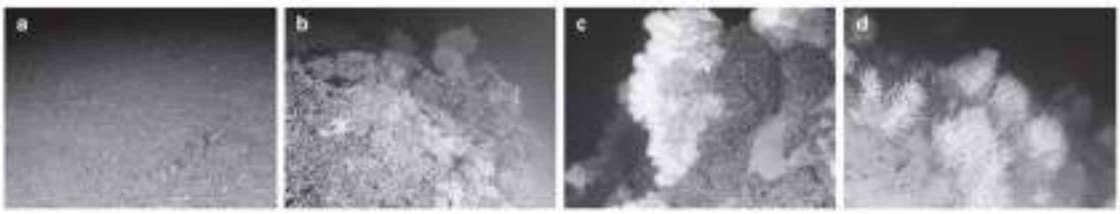

Fig.2. ROV images of the coral areas identified in the present study (a, Genoa mound, b, Portofino bioherm, c. Deiva Marina reef, d, Monterosso mound)

Genoa Plateau

SSS profiles identified no conspicuous topographic relief in any of the four Fusco's coral areas of this region, with the exception of a 20 m-large circular mound localized SE of F5 at 690 m (Fig. 1). Here, ROV exploration confirmed the occurrence of a coral mound formed by heavily fragmented rubble of *L. pertusa* (Linnaeus, 1758), no more than 15 cm high on a sub-horizontal muddy bottom (41% of the substrate coverage) (Fig. 2a). Heavy trawling traffic and deep trawling marks characterized this site.

Portofino Promontory and Entella Canyon

SSS profiles identified a massive relief, 5 km SE of the eastern cape of the Portofino Promontory. The bioconstruction stretches for about 4 km from 620 to 960 m and crosses F7 on its SE side (Fig. 1). The investigated portion of the bioconstruction is formed for the 95% by a dead *L. pertusa* bioherm, reaching up to 5 m high. Living colonies have also been observed. The bioherm is colonized by dense forests of habitat-forming alcyonaceans (Fig. 2b).

Deiva Marina Canyon

SSS profiles revealed the occurrence of two large reliefs, each about 2 km long, in the proximities of both Fusco's areas (F9 and F10) bordering the Deiva Marina Canyon. The areas are only marginally impacted by trawling because reefs mainly develop on the sloping and vertical walls of the reliefs up to the sub-horizontal crests. The complex living reefs, up to 3 m high and covering up to 62% of the investigated seafloor, are located between 480 and 710 m and are dominated by living *M. oculata* (Fig. 2c). This area, and in particular the rubble and the nearby mud, is also dominated by dense pluri-specific forests of habitat-forming alcyonaceans and massive sponges.

Levante Canyon

SSS profiles confirmed the occurrence of no major relief in F11 and F12 (Fig. 1), however, south of F12, in the Levante Canyon, two coral areas (300 and 500 m long), formed by numerous living *M. oculata* mounds up to 1 m high, were detected beside the one already reported by Fanelli *et al*. (2017) (Fig. 2d). One is located on the southern canyon wall, at few hundreds of meters from the known one, and the other is located on a silted terrace on the northern wall of the canyon. The area is interested by major currents and high turbidity levels, similarly to what observed in the Deiva Marina Canyon.

Discussion

The present study provides new information regarding the real extent and complexity of the eastern Ligurian CWC bathyal bioconstructions. The Fusco's coral areas represented important indications to start the research, however, with some exceptions, they proved to be not completely reliable. This could be because they were mainly based on bycatch coral rubble, which could have been dragged from nearby bioconstructions when the nets move accidentally too close to the sites, as already pointed out in the Portofino area (Enrichetti *et al*., 2018) and as plausible also for the areas of Deiva Marina. It could be also possible that in certain plateau areas, heavily impacted by trawling, coral mounds could have been swept away in time. The use of modified trawl nets with rubber rollers was suggested for the Levante areas (Fanelli *et al*., 2017) and the current status of the Genoa coral mound leaves no doubt about the nature of the impact.

The localization of the bioconstructions suggests the existence of a CWC belt, sustained by the high hydrodynamic flux moving into the Levante Canyon, along the entire eastern Ligurian basin. Further studies will help to fill the knowledge gap in the western sector, however, so far, the investigated area accounts for about 9 km^2 of both bioherms and

living reefs. This value is likely to increase because the coral occurrence in the Entella Canyon is still to be verified and the calculations in Deiva Marina need to better take into account the extension of the reefs along the slopes of the sites, only marginally considered here. These data support the existence of a distinct Ligurian CWC province as suggested for other areas (Angeletti *et al*., 2020).
Additional studies will investigate the associated diversity and the role of these important deep biogenic habitats as poles of attraction for other habitat-formers. The use of the coral skeletons as paleoenvironmental archives and the relationship with the environmental features will provide insights on the past and present functioning of these environments. Finally, further studies will shed light into the effects of artisanal and recreational fishing activities on the most heterogeneous portions of the bioconstructions, to better assess the environmental status of these important habitats.

Acknowledgements
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ORAL COMMUNICATIONS

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DISTRIBUTIONS OF SOME POORLY KNOWN ATLANTO-MEDITERRANEAN DEEP-SEA ANTHOZOANS IN THE WESTERN MEDITERRANEAN

Abstract
An increase in deep-sea research in the Mediterranean Sea has brought to light the occurrence of species not previously known to occur there. Such discoveries cover a wide array of taxa, including anthozoans, which are key species for benthic habitats. Anthozoans previously thought to be endemic to the Atlantic Ocean are now known to occur within the Mediterranean Sea. While the Alboran Sea is the most likely area for such species to occur, some Atlantic species have been found to extend further into Mediterranean waters. During the last 20 years, Oceana has researched the deep seafloor of the Mediterranean Sea, primarily using remotely operated vehicles (ROVs) to collect information on habitat-engineering species. These surveys have provided new data on the distribution of anthozoans that have traditionally been considered 'Atlantic', including species of Antipatharia (e.g., Phanopathes rigida), Scleractinia (e.g., Anomocora fecunda), Alcyonacea (e.g., Nicella granifera and Anthomastus sp.) and Pennatulacea (e.g., Protoptilum carpenterii). Some of these species can appear in large aggregations that form habitats (e.g., P. rigida, A. fecunda, N. granifera), while others are part of well-established benthic communities. These findings suggest that future surveys will likely reveal additional records of these species in the deep Mediterranean Sea.

Key-words: Anthozoans, deep-sea, Mediterranean Sea, habitat-forming species, VMEs

Introduction

Deep-sea surveys in recent years have identified the occurrence of species not previously recorded from the Mediterranean Sea. Most of these observations are of species whose distributions were previously recorded as extending from the Atlantic Ocean close to the Gibraltar Strait or slightly further, to the western African coast and to northern Europe. For example, reported findings of deep-water 'Atlantic' species in the Mediterranean Sea include sponges (Sitjà & Maldonado, 2014; Boury-Esnault *et al.*, 2015), echinoderms, (Evans *et al.*, 2018), and bryozoans (Harmelin & D'Hondt, 1993; Mastrototaro *et al.*, 2017). Such species have most commonly been found in the Alboran Sea, due to its proximity and direct connection to the Atlantic Ocean. However, some presumed Atlantic species have reached areas much further east, such as waters off Italy and Malta (Mastrototaro *et al.*, 2015; Evans *et al.*, 2018).

Among the 'Atlantic' species recorded in deep Mediterranean waters are a variety of anthozoans, which are of particular interest given their role as habitat engineers (Aguilar *et al.*, 2017) and concerns about their conservation status (Otero *et al.*, 2017). The average level of marine endemism in the Mediterranean Sea is very high (20-30%; Boudouresque, 2004), and some anthozoans do appear to be true Mediterranean endemics (e.g., *Cladocora caespitosa, Eunicella cavolini* and *Paramuricea macrospina*). However, most have a wider distribution, occurring both in the Atlantic Ocean and in the Mediterranean Sea. Nor do such species occur only sparsely in the Mediterranean; some already form

habitats, including within vulnerable marine ecosystems (VMEs) (for example *Viminella flagellum*; Chimienti *et al.*, 2022).
Here, we provide an overview of Oceana's observations of deep-sea 'Atlantic' species of anthozoans in the Mediterranean Sea, during the last two decades. Identifying such cases is useful for highlighting the prevalence of potential Atlanto-Mediterranean anthozoans that may form large colonies and habitats within the Mediterranean Sea, and therefore may play an important role in Mediterranean deep-sea ecosystems.

Materials and methods

Between 2006 and 2022, Oceana carried out 18 deep-sea expeditions in the Mediterranean Sea, from Spain to Lebanon. Most of these studies were conducted in the Western Mediterranean Sea, with a special focus in the Alboran Sea.
Video information was gathered during a total of more than 500 ROV dives around the Western Mediterranean Sea. All the ROV surveys were performed from the ketch catamaran *Ranger*, using a Phantom H2+2 ROV (camera-resolution 750 lines, F1.2 lens, 1:12 zoom) between 2006 and 2009; a Seaeye Falcon DR ROV (high-definition video (HDV) camera of 1920 x 1080 resolution, 1/2.9" Exmor R CMOS Sensor, minimum scene illumination of 3-11 Lux, and a 4.48 mm, f/1.8 3.4 zoom lens I) between 2010 and 2018; and a Nido Robotics Sibiu Pro (12 MP video camera recording at 4K with 60 fps, lens FOV 1/1.8" D108° H90° V59°, focal distance 18 mm) between 2019 and 2022.
The position of the ROV was continuously recorded using a LinkQuest Tracklink USBL transponder, with up to 0.25° accuracy. In some cases, samples of colonies were taken with the robotic arm of the ROV, to allow for later confirmation of taxonomic identification in the laboratory.

Results

In total, nearly 150 species of anthozoans were observed during the expeditions. While most of these species were previously well known from the Mediterranean, certain species observed are either less common or have only recently been recorded in this sea. They are described below.

1) *Nicella granifera*. In recent years, this coral species has been recorded from sites across the Atlantic, including the Azores (Braga-Henriques *et al.*, 2013), the Canary Islands (Álvarez *et al.*, 2015), and various seamounts (Grasshoff, 1985; Brenke, 2002; Oliveira *et al.*, 2017). Prior to these discoveries, however, it was already recognised as a Lusitanian-Mediterranean species (Watling & Auster, 2005). In the Mediterranean, Oceana has recorded *N. granifera* mainly below 150 m depth, on seamounts and marine elevations such as the Avempace, Cabliers, and Chella Banks in the Alboran Sea (Ocaña *et al.*, 2017), and the Emile Baudot escarpment in the Balearic Islands (Maldonado *et al.*, 2015). Recent research around the Balearic Islands has also listed this species among the area's benthic anthozoans (Massutí *et al.*, 2022).
   From Oceana's surveys, live colonies of *N. granifera* are white and reach roughly 15-20 cm in height. Polyps are sparsely distributed along branches, sometimes forming a zig-zag pattern. They grow on rocks and on calcareous debris. They appear to resist a certain level of sedimentation, growing on sedimented rocks on steep walls. While some colonies are isolated or in small patches, they can also create dense facies.
2) *Anthomastus* sp. Deep-sea species belonging to this genus of Alcyonacea are distributed in the eastern and mid-Atlantic Ocean (Molodtsova, 2013; WoRMS, 2022). Specimens found in the Mediterranean are normally red in colour, although Oceana

has also observed some that are white or slightly pinkish. All examples of *Anthomastus* species found by Oceana were living on dead corals and calcareous debris on Mediterranean seamounts (e.g., the Chella and Cabliers Banks), at 350-450 m depth. They were observed to form part of the deep-sea coral communities in those sites.

3) *Protoptilum carpenterii* is widely distributed in the northern and central Atlantic, in both the eastern and western basins (WoRMS, 2022), and has only been recently reported from the Mediterranean Sea (Mastrototaro *et al*., 2015). Oceana has filmed this species in Chella Bank, South Formentera, and in pockmarks close to Ses Olives seamount (Mastrototaro *et al.*, 2017). It was recorded from muddy bottoms below 300 m depth, where it formed mixed fields of pennatulacean. Many colonies have an intense red colour with large polyps, while others appear paler, with a pinkish/beige or darker red tone. They can reach over 50 cm height above the seabed. They may have passed unnoticed in some surveys due to possible confusion with *Funiculina quadrangularis*, a much more common species of sea pen in the Mediterranean Sea (Bastari *et al.*, 2018), or due to its rapid withdrawal behaviour into the sediment (Ambroso *et al.*, 2021).
4) *Phanopathes rigida*. This species is found in the Atlantic Ocean, mainly in the western central basin (WoRMS, 2022) and was also recently discovered in the Mediterranean Sea (Bo *et al.*, 2018). Oceana documented forests of *P. rigida* in the Cabliers Bank, where this species is the most important habitat-building black coral species (Corbera *et al.*, 2019). These findings are unique for the Mediterranean Sea and the Western Atlantic. Colonies of *P. rigida* are roughly 30-40 cm high, white in colour, with a bushy shape and very straight branches. Oceana has also documented it on the Chella Bank, but only as isolated colonies.
5) *Anomocora fecunda*. This scleractinian coral is very well know from the Macaronesian region and other Atlantic areas in both basins (WoRMS, 2022). Oceana has filmed this species in southern Portuguese waters, but also inside the Mediterranean Sea, on the Chella Bank (Ocaña *et al.*, 2017) and possibly also in the Balearic Island (pending confirmation). The species is commonly found forming caespitose colonies over rocky bottoms in waters deeper than 200 m.

In addition to the species above, Oceana has recorded other habitat-forming anthozoans in the western Mediterranean Sea that may have Atlanto-Mediterranean distributions. However, their taxonomic identity has not been confirmed. One of these species is a gorgonian, possibly of the genus *Placogorgia*, from deep rocky beds around the Alboran Sea and in the Balearic Islands. It is large, with yellow colonies, and differs from other Alcyonaceans known in the Mediterranean Sea; it is much taller (over 50 cm in height), thicker, and has more robust branches. The other species belongs to the suborder Alcyoniina, possibly of the genus *Siphonogorgia*, and has been found primarily on seamounts in the Alboran Sea. This soft coral is dark red to white in colour, and is taller and more highly branched than most Mediterranean Alcyoniina species. It settles on sedimented rocky bottoms or on coral/calcareous debris.

Discussion

Increasing knowledge about deep-sea biology in the Mediterranean is providing new information on the distribution of some ‘Atlantic’ habitat-forming species and extending their known ranges to the Mediterranean Sea. Oceana’s observations from the western Mediterranean Sea of anthozoan species that have typically been considered Atlantic add to this growing understanding. While these species have not been considered part of the benthic ecology of this sea, recent findings suggest that the presumed endemism of 'Atlantic' species should be reconsidered.

A useful starting point for such a reassessment would be to focus on those species that are known to occur on the Atlantic side of the Gibraltar Strait. For example, recent research has shown that *Radicipes gracilis* has been recorded in the Gulf of Cadiz, close to the Gibraltar strait (Grinyo *et al.*, 2022), while the Atlantic gorgonian *Acanthogorgia armata* already appears to have entered the Alboran Sea (Altuna & Poliseno, 2019). Beyond just adjacent waters, however, other observations highlight the possibility of more distant presumed Atlantic species reaching Mediterranean waters. For instance, the Macaronesian black coral *Antipathella wollastoni* has been recorded from the Mediterranean side of the Gibraltar Strait, possibly indicating a climate-drive range extension (Ocaña *et al.*, 2007).

It is worth noting that while this study focuses on the western Mediterranean basin, the distributions of some Atlanto-Mediterranean anthozoan species extend much further east in the Mediterranean Sea. New, but not yet published, records of species like *Protoptilum carpenterii* and *Placogogia* sp. will further increase their known distribution ranges inside the Mediterranean Sea in the near future, and will provide new data for evaluating their ecological importance.

As detailed here, deep-sea 'Atlantic' species like *A. fecunda*, *N. granifera,* and *P. rigida* already form monospecific habitats in the Mediterranean Sea, while others comprise part of important ecosystems like coral reefs and sea pen fields. Therefore, they should be considered Mediterranean habitats and also indicators of VMEs in Mediterranean waters. As components of fragile ecosystems that support high levels of biodiversity in the Mediterranean deep-sea, the importance of these newly-recorded Atlanto-Mediterranean species should be recognised.

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EXPLORATORY BASELINE SURVEY OF CAVE HABITATS WITHIN A MARINE PROTECTED AREA IN THE AEGEAN SEA

Abstract
Sea caves constitute a marine habitat of particular importance, hosting a significant amount of biodiversity and acknowledged as a biodiversity hot spot. The Aegean Sea (Eastern Mediterranean) hosts more than 600 marine caves, the majority of which are in the island-dominated South Aegean Sea. The marine protected area (MPA) of the Dodecanese archipelago incorporates the islands of Karpathos and Saria, both characterized by a complex rocky coastline with steep slopes and a high representation of priority habitats, as well as rare and protected species. An extended survey was performed to map and assess the cave habitats within the MPA jurisdiction, extending over approximately 25 nautical miles of coastline. The survey comprised collection and assessment of information from previous, partial surveys and local stakeholders, along with an expedition of a team of 5 divers to assess the habitat's presence along the rocky coasts of the MPA. In total, 57 distinct point locations were identified and mapped, according to international guidelines. Out of those, 30 were selectively assessed at 3 different levels of effort: (a) topographic characteristics, including entrance dimensions, orientation, and total length, (b) qualitative biodiversity assessment with visual census, (c) quantitative benthic community assessment using non-destructive photo sampling. The collected data will serve to: (a) establish a baseline of benthic community composition of these priority habitats, including typical, rare, protected, and introduced species, (b) evaluate their conservation status through relevant indices and a census of pressures and threats, (c) propose specific management, conservation, and restoration actions. The necessity for the development of methodology to facilitate large-scale mapping of marine cave habitats is highlighted.

Key-words: mapping; benthic assemblages; scientific diving; MPA; Eastern Mediterranean

Introduction
Submerged or partially submerged sea caves constitute a marine habitat of particular importance, hosting a significant amount of biodiversity and acknowledged as a biodiversity hot spot (Gerovasileiou & Bianchi, 2021). At the same time, they are sensitive and susceptible to a multitude of pressures despite the fact that they are often remote or difficult to access. Lately, there is a pronounced indication for impacts on the biotic components of marine caves, deriving either directly from human activities, or indirectly (e.g. climate change) (Gerovasileiou *et al*., 2016; Sempere-Valverde *et al*., 2019). Marine caves are included in the EU Habitats Directive (Code 8330) and are protected by Barcelona Convention under the Dark Habitats Action Plan (UNEP/MAP-RAC/SPA, 2015).
The Aegean Sea (Eastern Mediterranean) hosts more than 600 marine caves, the majority of which are in the island-dominated South Aegean Sea (Sini *et al*., 2017). However, a relatively small number of marine caves have been studied for their geomorphology and diversity (Gerovasileiou *et al*., 2013; 2015). Despite marine caves being known from 33

out of 62 Mediterranean Marine Protected Areas (MPAs), the exact number of sea caves in MPAs remains unknown (Abdulla *et al*., 2008) and no specific management plans or regulations are implemented for this habitat in most countries (Ouerghi *et al*., 2019). The aim of this work was to map and assess, for the first time, marine cave habitats within an MPA of the South-Eastern Aegean Sea, characterized by a complex rocky coastline with steep slopes and a high representation of priority habitats, in order to propose specific management and conservation actions.

Materials and methods

The exploratory survey was conducted over seven consecutive days between 27 October and 2 November 2019, extending along 45 kilometers of coastline within the area of jurisdiction of the MPA of North Karpathos and Saria Islands. This MPA is located in the south-eastern Aegean Sea (Greece) and is included in the Greek NATURA 2000 Network (code GR4210003). The survey was performed by boat, at close proximity to the coast, observing geological characteristics that could indicate the presence of partially or fully submerged caves (e.g. ridges, fractures and overhangs near the sea level). Additionally, information on potential presence of fully submerged caves was acquired by local fishers and divers. Three levels of evaluation were employed: (a) observation from the boat, (b) assessment with snorkeling and free diving, and (c) assessment with scuba diving. The surveys were performed by a team of five diving scientists with the support of the personnel of the Management Agency of Dodecanese Protected Areas. For cave formations fulfilling the criteria for characterization as 8330 Habitats (SPA/RAC–UN Environment/MAP & OCEANA, 2017), a minimum level of topographic data was collected (i.e. geolocation data, cave entrance dimensions, entrance orientation, cave length, existence of benthic zonation patterns). For sea caves hosting a typical sciaphilic community, a qualitative biodiversity assessment was performed by visual census. Lastly, for a sub-set of large representative caves, a full assessment of the biodiversity and the ecological status was performed, based on non-destructive photoquadrat sampling and application of ecological quality indices, respectively.

Results

In total, 57 locations with indicated or suspected presence of sea caves were surveyed along 45 kilometers of the MPA coastline (Fig. 1). Five of these locations were inspected with observation from the vessel only, while 20 cave formations were surveyed with scuba diving and 32 with snorkeling or free diving. Out of the 57 total examined locations, 27 did not comply with the criteria for the 8330 Habitat (i.e. were small-sized crevices, pass-throughs, overhangs, or walls with vertical or negative inclination) and no further assessment was performed apart from recording the location coordinates. For the remaining 30 cave formations complying with the 8330 Habitat criteria, the majority (20 caves, 67%) were partially submerged, meaning that a part of the entrance and the cave length was above seawater level, while 10 caves (33%) were fully submerged (i.e. their entrance was below sea level, although some had internal air chambers). The seafloor depth of the cave entrances ranged from -1 to -16 m (Fig. 2A), with a median of -7 m ± 4.5 SD. The length of the caves (meaning the extent of the cave formation that is covered by ceiling, from entrance to end point) ranged from 2 to 37 m (Fig 2B), with a median of 15 m ± 7.6 SD.

Regarding the biodiversity of the assessed 30 caves, 68 taxa were recorded in total, belonging to 14 major taxonomic groups, namely 2 plant, 12 invertebrate, and 2 vertebrate phyla. Overall, a rich biodiversity was recorded, including the typical components of the endemic Mediterranean coralligenous habitat (e.g. *Mesophyllum* sp., *Peyssonnelia* sp.) which were characteristic at the cave entrances, as well as aggregations of species representatives of the dark and semi-dark Mediterranean assemblages (e.g. the sponges *Diplastrella bistellata* and *Plakina* sp., the yellow cup coral *Leptopsammia pruvoti*, the narwal shrimp *Plesionika narval* and the cardinal fish *Apogon imberbis*). Moreover, several typical Eastern Mediterranean cave-dwelling organisms were commonly recorded, including the bryozoan *Hippaliosina depressa* and the coral *Madracis pharensis.* Among a rich motile fish fauna, five non-indigenous species (NIS) were commonly recorded at the examined caves. Out of those, *Pempheris rhomboidea*, *Pterois miles*, and *Sargocentron rubrum* were recorded at the semi-dark and dark parts of the caves, while *Enchelycore anatina* and *Siganus luridus* were observed mainly near the cave entrance zone. Pressures were recorded in some caves, in the form of marine litter, but also as partial necroses and mortality of sponges and fragmentation of fragile species (mainly bryozoans and corals). Detailed biodiversity and ecological quality assessment of the seven representative caves are presented in Digenis *et al*. (2022).

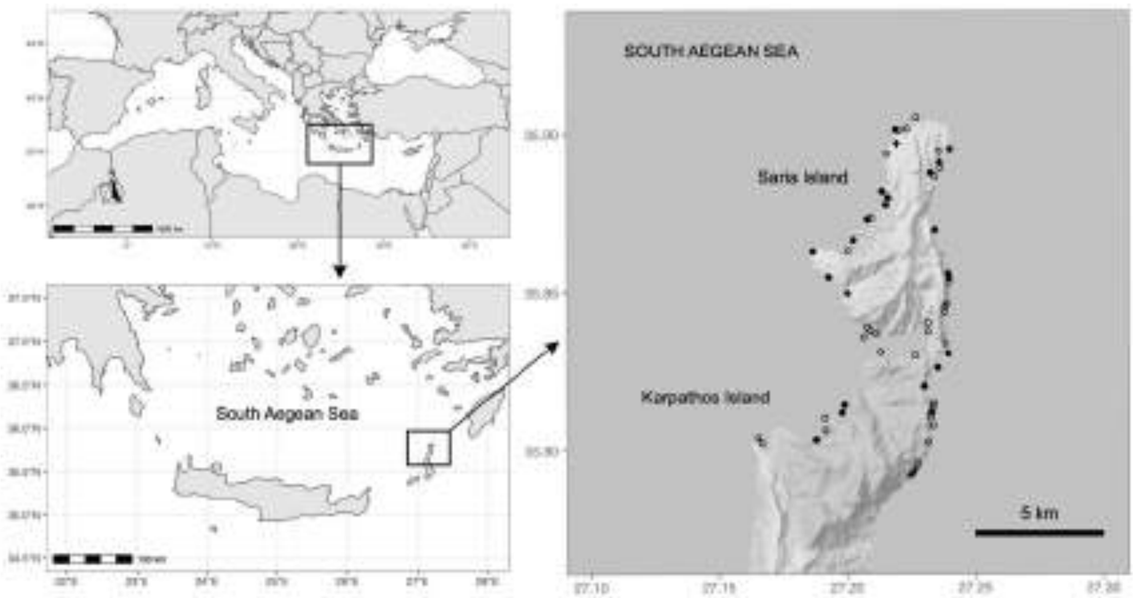

Fig.1. Map of the Marine Protected Area of North Karpathos and Saria Island, indicating the 57 point locations where the presence of fully or partially submerged sea caves was surveyed. Different symbols indicate surveying method: black circle = scuba diving; transparent circle = snorkeling and free diving; cross = observation from vessel.

Tab. 1: Location names and geographic coordinates of the 30 surveyed sites complying with the 8330 Habitat criteria. Survey method (SD: scuba diving; FD: free diving and snorkeling) and collected data (Topo: topographic characteristics; Bio: biotic data through visual census; PhQ: non-destructive photoquadrat sampling for comprehensive biodiversity analysis) are also listed for each cave.

Location name	Coordinates		Survey method		Collected data		
	Lat	Lon	SD	FD	Topo	Bio	PhQ
Troulakas	35.811	27.233	•		•	•	•
Alona	35.820	27.230	•		•	•	•
Alimounta	35.896	27.241	•		•		•
Platoma	35.870	27.234	•		•		
Monaxios	35.830	27.240	•		•		
Ammoui	35.825	27.236	•		•		
Yeros	35.802	27.232		•	•	•	
Palatia	35.887	27.233	•		•		•
Agios Panteleimonas	35.855	27.193	•		•	•	•
Melouros 1	35.803	27.188	•		•	•	
Yaftes 2	35.813	27.199	•		•		
Yaftes 3	35.811	27.198	•		•	•	
Linovrochi	35.837	27.232		•	•		
Yiourious 2	35.866	27.203	•		•	•	•
Plaka 3	35.882	27.214	•		•	•	
Oxonisos 2	35.901	27.219	•		•	•	•
Alimounta 2	35.895	27.236		•	•	•	
Kisolia	35.889	27.236		•	•	•	
Palatia 1	35.887	27.235		•	•	•	
Imera Athymaria 2	35.854	27.240	•		•		
Asprouas	35.853	27.240		•	•	•	
Spathareas 1	35.845	27.239		•	•		
Spathareas 2	35.844	27.239		•	•		
Agia Aikaterini	35.830	27.227		•	•	•	
Troulakas 1	35.814	27.234		•	•		
Arelia 2	35.809	27.233		•	•	•	
Nisi tis graias	35.795	27.228		•	•		
Chalavronta 1	35.837	27.212		•	•	•	
Chalavronta 2	35.839	27.208		•	•	•	
Pountes	35.831	27.213		•	•	•	

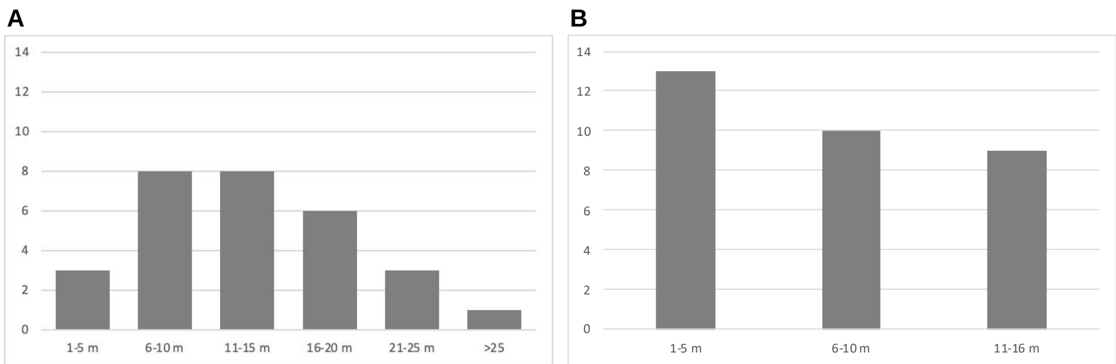

Fig.2. (A) Number of caves grouped in six categories according to cave length; (B) Number of caves grouped in three categories according to entrance depth.

Discussion and conclusions

The present study constitutes a detailed assessment of the presence and distribution of marine cave habitats (Habitats Directive type 8330) within an Eastern Mediterranean MPA, along with a preliminary evaluation of their topographic and biotic characteristics. The collected and analyzed data serve to: (a) provide a catalogue of the presence and extent of marine cave habitats within the MPA; (b) establish a baseline of benthic community composition; and (c) serve as a basis for sound management, conservation and restoration actions. It is notable that marine caves, although a priority habitat in the Mediterranean Sea, are not exhaustively mapped and assessed within Mediterranean MPAs, in contrast to other marine habitats listed in the EU Habitats Directive, such as *Posidonia* seagrass meadows (habitat type 1120), submerged sandbanks (habitat type 1110), and reefs (habitat type 1170). This is contradictory to their generally accepted important role as biodiversity hotspots and refugia (Gerovasileiou *et al*. 2015) and their sensitivity to ongoing and future pressures (UNEP-MAP-RAC/SPA, 2015). This gap is relevant to inherent difficulties in broad-scale mapping and remote sensing, i.e. there is presently no way to effectively identify submerged cave habitats by high-throughput methods such as acoustic and satellite data, due to their cryptic nature. This is augmented by the innate fragmentation of this habitat, as well as regional heterogeneity (Giakoumi *et al*. 2013). Our survey, though comprehensive and detailed, has limitations in regard to bathymetry (limited to 16 m) and its exhaustiveness, since it is based on indications from geomorphological features and scarce local ecological knowledge in order to focus the exploratory actions in areas of interest. At the same time, surveys of this nature are particularly resource-intensive, especially when applied over extended lengths of coastline. Future priorities towards the mapping and assessment of marine caves in Mediterranean MPAs should concentrate in the exploration and development of high-throughput methods to this end, including: (a) use of Diver Propulsion Vehicles (DPVs) to extend the exploratory survey range; (b) development of methodologies based on Remotely Operated Vehicles (ROVs) and possibly Autonomous Underwater Vehicles (AUVs) to enhance observation ranges overcoming the limitations of diving in terms of depth and operational time; (c) investigation of the potential of acoustic methods such as multi-beam sonar to indicate the presence of cave formations, and (d) employment of 3D photogrammetry analysis to increase the efficiency and resolution of the assessment of biotic and abiotic factors.

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EFFECTS OF GLOBAL AND LOCAL PRESSURES ON THREE SUBMERGED MARINE CAVES IN THE WESTERN MEDITERRANEAN SEA

Abstract
Submerged marine caves, priority habitats under European directives, are subjected to a combination of global and local human pressures. Although information about the effects of global warming on marine caves is seldom available, there is still very little information about the effects of local pressures on these unique habitats. In this study we evaluated and compared change over time in benthic communities experienced by three submerged marine caves in the Western Mediterranean Sea: Ventimiglia and Grotta Marina of Bergeggi in the Ligurian Sea and Grotta Azzurra of Capo Palinuro in the Tyrrhenian Sea. Photoquadrats have been sampled underwater in different stations along the caves and in distinct years, providing long series of data spanning from 10 to 30 years. On photoquadrats, the percent cover of non-taxonomic benthic descriptors, grouped into growth forms, was visually estimated. Euclidean Distance was used to measure change over time in benthic communities. Results showed that global pressures did not significantly change the cave benthic communities over the long time-period, whilst local pressures due to infrastructure building caused the most significant change in a comparatively shorter time. The three marine caves displayed different behaviours, thus confirming the singularity of each cavity system. The observed vulnerability of the three caves investigated urgently asks for including these habitats in monitoring plans to assess the effects of local human pressures.

Key-words: Mediterranean Sea, marine caves, non-taxonomic descriptors, trophic guilds, time-series

Introduction

Marine ecosystems are changing under the combined effect of local and global pressures (Gissi *et al.*, 2021). Evidence of community shifts has already been reported in many subtidal ecosystems of the north-western Mediterranean Sea, due to the synergic effects of climate change and local disturbances, which caused significant alterations, often irreversible, in community structure and composition (Gatti *et al.*, 2017).
Submerged marine caves are unique and vulnerable habitats protected by the European Community (Habitat Directive 92/43 EEC). They exhibit high biodiversity and are threatened by multiple pressures (Parravicini *et al.*, 2010; Montefalcone *et al.*, 2018). Their sessile communities are highly heterogeneous, reflecting the cave topography and gradients, so that the effects of pressures can vary considerably according to geographical location and environmental conditions. Local disturbances deriving from coastal interventions may cause the major changes in both semi-submerged and submerged caves, acting synergically with climate change and water warming (Montefalcone *et al.*, 2018). Diachronic inventories are rarely carried out and time series are of prime

importance to detect marine ecosystem change, even in the absence of direct human impacts (Bianchi *et al*., 2022). There is a few studies about time series on marine caves (Gerovasileiou & Bianchi, 2021), nevertheless in the NW Mediterranean Sea three iconic examples of caves monitored for decades exist: the Grotta Marina of Bergeggi, the cave system of Ventimiglia, and the Grotta Azzurra of Capo Palinuro, all located in Italy. This study represents the first attempt to compare the change over time (10 to 30 years) experienced by benthic sessile communities in these three marine caves to evaluate i) effects of both local and global pressures on cave ecosystems, and 2) similar patterns of change in response to anthropogenic pressures.

Materials and methods

The Grotta Marina of Bergeggi is located in the western Ligurian Sea (Savona, North-West Italy) within the Marine Protected Area 'Isola di Bergeggi', established in 2007. It is a semi-submerged cave. The submerged portion reaches 7 m depth, is a tunnel-shaped cave and has a limited development but a remarkable morphologic complexity, which creates strong gradients (Montefalcone *et al*., 2018). The main local human pressure on this cave originates from the nearby Vado Ligure commercial harbour, being the cave located a few kilometres downstream this harbour, which was hugely enlarged between 2012 and 2018. A long series (1969, 1971, 2005, 2008, and 2011) of beach nourishment interventions were also carried out around the Bergeggi coastal area, which enhanced sedimentation on many coastal ecosystems (Parravicini *et al*., 2010).

The cave system of Ventimiglia is located in the western Ligurian Sea (Ventimiglia, North-West Italy) and is made by two adjacent blind-ended caves (Grotta Grande and Grotta Piccola), considered as "twin cavities" (Gerovasileiou & Bianchi, 2021): a larger semi-submerged cave with a maximum depth of 6 m (Grotta Grande), and a smaller submerged cave with a maximum depth of 4 m (Grotta Piccola). The main local human pressure affecting this cave system is represented by the recent construction of the new marina in Ventimiglia, which started in 2010 and ended in 2019. The main dam and the entrance of the touristic harbour is nearby the entrance of the Grotta Piccola.

The Grotta Azzurra of Capo Palinuro is located in the Tyrrhenian Sea (Salerno, South-West Italy). It is a wide and complex semi-submerged cave, reaching a maximum depth of 30 m; with two distinct entrances and a wide lateral chamber the cave has both the characteristics of a tunnel-shaped and of a blind-ended cave. The coastline of Capo Palinuro is not significantly affected by major local human activities, and the only putative stressor may be linked with the summer frequentation by touristic boats that enter the cave.

In the Grotta Marina of Bergeggi a total of 8 sampling stations were selected randomly on walls in the different sectors of the cave and at the same depth of about 6 m (Montefalcone *et al*., 2018). In each station five replicates of wire-frame photography were collected in four distinct periods (1986, 2004, 2009, and 2013), for a total of 40 photoquadrats per year. In the Ventimiglia cave system, 9 sampling stations were selected randomly on walls at about 5 m depth and at a constant distance from each other, starting from the entrance to the inner portions of the two caves (Nepote *et al*., 2017). In each station three replicates of wire-frame photography were collected every year, from 2010 to 2018, for a total of 27 photoquadrats per year. In the Grotta Azzurra of Capo Palinuro, 17 sampling stations were selected randomly on walls in the different sectors of the cave and at about 10 m depth (Montefalcone *et al*., 2022). In each station three replicates of

wire-frame photography were collected in two distinct periods (1992 and 2018), for a total of 51 photoquadrats per year.
Growth forms have been adopted as non-taxonomic descriptors to assess change over time in benthic communities of the three marine caves (Parravicini *et al.*, 2010), which are informative about the ecosystem structure and the habitat occupancy strategy adopted by sessile organisms (Nepote *et al.*, 2017). Morphology of organisms is also a simpler descriptor to be employed and identified during laboratory analyses (Montefalcone *et al.*, 2018). Growth forms include borers, hemispherical determinate mounds, domed determined mounds, flattened indeterminate mounds, cylindrical prostrate indeterminate mounds, prostrate plates, erect plates, trees, runners, determinate sheets, indeterminate sheets, vines, and pedunculate sponges (see Nepote *et al*., 2017 and Montefalcone *et al.*, 2018 for further details).
On photoquadrats the percent (%) cover of each growth form was visually estimated, also considering the cover of turf and sediment. Mean values (±s.e.) of each descriptor were obtained averaging the photoquadrats on each station. Cover data were then arcsine $\sqrt{}$ (x/100) transformed. Change over time in the occupational strategy in each cave was evaluated by computing Euclidean Distances between the photoquadrats of a given year and the centroid of the photoquadrats of the first year of the time series. Euclidean Distances were chosen as a measure of dissimilarity among communities, as they emphasize differences in the proportional species composition and account for joint-absence information. The rate of change in the benthic communities of each cave was computed as the difference between Euclidean Distances over years, and then expressed as percentage. All statistical analyses have been performed by using the software R Development Core Team (2011) and the software IBM SPSS Statistics (v. 21).

Results and Discussion

The benthic community in the Grotta Marina of Bergeggi maintained virtually stable between 1986 and 2009, while it changed between 2009 and 2013 (Fig. 1). The Ventimiglia cave system showed a sharp change between 2010 and 2013 and then it maintained almost stable in the last sampling years (i.e., between 2013 and 2018). The Grotta Azzurra of Capo Palinuro maintained nearly stable between 1992 and 2018.
The synoptic comparison among the three caves shows that the Grotta Marina of Bergeggi displayed a low rate of change (0.03%) between 1986 and 2009, similar to that experienced by the Grotta Azzurra of Capo Palinuro (0.2%). On the contrary, the rate of change of the Grotta Marina between 2009 and 2013 was comparatively higher (2.8%), and similar to that observed in the Ventimiglia cave system, this latter showing the highest rate of change (4.6%) in the shortest time period.
The analysis of long time series of quantitative information about sessile communities in marine caves allowed elucidating the evolution of the Bergeggi, Ventimiglia, and Palinuro cave ecosystems during the last 30 years. Notwithstanding the dearth of long-term ecological studies in the Mediterranean Sea, little research effort has been invested on marine caves in comparison to other marine habitats (Gerovasileiou *et al.*, 2015) and the historical series of data analysed in this study may thus represent a precious and unique baseline for understanding the vulnerability of these fragile ecosystems to global and local pressures. The non-taxonomic descriptors here adopted (i.e., growth forms) were effective indicators of the benthic community alteration occurred within the caves. The Grotta Marina of Bergeggi experienced a slow but continuous decline in the cover of erect organisms in the last thirty years, matched by an increase in turf and sediment. 3-

dimensional growth forms have been replaced by 2-dimensional ones, leading to a general homogenisation of the cave communities (Parravicini *et al.*, 2010). The first evidence of change in this cave has been reported since the 1990s, which was likely to be due to the synergic effects of both local pressures and climate change (Montefalcone *et al.*, 2018). Beach nourishments were considered as the main source of impact, especially those major interventions occurred in 2005, 2008, and 2011. The Vado Ligure harbour enlargement (started in 2011) enforced the pressure of local disturbances affecting this cave, as resulted from the huge increase in the benthic community dissimilarity observed between 2009 and 2013. The human interventions that occurred along the coast of Bergeggi in recent years may have changed the natural hydrodynamic and sedimentary regimes, which, in turn, could have detrimental effects on the cave assemblages because of the increased water turbidity and the alteration in the sedimentation rate (Rovere *et al.*, 2015).
The Ventimiglia cave system has the comparatively shorter time series, but the most representative in terms of sampling frequency, as it was specifically addressed to evaluate the impact of the construction of the touristic harbour on the cave ecosystems (Nepote *et al.*, 2017). Since the beginning of the harbour construction, started in 2011, the benthic communities in the Ventimiglia caves showed significant change over time, as most of the 3-dimensional forms have been replaced by turf and sediment.
The very low rate of change experienced by the Grotta Azzurra of Capo Palinuro in the last three decades clearly reflects the virtual absence of major local human activities along the coastline, being climatic disturbance the major driver of the change observed (Gambi & Barbieri, 2012).

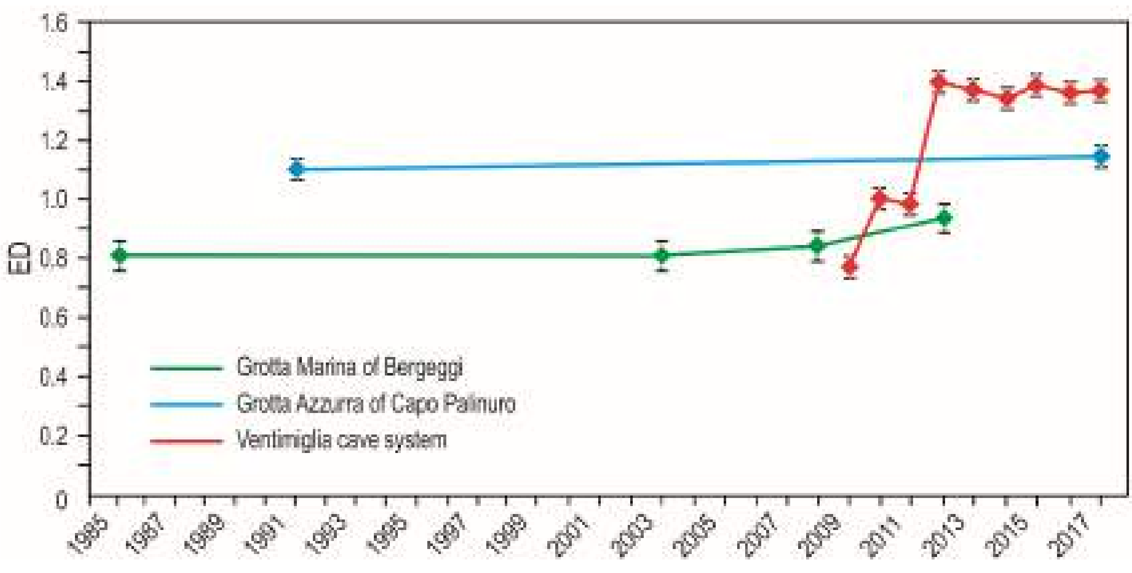

Fig. 1: Growth forms change over time, expressed as mean Euclidean Distance (ED ± s.e.) in the three marine caves investigated.

Conclusions

The synoptic analysis among the three marine caves showed that global pressures did not significantly change the cave benthic communities over the long time-period, whilst local pressures due to infrastructure building caused the most significant change in a comparatively shorter time. The greatest changes were experienced by those caves affected by local human pressures, such as the touristic harbour construction in

Ventimiglia and the commercial harbour enlargement in Bergeggi. Both Ventimiglia and Bergeggi caves displayed a similar rate of change, which was remarkably higher than in Palinuro. The three marine caves displayed different behaviours, thus confirming the singularity of each cavity system.
Although a slow but gradual change has already been described in cave ecosystems because of climate change and water warming (Gambi & Barbieri, 2012; Parravicini *et al.*, 2010; Montefalcone *et al.*, 2018), the synergic occurrence of local human pressure may cause the most detrimental effects on the resilience of these vulnerable ecosystems. Assessing the effects of human pressures on biodiversity is a prerequisite for effective marine spatial planning, harmonizing conservation purposes with sustainable development. The observed vulnerability of the three caves investigated urgently asks for including these habitats in monitoring plans to assess the effects of multiple pressures.

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COMPARISON OF BENTHIC ASSEMBLAGES FROM SIX MARINE CAVES IN A PROTECTED AREA OF THE SOUTH-EASTERN AEGEAN SEA, GREECE

Abstract
Despite the large number of marine caves which have been recorded along the Greek coasts of the Aegean Sea, only few have been studied for their biodiversity. In this study, the benthic assemblages of six marine caves in a Protected Area of the South-Eastern Aegean Sea (Karpathos and Saria Islands, Greece) were quantitatively studied for the first time. A total of 120 photoquadrats were collected and analysed, covering the entrance and semi-dark zones of the caves. Motile taxa were qualitatively recorded with visual census. Our study yielded 81 sessile and 45 motile taxa, including 12 protected and 10 non-indigenous species. Multivariate analysis showed that different geomorphological and topographic features of the studied caves significantly affected the structure of the benthic assemblages. In addition, several pressures and threats were recorded in all studied caves. The results of this baseline survey are essential to the development of specific management and conservation actions for the protection of marine caves and their biota.

Key-words: benthic communities, motile fauna, morphological variables, MPA, Eastern Mediterranean

Introduction
Marine caves have been characterized as “biodiversity reservoirs” or “refuge habitats” due to the high number of taxa which find refuge in their interior, including several endemic, rare and protected species (Harmelin *et al*., 1985; Gerovasileiou & Voultsiadou, 2012; Gerovasileiou & Bianchi, 2021). Therefore, they are protected by the European Union’s Habitats Directive (92/43/EEC) and the Barcelona Convention under the Dark Habitats Action Plan (UNEP/MAP-RAC/SPA, 2015). Sea caves constitute a typical feature of the Mediterranean Sea with more than 3,000 caves recorded along its rocky coastline (Giakoumi *et al*., 2013). According to the latest census, 622 sea caves are known from the Greek coasts of the Aegean Sea with their majority distributed at its southern, island-dominated sector (Sini *et al*., 2017).
Marine caves exhibit a notable biotic heterogeneity, also known as “individuality”, which is commonly attributed to different geomorphological and topographic features (e.g. cave morphology, depth, and orientation of the entrance) generating steep environmental gradients (Riedl, 1966; Bussotti *et al*., 2006; Gerovasileiou & Bianchi, 2021) which directly affect the structure of hard substrate benthic communities. Herein, a quantitative description of hard-substrate benthic assemblages and a qualitative assessment of motile fauna are provided for six marine caves in a Marine Protected Area (MPA) of the Eastern Mediterranean Sea. Furthermore, we investigated the association of different geomorphological and topographical factors of the caves with the structure of benthic assemblages.

Materials and Methods

Six marine caves of the MPA of North Karpathos and Saria Islands (NATURA 2000 code GR4210003) at the South-Eastern Aegean Sea, Greece (Fig. 1A) were surveyed by diving scientists in October 2019. Two of the studied caves (Alona and Troulakas) were fully submerged, located at the north-eastern coast of Karpathos Island, while the other four caves (Giourious, Oxonisos, Palatia and Panteleimonas) were semi-submerged, located along the northeast to western coastline of Saria Island (Fig. 1B). All examined caves were blind-ended (i.e. with a single opening/entrance) with a total length of 16 to 37 m, while their maximum depth ranged from 4 to 11 m for fully submerged and 9–16 m for semi-submerged caves.

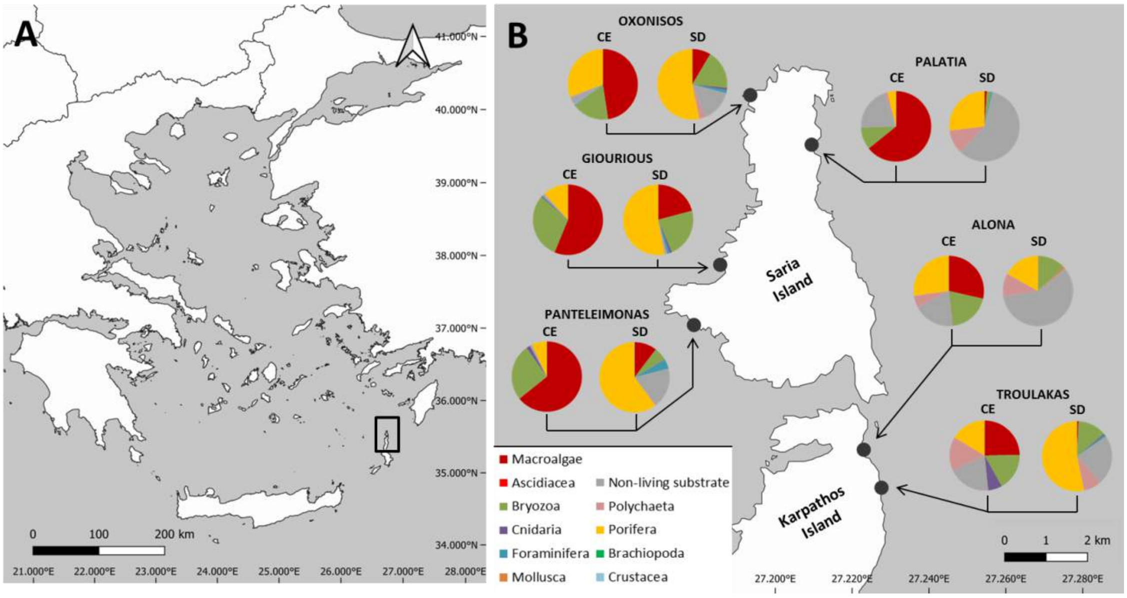

Fig.1. Location of the study area in Greece, Eastern Mediterranean (A) and the studied caves with the percent coverage of sessile taxa and non-living substrate for each ecological cave zone (CE: cave entrance, SD: semi-dark zone) (B).

For the study of hard substrate benthic assemblages, a series of 10 randomly placed quadrats (25x25 cm) was photographed from the opposite walls of each cave zone, resulting in a total of 120 photoquadrats for the six surveyed caves. Since none of the studied caves had a distinct completely dark zone, the zones examined were the cave entrance (CE) and the semi-dark zone (SD). Photographs were taken at 4,608 by 3,456 pixel resolution with a digital camera coupled with two external underwater strobes. Qualitative identification of motile species was performed with visual census, with emphasis on protected and non-indigenous species (NIS).

The percent cover of sessile taxa in the photoquadrats was calculated with PhotoQuad software by overlaying 100 uniformly stratified points (Trygonis & Sini, 2012). An arbitrary value of 0.5% cover was given to those taxa that were present in photoquadrats but did not fall below an assignment point. Multivariate, non-parametric resemblance analysis of the biotic coverage data (transformed under the square root formula) was performed with the software PRIMER-6. Non-metric multidimensional scaling (nMDS), one-way analysis of similarity (ANOSIM) and similarity percentages analysis (SIMPER) were used to investigate benthic assemblage structure and its association with different geomorphological and topographic factors of the studied caves.

Results

A total of 81 sessile taxa were identified, classified into Porifera (37), Bryozoa (13), Macroalgae (10), Cnidaria (6), Ascidiacea (6), Brachiopoda (3), Mollusca (3), Foraminifera (1), Polychaeta (1), and Crustacea (1). Both the cave entrance and the semi-dark zone of the studied caves were rich in terms of biodiversity, with 71 and 68 recorded taxa from each zone, respectively. Macroalgae, mostly rhodophytes, dominated in terms of percent cover at the entrance of all caves while sponges dominated at the semi-dark zone (Fig. 1B). The total number of taxa varied among the surveyed caves, from 43 in Alona and Palatia caves to 59 in Troulakas cave.

All studied marine caves and ecological zones within these caves were significantly differentiated. The results of one-way ANOSIM analysis showed that the examined geomorphological and topographic factors (i.e. cave type, entrance depth, entrance area, and entrance orientation) had significant effect on the resemblance patterns of benthic assemblages structure (p-value < 0.01 in all cases). This heterogeneity is particularly evident for photoquadrats from different ecological zones, cave types and entrances with different size and depth, which appear to be grouped in different clusters in the nMDS plots (Fig. 2).

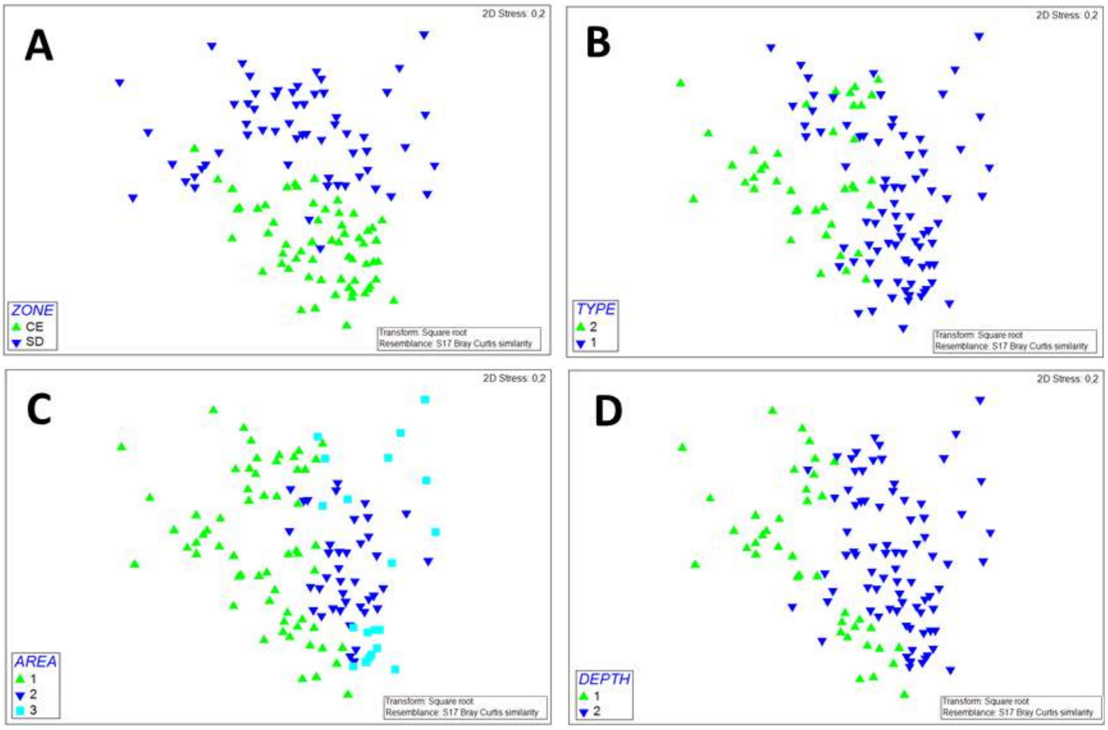

Fig.2. Non-metric multidimensional scaling (nMDS) plots showing the similarities among the analysed photoquadrats from all studied caves. Photoquadrat samples have different shapes and shades for different factors. (A) Ecological zone: cave entrance (CE) or semi-dark zone (SD); (B) Cave type: semi-submerged (1) or submerged (2); (C) Entrance area: 15–40 m^2 (1), 110–230 m^2 (2), or 630 m^2 (3) and (D) Entrance depth (max): 0–10 m (1) and 10–20 m (2).

SIMPER analysis results showed that different taxa had different percent contribution to the average similarity for every studied factor. Nevertheless, the average similarity of equivalent ecological zones from different marine caves was higher (46.0% for the entrance and 37.2% for the semi-dark zone) than the average similarity between the different zones (28.8%).

Regarding motile fauna, 45 taxa classified as Pisces (24), Crustacea (10), Echinodermata (6), Polychaeta (2), Mollusca (2), and Mammalia (1) were recorded, including five protected and 10 non-indigenous species (NIS). In addition, several pressures and threats were recorded in the studied marine caves (Fig. 3). NIS were observed in all caves, varying from one to seven taxa in a single cave. Litter, mainly consisting of plastic waste (e.g. fishing lines) was observed in most of the caves (Fig. 3B).
Partial necrosis and broken fragments of different taxa were also observed in five caves (Fig. 3C), specifically of encrusting rhodophytes, massive sponges (*Agelas oroides*, *Ircinia oros* and *Spirastrella cunctatrix*), scleractinian corals (*Leptopsammia pruvoti*) and erect bryozoans (*Myriapora truncata* and *Reteporella* sp.).

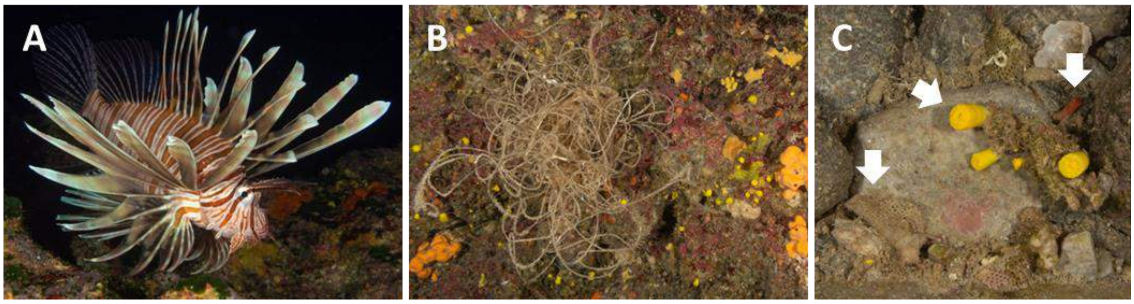

Fig.3. The alien lionfish *Pterois miles* from the semi-dark zone of Oxonisos cave (A), fishing line attached to the walls at the semi-dark zone of Troulakas cave (B) and fragments of scleractinian corals and erect bryozoans (with arrows in C). Photos by T. Dailianis.

Discussion and conclusions

This quantitative and qualitative baseline survey underlines the rich biodiversity of six marine caves belonging to the MPA of Karpathos and Saria Islands in the South-Eastern Aegean Sea. While most of the sessile species identified are commonly found in the interior of marine caves and other marine habitats of the Greek Seas (Gerovasileiou *et al*., 2015), 10 species were recorded for the first time as part of the marine cave fauna of the Aegean Sea (the sponge *Haliclona aquaeductus*, the gastropod *Cerithium scabridum*, the bryozoans *Caberea boryi*, *Reptadeonella violacea* and *Patinella radiata*, the echinoderm *Diadema setosum*, the ascidian *Pycnoclavella nana*, and the fishes *Atherina* sp., *Parupeneus forsskali*, and *Torquigener flavimaculosus*).
Multivariate resemblance analysis showed that the six studied marine caves were significantly differentiated, mainly due to location-specific geomorphological features, resulting in high levels of individuality (Bussotti *et al*., 2006; Gerovasileiou & Bianchi, 2021). In addition, the studied caves presented higher similarity when equivalent ecological zones were compared, mainly due to the dominance of rhodophytes and sponges in the entrance and semi-dark zone, respectively (Gerovasileiou & Voultsiadou, 2012; Gerovasileiou *et al*., 2017).
Marine caves are difficult to assess over extended geographic areas or with remote sensing, requiring dedicated surveys by experienced scientists for their exploration and assessment. As a consequence, they constitute an under-reported habitat type, even within protected areas and despite their ecological importance. The high spatial heterogeneity in the composition of benthic assemblages coupled with the finding of several NIS and other threats in the studied sea caves shows that further monitoring, management and conservation actions are needed for this vulnerable marine habitat in the Eastern Mediterranean Sea.

Acknowledgments

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SUBSTRATE-RELATED FACIES OF *DENDROPHYLLIA CORNIGERA* (LAMARCK, 1816)

Abstract
Dendrophyllia cornigera (Lamarck, 1816) is a bright yellow scleractinian considered a typical hard bottom species from the NE Atlantic Ocean and the Mediterranean Sea. Some evidence suggests that this coral displays wide adaptability to environmental constraints, including the ability to create aggregations on soft bottoms.
To fully investigate D. cornigera ecological requirements, we present a comprehensive revision of the available literature on its distribution and ecology, including new ROV records along the Italian coast. Results indicate that this species can create three main facies, characterized by a specific combination of substrate type, inclination, and depth: (1) scattered living colonies on a wide array of hard bottoms, presenting a wide geographical and bathymetrical distribution; (2) dense aggregations occurring on sub-horizontal soft bottoms, reported only from mesophotic depths in two sites; (3) Dendrophyllia thanatocoenoses resulting widespread on upper bathyal rocks and soft bottoms. Dead branches are often intermixed with scattered living polyps and can form extended frameworks, enhancing seafloor complexity. Differences in colony size and associated fauna composition have been detected among the three facies.
This study highlights the wide adaptability of D. cornigera in terms of environmental settings, changing the traditional view of its substrate preference and providing valuable insights for the implementation of international deep-sea habitat classification schemes.

Key-words: Dendrophylliidae, Mediterranean Sea, mesophotic, thanatocoenoses, upper bathyal

Introduction
Coral forests are considered key habitats in mesophotic and deep-sea environments, providing shelter and food for a rich associated fauna and playing a fundamental role in marine ecological processes (Rossi *et al.*, 2017). The conservation of these habitats is of primary interest, but their geographical distribution and the ecological requirements of the structuring species have been only partially addressed. The yellow coral *Dendrophyllia cornigera* (Lamarck, 1816), for example, presents a NE Atlantic and Mediterranean distribution (Gori *et al.*, 2014; Castellan *et al.*, 2019), and it is considered a typical hard bottom habitat-forming species (Pérès and Picard, 1964; Zibrowius, 1980; Chimienti *et al.*, 2019). Several studies suggest that it displays wide adaptability to environmental features, including depth, temperature, and turbidity (Roberts *et al.*, 2006; Naumann *et al.*, 2013; Castellan *et al.*, 2019; Reynaud *et al.*, 2021). In addition, *D. cornigera* has been reported also on soft bottoms, indicating that its broad adaptability can be extended to its substrate preferences (Bo *et al.*, 2014; Michez *et al.*, 2014; Enrichetti *et al.*, 2019).
Aiming to better understand *D. cornigera* occurrence and ecological requirements, we present a comprehensive revision of the available information on its distribution and

ecology, with a specific focus on the different biocoenoses it participates in and the substrate type on which they develop.

Materials and methods

To investigate *D. cornigera* ecological requirements throughout its whole distribution range, a large dataset was created including information from i) an extensive bibliographic research and ii) 645 sites explored from 40 to 1800 m by means of ROV (Remotely Operated Vehicles) along the Italian coast.

Bibliographic material and ROV footages were analyzed searching for information regarding *D. cornigera* spatial and bathymetrical distribution, substrate type, inclination, and the biocoenosis of which is part. In addition, the ROV video was analyzed to obtain data about the structure of the populations of *D. cornigera* recorded in different situations.

Results

The final dataset accounted for 383 *D. cornigera* records, spanning from the Celtic Sea to Senegal and from the Azores Islands to the Aegean Sea. 83% of the records come from hard substrates, including outcropping and sub-outcropping rocks, coralligenous rocks, and dead *Lophelia/Madrepora* frameworks. Records on soft bottoms result common (17%) both in the NE Atlantic Ocean and the Mediterranean Sea (Fig. 1). Sloping substrates, with inclinations ranging between 20° and 70°, are the most common (55%), followed by horizontal (37%) and vertical substrates (8%).

Three main *Dendrophyllia* facies have been identified, each one characterized by a specific combination of substrate, inclination, and depth (Fig. 2).

Living colonies on hard bottoms (Fig. 2a) present a wide geographical and bathymetrical distribution (30-1200 m) and are a component of different biocoenoses. Density generally ranges from 0.6 to 2 colonies m^{-2}, and average size results in 9.2 ± 0.2 (SE) cm. Within these biocoenoses, *D. cornigera* is generally associated with sponges (e.g., *Pachastrella monilifera*), gorgonians (e.g., *Eunicella cavolini*), or black corals (e.g., *Antipathella subpinnata*).

Dense aggregations on sub-horizontal soft bottoms (Fig. 2b) have been reported only from mesophotic depths (90-140 m) in two sites of the Ligurian and Ionian seas, where density generally ranges from 11 to 36 colonies m^{-2}. Colonies' size is slightly reduced (8.3 ± 0.2 cm). Typically-associated fauna includes cerianthids, the sabellid *Myxicola* sp., and holothurians.

Dendrophyllia cornigera thanatocoenoses (Fig. 2c) result quite widespread on upper bathyal (140-800 m) rocks and soft bottoms. Dead branches are often intermixed with scattered living polyps and can form extended frameworks, greatly enhancing seafloor complexity. Associated fauna includes several bathyal sponges (e.g., *Hamacantha (Vomerula) falcula*), small gorgonians (e.g., *Bebryce mollis*), and brachiopods (*Megerlia truncata*).

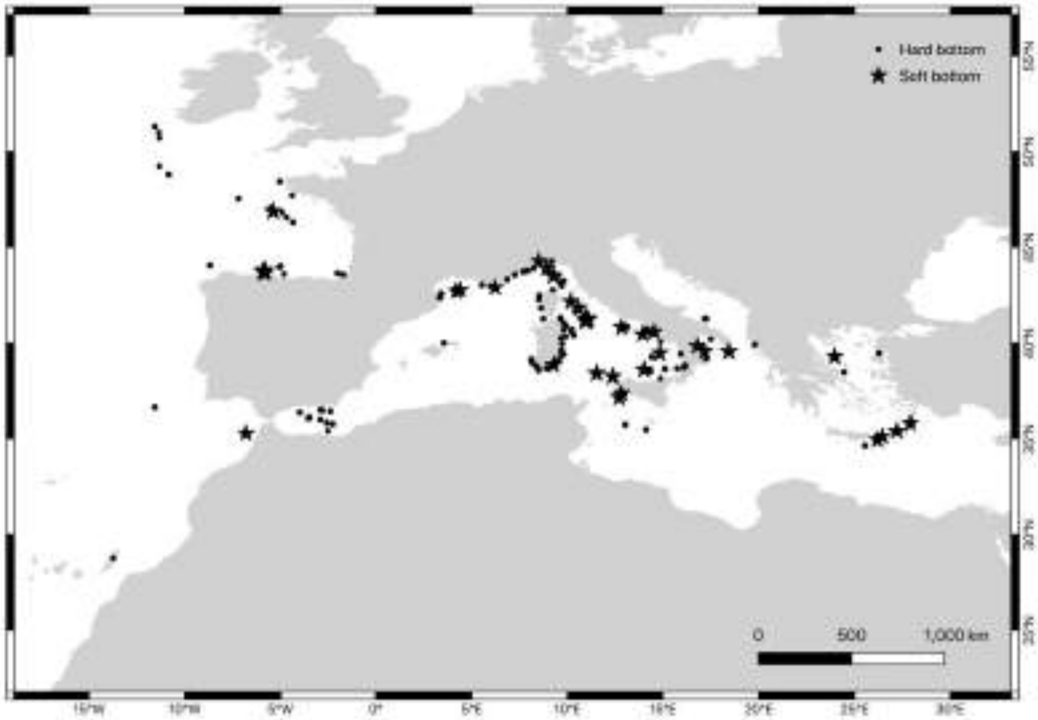

Fig. 1. ***Dendrophyllia cornigera* geographical distribution according to the substrate type**

Fig.2. Images of the three main biocoenoses created by *D. cornigera*: (a) scattered isolated colonies on hardgrounds (SW Sardinia), (b) dense meadow on soft bottom (Ligurian Sea), and (c) extended thanatocoenosis on upper bathyal soft bottoms (NW Sicily).

Discussion

Dendrophyllia cornigera presents wider adaptability if compared to other cold-water corals, especially regarding its broad bathymetrical distribution, and thermal and turbidity tolerance (Roberts *et al.*, 2006; Naumann *et al.*, 2013; Gori *et al.*, 2014; Castellan *et al.*, 2019; Reynaud *et al.*, 2021). The present study further enlarges the combination of environmental constraints suitable for this species, including substrate inclination (from horizontal to vertical) and substrate type (including hard and soft bottoms).

The majority of the records were reported from hardgrounds, according to the common view of *D. cornigera* as a typical hard bottom species (e.g., Chimienti *et al.*, 2019). Indeed, this species is included in various categories of the updated Barcelona Convention (BC) classification of marine benthic habitat types for the Mediterranean Sea (Montefalcone *et al.*, 2021), where it participates in different biocoenoses, including those

dominated by sponges, gorgonians, black corals, and deep-sea scleractinians (Bo *et al.*, 2012; Castellan *et al.*, 2019; Chimienti *et al.*, 2019; Enrichetti *et al.*, 2019).
The formation of soft bottom facies is probably linked to the ability of *D. cornigera* larvae to settle on small pebbles or shell fragments. Interestingly, the congeneric species *Dendrophyllia ramea* (Linnaeus, 1758) is also known for creating aggregations on soft bottoms (Orejas *et al.*, 2019), indicating that the affinity for this substrate could be widespread within the genus. The wide occurrence of *D. cornigera* on soft bottoms, with at least two high-density populations, supports the existence of a proper *Dendrophyllia* facies developing on this substrate, despite no specific category is available yet in the BC classification system (Montefalcone *et al.*, 2021). However, this classification presents a proper category for soft bottom *Dendrophyllia cornigera* thanatocoenoses, as a result of their wide occurrence in the offshore circalittoral and upper bathyal of the NE Atlantic Ocean and the Mediterranean Sea.

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MONITORING ITALIAN CIRCALITTORAL AND UPPER BATHYAL BIOGENIC REEFS WITHIN THE EUROPEAN MARINE STRATEGY FRAMEWORK DIRECTIVE (MSFD)

Abstract
Through the implementation of the European Marine Strategy Framework Directive (MSFD), Italy is monitoring coralligenous, off-shore rocky habitats and cold-water corals to evaluate the Good Environmental Status. This, because of the ecological importance and vulnerability of these habitats. The national monitoring is carried out by the national Agencies for the Environmental Protection, between 30m and 100m, and by ISPRA in deeper sites. This study presents the results of the monitoring carried out from 2020 to date for the deeper habitats by ISPRA in five Tyrrhenian and Ionian areas. The overall area covered by multibeam was 74 km^2. The ROV-imaging data analysis allowed to identify more than 100 megabenthic taxa per area. Collected information confirms the importance of monitoring to assess the MSFD measures efficacy and to define new conservation actions.

Key-words: MSFD, CWC, ROV-Imaging, marine litter, Mediterranean Sea

Introduction

The Mediterranean deep benthic habitats are increasingly threatened by growing human pressures, such as fishing, pollution, climate change, littering, oil and gas exploration and production, and the construction of marine infrastructure (Fanelli *et al*., 2021). For these reasons several international and European agreements and Directives provide the basis for the protection of deep-sea ecosystems. In addition, since 2008, the Marine Strategy Framework Directive (MSFD) requires the achievement of the Good Environmental Status (GES) of marine waters throughout Europe. To this end, in 2012, EU Member States completed an initial assessment of their marine waters, based on the available data, and defined targets and indicators for the following monitoring phases; the current one will end in 2026. The GES achievement of marine waters is assessed considering 11 descriptors. Descriptor 1 "Biodiversity" considers, as well as several other components, the environmental status of benthic habitats by combining it with the objectives and criteria of descriptors 6 (D6 - Seafloor integrity) and 10 (D10 - Marine litter). In this framework, Italy has identified coralligenous habitats, off-shore rocky habitats and Cold-Water Corals (CWCs) as important habitats to monitor due to their ecological importance and vulnerability to anthropogenic impacts. The national monitoring is carried out by the national Agencies for the Environmental Protection (ARPAs), in the 30-100 m depth range, and by ISPRA in deeper sites. The MSFD envisages an assessment approach per individual European sea, which in turn is subdivided into sub-regions. Specifically, Italian marine waters belong to three different subregions: the Western Mediterranean (MWE), the Adriatic Sea (MAD), the Ionian and the Central Mediterranean Sea (MIC). This paper presents the results of the monitoring of deep habitats in the range between 100 m and 500 m, applying a methodology fully in line with the Ecosystem Approach Process

(EcAp) of the UNEP/ MAP Barcelona Convention, by which the Contracting Parties committed to achieve the GES of the Mediterranean Sea.
The monitoring activities were carried out on assemblages characterized by habitat-forming species of ecological importance, living in conditions of low or no light and stable low temperatures (Chimienti *et al.*, 2018). These are mainly composed by anthozoan structuring species living both on coarse and hard bottoms, often inside canyon systems or in areas of high hydrodynamism. These species are one of the main components of Vulnerable Marine Ecosystems (VMEs), which are considered hotspots of biodiversity and ecosystem functioning in the deep sea (FAO, 2009). VMEs are also characterized by high vulnerability to mechanical disturbance and low recovery ability due to the fragility, uniqueness, structural complexity, and life history of their component species. Because of this, Italy defined a specific monitoring program of these habitats within the MSFD framework. VMEs, because of their limited accessibility, have long been poorly studied. Currently, the availability of innovative techniques that include the use of Remotely Operated Vehicles (ROVs), has enabled a significant increase in their knowledge, allowing accurate and quantitative descriptions of community composition and associated fauna with a non-invasive approach. Nowadays, data collection through ROVs is also complemented by other monitoring methodologies such as bycatch study of fisheries, photogrammetry technologies and habitat prediction. The use of ROVs was applied to monitoring activities of circalittoral and bathyal environments in areas known for the presence of animal forests and CWCs during the first Italian MSFD cycle. This paper describes the preliminary results of deep habitat monitoring activities from 2020 to present, with the aim of establishing a baseline assessment of the ecological status of the circalittoral animal forests and scleractinian CWCs for future MSFD monitoring activities.

Materials and methods

During the summer-autumn 2020 and 2021, five research campaigns were conducted on board the ISPRA research vessel *Astrea*. The study areas were selected because they were known to harbor important circalittoral animal forests and upper bathyal areas dominated by scleractinian CWCs (e.g., Taviani *et al*., 2019; Angeletti *et al*., 2020; Romagnoli *et al*., 2021; EU-ENPI Project). In particular, the monitoring activities were carried out considering three main different typologies of assemblages characterized by habitat-forming species: CWCs scleractinian reefs, black corals forests, and areas characterized by soft bottom with scattered rocky boulders. In August and September 2020, an offshore area located in the Corsica Channel (CC) and the Dohrn Canyon (DC) were studied for the presence of CWCs, while, in August-September 2021, the waters around the islands of Ponza (PO) and Linosa (LI) were studied focusing on black corals, and an area off Syracuse (SI) was explored to monitor the third habitat type. All areas belong to the MWE subregion, except LI and SI, which are part of the MIC subregion (Fig. 1).

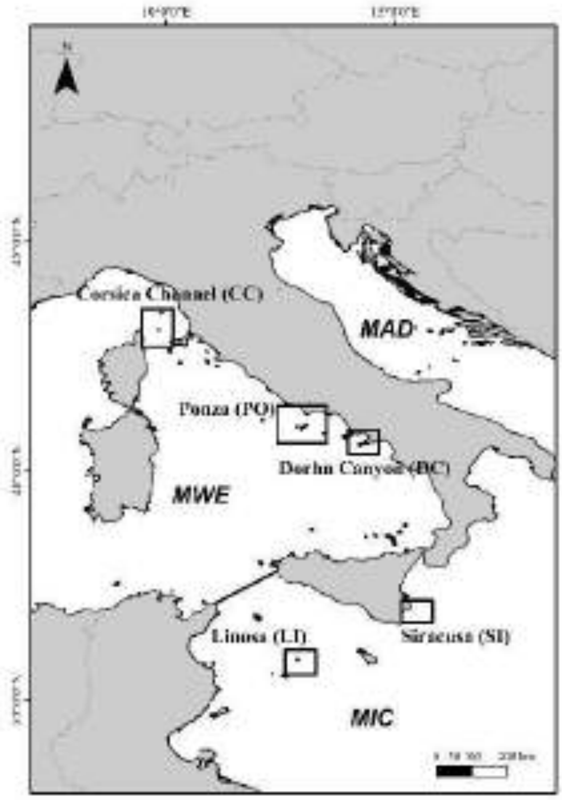

Fig. 1: Localization of the five investigated areas belonging to the two MSFD MWE and MIC subregions.

The offshore area CC is a narrow (10-35 km wide) and shallow (maximum depth of about 900 m) channel between Corsica Island and the Tuscan Archipelago (Angeletti *et al*., 2020). The PO study area is in the Pontine Archipelago and encompasses the waters around Ponza and Ventotene islands. This Archipelago comprises two groups of volcanic islands: Ventotene and Santo Stefano to the southeast and the three islands: Ponza, Palmarola and Zannone to the northwest (Barberi *et al*., 1967). The DC is located in the Gulf of Naples and, with its forked structure, extends perpendicularly to the coastline from a depth of about 250 m to a depth of about 1300 m, about 12 NM from the near shore (Taviani *et al*., 2019). The SI study area, situated at about 1.5 NM offshore the nearest coast, is in the southeastern part of the Sicilian continental margin and includes the so-called Apollo-bank, recently described by Angiolillo *et al*. (2022). The LI study area is located south of Sicily, between Malta and Tunisia; it belongs to the Pelagie Archipelago and encompasses the waters around the island. All the areas were investigated with a ROV (Perseo, L3 Calzoni) equipped with a Kongsberg high-definition video-camera, two laser beams 16 cm apart used as a metric scale, a robotic arm to take species samples and an ultrashort baseline (USBL) to acquire the ROV position every 2 seconds. The ROV was moving at a speed of c.a. 0.5 knots keeping a fixed distance from the seabed of c.a. 1.5 meters. When these requirements were not met such as, for example, during the ascent and descent phases of the ROV, when there was poor visibility, or when the ROV was stationary for sample collection or close-up video recording, the corresponding video frames were not considered in the analyses. When possible, high-resolution bathymetric data were collected using a hull-mounted Kongsberg EM2040 multibeam echo-sounder, to reconstruct very high-resolution digital elevation models

(DEMs) and backscatter maps of the seafloor, useful for identifying the potential presence of hard bottom to investigate with ROV. The data collected were analyzed to classify, to the lowest possible taxonomic level, all megafaunal taxa visible along each video transect using the free internet software VLC. Particular attention was paid in the identification of the structuring anthozoan species to determine coral abundance, size structure and condition. In addition, the marine litter observed was classified according to the MSFD Joint List (Fleet *et al*., 2021), giving an estimation of the impact caused to erect species (entanglement).

Results

A total of 46 dives were performed with ROV during the monitoring activities: 4 in CC, 11 in DC, 23 in PO, 6 in LI and 13 in SI. Overall, the activities conducted resulted in 85 hours of ROV surveys on the seafloor, covering a linear distance of 33 km, allowing to identify more than 100 megabenthic and demersal species.

Monitoring activities have confirmed the presence, in the bathyal areas of CC and DC, of significant assemblages of CWCs and associated species. In both areas, cnidarians were the most important contributor to the overall diversity (around 30%). In DC area, the two CWC scleractinian species, *Desmophyllum pertusum* and *Madrepora oculata*, were present throughout the bathymetric range explored (322–452 m) with average densities of 0.87–2.01 col. m^{-2} and 2.46–4.30 col. m^{-2}, respectively. There are numerous charismatic species associated with the CWC scleractinian *facies*, such as *D. dianthus*, which forms dense aggregations (up to 33 individuals m^{-2}) and *Javania cailleti* with 243 individuals m^{-2} along the NW wall of the canyon. Here, are also found the largest populations of *Neopycnodonte zibrowii* and *Acesta excavata* (up to 25 and 22 individuals m^{-2}, respectively). The only conspicuous and structuring species that is well distributed in DC is the black coral *Parantipathes larix*. In CC, CWC scleractinians formed mounds at 341–457 m with average densities of 0.44–2.16 colonies m^{-2} of *D. pertusum* and 1.18–4.78 colonies m^{-2} of *M. oculata*. Moreover, different assemblages of gorgonian species were observed: on the flank of the mounds mixed forests of *Viminella flagellum*, distributed in dense patches with up to 21 colonies m^{-2}, and *Callogorgia verticillata* were dominant, while on the top of the mounds, smaller colonies of *Paramuricea hirsuta*, *Villogorgia bebrycoides*, *Muriceides lepida*, and *Swiftia dubia* settled on the scleractinian coral bioconstruction. Noteworthy is the presence of colonies of *Isidella elongata* in the muddy areas around the mounds. Marine litter accounted for 138 items in the two study areas, mainly made of lost fishing gear. This caused entanglement events (32-68%) mainly on *M. oculata*, *D. pertusum, M. lepida* and *P. larix*. In the PO area, ROV observations were mainly conducted on rocky outcrops along the continental slope. The biological cover of the seabed was predominantly dominated by sponges in moderately sedimentary conditions and only partially by coralline algae. In almost all ROV dives, the black coral *P. larix* formed notably large forests. In this area, stretches of seabed characterized by *Dendrophyllia cornigera* and walls with *Corallium rubrum* were found. Also, of great interest were the lithistid sponges still poorly known in the Mediterranean Sea. Data collected in the LI area confirmed the presence of a rich benthic community dominated by black corals and small gorgonians (*Bebryce mollis* and *V. bebrycoides*), found between 115 m and 345 meters depth. Moreover, rich low-circalittoral coralligenous communities made of lush colonies of *Eunicella cavolini*, *Paramuricea clavata* and *Antipathella subpinnata* were observed. The SI area was characterized by the occurrence of vulnerable species typical of soft bottoms such as *Funiculina* *quadrangularis* and *Virgularia mirabilis*. In the area several trawl marks were observed. In the shallower and coastal site, known as “Apollo bank”, ROV dives, carried out between 70 and 80 meter depth on a soft bottom with scattered boulders, revealed a lush population of *Dendrophyllia ramea* and the presence of several colonies of *C. rubrum*. A total of 171 colonies of *D. ramea* were counted over an area of approximately 350 m^2, with a mean density of 0.17-0.88 col. m^{-2} (up to 8 col. m^{-2}). 97% of the observed colonies were under 40 cm in size and about 50% of the overall colonies showed single corallites. Moreover, a total of 30 litter items were counted highlighting that the dominant litter types (96.6%) were related to fishing activities, 26.6% of which was observed to entangle sessile invertebrates. Entanglement affected 44% of the largest colonies of *D. ramea* that, occasionally, presented epibiosis (Angiolillo *et al*. 2022).

Discussion and conclusions

High- resolution image data collected provide a preliminary assessment of the ecological status of circalittoral animal forests and scleractinian CWCs, showing the different effects of anthropogenic impact on the considered vulnerable habitats. The main pressure is marine litter (mainly lost fishing gear) and consequent entanglement of structuring species. Data collected confirmed the importance of CC and DC areas for the presence of scleractinian CWCs and allowed the collection of important data on these communities, discovering some new sites in very good health status. The considered target scleractinian CWC species are classified as endangered by the IUCN Red List and listed in Annex II of the SPA/BD Protocol (Barcelona Convention). Black corals (EN, NT, Annex II) and some habitat-forming gorgonians (CR, DD, Annex II), important components of these assemblages, confirming that the investigated areas fully meet the criteria to be classified as Vulnerable Marine Ecosystems (VMEs) (Morato *et al*., 2018). Moreover, both areas, host benthic species known to reach more than 500 years of age, such as *N. zibrowii, L. glaberrima* and *I. elongata* (Bo *et al*., 2015; Montero-Serra *et al*., 2018); in addition, the DC area is characterized by the presence of a biocenosis, unique to the Mediterranean, dominated by bivalves (Taviani *et al*., 2019). In all areas there is a high predisposition of the dominant species to create a three-dimensional habitat complex, both as a carbonate bioconstruction and as an animal forest. In addition to this, a high density of large habitat-formers was recorded in CC, probably due to the specific hydrodynamics of the area. Data collected in PO and LI areas confirmed the presence of benthic assemblages dominated by structuring species of great ecological value, harboring the typical antipatharians species of Mediterranean mesophotic zone. The extensive forests of the black coral *P. larix* in the PO area are confirmed (ENPI-EU, 2014; Ingrassia et al., 2016). Regarding the SI area, the presence of a large population of *D. ramea* is worthy of attention also because of the peculiarity of the co-presence of this species with *C. rubrum*. The activities conducted at the five areas made it possible to confirm the presence of habitats of special conservation interest and to increase the information available on their distribution, composition and vulnerability to certain human activities (i.e., marine litter and fishing activities). The information gathered makes it clear how important it is to urgently implement measures to protect deep-sea habitats through the creation of marine protected areas, starting with the CBD's requirement to protect 30% of the seas by 2030, and the European Biodiversity Strategy 2030, which calls for 10% of waters to be under strict protection by that date.

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BATHYAL MEGABENTHIC DIVERISTY ON THE AJACCIO AND VALINCO SUBMARINE CANYONS (WEST CORSICAN MARGIN)

Abstract
Deep-sea environments in the Mediterranean Sea have been widely degraded over the past decades and thus there is an urgent need to implement protection measures. Within European Union's Mediterranean waters vast extensions of the deep-sea ecologically relevant ecosystems have been protected by integrating them to the Natura 2000 network. Inside the French Exclusive Economic Zone, this network is currently expanding to offshore areas, beyond 12 nautical miles. This study is focused on the megabenthic biodiversity on a volcanic promontory on the south flank of the Ajaccio submarine canyon and a wall of the Valinco Canyon (SW Corsica), between 1690 and 2250 m depth. We addressed differences in organism abundance and megabenthic assemblage composition between substrates and characterized lebensspuren and benthic litter in these two sites. By means of a photogrammetric analysis, four megabenthic assemblages segregated by substrate were identified. Hard substrate assemblages were characterized by the presence of sponges, the squat lobster Munida tenuimana, polychaetes and gastropods while soft sediment assemblages were characterized by the elpidiid holothurian Penilpidia ludwigi and an unidentified sabellid Lebensspuren likely derived from the activity of epibenthic, infaunal and bathypelagic organisms. Litter was not abundant at these offshore sites. Litter at the Valinco site, had both a land-based and marine origin while the one at the Ajaccio site had a marine origin. This study provides new understanding on the ecology and diversity of Mediterranean deep-sea environments in the 2000 m bathymetric zone, stressing the persistent knowledge gap surrounding deep-sea escarpments in the basin.

Key-words: deep-sea, megabenthic assemblages, lebensspuren, marine litter, Mediterranean Sea

Introduction

The study of megabenthic assemblages in the Mediterranean Sea has been mainly focused on the study of three-dimensionally complex sessile suspension feeder communities (Bo *et al*., 2014; Mastrototaro *et al*., 2017; Santín *et al*., 2018). This research has mostly been developed from the lower circalittoral to the upper bathyal, with few studies venturing into the lower bathyal zone. Most studies conducted in lower bathyal Mediterranean environments were conducted by means of bottom contact gears on soft bottoms (Cartes, 1993; Sardà *et al*., 2004; Ramírez-Llodra, 2008) These gear's operational limitations on complex hard bottoms has greatly limited knowledge on megabenthic assemblages on lower bathyal canyon walls and seamounts.
Recently, the anthropogenic pressures on deep Mediterranean communities have substantially raised, including bottom trawling, marine pollution and exploration for hydrocarbons. Therefore, there is a pressing need to establishing baselines for future conservation and management plans, which are now lacking. Within EU Mediterranean waters, large areas of the continental shelf and slopes have been protected through their inclusion in the Natura 2000 network (EC 2020/96) and, currently, the Natura 2000

network has expanded to offshore areas (>12 nautical miles). Within the French exclusive economic zone, a new site known as the "Reefs of the Ajaccio Seamount and of the Valinco rocky outcrops" have been included. This site hosts rocky outcrops and coral thanatocoenoses associated to submarine canyons and volcanic promontories. This study's main goals are to assess the bathymetric distribution of megabenthic species, tackle standing stock variability among different substrates, characterize the composition of the megabenthic assemblages, as well as characterize and quantify lebensspuren and benthic litter in the area.

Material and Methods

This study's main target was the top and side of a volcanic promontory located at 2060 - 2250 m depth, 27 miles off Ajaccio. The second target was located on a steep wall of the Valinco canyon, between 1690 - 1990 m depth. Four ROV transects were carried out. Three transects were recorded on the volcanic promontory on the Ajaccio canyon and one single transect was recorded on a vertical wall of the Valinco canyon. The Hybrid Remotely Operated Vehicle (H-ROV) Ariane (Ifremer), specifically designed to perform georeferenced photographic surveys of the seafloor to develop 3D mosaics, was used. A total of 1041 HD (6000 x 4000 pixels) still images were recorded, covering a surface of 7714 m^2. The Agisoft PhotoScan professional edition (v.1.2.5) software was used to build 3D image reconstructions. The 3D reconstructions were used for fine-scale spatial positioning of all megafaunal species, lebensspuren formations and marine litter items. Megafauna was identified to the lowest possible taxonomic level, yet morphospecies categories were established since most organisms could not be identified to species. Lebensspuren formations were classified based on their potential origin and morphology (Bell *et al*., 2013). Litter items were classified based on their origin and the material they were made. For each 3D photogrammetric reconstruction, morphospecies abundances were standardized by dividing the abundances registered in a certain substrate patch by its surface resulting in a density value. A non-metric multidimensional scaling ordination (nMDS) was used to identify megabenthic assemblages. Adonis permutation multivariate analysis was used to test for significant differences between assemblages. The indicator value index (IndVal) was used to identify each assemblage representative morphospecies.

Results

A total of 4508 organisms were observed on the explored area. Twenty morphospecies were identified in the Ajaccio volcanic promontory. Porifera and Polychaeta were the most diverse and abundant groups, being represented by 4 and 5 morphospecies, which accounted for 89% and 8.9% of all observed organisms, respectively. Eleven morphospecies were identified in the Valinco Canyon wall. Polychaeta was the most diverse group the most abundant (59.3% of all observed morphospecies), followed by Echinodermata (31.6%).

Within the explored bathymetric range, morphospecies densitiesly ranged between 1 and 25 individual m^2. However, at 2030 to 2070 m depth Porifera disclosed an abrupt increment in abundance reaching values of 174 individuals m^{-2}, mainly corresponding to Porifera sp.1 patches. This phylum presented the narrowest bathymetric distribution, occurring between 2000 and 2190 m depth. Conversely, Polychaeta and Echinodermata showed the widest bathymetric distribution.

Overall morphospecies density was variable across the different substrates with average values ranging between 0.20±0.07 ind. m^{-2} (SD) on compacted muds to 47±18 ind. m^{-2} (SD) in serpulid thanatocoenosis.
The nMDS analysis and subsequent permutation multivariate allowed to identify four megabenthic assemblages differentiated by the substrate on which they occurred.
Bare rock assemblage: this assemblage occurred on oxide coated rocky substrates and to a lesser extent compacted mud. Twelve morphospecies formed this assemblage which was characterized by an unidentified Gastropod and an amphinomid Polychaeta.
Coral thanatocoenosis assemblage: this assemblage was composed of 8 morphospecies, six of which were sessile. It was characterized by the squat lobster *Munida tenuimana*.
Serpulid thanatocoenosis assemblage: this assemblage was formed by 5 morphospecies which were characterized by an unidentified incrusting sponge that represented 98% of all observed organisms achieving maximum densities of 180 patches m^{-2}.
Soft sediment assemblage: this assemblage was formed by 5 morphospecies. An unidentified sabellid and the holothurian *Penilpidia ludwigi* were the dominant ones, representing 48% and 37% of all observed organisms, respectively.
Five distinct lebensspuren categories were identified: crawl marks, decapod tracks, depressions, mounds, and pocks (small holes). In the Ajaccio promontory 226 lebensspuren formations were observed, pocks and mounds were the most abundant (48% and 23%, respectively). In the Valinco canyon 282 lebensspuren formations were found, mounds and crawl marks were the most abundant formations (67% and 18%, respectively). Lebensspuren densities were significantly higher in the Valinco canyon, except for pocks and decapod tracks.
Twelve litter items, belonging to 5 different categories, were observed on the Ajaccio promontory. Glass bottles, military and textile items were the most abundant categories (25% of all litter items). Six marine litter items, belonging three categories were observed, in the Valinco canyon. Plastic debris was the most abundant category (50% of observed items). Litter densities reached values of 0.001 items m^{-2} and 0.006 items m^{-2}, in the Ajaccio and Valinco areas respectively.

Discussion and conclusions

Our observations are in accordance with previous species richness values from Mediterranean lower bathyal environments (Ramírez-Llodra et al., 2008). Porifera and polychaeta were the most diverse and abundant groups, representing 87.4% and 9.7% of all observed organisms, respectively. This contrasts with earlier observations on Mediterranean megabenthic assemblages bound between 1200 m and 2200 m depth, where these groups are thought to be scarce (Cartes *et al*., 2009).
Anthozoans were rather scarce, being limited to two species and four observations. This contrasts with Mediterranean bathyal environments above 1500 m depth where anthozoans can account for a considerable proportion of megabenthic assemblages (Bo *et al*., 2014). Furthermore, rare morphospecies (≤ 3 records) significantly contributed to species richness, representing 33.3% and 66.6% of all morphospecies in Ajaccio and Valinco, respectively. This appears to be a shared feature of megabenthic assemblages on bathyal and abyssal environments in which rare taxa can make up for a large proportion of megabenthic assemblages (Durden *et al*., 2015).
Megabenthic species abundance varied across the explored bathymetric range, experiencing an abrupt increment between 2030 and 2075 m depth where highest densities were registered (180 encrusting sponge patches m^{-2}). Although it is entirely due

to the occurrence of the numerous patches of the peculiar Porifera sp.1, this pattern contrasts with the common conception that megabenthic organisms' density decreases with depth (Rex *et al*., 2006). However, in seamounts and canyons in other areas of the world similar situations have been observed (Santín et al., 2018).

Megabenthic assemblages were separated by substrate type. Three different megabenthic assemblages were found on hard substrates. Bare rock assemblages were found on both Fe-Mn oxide-coated rocks (95.6%) and compacted muds (4.4%).

Bare rock assemblages occurred on Fe-Mn oxide-coated rocks (96%) and compacted muds (4%), contrasting with previous studies on seamounts, which differentiated between assemblages occurring on polymetallic crusts from those occurring on other hard substrates (Schlacher *et al*., 2014). Furthermore, assemblages occurring on polymetallic crusts on Pacific seamounts presented lower densities than those occurring on other adjacent hard substrates (Schlacher *et al*., 2014). This differs from the present study as overall organism density on Fe-Mn coated rocks and compacted muds did not significantly differ. This assemblage was characterized by the presence of gastropods and polychaetes and several sponge species, but other groups characteristic of shallower Mediterranean Fe-Mn oxide coated environments such as bryozoans and bivalves were completely absent (Toscano & Raspini, 2005).

In the Mediterranean Sea, thanatocoenoses are omnipresent on hard substrate between 200 and 3500 m depth. Depending on the subfossil remains that form thanatocoenosis benthic assemblages may differ in terms of abundance and species composition (Rosso *et al*., 2010). This was the case of both benthic assemblages occurring on serpulid and coral thanatocoenoses in the study area. On solitary coral thanatocoenosis assemblages presented higher species richness than those occurring on serpulid thanatocoenosis and where characterized by *Munida tenuimana*. This squat lobster is a common inhabitant of bathyal environments where it has been observed to occur on both live and dead coral assemblages (Mastrototaro *et al*., 2010). Furthermore, it should be mentioned that 85% of organisms were sponges contrasting with previous reports on solitary coral thanatocoenosis where this group was quite scarce (Sartoretto & Zibrowius, 2018). Similarly, on serpulid thanatocoenosis, sponges were the most abundant group. This assemblage was dominated by Porifera sp.1, which represented 98% of all observed organisms forming dense aggregates that reached densities of 180 encrusting sponge patches m^{-2} (62 ± 58, mean ± SD). This may suggest that serpulid thanatocoenosis in deep bathyal environments provide a rather suitable substrate for this particular encrusting sponge.

Assemblages on soft sediments were represented by *Hymenodicus coronata*, *Bathypterois dubius* and *Plesionika acanthonotus,* typical inhabitants of Mediterranean bathyal muds (Cartes *et al*., 2009). This assemblage was characterized by an unidentified species of sabellid and the elpidiid holothurian *Penilpidia ludwigi*. This unknown sabellid was sparsely distributed, reaching maximum densities of 0.4 ind. m^{-2}, resembling previous observations on bathyal sediments in submarine canyons of the Catalan margin (Mamouridis *et al*., 2011). Lastly, *P. ludwigi* has been recently described to occur across the entire Mediterranean, covering a wide bathymetric range (480 – 4760 m depth) (Chimienti *et al*., 2019). This species have been described to form dense aggregates reaching densities of 300 ind. m^{-2} yet, in the study area it was quite scarce, reaching maximum densities of 2 ind m^{-2} (Chimienti *et al*., 2019).

In both sampling locations lebensspuren presented a similar composition but densities in Valinco were generally higher. Lebensspuren density is considered to result from a

combined effect of both the bioturbation capacity of a benthic community and physical processes that regulate lebensspuren residence time (Bell *et al*., 2013). Based on organic carbon flux patterns in submarine canyons it is likely that Valinco that covered a shallower bathymetric range is closer to the canyon head, thus receiving higher organic carbon fluxes which promotes lebensspuren formation (Smith *et al*., 2008). Lebensspuren likely derived from biogenic processes exerted by epibenthic (crawl marks), infaunal (pocks and mounds) and bathypelagic (depressions) organisms.
Marine litter was observed in all dives but in small quantities compared to previous studies in the Mediterranean Sea. Densities values agreed with previous observations on deep bathyal environments in the Mediterranean (Tubau *et al*., 2015). Litter composition in both sampling stations differed. In Ajaccio glass, textile and military debris were the most abundant components. Conversely, Valinco presented a more homogeneous composition with single use plastic items (plastic bags) and glass bottles (beer bottles) as the most abundant component, being in line with previous observations in Mediterranean bathyal environments (Cau *et al*., 2019).

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THE SEA CAVES OF THE MARINE PROTECTED AREA OF PORTOFINO (LIGURIAN SEA): LACK OF KNOWLEDGE AND MANAGEMENT

Abstract
Sea caves are priority habitats in need of protection according to the Habitat Directive of the European Union and to the Mediterranean Action Plan of the United Nations Environment Programme, and most Mediterranean Marine Protected Areas (MPAs) include sea caves. The rocky cliffs of the Portofino MPA (Ligurian Sea) host a number of sea caves, which are habitual destinations for recreational scuba divers. This notwithstanding, these caves have never been the subject of dedicated studies, and no specific management plans exist. The aims of this paper are to take stock of present knowledge, to report on the results of expeditious topographic surveys of some cavities, and to provide a first inventory of the most conspicuous species that inhabit them. The sea caves of Portofino MPA are essentially rockfall and/or marine erosion cavities, with few or no speleothems due to the geological nature of the rock (conglomerate). Some of Portofino sea caves reach the surface and include an emerged part; most of them, however, are completely submerged, and open at depths that can be grouped in three levels: 7-10 m, 20-25 m, and 33-38 m. All are rather small, their lengths being mostly comprised between 10 m and 20 m. Among the conspicuous species inhabiting the caves, Corallium rubrum, Homarus gammarus, Palinurus elephas, *and* Sciaena umbra *are of conservation interest. Dearth of knowledge, divers' safety and need of protection for habitats and species combine to claim for developing management plans for the sea caves of Portofino MPA.*

Key-words: Expeditious survey, *Corallium rubrum*, *Homarus gammarus*, *Palinurus elephas*, *Sciaena umbra*

Introduction

Mediterranean sea caves are ecosystems of great scientific interest (Bianchi *et al*., 1996) and represent major biodiversity hot spots (Gerovasileiou & Voultsiadou, 2014); yet, they are vulnerable to many anthropogenic threats (Rastorgueff *et al*., 2015; Montefalcone *et al*., 2018), and are therefore classified as in need of protection by the Habitat Directive of the European Union and the Mediterranean Action Plan of the United Nations Environment Programme (Giakoumi *et al*., 2013; Gerovasileiou & Bianchi, 2021). Most Mediterranean Marine Protected Areas (MPAs) include sea caves (Abdulla *et al*., 2008). The rocky cliffs of the Portofino MPA (Ligurian Sea) host a number of sea caves (Bavestrello *et al*., 2022), which are well known to recreational scuba divers but have never been the subject of topographic surveys and/or detailed biospeleological studies (Canessa *et al*., 2014). Consequently, no management plan (monitoring, access regulations, etc.) for Portofino sea caves is available.
This paper aims at: i) taking stock of present knowledge on Portofino sea caves; ii) reporting on the results of expeditious topographic surveys of some of the cavities; and

iii) providing a first inventory of the most conspicuous species that inhabit them, with special attention to those of conservation interest according to European Directives and other international agreements (Relini, 1999).

Materials and methods

Information on the sea caves of the Portofino MPA has been collated from the literature and direct field observations by scuba diving. Expeditious topographic surveys have been carried out in some of the caves, and schematic plans and sections are provided, in which depths are measured instrumentally (dive computer) while lengths and width have been estimated by eye, fin-strokes and/or arms stretch. The inventory of the most conspicuous species has been undertaken visually.

Results and Discussion

Some of the sea caves of Portofino reach the surface and include an emerged part; almost all of them are mentioned in the volume of Bixio (1987) and listed, without a cadastral number, in the census of Ferrari (2003). Most of the sea caves of the Promontory, however, are completely submerged or include a major submerged part, and are frequently visited by recreational divers. From west to east, they include (Tab. 1): the Shrimps Cave (Grotta dei Gamberi); the Dragon Tunnel (Tunnel del Dragone); the Tortonese Cave (Grotta Tortonese); the Armatum Cave (Grotta dell'Armato); the Marcante Cave (Grotta Marcante); the Ravioli Rock Caves (Grotte dello Scoglio del Raviolo) I and II; the Halocline Cavern (Caverna dell'Aloclino); the Saint George Church Cave (Grotta della Chiesa di San Giorgio); the Paraggi Castle Cave (Grotta del Castello di Paraggi); and the Paraggi Crib Grotto (Grottina del Presepe di Paraggi). Other smaller cavities, however, are widespread throughout the Promontory.

Tab. 1: Topographic features of the main submerged cavities of the Portofino Marine Protected Area, listed from west to east. Length and width measurements are approximate.

Name of the cavity	Depth (m)	Length (m)	Width (m)
Shrimps Cave	38-32	15	3
Dragon Tunnel	25-10	15	2
Tortonese Cave	10-0	20	3
Armatum Cave	24-20	10	1
Marcante Cave	36-31	10	5
Ravioli Rock Cave I	28	10	1.5
Ravioli Rock Cave II	35	10	2.5
Halocline Cavern	7-5	4	5
Saint George Church Cave	10	15	5
Paraggi Castle Cave	7-15	24	2
Paraggi Crib Grotto	20	2	2

The Shrimps Cave takes its name from the huge numbers of the unicorn striped shrimp *Plesionika narval* that find shelter in the dark part of this blind-ended cave (Fig. 1). The semi-dark portion of the cave is home to several individuals of the forkbeard *Phycis phycis*. The sponge *Axinella verrucosa* colonizes the rocky portions of the otherwise sandy pavement, while encrusting sponges (*Petrosia ficiformis* and others) and serpulid tubeworms are the main space occupiers of the walls. Schools of the cardinal fish *Apogon* *imberbis* occur through the cave. The cave also harbours juveniles of the European lobster *Homarus gammarus* and of the spiny lobster *Palinurus elephas*.

P. narval is also found in the two caves of Paraggi, located at the opposite end of the Promontory. The Paraggi Castle Cave is a blind-end cavity (Fig. 1), whose walls are colonized by massive sponges, ascidians and hydrozoans in a first zone after the entrance and then by encrusting sponges, while most of the cave has almost bare walls, with serpulids only. In addition to the shrimp, the motile fauna is represented by the common prawn *Palaemon serratus*, the brown meagre *Sciaena umbra* and the cardinal fish *A. imberbis* (Bianchi & Morri, 1994). Not far from this cavity is the small Paraggi Crib Grotto, with a pavement of fine sediment and the walls covered by massive sponges. The motile fauna includes, besides *P. narval*, the golden coral shrimp *Stenopus spinosus* and the leopard-spotted goby *Thorogobius ephippiatus*. The squid *Loligo vulgaris* visits the grotto, as evidenced by the presence of the typical ootheca hanging from the ceiling (Bianchi & Morri, 1994).

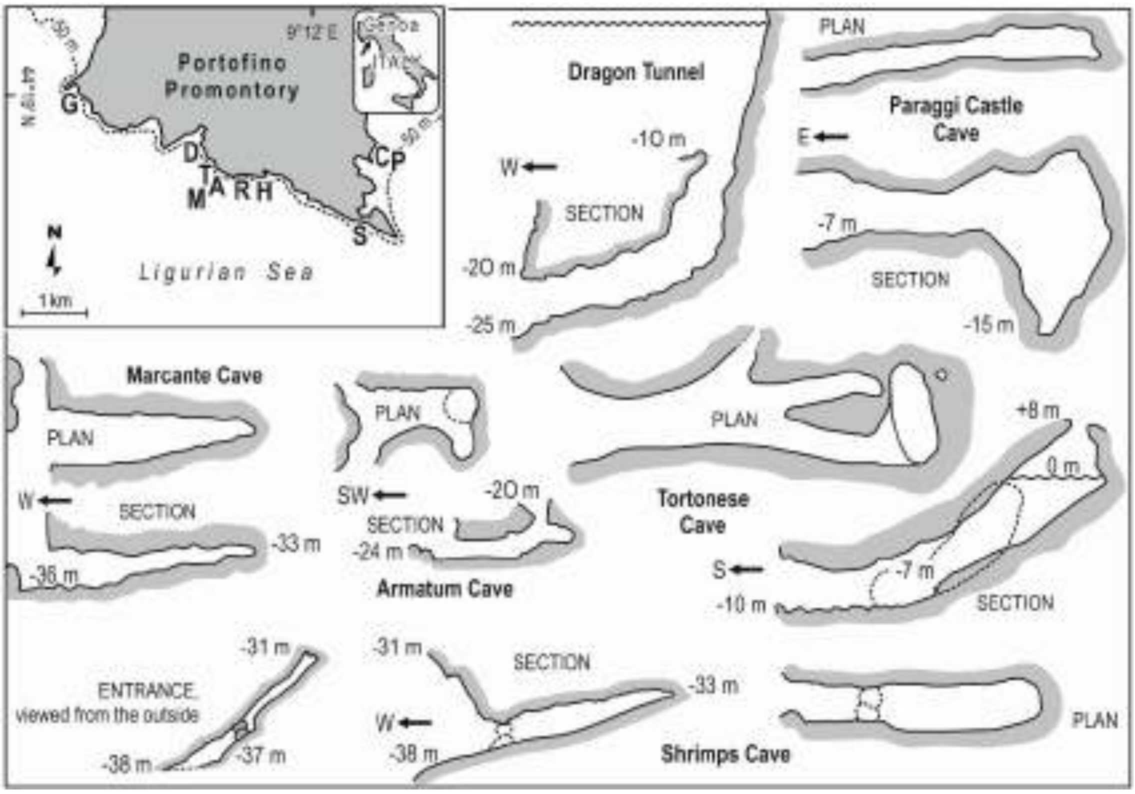

Fig. 1: Location of the main sea caves of the Portofino Promontory, and expeditious survey schemes of some of them (horizontal scale approximate). A) Armatum Cave; C) Paraggi Castle Cave; D) Dragon Tunnel; G) Shrimps Cave; H) Halocline Cavern; M) Marcante Cave; P) Paraggi Crib Grotto; R) Ravioli Rock Caves (I and II); S) Saint George Church Cave; T) Tortonese Cave.

A major system of cavities is represented by the three Dovecot Caves (Grotte delle Colombara), located at different depths on the same rock spur. The shallowest is the Tortonese Cave (Fig. 1), named in honour of Enrico Tortonese (1911-1987), who first cited it in a study on the benthos of the region (Tortonese, 1961). The yellow cluster anemone *Parazoanthus axinellae* and the calcareous sponge *Ascandra contorta* abound

at the entrance, while the ceiling is colonized by the sunset cup coral *Leptopsammia pruvoti*. In the inner part, the rock is almost bare, apart from scattered encrusting sponges. *H. gammarus*, *S. spinosus* and *A. imberbis* are the commonest motile species. The eastern branch of the cave surfaces to an inner 'lake'. The emerged walls of the lake show some modest speleothems due to the percolation of meteoric water and the vault has a window through which the sky and the terrestrial vegetation can be seen. The submerged walls are colonized only by serpulids, while the midlittoral rock of the lake is encrusted by the barnacle *Microeuraphia depressa*; *P. serratus* swims in the water. The Armatum Cave occurs deeper: is a tunnel-shaped passage between boulders (Fig. 1). The entrance arch is characterized by the abundance of the hydrozoan *Eudendrium armatum*, from which the cave takes its name. The moray eel *Muraena helena*, attended by the cleaner shrimp *Lysmata seticaudata*, may be encountered. *L. pruvoti* colonizes the ceiling, while the walls are populated by *Agelas oroides*, *P. ficiformis* and other massive or encrusting sponges; a small chamber on the eastern side of the tunnel has bare rock with scattered serpulids. The deep Marcante Cave was first mentioned by Tortonese (1958), which illustrated its location thanks to the indications of the diver Duilio Marcante (1914-1985). It is a wedge-shaped cavity (Fig. 1), which houses a rich population of the red coral *Corallium rubrum* on the ceiling (Cattaneo-Vietti *et al*., 1993; Bavestrello *et al*., 1994), together with *L. pruvoti* and *P. ficiformis*; the terminal portion of the cave has almost completely bare rock, with few serpulids.
C. rubrum also occurs on the ceiling of the Dragon Tunnel (Fig. 1), which is however mostly characterized by the scleractinians *L. pruvoti*, *Madracis pharensis* and *Polycyathus muellerae*; the sponge *P. ficiformis* is also abundant. *P. phycis* takes refuge inside the tunnel.
The Halocline Cavern is a large and low blind-ended opening in the rocky cliff, which has not been surveyed yet. The biota is poor overall: the pavement is colonized by *Chondrosia reniformis* and *P. ficiformis*, the ceiling by *L. pruvoti*, the walls by *Spirastrella cunctatrix* and *Protula tubularia*. In the terminal part, the percolation of meteoric water through the cracks of the rocky cliff creates a freshwater lens floating on seawater, with a permanent halocline easily perceived by divers.
Neither topographical surveys nor faunal inventories are available for the remaining sea caves of the Portofino MPA.

Concluding remarks

Combining literature data and field observations, it has been possible to provide preliminary data on eleven main sea caves of the Portofino MPA; information on the biota inhabiting eight of them has been collated, and expeditious topographic surveys have been provided for six of them.
These caves are essentially landslide and/or marine erosion cavities, with rare or absent speleothems due to the geological nature of the rock (puddinga). The major sea caves in Liguria are located in karst areas, and originate from the marine entry into pre-existing terrestrial cavities (Canessa *et al*., 2014).
Only a few caves reach the surface and include an emerged part; most are completely submerged, and open at depths that can be grouped into three levels: 7-10 m, 20-25 m, and 33-38 m, which may represent the outcomes of sea-level stands during the Holocene transgression.
All are quite small, mostly between 10 m and 20 m in length and only few meters wide. The small size can represent a problem for diving visitors, who may remain entrapped,

especially in presence of undertow. There have been cases of rescue intervention (three people in Tortonese Cave), and unfortunately fatalities occurred among recreational divers (four people in Saint George Church Cave).
Among the conspicuous species that inhabit the sea caves of Portofino, the red coral *Corallium rubrum*, the European lobster *Homarus gammarus*, the spiny lobster *Palinurus elephas*, and the brown meagre *Sciaena umbra* are of conservation importance, in accordance with EU directives and international agreements (Relini, 1999; Ouerghi *et al*., 2019). This short list is surely far from complete.
Dearth of knowledge, divers' safety, and need of protection for habitats and species combine to urge the development of management plans for the sea caves of the Portofino MPA. Diving centres should be provided with maps of the caves and diving guides and instructors should be informed about the vulnerability of the cave habitat and species to visitation by divers.

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EFFECTIVE MANAGEMENT MEASURES TO REDUCE THE BYCATCH OF COLD-WATER GORGONIANS AND SUPPORT SUSTAINABLE ARTISANAL FISHING ON THE MEDITERRANEAN CONTINENTAL SHELF

Abstract
Cold-water coral (CWC) gardens, mainly composed of gorgonians, soft corals and black corals, support a highly diversified associated mobile fauna, representing a key benthic habitat on the Mediterranean continental shelf. These communities are vulnerable to bottom-contact fishing gears, including trawling and small-scale fishing. In the frame of a collaboration with local fishers, the present study aims to identify the most effective local management measures to significantly reduce impacts while supporting sustainable fishing activity on the continental shelf of Cap de Creus (North-Western Mediterranean Sea). The number and size of gorgonian colonies accidentally captured by trammel nets were quantified, being most of them large colonies. The mean size of bycatch gorgonians was significantly higher. Thus, bycatch effects could lead to changes in population structure and overall ecosystem functioning. However, most bycatch gorgonians came from specific areas, between a specific bathymetric range and were accidentally fished during long soaking time and with high waves conditions. Consequently, avoiding fishing in a restricted selected area when weather conditions are rough may help to significantly reduce the gorgonian bycatch, promoting the conservation of these CWC gardens.

Key-words: Cold-water coral gardens, fishing impact, continental shelf, conservation, artisanal fishing

Introduction
Fishing activity is a serious and worldwide threat to marine ecosystems and their living resources (Roberts, 2002; Morato *et al.,* 2006), severely affecting continental shelves (50-200 m depth) (Halpern *et al.,* 2008). In those environments, under strong currents and food supply, cold-water coral (CWC) species provide reproductive, nursery and feeding grounds for highly diversified associated fauna (Freiwald & Roberts, 2005; Roberts *et al.,* 2009), several of them of commercial interest (Henry & Roberts, 2007). CWC species, encompassing reef-forming and garden-forming species (ICES & Hall-Spencer, 2007; Roberts *et al.,* 2009), are classified as Vulnerable Marine Ecosystems (VEMs) (FAO, 2009). They are species with long lifespan, slow growth, late sexual maturity, and low recruitment rates (Andrews *et al.,* 2002; Orejas *et al.,* 2011), with low recovery capability. Thus, impacts on theirs populations can have severe consequences, also for all the associated fauna they support (Williams *et al.,* 2010; Huvenne *et al.,* 2016).

Habitat destruction, mortality and removing of non-target species, and changes in ecosystem structure and functioning are ones of the impacts described, mainly cause by bottom trawling (Hiddink *et al.,* 2006; Pusceddu *et al.,* 2014). This is the most destructive fishing gear worldwide, however artisanal fishing also interacts with the benthic ecosystem, with less severe effects but with potential important consequences (Chuenpagdee *et al.,* 2003). Hence, the aim of this study is to assess the impact of artisanal trammel net fishing on CWC gardens located on the continental shelf of Cap de Creus, by analyzing the bycatch of gorgonian colonies, to recommend specific management measures to improve the conservation of CWC gardens and their associated biodiversity.

Material and methods

The study area is located on the continental shelf (60–130 m depth) on the north and east of the Cap de Creus peninsula (*North-Western Mediterranean Sea;* 42° 19′ 12″ N; 003° – 19′ 34″ E; Fig. 1). The area is recognized as one of the most productive regions among the Mediterranean Sea (Orejas *et al.,* 2009; Gili *et al.,* 2011). Benthic habitats on the continental shelf are mainly composed by sponges, gorgonians, corals and sea pens, forming CWC gardens (Gili *et al.,* 2011; Dominguez-Carrió, 2018). Due the rich and productive community associated to CWC gardens, the marine area of Cap de Creus has been traditionally exploited by the professional fishing fleet (Gómez *et al.,* 2006; Lloret & Riera, 2008). However, due to their vulnerability and high ecological value, the area is now recognized under two protection figures: the Natural Park of Cap de Creus, and the South-West Gulf of Lions Canyons System Special Area of Conservation (Fig. 1). Trawling fishing is the main fishing activity in the Special Area of Conservation, but it is forbidden inside the Natural Park, where artisanal fishing coexists with other recreational activities as diving and boating. Trammel nets, longlines and gillnets dominate the artisanal fishery in the study area (Purroy *et al.,* 2014).

During all fishing season (from April to August) of 2018 and 2019, the impact of artisanal fishing on gorgonian populations in the study area was evaluated by quantifying bycatch of the gorgonian *Eunicella cavolini* (Koch, 1887) from bottom trammel nets (107 nets in total). For each fishing event, location, depth, net length, height, material, mesh size, soaking time, and target species were recorded.

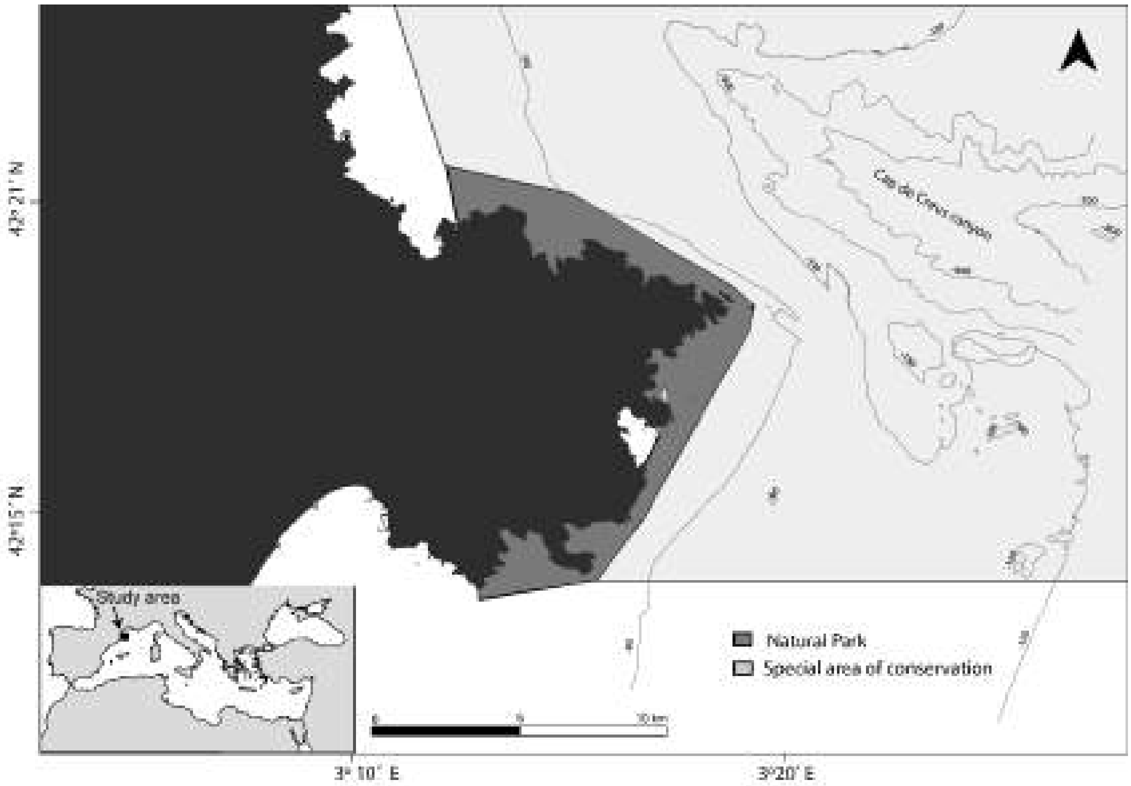

Fig. 1. Map of the study area showing boundaries of protection areas (UTM31 WSG84)

Results

Overall, around 360 *E. cavolini* bycatch colonies, including fragments and entire colonies, were recorded in 107 trammel nets evaluated.

The nets most frequently employed were 200 m long of nylon material and with an inner mesh size of 3–3.5 cm. On average, trammel nets had a soaking time of 48h and the gorgonian bycatch significantly increased when soaking time exceeded three days. Most of *E. cavolini* catches occurred in the north of the Cap de Creus peninsula, specifically from 50 m to 120 m depth, with most catches concentrated at 70–100 m depth, and from June to August.

Discussion and conclusions

Gorgonian bycatch in the study area is concentrated in the northeast area of the Cap de Creus peninsula, between 70 and 100 m depth. More specifically, although artisanal fishing effort was primarily concentrated at shallower depth, it is at 90–100 m depth where it had the most impact, since gorgonian bycatch is high despite a much lower fishing effort. This area is highly exposed to wave action and strong currents, and consequently is mainly fished in the last three months of the fishing seasons (June to August) when environmental conditions are good. However, when weather is rough, trammel nets are likely to move on the sea floor, increasing the probability of entanglement with gorgonians (Batista *et al.,* 2009). When trammel nets soaking time are long, gorgonian bycatch increases.

The present study reveals that trammel net fishing in the continental shelf of Cap de Creus affects *E. cavolini* populations, however with significantly less impact to benthic ecosystems than bottom trawling fishing (Pham *et al.,* 2014; Catanese *et al.,* 2018).
Trammel nets primarily caught medium and large colonies. The size structure of bycatch colonies was clearly biased toward larger colonies, confirming that nets remove more easily larger and morphological complex colonies (Pham *et al.,* 2014). By reducing the abundance of large colonies, it could have consequences on ecosystem functioning, limiting the role of coral gardens as a refuge, feeding and breeding area for associated fauna (Enrichetti *et al*., 2019). It could also have negative consequences on the long-term survival of the population since reproductive success could be compromised (Coma & Lasker, 1997). Most bycatch gorgonians were alive and entire colonies, which evidences that colonies are easily removed by trammel nets without causing *in situ* mechanical damage.
From a management perspective, reduce soaking times, avoid adverse weather conditions, fishing shallower, avoiding critical range depth and specific locations with known high densities of gorgonians would significantly reduce gorgonian bycatch in the area. Results of this study evidences the importance to manage small-scale fisheries efficiently by implementing specific measure for this study area.

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

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.

Key-words: Cold Water Corals, MaxEnt, species distribution modelling, habitat suitability

Introduction
Cold Water Corals (CWCs), are cold-affinity azooxanthellate cnidarians and bioengineering species able to form three dimensional structures (Chimienti *et al*., 2019). These species have a wide bathymetric distribution, generally deeper than 50 m depth, and are able to live both on hard and soft substrata. (Angiolillo *et al.*, 2018; Chimienti *et al*., 2019). They are also sensitive to environmental modification and anthropogenic pressure (Sundahl *et al*., 2020). Therefore, CWC communities are categorized as Vulnerable Marine Ecosystems (VEMs). These habitats play a key ecological role for a variety of fish species and are also classified as Essential Fish Habitats (EFHs) (D'Onghia 2019). Understanding the spatial distribution of CWCs ecosystems is the best means of applying the correct management measures. Habitat Suitability Models (HSMs) are statistical models that predict the distribution of generally suitable habitat ranges for species in relation to significant environmental variables and are considered a useful tool for managing marine ecosystems (Anderson *et al*., 2016). The aim of the present work is to describe the principal environmental and anthropogenic variables that play a role in shaping the distribution of six sensible CWC species belonging to three different orders: Scleractinia (*Madrepora oculata, Desmophyllum pertusum* and *Dendrophyllia*

cornigera), Antipatharia (*Leiopathes glaberrima*) and Alcyonacea (*Callogorgia verticillata* and *Isidella elongata*).

Materials and methods

The study area of 1650 km^2 is located in the Northern part of Strait of Sicily (38°0,587'N; 11°19,329'E), between 130 and 1000 m of depth. During the survey (conducted from August to November 2021), seafloor morphology has been acquired by a Multibeam echosounder Kongsberg EM2040 for shallow water and Kongsberg EM710-MK2 for deeper water. Video survey was carried out using a Tomawok ROV Light Work Class equipped with a high definition (6K camera) and laser beams. A total of 140 transects were carried out for a total of 129,507 km in length. The georeferenced presence data of the six species was derived from video analysis (*M. oculata* n=4342, *D. pertusum* n=582, *D. cornigera* n=192, *L. glaberrima* n=375, *C. verticillata* n=3221, *I. elongata* n=10378). Oceanographic parameters were collected in 97 stations using a Rosette and CTD (Sea Bird Scientific SBE 911 Plus V2). Ten environmental variables subdivided in: morpho-bathymetrics (depth, slope, roughness, aspect, flowdir), oceanographic (temperature, salinity, current North, current East) and AIS fishing effort (Astra paging Ltd) were considered in the models. Habitat suitability maps for the six species of CWCs were performed using Maximum Entropy modeling (MaxEnt), a modeling approach used to identify probability distribution with the highest level of entropy when only species presence data are provided (Etnoyer *et al*., 2017). The MaxEnt model was trained using batch files that allowed us to generate multiple models. Model generation has been automated with command-line arguments, avoiding the need to click and repeatedly type the program interface (Philips, 2005). For each species were 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. This is a key parameter because the higher the value, the more widespread and less localized the forecast will be (Philips, 2005), reducing the overfitting of the data (Bargain *et al*., 2018). Jackknife test was used to highlight the percentage of the contribution of each variable contributed to the final MaxEnt habitat suitability model. The accuracy of prediction of the models was calculated by Receiver Operating Characteristics (ROC) analysis through comparison of the Area Under the ROC Curves (AUC). The AUC value is related to the reliability of the model with respect to 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 value of 1 indicates an ideal model result (Bargain *et al*., 2017). The obtained probability maps of CWC distribution have been processed in R software to visualize the probability of presence in the study area.

Results

Mean AUC values of MaxEnt model for *I. elongata* and *M. oculata* are 0.744 and 0.879, respectively. For the other species this value is more than 0.90. For all species, slope represents the most important predictive variable that explains coral distribution, which is more than 60% with the exception of *I. elongata*. All the species increase linearly their preference until a slope between 20-30° reaching a plateau. *M. oculata* and *C. verticillata* show an optimum range of slope between 20-50° then their preference decrease (Figure 1). *I. elongata* is the only species with a 60% contribution represented by three different variables: slope (26.1%), chlorophyll-*a* (25.1%) and bathymetry (13.5%) (Figure 1). This species shows high preference of slope from 20°, an optimum bathymetry range between

450 and 700 m depth and highest preference for chlorophyll-*a* around the value of 0.077 mg m^{-3}. Habitat suitability maps (Figure 2) for *M. oculata* (South and South-West) and *L. glaberrima* (North, West and South-West) show the presence of small patches in delimitated geographical areas. *D. pertusum*, *D. cornigera*, and *C. verticillata* are more randomly distributed, while *I. elongata* has a suitable habitat extended throughout the study area, with a higher probability in the South-East area.

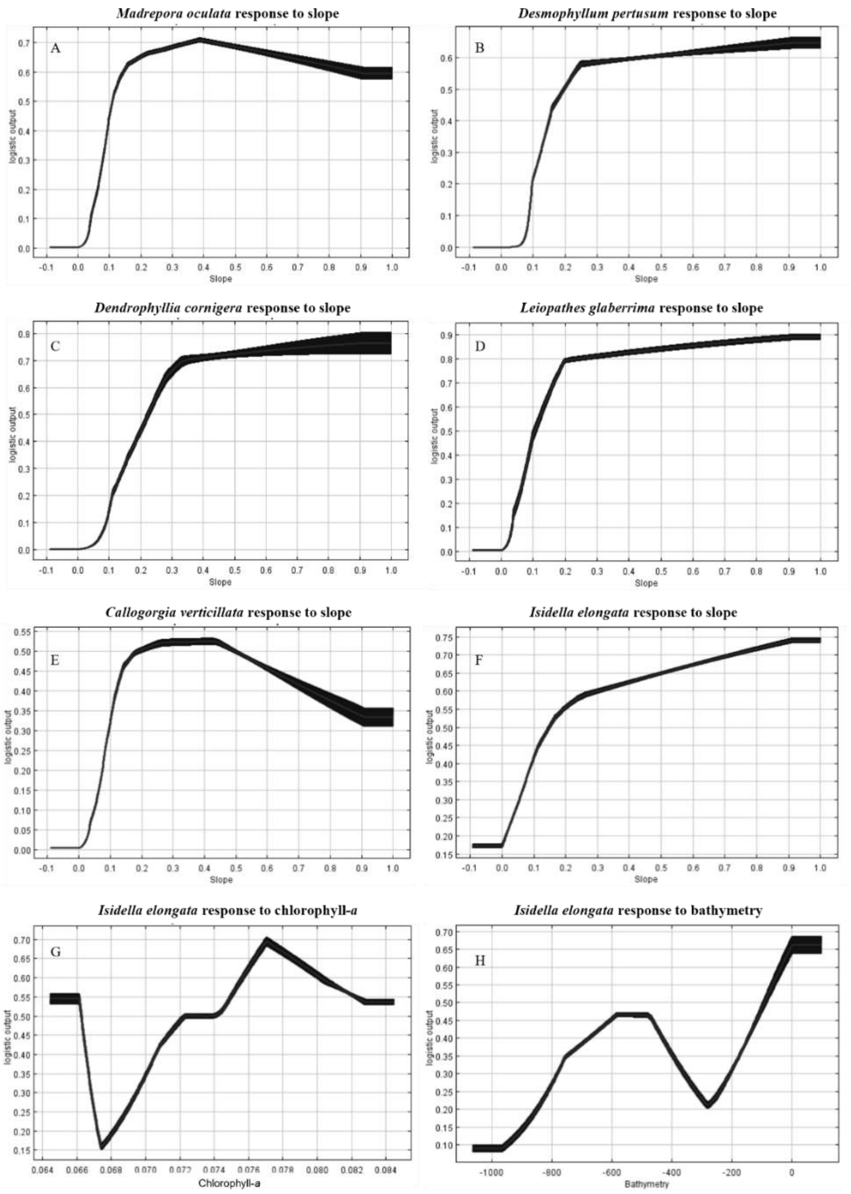

Fig 1: Individual response curve of the main predictive environmental variables for *Madrepora oculata* (A), *Desmophyllum pertusum* (B), *Dendrophyllia cornigera* (C), *Leiopathes glaberrima* (D), *Callogorgia verticillata* (E) and *Isidella elongata* (F-H).

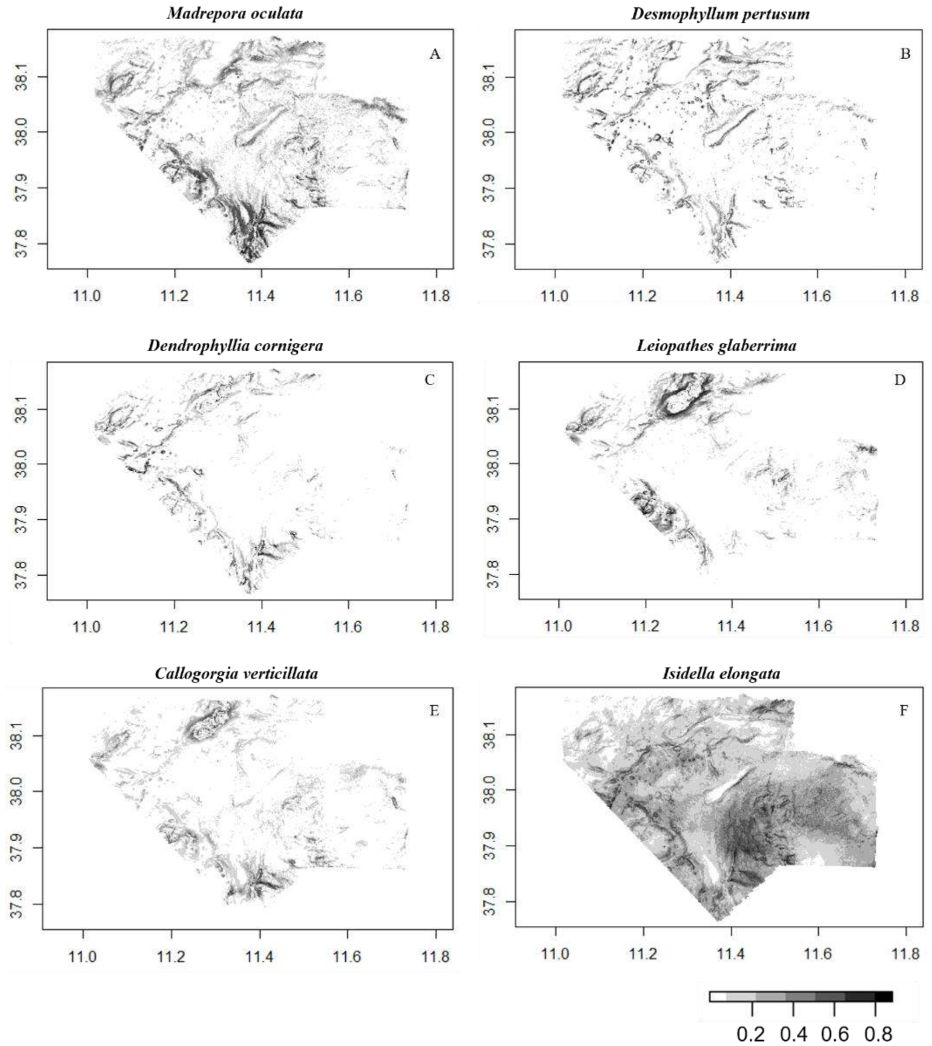

Fig 2: Habitat suitability map for *Madrepora oculata* (A), *Desmophyllum pertusum* (B), *Dendrophyllia cornigera* (C), *Leiopathes glaberrima* (D), *Callogorgia verticillata* (E) and *Isidella elongata* (F) on the Northern part of the strait of Sicily using MaxEnt model.

Discussions

The protection and management of VMEs and VME indicator taxa need detailed spatial data. These data could be difficult to obtain or collect in some challenging and large areas. Therefore, the prediction of their distribution using suitable habitat models is becoming a useful tool for decision makers (Kinlet *et al*., 2022). Identifying the probable species occurrence over wide areas is fundamental for marine spatial planning. Within this study, habitat-modelling techniques were used to create predictive maps of six CWC species of conservation concern in the study area. The present study had the possibility to use a large observation and detailed bathymetry dataset that is fundamental to produce robust habitat suitability models (AUC > 0.7) and consequently useful predictions of VMEs occurrence (Anderson *et al*., 2016).

The MaxEnt variable response curves gave us a picture of how the variables influenced the potential suitability of the species reported in the maps. The slope was the variable that most significantly influenced the distribution of all the analyzed species, with a positive linear relationship. This variable provides information about the topography of the bottom, and increasing slope value is related to an increase in terrain complexity (Bargain *et al*., 2018). In general, high slope values are associated with steeply areas, whereas low values are associated with flat areas (Wilson *et al*., 2007). All the species prefer step slope (> 25°) except for *C. verticillata* that decreases its preference for slope > 50°. Usually, CWC species show a preference for high terrain complexity (Pearman *et al*., 2020). As reported by Angiolillo *et al*. (2021), for example, *M. oculata* and *D. pertusum* prefer vertical walls slope that correspond usually to areas exposed to high water circulation and low sedimentation rates. From our model, these species are influenced by bathymetry variable preferring a range between 450 and 700 m. Indeed, the bathymetric range of occurrence for these species is between 500 and 1650 m depth (Chimienti *et al*., 2019). In the Mediterranean Sea, *I. elongata* forests are EFHs for red shrimps *Aristeus antennatus* and *Aristaeomorpha foliacea*, and are considered extremely vulnerable to trawling, which has caused a population decline in the Mediterranean Sea (Pierdomenico *et al*., 2018). The new valuable information available from the present study will be useful for the management of fishing activity and for the possible selection of new Fishery Restricted Areas (FRAs) aiming at the conservation and restoration of the area.

Conclusions

The habitat suitability modeling information presented in this study is a key step to better understanding the distribution of CWCs in the Northern part of the Strait of Sicily. Furthermore, it provides essential information to guide future surveys and conservation plans.

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LITHISTID SPONGES FROM SUBMARINE CAVES OF CRETE ISLAND: HIDDEN DIVERSITY AND FALSE ENDEMICITY

Abstract
Recent exploration in marine caves of Crete (Greece, Eastern Mediterranean Sea) brought to light several taxa of lithistid sponges, some of them important volumetrically. Further examination of sponge samples from already investigated and newly explored caves of Crete Island revealed the presence of four lithistid taxa: Neophrissospongia endoumensis, Microscleroderma lamina, Gastrophanella phoeniciensis and numerous specimens of an undescribed phymaraphiniid reported for the first time in the Mediterranean Sea. The presence of four different species of lithistid sponges in five marine caves of Crete, including taxa known only from the Western Mediterranean (or even Atlantic Ocean) and Lebanon contradicts current ideas about their diversity, distribution and origin. Through the current study it is shown that targeted sampling and taxonomic identification based on spicules can overcome misidentifications, underestimations of diversity and false patterns of endemism.

Key-words: Crete, Eastern Mediterranean, lithistid demosponges, biodiversity, taxonomy

Introduction
Although rock sponges (also known as lithistids or desma-bearing demosponges) are mostly known from the deep sea, they have been reported in shallow waters around the globe only from marine caves (Schuster *et al*., 2021 and references therein), especially in the northern and eastern Mediterranean Sea (Perez *et al*., 2004; Pisera & Vacelet, 2011; Pisera & Gerovasileiou, 2021). In most cases, particular caves harbour a single species that is usually unknown from other caves or habitat types. Numerous lithistids that belong to the species *Neophrissospongia endoumensis* (family Corallistidae) were recently reported to form large masses in marine caves of Crete (Greece), associated with freshwater influx (Pisera & Gerovasileiou, 2021). Further studies of these caves and more detailed scrutiny of already known specimens revealed higher lithistid diversity and past misidentifications.

Materials and methods
During summers of 2016, 2017 and 2021 several marine caves were surveyed with SCUBA diving on the south and northwest coast of Crete (Greece, Eastern Mediterranean). Lithistids were found and collected from three semi-submerged and two submerged caves with internal air chambers and freshwater springs at their innermost dark part. The depth of the studied caves ranged between 0 and 15 meters.
Sponge specimens were studied under binocular stereomicroscope in reflected light in order to choose representative areas not overgrown by other sponges, for further SEM analysis. Selected fragments were boiled in concentrated nitric acid for organic tissue removal and to obtain clean siliceous spicules. Loose spicules and fragments of choanosomal skeleton were studied under SEM.

Results

Neophrissospongia endoumensis was the most common and volumetrically important lithistid sponge in the studied caves (Fig. 1D, 2A). This sponge belongs to family Corallistidae and is characterized, among others, by the presence of strongly sculptured desmas known as dicranoclones (see Pisera & Gerovasileiou, 2021, and Fig. 2A herein). The second most common lithistid was a new taxon of the family Phymaraphiniidae (Fig. 1E), which had characteristic trider desmas (Fig. 2B), phyllotriaenes as ectosomal spicules, spinose microrhabds and amphiasters as microscleres (not shown in Fig. 2B). This sponge was known for a long time from those caves, but it was misidentified as discoloured *Petrosia* sp. based solely on underwater photos, due to its similar shape, size and colour inside dark caves. Recent studies of spicules in SEM, revealed its real taxonomic position.

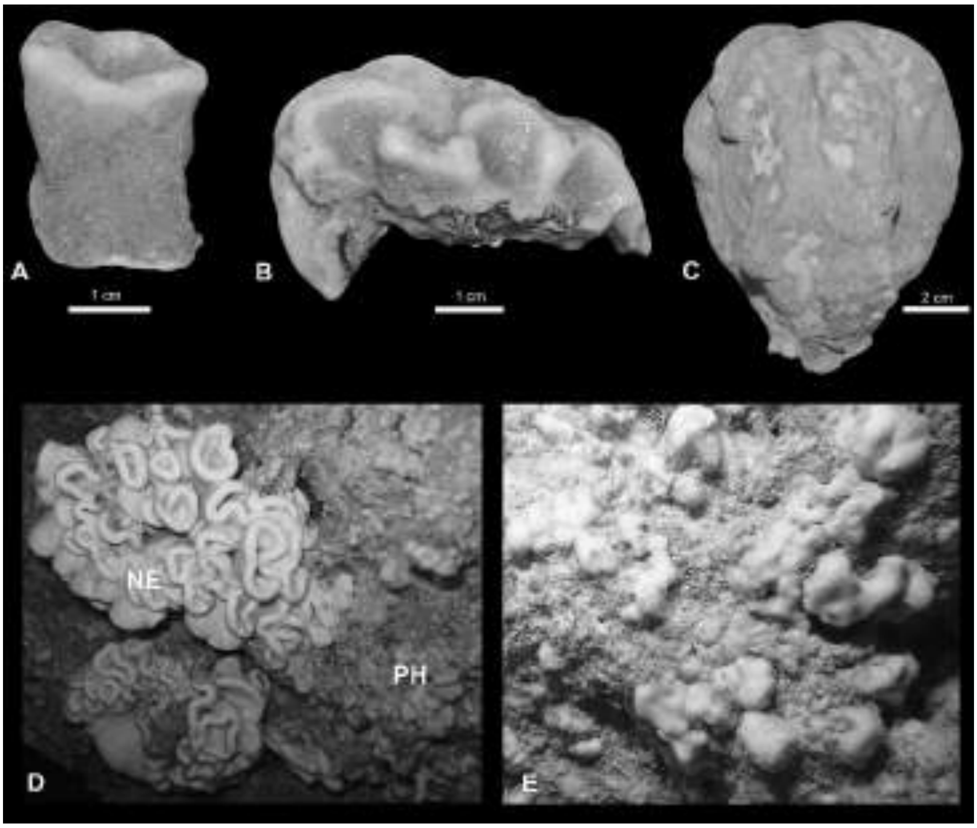

Fig.1. Morphology of the studied lithistid sponges: (A-B) *Microscleroderma lamina*; (C) *Gastrophanella phoeniciensis*; (D) Underwater photo of *Neophrissospongia endoumensis* (NE) and the undescribed phymaraphiniid (PH); (E). Underwater photo of the undescribed phymaraphiniid sponge.

Microscleroderma lamina (family Scleritodermidae) was recorded in a semi-submerged cave in northern Crete, forming contorted laminae, similarly to *N. endoumensis*, but thinner, smaller, brown in colour (when alive) and with “hairy” outer surface. Due to its habits, it could be easily mistaken for *N. endoumensis*, which however has thicker walls and is white when alive (but necrosed parts are brownish). It is characterized by rhizoclone choanosomal desmas (Fig. 2C) and sigmaspire microscleres (not shown in Fig. 2C). Last but not least, *Gastrophanella phoeniciensis* (family Siphonidiidae) was found for the first time in caves on the northern coast of the island. It constitutes a large, slightly club-shaped sponge (Fig. 1C) with rhizoclone choanosomal desmas (Fig. 2D) and tylostyle microscleres (not shown in Fig. 2D).

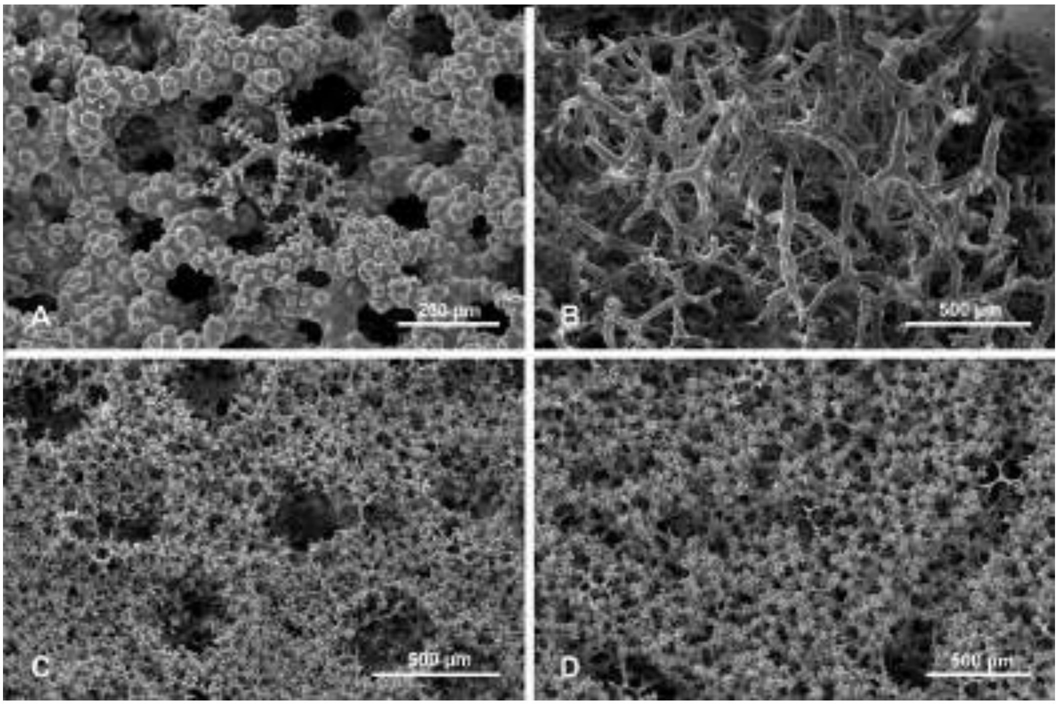

Fig.2. Desma skeleton of the studied lithistid sponges showing clear differences in character of their choanosomal skeleton: (A) *Neophrissospongia endoumensis*; (B) undescribed phymaraphiniid; (C) *Microscleroderma lamina*; and (C) *Gastrophanella phoeniciensis* (D). Different taxa bear different microsclere spicules (not shown here).

All four lithistid taxa were found in the inner dark chambers of shallow caves, at a depth of 0.5-1 m, just below freshwater masses (halocline) or nearby springs of freshwater influx. *N. endoumensis*, which formed large masses in a marine cave in Sfakia area (Fig. 1D), was also collected from a deeper marine cave in the same area, at 15 m, but always next to freshwater springs. The undescribed phymaraphiniid was found in both semi-dark and dark cave parts and had a mean cover of 22.7% ± 5.4 (SE) on the cave walls (as measured in 16 randomly placed photoquadrats of 25 x 25 cm), thus representing an important colonizer of the cave (Fig. 1E).

Discussion and conclusions

The presence of the four lithistid taxa – sometimes in high abundances and large masses – in dark cave sections with freshwater springs, corroborates previous suggestions that their presence could be linked to the higher concentration of silicate in the seawater due to freshwater influx, which resembles conditions in the deep sea (Pisera & Gerovasileiou, 2021). This is also supported by observations from four other caves in different islands of the Aegean and Levantine Seas (VG & MD, unpublished data) which are still under investigation. To our opinion, the most probable source of propagules for shallow sea caves are unrecognized deep-water populations of lithistid sponges (Pisera & Gerovasileiou, 2021). This could – at least partly – explain their fragmented distribution pattern.

The species *N. endoumensis* was originally described from a Western Mediterranean marine cave near Marseille (Pisera & Vacelet, 2011), but most probably occurs also in marine caves of Tenerife in the Atlantic Ocean (AP, unpublished observations). *Microscleroderma lamina* and *G. phoeniciensis* were previously known only from a shallow marine cave of Lebanon (Perez *et al*., 2004). It cannot be excluded that the species *Aciculites mediterranea*, reported from a marine cave of Sardinia (Manconi *et al*., 2006) could be conspecific with *G. phoenicensis*. On the other hand, phymaraphiniid sponges have never been reported from the Mediterranean Sea but are known from the Atlantic Ocean (Carvalho & Pisera, 2019; Carvalho *et al*., 2020). Mediterranean forms are different from those of the Atlantic genus *Exsuperantia* and constitute without doubt a new species (probably also a new genus). Occurrence of four different species of lithistid sponges in five marine caves of Crete, including taxa known only from the Western Mediterranean (or even Atlantic Ocean) and Lebanon, contradicts earlier findings which concluded that every marine cave may harbour a single species that is unknown from other caves (i.e., suggesting its origin in each cave). Our study clearly shows that such pattern is caused by limited exploration, regional lack of expertise and superficial examination that often leads to misidentification, suggesting inexistent patterns in sponge diversity and endemicity.

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NEW INSIGHT INTO THE BRYOZOAN DIVERSITY OF MARINE CAVES OF THE AEGEAN SEA (EASTERN MEDITERRANEAN)

Abstract

Bryozoans are among the main colonizers of marine caves. Nevertheless, their diversity in sea caves of the Eastern Mediterranean Sea remains largely understudied. During the last decade several marine caves were surveyed with SCUBA diving in insular areas and rocky peninsulas of the Aegean Sea in the framework of different research expeditions. Numerous qualitative samples were collected in order to enrich taxonomic accounts for this phylum from this area. Examination of the collected material revealed 58 taxa from 15 caves across seven Greek islands (Agios Efstratios, Antikeri, Pantieronissi, Polyaigos, Crete, Karpathos and Saria) and Chalkidiki Peninsula in North Greece. The majority of the identified taxa belonged to Cheilostomatida (43), followed by Cyclostomatida (13) and Ctenostomatida (2). Four taxa constitute new records for the bryozoan fauna of Greece, while several are reported for the first time from marine caves of the Aegean Sea. In addition, some unidentified taxa constitute candidate new records for this marine area or possibly undescribed species.

Key words: Benthic invertebrates, biodiversity, taxonomic identification, hard substrate, sea caves

Introduction

Bryozoans are among the main colonizers of hard substrates in Mediterranean marine caves (e.g., Harmelin, 2000; Gerovasileiou & Bianchi, 2021, and references therein) that host a relevant proportion of the bryozoan biodiversity of the Mediterranean Sea (Rosso & di Martino, 2016). However, bryozoan diversity in marine caves of the Eastern Mediterranean Sea remains largely understudied. Few isolated records of cave bryozoans occur in historical literature (see Gerovasileiou & Rosso, 2016). Some recent papers also contributed with information from a few marine caves, geographically scattered in the region, specifically small caves and overhangs in Lebanon (e.g., Harmelin, 2014, Harmelin et al., 2016), two submarine caves in the Island of Lesvos (Rosso et al., 2019) and other caves of Greece (Gerovasileiou et al., 2015) and one submarine cave in Cyprus (Guido et al., 2017; Achilleos et al., 2020). These investigations allowed for the discovery of species new to science as well as species originating from outside the Mediterranean Sea (Rosso et al., 2020a, 2020b).
During the last years several marine caves were surveyed with SCUBA diving in different insular areas and rocky peninsulas of the Greek Aegean Sea in the framework of different research expeditions. Numerous qualitative samples of sessile benthos were collected for taxonomic identification of small-sized taxa, including bryozoans. This material has been

examined aiming at increasing present knowledge on bryozoan diversity of marine caves in the Eastern Mediterranean and especially in the Aegean Sea.

Materials and Methods

The studied material was collected from 15 marine caves (nine semi-submerged and six entirely submerged with internal air chambers) from seven different islands of the Aegean Sea (i.e., Agios Efstratios, Pantieronissi, Polyaigos, Antikeri, Crete, Saria and Karpathos) and the Chalkidiki Peninsula. The study area covers the entire Aegean ecoregion, with the northernmost cave located in Chalkidiki and the southernmost on the southern coasts of Crete (Fig. 1, Table 1). Most caves (7) are located in the western sector of Crete (four on the northern and three on the southern side), while 1-3 caves were surveyed in each of the other areas.

Samples were collected with SCUBA diving from different ecological cave zones (i.e., entrance, semi-dark and dark zone), but mostly from semi-dark cave sectors. Samples were either fixed in 96% ethanol or preserved dried. Sampling events took place between 2009 and 2021. Material was examined under a stereomicroscope and selected colonies were observed both untreated and washed, but uncoated under a Tescan LMU2 Vega Scanning Electron Microscope at the University of Catania Microscopy Laboratory.

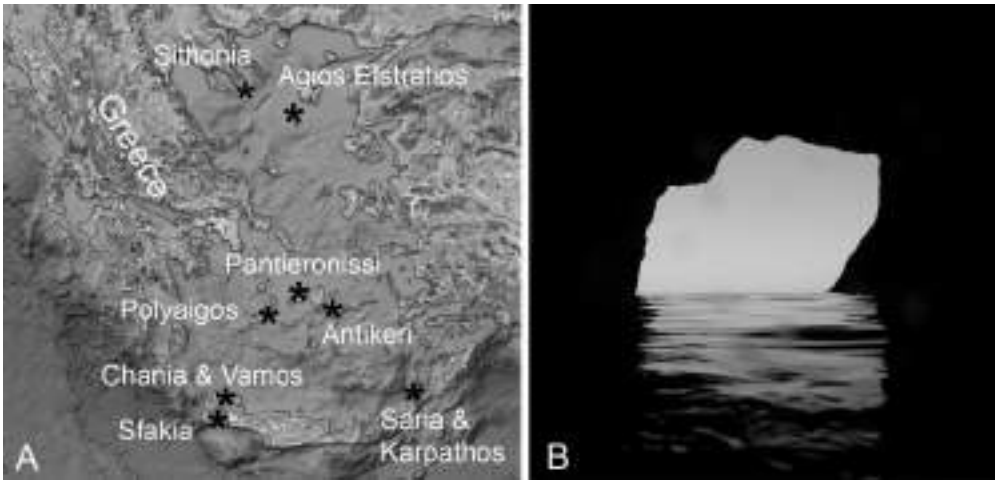

Fig.1. Location of the studied marine caves in the Aegean Archipelago, Eastern Mediterranean Sea (A) and the entrance of the semi-submerged Trypia Spilia Cave in Agios Efstratios Island (B).

Table 1. Investigated caves and localities, and their main features. CE: cave entrance; SD: semidark zone; D: dark zone. Numbers after + indicate species found only as dead colonies.

Geographical location	Sithonia, Chalkidiki	Agios Efstratios Island	Pantieronissi Island	Polyaigos Island	Antikeri Island	Chania & Vamos, Crete	Sfakia, Crete	Saria & Karpathos Islands
Number of caves	1	1	1	1	1	4	3	3
Depth range	0-12 m	0-5 m	0-8 m	0-4 m	15 m	0-10 m	0-17 m	0-16 m
Number of samples	1	5	1	3	1	5	6	4
Cave zone	SD	CE, SD	SD	SD, D	SD	CE, SD, D	CE, SD	SD
Number of species	1	10+8	3	9	14	7	30+2	23

Results

A total of 52 living species were identified (some shown in Fig. 2). The majority belonged to Cheilostomatida (38 taxa), followed by Cyclostomatida and Ctenostomatida (12 and 2 taxa, respectively). This list increases to 58 taxa if we also consider one further cyclostomatid and five cheilostomatid species found only as dead colonies.

From this preliminary survey, the highest number of species (32) was found in Sfakia area in South Crete, and mostly in the Italian Cave (27 species), which received higher sampling effort compared with the other study sites. The semi-submerged Trypia Spilia cave of Agios Efstratios Island (Fig. 1) was found to host 18 species (10 living + 8 dead), followed by the submerged cave in Antikeri Island (14 species), two semi-submerged caves of Saria Island (Agios Panteleimonas and Oxonisos caves with 14 and 13 species, respectively) and 1-HT_8330_13 cave in Sfakia (11 living + 2 dead species). All other caves had less than ten species in the collected material.

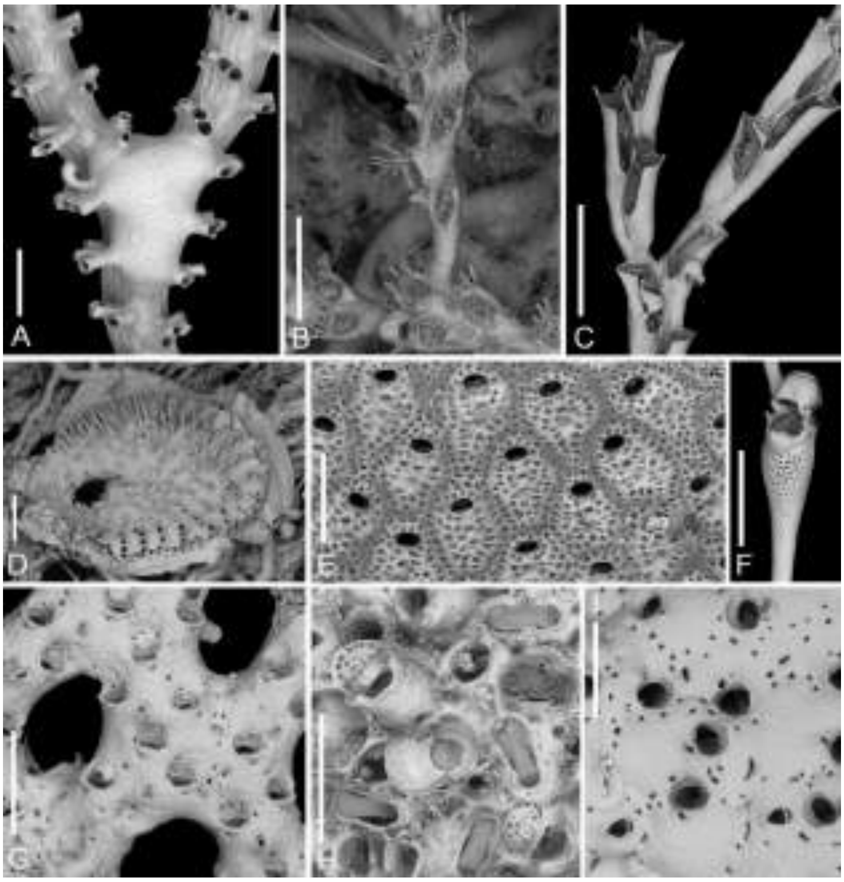

Fig.2. Some cyclostomatid (A, D) and cheilostomatid (C, C, E-I) bryozoan species from the studied Aegean caves. (A) *Idmidronea triforis*; (B) *Cradoscrupocellaria hirsuta*; (C) *Bugula germanae*; (D) *Plagioecia sarniensis*; (E) *Reptadeonella* cf. *violacea*; (F) *Savignyella* cf. *lafontii*; (G) *Reteporella grimaldii*; (H) *Celleporina caliciformis*; (I) Celleporidae sp. Scale bars: 500 µm for all species, except for D: 1 mm.

Most species were present in different marine caves with only a single or a small number of colonies, except for *Savignyella* cf. *lafontii* (forming extensive mats of several colonies in the Antikeri and the Italian caves), *Idmidronea triforis* and *Crisia* spp. (more than 20 and 10 colonies, respectively, in the Italian Cave), *Chlydonia pyriformis* (with a dozen of colonies in the Blue Cave of Chania, Crete) and *Turbicellepora* cf. *coronupus* (with a dozen of colonies in Trypia Spilia and Oxonisos caves). None of the identified species was found to occur in all the surveyed caves and the most frequent species (i.e. *Chlydonia pyriformis*) was shared by 7 out of 15 marine caves. *Cradoscrupocellaria hirsuta* and *Turbicellopra* cf. *coronopus* were found in five caves while *Caberea boryi*, *Escharina vulgaris* and *Celleporina* cf. *caliciformis* were found in four caves.

Discussion and conclusions

Despite the qualitative nature of our sampling and variable effort among study sites, this preliminary survey revealed a high number of species. All caves were studied for the first time for their bryozoan fauna and a considerable number of species (ca. 20 species - 35%) were reported for the first time as elements of the marine cave fauna of the Aegean Sea (Gerovasileiou et al., 2015; Rosso et al., 2019). To our knowledge, this is also the first finding of extensive mats of the cheilostomatid *Savignyella* cf. *lafontii* in marine caves of the Eastern Mediterranean Sea (found at the entrance of two submerged caves, at 15-17 m depth). The maximum number of species identified in a single cave, i.e. 27 species in the Italian Cave (Sfakia, Crete), was quite high when compared to that of the well-studied Fara Cave of Lesvos Island, where systematic quantitative sampling was applied (Rosso et al., 2019). Both caves are located at a similar depth range (Italian Cave: 8-18 m; Fara Cave: 14-18 m) and host a much lower number of species compared to the deeper, well-studied Agios Vasilios Cave (24-40 m, 63 species) of Lesvos Island (Rosso et al., 2019). Four taxa, namely *Cradoscrupocellaria hirsuta*, *Schizomavella* cf. *subsolana*, *Celleporina caliciformis* and juvenile colonies possibly belonging to the genus *Lagenipora* are reported for the first time from Greece (Gerovasileiou & Rosso, 2016). *Cradoscrupocellaria hirsuta*, first described from the Azores, has only recently been reported from several submarine caves in Italian and Croatian waters by Rosso et al. (2016) who suggested that this species is either introduced in the Mediterranean or cryptogenic, but possibly widespread in this basin.
Several taxa are reported for the first time from marine caves of the Aegean Sea. Among them, some (e.g., *Walkeria tuberosa*, *Bugula germanae*, *Synnotum egyptiacum*, *Caberea boryi*, and *Chlydonia pyriformis*) develop small erect flexible colonies and are usually rare within caves. In contrast, the erect rigid species *Reteporella grimadii* forms large convolute reticulate laminar colonies up to some centimetres wide. *Bugula germanae* is a rarely reported species that was described from Corsica in the beginning of the 20th century. So far it is known from a wide depth range (10-60 m), associated with algae and *Posidonia* meadows in the Western Mediterranean, and from 180 m on a biogenic sandy bed near Crete in the Aegean Sea (Harmelin, 1969; Zabala, 1986). This is its first documented finding in sea caves.
In addition, some taxa in the studied material were identified only at the genus/family level, or as similar to already established species (cf.) and constitute candidate new records for this marine area or possibly undescribed species. Further qualitative and quantitative surveys in marine caves of the Eastern Mediterranean Sea are expected to increase the bryozoan diversity of this understudied area.

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ECOLOGICAL RESTORATION OF HUMAN-IMPACTED MEDITERRANEAN BENTHIC MARINE ECOSYSTEMS THROUGH ACTIVE STRATEGIES AND PARTICIPATORY APPROACH: THE LIFE PROJECT ECOREST

Abstract
The negative effects from recurrent industrial fishing are widespread in the Mediterranean Sea waters, being the conservation status of the benthic ecosystems especially critical in the Catalan continental margin (NW Mediterranean Sea), which is identified as a hotspot of ecological importance due to the high concentration of endangered, threatened or vulnerable sessile and demersal species. Here, more than 90% of the sea bottom between 50 and 800 m depth has been affected by bottom otter trawling. This high fishing pressure is triggering a reduction in the structural complexity of the benthic habitats and an impoverishment of the associated biodiversity, affecting most of the Ecosystem Services provided. In the northern and central Catalan margin, 14 no-take fishing areas (29.022 ha), have been established and supported by fisher's guilds to permanently restrict fishing and help recover and restore the benthic communities and fishing resources. However, the ability of these benthic habitats to recover from human impacts is extremely slow since they are formed by long-lived, slow-growing sessile species, with delayed sexual maturity and limited recruitment. Natural recovery of these species may take decades to centuries, so it is of key importance to actively accelerate their recovery and revert their degradation. Ecological restoration aims to return the damaged ecosystems to a state that is within some acceptable limits relative to a less disturbed status, in order to recover its ecosystem services and functionality.
Up to date, most restoration actions beyond the littoral zones have been far to match with the scale of ecosystem damage. The project Life ECOREST aims at scaling up the spatial and temporal frames of restoration actions performed by current scientific efforts on continental shelf and slope deep-sea environments, making them technologically and economically affordable while, at the same time, doing so in close cooperation with fishers' guilds and relevant stakeholders.

Keywords: Active restoration, long-term monitoring, researchers & fishers' collaboration, Vulnerable Marine Ecosystems, Western Mediterranean Sea.

Background

The highly diverse Mediterranean Sea ecosystems host around 7-10% of the world's marine biodiversity (Bianchi & Morri, 2000; Cuttelod *et al*., 2008). In this sense, shelf waters represent 20% of Mediterranean waters, playing an important role for biodiversity compared with the 7.6% share in the world oceans (Gori *et al*., 2017). However, the marine communities inhabiting the continental shelves and slopes are in a poor conservation state, mainly caused by decades of human impacts (Ramirez-Llodra, *et al.*, 2011). Bottom trawling has greatly contributed to the degradation of deep-water habitats to a point where the abundance of megafauna has declined dramatically (Althus *et al.*, 2009; Pusceddu *et al.*, 2014). This has caused the rarefaction and local extinction of multiple species and the simplification of habitats, now mainly formed by vast extensions of barren sediments with low structural complexity and populated by a reduced number of species that tolerate human impacts (e.g. motile or burrowing species).

The negative effects from recurring industrial fishing are widespread in all the Spanish Mediterranean coast. The conservation status of the benthic habitats is especially critical in the Catalan continental margin (north-western Mediterranean Sea) which is identified as a hot spot of ecological importance due to the high concentration of endangered, threatened or vulnerable species (Bianchi & Morri, 2000). Here, more than 90% of the sea floor between 50 and 800 m depth is severely exploited and depleted by different practices, trawling being the most extended (Aguzzi *et al.*, 2022). In rocky areas, the high complex topography of bottoms prevents trawling access, however, benthic ecosystems are here threatened by artisanal fisheries, which can annually remove more than 1,300 structuring species per boat (Santín *et al.*, 2022). This high fishing pressure is triggering a reduction in the structural complexity of the benthic habitats and an impoverishment of the associated biodiversity, affecting most of the Ecosystem Services (ES) provided. Three-dimensionally complex benthic ecosystems maintain nursery grounds and are crucial habitats for a myriad of marine organisms to complete their life cycle, find food or hide from predators (Gori *et al.*, 2017). Additionally, they are key components in the transfer of energy and matter playing a paramount role in nutrient cycles in the deep sea. The loss of ES ultimately affects several economic sectors depending on the sea (Cuttelod *et al.*, 2008).

Benthic habitats such as sponge grounds, coral gardens or cold-water coral reefs are considered by FAO as Vulnerable Marine Ecosystems (VMEs): groups of species, communities or habitats highly vulnerable to fishing impacts (UNGA Resolution 61/105). They are also protected by the Convention on International Trade in Endangered Species and by the EU Habitats Directive (92/43/ECC). Specifically, Reef formations corresponding to the Habitat of Community Interest 1170 “Reefs” (Annex I) occur in several fishing grounds of the continental shelf and shelf edge on the Catalan margin. Based on remotely operated vehicle observations (ROVs) and artisanal fishing by-catch, it is known that these reefs host densely populated coral gardens (15-20 colonies m^2) and sponge grounds (14 individuals m^2) (Gili *et al.*, 2011). However, reef sites have been widely impacted by fishing activities. In reef sites on the Cap de Creus, lost fishing gear reached densities of 25 items per 100 m, up to 15% of gorgonians showing epiphyted or necrotic sections and 10% of colonies were entangled in fishing gears. Although Cap de Creus is a Site of Community Interest (SCI), fragile habitats in this area are still being impacted by fishing and need the implementation of conservation measures (Gili *et al.*, 2011).

In the central and northern Catalan margin, there are 15 no-take areas ($315km^2$) where the fishing activity has been recently permanently restricted to recover fish stocks (Orden APA/1212/2020). No-take restrictions have been officially declared (8 areas) or agreed with fisher associations (7 areas) (Fig. 1). However, the ability of these habitats to recover from human impacts is extremely slow since they are formed by long-lived, slow-growing species, with a delayed sexual maturity and limited recruitment. Natural recovery may take centuries, so it is of key importance to actively initiate or expedite recovery and revert degradation (Montseny *et al.*, 2021). Ecological restoration is an attempt to return a damaged system to an ecological state that is within some acceptable limits relative to a less disturbed system, in order to recover a natural range of ecosystem structure and dynamics. Up to date, most restoration actions beyond the littoral zone have been far to match with the scale of ecosystem damage. The project Life ECOREST aims at scaling up the spatial and temporal frames of restoration actions performed by current scientific efforts on continental shelf and slope, making them technologically and economically affordable.

The Life ECOREST project

In order to contribute towards the recovery of benthic habitats, it is proposed to restore the existing no-take areas (315 km^2) in the continental shelf and slope (50 - 400 m depth) of the Catalan continental margin (Fig. 1) through the recovery and transplant of ecosystem engineering benthic species accidentally caught by fishers in adjacent zones, this being the first restoration project in the deep sea with such a geographical scope at the EU level. Although during the last 20 years the restoration of marine ecosystems has increased substantially worldwide, most restoration actions to date have been carried out in shallow environments, mainly focusing on structuring species such as oysters, mangroves, seagrass or tropical coral reefs, which play a paramount role in the formation of a habitat while, in contrast, only seven active restoration actions focusing on deep-sea sessile structuring organisms (e.g. *Oculina varicosa* Le Sueur, 1820 and *Desmophyllum pertusum* (Linnaeus, 1758)) have been performed in intermediate (50 - 200 m depth) to deep-sea (> 200 m depth) environments (Montseny *et al*., 2021). Owing to technical and logistic challenges and the high costs associated with working in deep-sea environments, these actions have been generally carried out over short periods of time (1 to 4 years) and covering small areas (2 - 20 m^2).

To overcome these limitations, a novel cost-efficient restoration technique for cold-water coral gardens (named the "*badminton method*") was recently developed, consisting on attaching accidently caught sessile structuring organisms (mainly gorgonians) to cobble supports and returning them to deep environments by gently tossing the transplanted organisms directly from a boat (Montseny *et al*., 2020). With a survival rate over 90 % on all transplanted individuals, this technique offers a low-cost methodology for the restoration of deep-sea sessile fauna, but the technique needs to still be validated for other sessile organisms (e.g. sponges), has so far only been tested in a small area (north of Cap de Creus, Fig. 1) and still needs long term monitoring and validation.

Parallelly, the insufficient exchange of information between researchers and fishers usually resulted in management regulations enforced on the community, and without the search for collaboration within collectives and multidisciplinary and participative approaches, government policies are often perceived by the resource users as alien restraints, ending up not followed as desired (Santín *et al*., 2022). Fortunately, this view is changing, and fishers and scientists are more open to listening and contributing to both collectives' benefit, pursuing a common goal: more diverse and well-conserved seafloors that translate into more productive seafloors. Furthermore, fishers' knowledge and expertise about local ecology and conditions should be considered as a valuable asset to conservationists and resource managers to be considered for fisheries legislation, policy-making, basic research, and environmental studies.

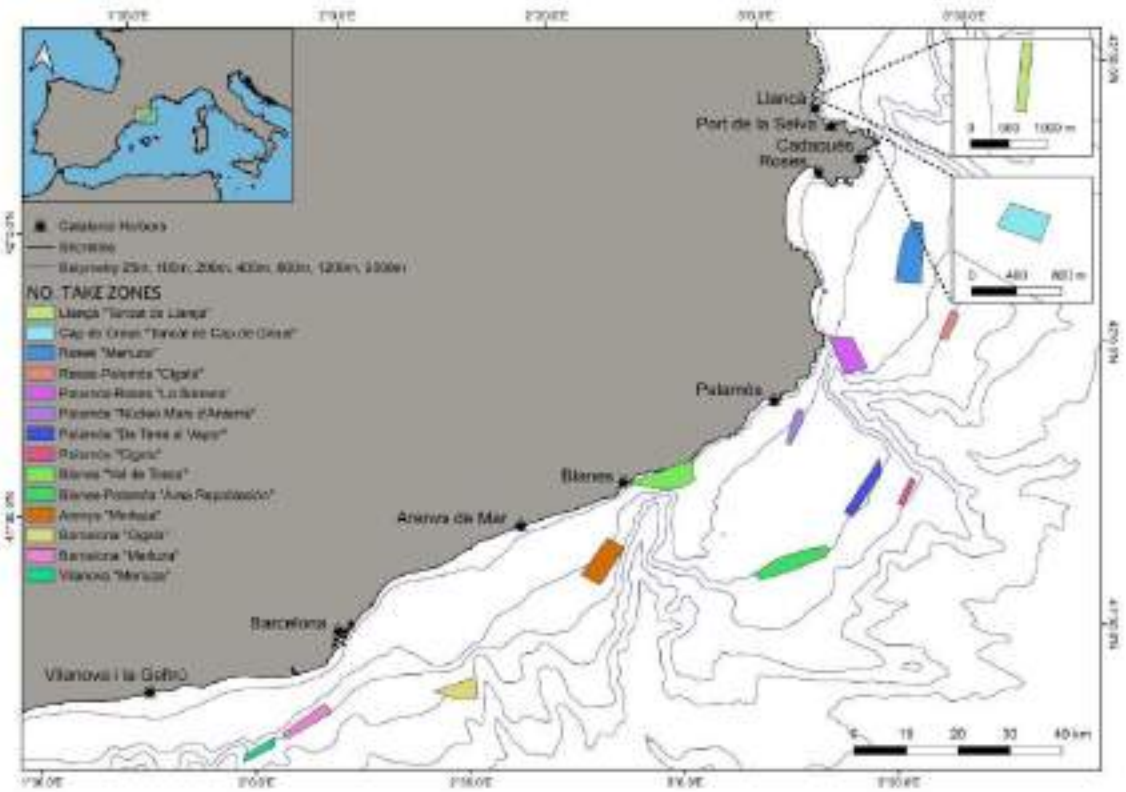

Fig.1. Map of the 16 no take areas in which the Life ECOREST project will be applying active restoration techniques

Therefore, the general objective of the Life ECOREST (2022 – 2026) project is to revert the poor conservation status of benthic habitats impacted by fishing activities and re-establish their main ES and functions through active restoration strategies, to be implemented in close collaboration among scientists, fishers and public administration. Accordingly, the project has set the following specific objectives to be achieved within the timeframe of the project and sustained beyond its end:

(I) To validate the efficacy of low-cost, low-tech restorations strategies of transplanting at large spatial scales to recover habitat structural complexity within the no-take zones.

(II) To evaluate the recovery of ES (nursery grounds and resting and breeding habitat functions) in restored areas and buffer zones by assessing variations in habitat complexity and associated biodiversity.

(III) To promote a participative fishery management by elaborating a collaborative and consensus based proposal of permanent no-take areas and management measures between partners and stakeholders to minimize the impact of fishing.

Hence, the main expected result of the Life ECOREST is to enhance prior and develop new methodologies and protocols that can be officially assumed by the competent administration and fishers for the active restoration of degraded marine communities. They will be jointly developed between scientists, fishers, public administrations and NGOs and will allow the maintenance of restoration actions by fishers in the years to come. During the project, fishers will actively participate in the development and application of restoration techniques gaining a unique experience that will be incorporated into their daily routine since aquaria facilities for the maintenance of daily bycatch will remain in the fisher associations. The governance mechanisms implemented during the project will guarantee an agreed decision about how restoration actions will continue after the end of the project.

Efforts from both researchers and fishers should be made and are being made, to bring mutual benefits. For scientists, fishers contribute with valuable data, experience, and knowledge on the field. For fishers, scientists can provide expertise on managing the resource sustainably, and explanations for the phenomena observed, enriching both collectives in a new and profitable way. The paradigm shift in the treatment and trust between fishermen and scientists is possible, and with the aim of long-term sustainability, could be enormously beneficial for society.

Acknowledgements

The Life ECOREST team would like to thank the personnel of EuroVértice Consultores, and specifically María Huertas, Marta Reguilón and Josmary Pérez for their dedication, help and hard work during the first steps of the project. The present contribution is part of the LIFE ECOREST project (LIFE20 NAT/ES/001270), which is funded by the LIFE programme, the EU financial instrument for the environment. The contents of this publication are the sole responsibility of the authors and do not necessarily reflect the opinion of the European Union.

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POSTERS

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DIVERSITY AND DISTRIBUTION OF ELASMOBRANCHS IN THE SE-IBERIAN PENINSULA (WESTERN MEDITERRANEAN) AS POSSIBLE INDICATORS OF HABITAT PRESENCE

Abstract
The Mediterranean Sea is considered a Elasmobranchii-rich basin but it has the highest proportion of threatened species in the world. There are currently 73 species and almost half of them are threatened. The Mediterranean elasmobranchii community has shown a decline in diversity and abundance mainly due to overfishing. Elasmobranch identification and distribution has been carried out from the MEDITS demersal surveys and other sources, in order to know its potential habitats in the SE of the Iberian Peninsula and viability as indicators of conservation status of deep habitats. 34 species (16 Selachii and 18 Batoidea spp.) have been identified. 93.2% of the total MEDITS samples presented some species. The most abundant species are Scyliorhinus canicula, Galeus melastomus and Etmopterus spinax. Most of these species are common in trawling or surface longline bycatch. The seabed of the SE of the Iberian Peninsula is of great ecological interest due to the convergence of different geomorphological formations and oceanographic features. All the species present in the study area have shown a distribution (geographical and bathymetric) in accordance with that described so far.

Key-words: Chondrichthyes, bycatch, distribution, diversity, vulnerable species

Introduction
The Mediterranean Sea is a biodiversity hotspot and a conservation area of global priority (e.g. Derrick *et al*., 2020). Many of the bottom trawl fisheries operating in this area are multispecific and their selectivity is low (Lleonart & Maynou, 2003). Elasmobranchii make up a significant proportion of the bycatch, although they represent a minor part of the landings (Guijarro *et al*., 2012). The objective of this work is to know the diversity and distribution of elasmobranchs in the SE of the Iberian Peninsula and determine the potential habitats of the main species analyzing their viability as indicators of presence and conservation status of deep habitats.

Material and methods
The study area covers from Torrevieja (Alicante) to Águilas (Murcia), between 40 to 845 m depth. The geomorphology of the seabed presents canyons, seamounts and pockmarks. Much of this area is included in the Natura 2000 Network (Fig. 1). The identification of elasmobranchs has been carried out from the MEDIterranean Trawl Surveys (MEDITS) (1994 to 2017) and has been completed with other sources (Tab. 1).

Results
In total, 34 species of elasmobranch have been found in the study area (Tab. 1). 93.2% of the total tows (279) made during the MEDITS surveys presented elasmobranchs. The most abundant species were *S. canicula*, *G. melastomus*, *E. spinax R. asterias* and *T.*

marmorata. The abundance captured of *S. canicula* has increased over the years, but there is a slight tendency to decrease in the average weight per specimen.

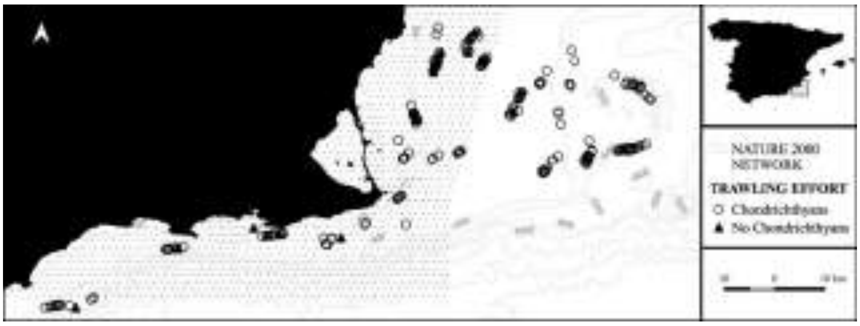

Fig. 1: Map of the elasmobranch sampling effort of the MEDITS surveys (1994-2017).

Tab. 1: Elasmobranch species identified. (A) Abundance (N.º indiv.) captured in MEDITS surveys; (D) Depth (m); (Pel) Pelagic, (Dem) Demersal; Mediterranean IUCN Category.

Specie	A	D	IUCN	Specie	A	D	IUCN
Heptranchias perlo	-	91-150	DD	*Leucoraja naevus*	12	41-256	NT
Hexanchus griseus	-	116-304	LC	*Raja asterias*	123	41-403	NT
Alopias vulpinus	-	Pel	EN	*Raja brachyura*	17	47-254	NT
Cetorhinus maximus	-	Pel	EN	*Raja clavata*	6	162-256	NT
Isurus oxyrinchus	-	Pel	CR	*Raja miraletus*	5	41-87	LC
Prionace glauca	-	Pel	CR	*Raja montagui*	33	41-402	LC
Galeorhinus galeus	-	Dem	VU	*Raja polystigma*	2	40-77	LC
Galeus atlanticus	1	393	NT	*Raja radula*	3	42-45	EN
Galeus melastomus	4324	254-845	LC	*Aetomylaeus bovinus*	-	Dem	CR
Mustelus mustelus	2	44-46	VU	*Dasyatis pastinaca*	-	Dem	VU
Scyliorhinus canicula	9609	41-598	LC	*Gymnura altavela*	-	Dem	CR
Scyliorhinus stellaris	2	44-46	NT	*Mobula mobular*	-	Pel	EN
Dalatias licha	6	408-543	VU	*Myliobatis aquila*	8	44-48	VU
Etmopterus spinax	328	92-596	LC	*Pteroplatytrygon violacea*	-	Pel	LC
Oxynotus centrina	1	252	CR	*Tetronarce nobiliana*	1	220-418	LC
Squalus acanthias	1	110	EN	*Torpedo marmorata*	93	41-455	LC
Rhinobatos rhinobatos	-	Dem	EN	*Torpedo torpedo*	-	Dem	LC

Discussion and conclusions

According to the FAO, the volumes of bycatch and its composition in species are scarcely documented and are rarely incorporated into statistics. It is worth noting the presence of *Galeus atlanticus* near the pockmark field, since its distribution in the Mediterranean has been described as far as Cabo de Gata (Rey *et al*., 2006).

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NEW RECORDS OF BRACHIOPODS FROM MARINE CAVES OF THE AEGEAN SEA, EASTERN MEDITERRANEAN

Abstract
Brachiopods, though common in marine caves, have been so far rarely reported from caves of the Eastern Mediterranean Sea. Herein we present new records of brachiopods collected in eleven marine caves located on eight Greek islands (Kastelorizo, Rhodes, Crete, Samos, Agios Efstathios, Polyaigos, Pantieronissi and Skyros). Four species (60 individuals) have been identified, representing 40% of the brachiopod fauna known from Mediterranean marine caves, i.e., inarticulate Novocrania turbinata *(Poli, 1795) and three representatives of the family Megathyrididae,* Megathiris detruncata *(Gmelin, 1791),* Argyrotheca cuneata *(Risso, 1826), and* Joania cordata *(Risso, 1826). Most samples originated from semi-dark and dark zones, except for one coming from the entrance. A few specimens were found as epibionts on sponges, corals and ascidians. Future research is expected to increase knowledge of the regional diversity of brachiopods.*

Key-words: Brachiopods, taxonomic identification, sea caves, Aegean Sea, Mediterranean

Introduction

Brachiopods are marine benthic, suspension-feeding invertebrates, preferring cryptic, low light environments such as marine caves. In the Mediterranean Sea, they constitute common members of the cave benthos (Gerovasileiou & Bianchi, 2021), although they have been so far rarely reported from caves of the Eastern Mediterranean (Logan *et al*., 2002). Recently their assemblage structure and spatial variability were quantitatively studied in two marine caves of Lesvos Island, Aegean Sea (Bitner & Gerovasileiou, 2021). During the last years, several marine caves were surveyed in insular areas of the Aegean Sea. Numerous invertebrate samples were collected for taxonomic identification of small-sized taxa, including brachiopods.

Material and methods

Brachiopods were collected in eleven marine caves in eight Greek islands (Kastelorizo, Rhodes, Crete, Polyaigos, Agios Efstathios, Pantieronissi, Samos and Skyros), spanning from the Levantine to the North Aegean Sea. None of these caves had been studied before for its brachiopod fauna. Qualitative samples (60 specimens) were collected from the walls of the semi-dark and dark zones of the studied caves, where most brachiopods usually occur. Only in one cave of Crete samples were also collected from the entrance.

Results

Four brachiopod species were identified (Fig. 1). *Novocrania turbinata*, an inarticulate brachiopod cementing by the ventral valve, was found in five caves. The remaining three species belong to Megathyrididae. *Megathiris detruncata* was found merely in three caves. *Argyrotheca cuneata* and *Joania cordata* dominated the collected material, being found in six and nine caves, respectively. They all constitute micromorphic forms, living attached to the substrate by a short pedicle with the shell oriented in a vertical position. A few specimens were found as epibionts on lithistid sponges, the coral *Madracis pharensis* (Heller, 1868) and the ascidian *Pyura dura* (Heller, 1877).

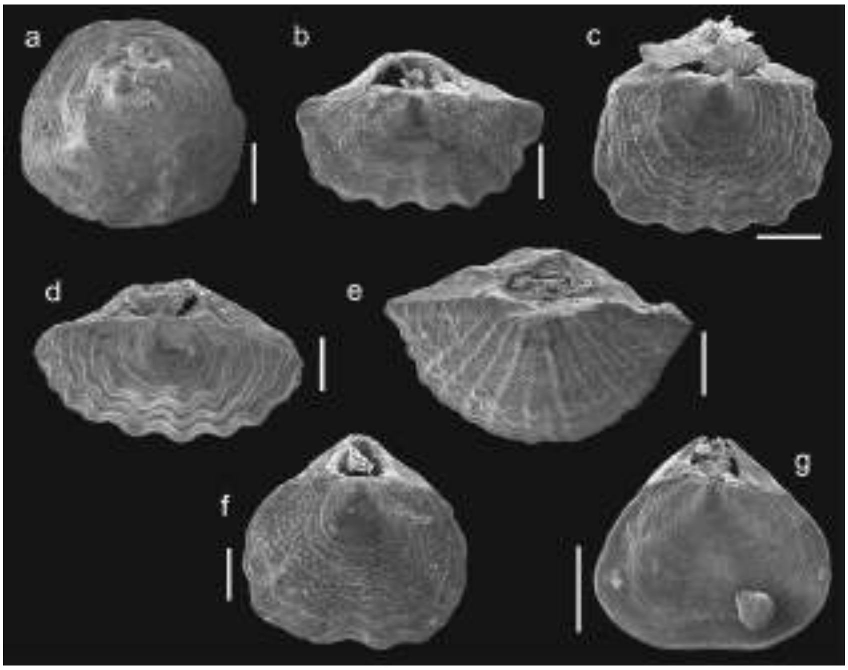

Fig. 1: Brachiopods from the studied marine caves, Aegean Sea, Eastern Mediterranean. a) *Novocrania turbinata*; b-c) *Argyrotheca cuneata*; d-e) *Megathiris detruncata*; f-g) *Joania cordata*. Scale bars: a, 2 mm; b-d, f, 500 µm; e, g, 1 mm.

Discussion and conclusions

Despite the small number of species found, they represent 40% of the brachiopods known from Mediterranean marine caves. All species are already known from Greek waters (Gerovasileiou & Bailly, 2016; Bitner & Gerovasileiou, 2021). Due to their small size (few mm) and limited research effort regarding cave biota, brachiopods are often overlooked in marine caves. Future research in other marine caves focusing also on thanatocoenoses from cave sediments, is expected to increase knowledge on the regional diversity of brachiopods in the marine cave habitat.

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UNVEILING THE REPRODUCTIVE BIOLOGY OF MESOPHOTIC AND BATHYAL MEDITERRANEAN BLACK CORALS

Abstract
Black corals (Cnidaria, Anthozoa, Antipatharia) are among the most charismatic habitat-forming species of the mesophotic and upper bathyal hardgrounds of the Mediterranean Sea. Many aspects of their ecology are known, however, reproductive traits, are mostly undisclosed due to logistic difficulties related with long-term deep-sea monitoring. This study gives the first insight into the reproductive ecology of seven Mediterranean black corals species, sampled through Remotely Operated Vehicles (ROVs) in various localities of the Italian seas, in different years and seasons. Histological analyses were conducted to determine the sex of the colonies, the stage of the gametogenesis, and the number and diameter of eggs and spermatic cysts. Independently from the species, Mediterranean black corals are gonochoric, with the gametogenesis starting in spring and showing only a single event of spawning in late summer. Variations in the size of eggs and sperm cysts are observed among species and depths. Both Antipathella subpinnata and Antipathes dichotoma may occasionally show hermaphroditic polyps.

Key-words: black corals, Mediterranean Sea, sexual reproduction, ROV

Introduction
In the Mediterranean Sea, black corals form the so-called animal forests (Rossi et al., 2017), playing crucial ecological roles at mesophotic and bathyal depths (50-2000 m). Various studies highlighted the phylogenetic relationships of Mediterranean black corals, their distribution, population structure, age, diet, and associated fauna. Reproductive traits, however, are more complicated to evaluate due to logistic difficulties related with long-term deep-sea monitoring. Preliminary observations conducted in aquaria suggest a high degree of plasticity in asexual reproduction (Coppari et al., 2019), but very little is known regarding sexual reproduction (Gaino & Scoccia, 2010). This study gives the first insight into the sexual reproduction of the seven Mediterranean black coral species.

Materials and methods
Black corals were collected by Remoted Operated Vehicle (ROV) in different years (from 2008 to 2018), seasons, and different areas of the Italian Seas. The species collected were the following: *Antipathella subpinnata* (Ellis & Solander, 1786), *Antipathes dichotoma* (Pallas, 1766), *Stichopathes* sp. (Brook, 1880), *Parantipathes larix* (Esper, 1790), *Parantipathes tetrasticha* (Pourtalès, 1868), *Leiopathes glaberrima* (Esper, 1792) and *Phanopathes rigida* (Pourtalès, 1880). Samples were analyzed with histological techniques after resin inclusion (Technovit 8100), and microscope observations to determine the sex of the colonies, the starting period or the stage of the gametogenesis, the spawning period, the number, and the dimension of eggs and spermatic cysts.

Results

A total of 112 samples were analysed, most of which (78) were samples of *A. subpinnata*. The majority of the species were fertile in the period between April and August (Fig. 1) with the only exception of *Leiopathes glaberrima* that was fertile until December. All the species were gonochoric showing only female or male gametes. However, 2 polyps out of the 1055 observed, belonging to *A. dichotoma* and *A. subpinnata*, respectively, were hermaphroditic (Fig. 2). The size and number of oocytes and spermatic cysts increased with the progression of the gametogenesis with the minimum and maximum size in all the species that were 18 µm and 500 µm, respectively. A size increase of both oocytes and spermatic cysts was observed with depth. The number of eggs and spermatic cysts per polyp varied among 2 and 80 considering all the studied species.

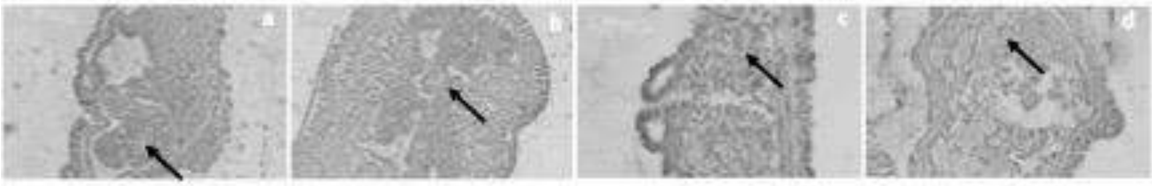

Fig. 1: *Antipathella subpinnata*: spermatic cysts in July indicated by the arrows (a), and in August (b), oocytes in July (c) and August (d).

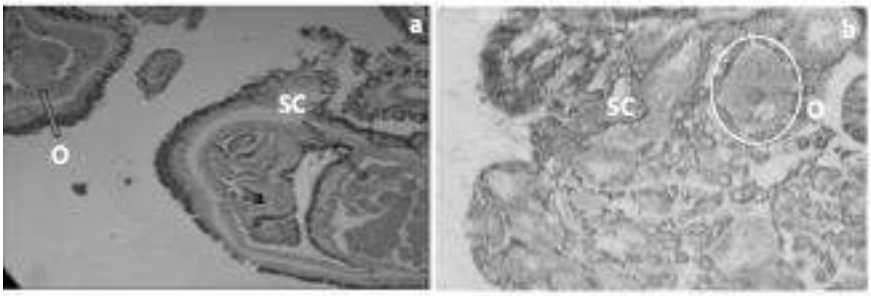

Fig. 2: Rare examples of hermaphroditic polyps: adjacent polyps of *A. dichotoma* showing an oocyte (O) and several spermatic cysts (SC) (a); male polyp of *A. subpinnata* showing an oocyte (O) surrounded by many spermatic cysts (SC) (b)

Discussion and conclusions

The knowledge of the reproductive biology of the species forming marine animal forests is of crucial importance to understand the population structure, the potential resilience of species against environmental and anthropic impact, including climate change, and it is therefore essential to delineate proper management and protection measures.

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SPONGES ASSOCIATED WITH WHITE CORALS FROM THE DEEP EASTERN LIGURIAN SEA

Abstract
Cold-Water Coral (CWC) reefs dominated by scleractinians are known to be biodiversity hotspots, however, the sponge assemblages associated with these habitats are still poorly studied in the Mediterranean Sea. Recent investigations of the eastern Ligurian canyons, carried out within the Curiosity Project (UniGe) between 450 and 750 m, unveiled dead and living reefs and rare biocoenoses. In the present work, seven white coral samples, collected from three coral areas, were used to investigate the associated sponge diversity. The taxonomic investigation revealed a high biomass occupancy (176 specimens in about 1500 cm^3 of material) and a high biodiversity with 27 species among which 1 new record for the Mediterranean Sea (Iotroata acanthostylifera), 2 new records for the Ligurian Sea, Hymedesmia (Hymedesmia) mutabilis and Plocamionida tylotata, and Acanthancora sp. which is a probable new species.

Key-words: CWC, Ligurian Sea, Porifera, white corals

Introduction

Cold-Water Coral (CWC) reefs are bathyal bioconstructions formed by a complex framework of living and dead skeletons of scleractinians (white corals), hosting a rich associated fauna. Sponges are known to inhabit the surface of the bioconstructions, the coral rubble in their surroundings, as well as the carbonatic matrix and the cavities of the framework (Bertolino *et al*., 2019). Despite they play a fundamental role in the trophic sustenance of this ecosystem, a comprehensive picture of their diversity, habit and distribution is still missing, with only few studies targeting the small cryptosponges, which account for the highest, yet less accessible, diversity. The Mediterranean CWC reefs accounts for 172 sponge taxa (Santìn *et al*., 2021), showing a high biomass occupancy of the frameworks and sharing numerous species with other sciaphilous, cavitary ecosystems, such as the coralligenous bioconstructions (Bertolino *et al*., 2019).
Recent investigations of the eastern Ligurian canyons, carried out within the Curiosity Project (UniGe) between 450 and 750 m, unveiled dead and living reefs and rare biocoenoses. The oceanographic uniqueness of the deep Ligurian Sea and the limited information available for the bathyal sponge community associated to the reefs, make the studies in this region extremely valuable. In the present work, seven white coral samples, collected from three coral areas, were used to investigate the associated sponge diversity.

Materials and methods

Samplings were carried out by means of the ROV Multipluto onboard of the vessel *Daedalus* (Fondazione AzioneMare) during summer 2021. Samples were collected from

the Portofino bioherm, the Genoa mound, and the Deiva Marina and Levante canyons (474-724 m depth) for a total of seven coral portions.
In laboratory, the dead coral colonies were first photographed and observed under a stereomicroscope to find the associated sponges, which were also all photographed and subsequently processed by standard methods for Porifera identification. Taxonomic decisions were made according to the classification present in the World Porifera Database (de Voogd *et al*., 2022).

Results
Six dead coral fragments were collected for a total volume of about 1500 cm^3. The taxonomic investigation revealed 176 sponge specimens divided into 27 sponge species among which 1 new record for the Mediterranean Sea, *Iotroata acanthostylifera* (Stephens, 1916), 2 new records for the Ligurian Sea, *Hymedesmia* (*Hymedesmia*) *mutabilis* (Topsent, 1904) and *Plocamionida tylotata* Brøndsted, 1932, and *Acanthancora* sp. which is a probable new species.

Discussion
Currently, over 172 potential sponge taxa have been found associated to Mediterranean CWC reefs (Santìn *et al*., 2021), well behind data published for the North Atlantic Ocean (ca. 260; van Soest & de Voogd, 2015). This could suggests that the sponge fauna associated with Mediterranean reefs is potentially still underestimated, despite the increasing effort to study it. This is partially due to a lower sampling effort in the Mediterranean basin, to taxonomic difficulties regarding Porifera, to the high heterogeneity of the frameworks (Buhl-Mortensen *et al*., 2010) making comprehensive collections difficult, and to the encrusting nature of most sponge species living here, alongside the paucity of the material.
Despite these difficulties and although our study is still preliminary, it confirms the role of CWC reefs as true biodiversity hotspots for sponges in the deep Mediterranean Sea. In fact, the taxonomic investigation revealed a high biomass occupation (176 specimens) compared to the relatively small coral volume investigated and a high biodiversity with 27 sponge species including 1 new record for the Mediterranean Sea, 2 new records for the Ligurian Sea, and one probable new species.

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USING LOCAL ECOLOGICAL KNOWLEDGE TO SUPPORT CONSERVATION OF THE WRECKFISH (*POLYPRION AMERICANUS*) IN THE LIGURIAN SEA

Abstract
The wreckfish, Polyprion americanus *(Polyprionidae), is a long-lived temperate fish undergoing a pelagic to bathydemersal transition over its lifetime. Juveniles are found close to the surface, below floating objects, while adults dwell in deeper habitats, down to 1000 m depth. In the Mediterranean Sea the species can be caught by commercial and recreational fishers. In 1974, an extraordinary catch of wreckfish occurred at the Ulisse Seamount, a previously unexploited fishing ground in the Ligurian Sea, then landings dramatically dropped. Deep ROV surveys that extensively explored Ligurian seamounts in 2017-2018 did not record the species. Overall, information about the life cycle and the status of the Ligurian wreckfish populations is fragmented and further investigations are needed to assess its conservation status, since there is concern that fishing might threaten its populations. To fill this gap, we used a local ecological knowledge (LEK) approach interviewing recreational and professional fishers, technical wreck divers, whale watchers and underwater technical operators. We found that the wreckfish is still being fished in the Ligurian Sea, although most interviewees reported it has been declining. Our results can i) help improve the available knowledge on the status of wreckfish in the Ligurian Sea, ii) clarify some aspects of its complex life cycle and iii) help build management tools tailored to its life cycle, highlighting the importance of LEK in conservation science.*

Key-words: *Polyprion americanus*, local ecological knowledge, seamounts, conservation, deep-sea fish

Introduction
Despite its worldwide distribution, little is known on the wreckfish *Polyprion americanus* (Polyprionidae), especially in the Mediterranean Sea, where the species is classified as Data Deficient in the IUCN Red List (Yokes *et al*., 2011). This grouper-like fish has a peculiar life history: juveniles are found close to the surface, under floating objects, while adults dwell on hard bottoms, such as seamounts and rocky terraces in canyons, and within white coral reefs, down to 1000 m. The wreckfish is a gonochoric species that matures late in life (>7 years), is long lived (>80 years), grows to large sizes (~200 cm, ~100 kg), and likely forms spawning aggregations (Sadovy, 2003). All these traits make this fish highly vulnerable to overfishing. In the Mediterranean Sea, the wreckfish has no relevant commercial interest due to its rarity, but it is an appealing prey for recreational fishers. In the Ligurian Sea (NW Mediterranean), as far as we know, the first greatest landing of wreckfish (~450 kg, 20 individuals) occurred at the previously unfished Ulisse Seamount in 1974. Then, landings dramatically dropped (Bo *et al*., 2021). This raises major concern about the vulnerability of the species to fishing. Deep ROV surveys, also, did not record this species in any of the Ligurian seamounts' tops explored in 2017-2018 (Bo *et al*., 2021), suggesting a significant decline in wreckfish abundances. This led us to investigate the

species occurrence through an alternative methodology to collect information of use for conservation purposes.

Materials and methods

Collection of past and present data on the occurrence of wreckfish in the Ligurian Sea has been carried out by means of a Local Ecological Knowledge (LEK) approach. Two structured questionnaires were administered to two main categories of sea users: 1) local fishers, comprising both recreational and professional fishers, and 2) "observers", comprising boaters, whale watchers and technical divers. From April to June 2022, a total of 28 interviews were administered: 21 to fishers (out of which 12 were recreational and 9 were professional fishers) and 7 to "observers".

Results

Most interviewees reported that juvenile and adult wreckfish are declining. Pelagic juveniles were often observed below floating objects from June to November, likely depending on the surface seawater temperature. Half-sunken objects providing shade and crossed by the light seem to be the most suitable shelters for juvenile wreckfish. Some interviewees have found early stages of fish (Mullidae) and amphipods in the stomachs of pelagic juveniles. Notably, fish of 2-4 kg have been fished in both shallow and deep waters (<150 m). This suggests that, around such sizes, wreckfish move from the surface to the demersal domain of the continental shelf, before descending to deeper habitats of the continental slope and seamounts, where only large-sized individuals (4-50 kg) are fished. Adult wreckfish are commonly caught close to white coral reefs, in line with literature evidence, and light baits are known to be effective in attracting them. Adult wreckfish are still being fished on Ligurian seamounts, where *Pagellus bogaraveo* (preyed on by wreckfish) is one of the main fishing targets.

Discussion and conclusions

Large slow-growing and late-maturing fishes are often highly vulnerable to fishing (Jennings *et al.*, 1998). Since the late 1990s, more advanced fishing gears have helped capture large deep fishes. Wreckfish populations are thus likely to keep declining if proper conservation measures are not adopted. By using LEK, our study shed new light on the complex life cycle of the wreckfish and its catches in the Ligurian Sea, which may help set proper conservation solutions for this and other data-deficient species.

Acknowledgments

We thank the interviewees for having made their time, experiences and knowledge available.

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A CITIZEN-BASED PROTOCOL TO ASSESS VULNERABILITY OF NARROW PASSAGES TO SCUBA DIVING

Abstract
*Scientific community could rely more and more on citizen science to assess marine biodiversity and evaluate its ecosystem services, as confirmed by 27 studies conducted in the Mediterranean from 2015 to 2019. Citizen scientists could also help to assess impacts on narrow and dark habitats where particularly fragile organisms develop even in shallow waters. Until now, most of the proposed methods to estimate level of disturbance of these fragile habitats are based on rigorous protocols that often are too costly to be replicated over time and too complicated to be applied by recreational divers. A simpler and cheaper approach —carried out with the contribution of dive centers and non-scientific divers— would enhance monitoring activities on narrow and dark passages and ensure the continuity of data collection. The objective of this study is to implement an easy-to-apply and standardized protocol to establish a continuous data recording system on fragile communities in these habitats. Recreational scuba divers and dive centers from the Mediterranean Sea will be engaged and trained to identify target species vulnerable to mechanical impact. An index to evaluate level of "**D**isturbance due to scub**A** divi**N**g in submerged **N**arrow passages (**DANNO Index**)" is also proposed.*

Key-words: Underwater canyons, caves, tunnels, arcs, mechanical damage

Introduction

Narrow passages in a dive route on natural rocky habitats include corridors of any length characterized by a width and/or a height <2 m, such as submerged canyons, tunnels, cave entrances, and arcs, where scuba divers are forced to move very close to substrate. Here, dim light conditions are generally present, allowing the development of sciaphilous and fragile species, which are extremely vulnerable to mechanical impacts. Scuba divers may disturb these communities through intentional or unintentional contact (with body parts or dive gears), or by sediment resuspension and bubbling (Di Franco *et al*., 2009, 2010; Luna-Pérez *et al*., 2011; Lucrezi *et al*., 2019). The main objective of this work is to propose a protocol to assess vulnerability of habitats where the diver frequentation should be regulated. The protocol will be presented to a few dive centers to be tested by marine citizen scientists and to address eventual weakness.

Materials and methods

To calculate the **DANNO** index and evaluate impact of scuba diving in narrow passages, 5 m x 1 m transects will be surveyed along target sites by scuba divers considering four factors and six target taxa. Factors are: A. abundance; B. elevation from the substrate; C. exposure level of vulnerable species; and D. signs of scuba diving impact (i.e., mechanical impact on colonies along the walls or large fragments on the floor, and presence of bubbles trapped in crevices/ceiling of a cave, arc or tunnel). The six fragile taxa are: 1. the bryozoan

Myriapora truncata; 2. other erect, branched, and brittle bryozoans; 3. reteporiform bryozoans; 4. *Corallium rubrum*; 5. other sessile animals protruding from the substrate (e.g. gorgonians, erect sponges, large serpulids, and scleractinians). A score varying from 0 (absence of vulnerable taxa) to 3 (presence of exposed fragile taxa) will be given to each factor for each target taxon, while presence of broken organisms and bubbles will receive a negative score (-2 and -1, respectively). A pre-set dive slate will be used to annotate data.

Results

The index results by summing all the attributed scores, the higher the total, the lower the impact in the dive route. Considering that the index will range between -3 and +45, five classes of the level of disturbance of the narrow environment subjected to impacts from scuba diving were empirically established: 45: Undisturbed, 35-44: Little disturbed, 25-34: Moderately disturbed, 15-24: Highly disturbed, and <15: Extremely disturbed environment.

Discussion and conclusion

Globally, most research assessing diving impact is focused on determining its mechanical consequences without considering prevention measures. In order to improve the underwater behavior of scuba divers and limit impact of marine citizen scientists and visitors, rules of good underwater conduct to be adopted in narrow passages should be mentioned before diving. Staff of the dive centers should be trained by marine biologists to learn how to identify the indicator taxa and to train, in their turn, recreational scuba divers frequenting their facility. This will ensure reliable data collection. Scuba diver frequentation data can be retrieved from dive centers' archives and compared with data collected underwater to test the hypothesis that there are no significant differences between sites (or periods within the same dive spot) with different levels of impact.

Particular attention should be dedicated to raise awareness of photographers and video-makers which, due to their cumbersome equipment, may have a higher impact on marine communities. A video showing scuba divers impacting benthic organisms could be presented to increase awareness about underwater behavior and prevent possible mechanical damage (Ajzen, 1991). The effectiveness of this awareness-raising measure could be tested by evaluating behavior of trained and non-trained divers moving in the same passage.

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ALIEN FISH IN MARINE CAVES OF THE AEGEAN SEA, GREECE

Abstract
Although distribution patterns of marine non-indigenous fish are widely studied in the Mediterranean Sea, little is known about their presence in marine caves. In the framework of the Research Program "Aliens in the Aegean – a sea under siege (ALAS)", an underwater visual survey protocol for recording motile species in marine caves was developed and applied for the first time. Both species richness and abundance of motile fauna were recorded within 3-minute visual surveys at the three cave ecological zones (entrance, semi-dark and dark zone) while SCUBA diving. Twelve shallow submerged and semi-submerged marine caves distributed across nine islands of the Aegean Sea (Greece) were surveyed. Eight alien fishes (25%) were found out of 32 recorded fish species, comprising up to 46% of the recorded population. Non-indigenous fish distribution patterns fit well with their known spatial distribution from other habitats in the Aegean Sea.

Key-words: sea caves, alien fish, Aegean Sea, motile fauna, visual census

Introduction
Although >200 alien species have been recorded in the Aegean Sea over the last decades (Katsanevakis *et al*., 2020a), little is known about their presence in dark habitats such as sea caves (Gerovasileiou *et al*., 2016). One of the main goals of the Research Program "Aliens in the Aegean – a sea under siege (ALAS)" (Katsanevakis *et al*., 2020b) is to investigate the establishment of alien species in marine caves of the Aegean Sea.

Materials and Methods
During the summer of 2020, a total of 12 marine caves, distributed across nine Greek islands in the Aegean Sea, were surveyed for the first time for their motile fauna. Two scientists recorded both the abundance and species richness of motile taxa during a 3-min visual survey in each of the three ecological zones (i.e., entrance, semi-dark and dark zone) of each cave while SCUBA diving. Species that were observed out of transect during the approximately 90-min dives were also recorded.

Results
A total of 32 fish species were recorded in the studied marine caves. Among them, eight (8) alien species were found, comprising 10% of the total fish abundance. The Indo-Pacific sweeper fish *Pempheris rhomboidea* exhibited the highest abundance, with more than 200 individuals observed at the dark interior and more than 200 juveniles at the cave entrance indicating its successful establishment at the southeasternmost studied cave in Kastelorizo Island. The lionfish *Pterois miles* was recorded at all cave zones, while all

the other alien and cryptogenic fishes (i.e., *Enchelycore anatina*, *Sargocentron rubrum*, *Siganus luridus*, *Siganus rivulatus*, *Torquigener flavimaculosus*, *Parupeneus forsskali*) were reported at the cave entrance or semi-dark zone. Alien fish comprised up to 46% of recorded fish individuals in the studied caves, with the highest ratio of alien/native species abundance found in the south-eastern Aegean caves (Fig. 1).

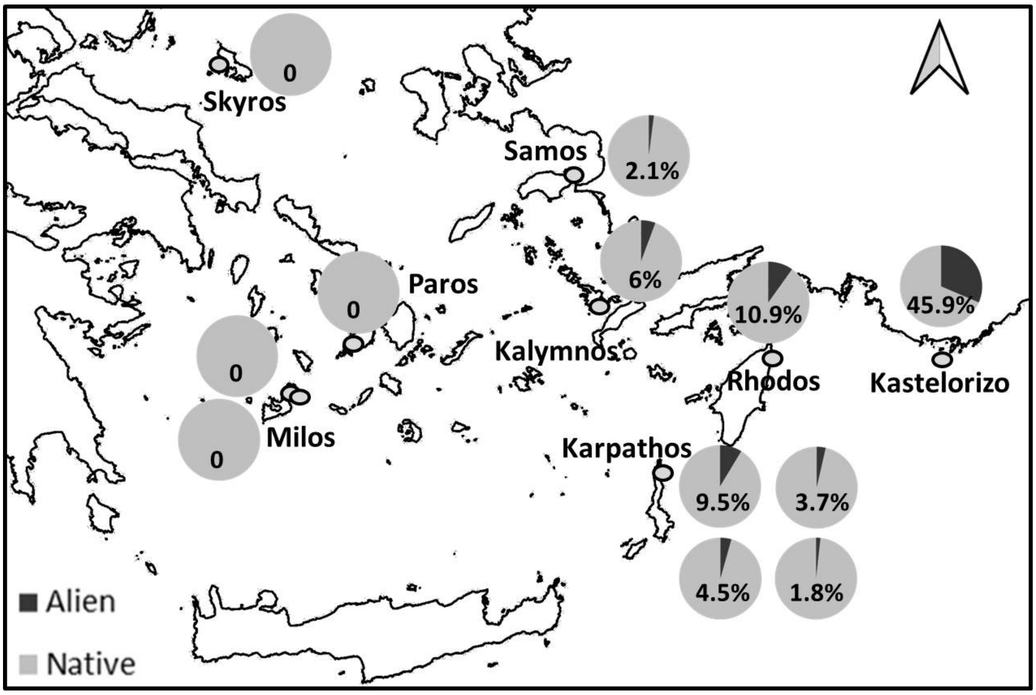

Fig. 1: Map of the studied area with alien / native abundance percent ratio of fish species.

Discussion and conclusions

This study shows that alien fish have successfully been established in the marine cave environment of the Eastern Mediterranean Sea, especially at the entrance and semi-dark cave zones. Their distribution pattern in marine caves fits well with their known distribution in other marine habitats in the Aegean Sea (Katsanevakis *et al*., 2020a). Better estimates of alien species' impact on marine cave ecosystems are expected to be provided via further analysis of the collected data and additional samplings.

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A MARINE CAVE AS ECOLOGICAL REFUGE FROM MACROALGAL INVASION

Abstract
Although Mediterranean marine caves have been characterized as biodiversity reservoirs and vulnerable habitats, little is known about the threats they may face. The invasive macroalgae Rugulopteryx okamurae is rapidly spreading along the Western Mediterranean Sea displacing native sessile competitors through space monopolization but also negatively affecting erect species, on which macroalgae's thalli are frequently entangled. In this study, the relevance of these two processes, as well as the impacts derived from the presence of R. okamurae surrounding marine cave habitats, were studied for the first time in the Mediterranean Sea. A semi-submerged cave located within a Marine Protected Area (MPA) of the northern Alboran Sea was biannually monitored. Photoquadrats were collected from six sites distributed along the entrance and inner semidark zone of the cave. Also, 20 colonies of the gorgonian Eunicella labiata located at the semidark zone were marked and macroalgae's thalli interacting with each colony were quantified. Preliminary results highlight the competitive superiority of R. okamurae at the cave entrance and its progressive monopolization of space through time. The sessile community structure of the semidark zone remained relatively undisturbed while no evidence of mortality was recorded for gorgonians' colonies. This study reinforces the role of marine caves as refuge habitat, but also claims the need to evaluate the conservation status of vulnerable habitats when linked to global change scenarios.

Key-words: marine cave, macroalgal invasion, gorgonians, monitoring, Western Mediterranean.

Introduction
The invasion of *Rugulopteryx okamurae* represents a huge concern in terms of biodiversity and community structure loss across the Strait of Gibraltar and adjacent waters. Given its successful space monopolization and its wide adaptability, the existence of key habitats that may act as ecological refuges may be vital for the conservation of native species. Marine caves are considered 'biodiversity reservoirs' of great conservation value (Gerovasileiou & Bianchi, 2021). This study aims to test the role of a marine cave within a macroalgae invasion event by two monitoring approaches that reflect different competitive strategies: spatial displacement and indirect effects of detached thalli.

Materials and methods
A monitoring of four seasonal samplings was applied (from July 2020 to February 2022) to a shallow semi-submerged cave of the Maro-Cerro Gordo Natural Park (Granada, Spain). The non-destructive method of photoquadrat sampling was applied to assess *R. okamurae*'s impact on the sessile marine cave community. Three random quadrats were collected from six cave sites (three from the entrance and three from the inner semidark

zone) and percent coverage of sessile taxa was obtained through the PhotoQuad software. In addition, the coverage of entangled *R. okamurae*'s thalli on twenty colonies of the gorgonian *Eunicella labiata* from the inner cave zone were monitored to quantify possible impacts from the invasive macroalgae.

Results

The sessile community structure significantly differed between the outer and inner zone for all sampling periods (p<0.01). Although the results did not reveal seasonal changes for the semidark community structure, the increasing dominance of *R. okamurae* at the entrance led to changes in the structure of the photophilic community with ongoing homogenization of the outer biota (Fig. 1). On the contrary, significant spatial differences found in the inner semidark zone did not vary through the different sampling periods (p=0.1269 for the factor 'Time'). Although *R. okamurae*'s thalli were repeatedly found entangled in the gorgonians' colonies during surveys, no evidence of mortality was obtained by the end of the study.

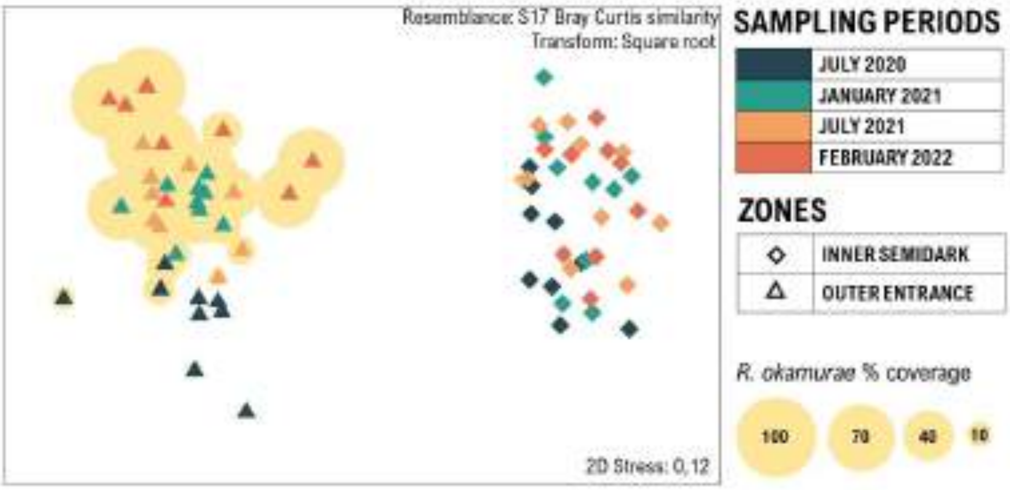

Fig. 1: MDS plot of the quadrats collected from the four sampling periods from the entrance and semidark zone of the studied semi-submerged cave. Percent coverage of *R. okamurae* per photoquadrat is represented by scaled bubbles.

Discussion and conclusions

This study reflects the consequences of the invasive success of *R. okamurae* in favorable habitats while revealing the role of a marine cave in buffering impacts from both direct (spatial competition) and indirect (entangled thalli) processes of macroalgal invasion. The patterns obtained reinforce the role of marine caves as natural refuges, while claiming the need for future and long-term monitoring to elucidate impacts linked to global change scenarios.

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VULNERABLE BENTHIC ASSEMBLAGES IN THE DEEP EASTERN MEDITERRANEAN: REVISITING UNDERWATER SURVEYS TO SHED LIGHT ON UNKNOWN DIVERSITY

Abstract
Benthic communities in the deep Eastern Mediterranean (EMED) have attracted limited research effort compared with other Mediterranean basins. Over the last two decades, the Hellenic Centre for Marine Research (HCMR) has coordinated and participated in numerous projects and expeditions investigating seabed features and wrecks in the EMED. Within the DEEPEASTMED project archive video material recorded by remotely operated vehicles (ROVs), towed video systems and a manned submarine was assessed for the occurrence of benthic fauna with a special focus on protected and indicator taxa for Vulnerable Marine Ecosystems (VMEs). The examined video material (250 hours) covered 36 sites spanning from the Eastern Ionian to the Levantine Sea and a bathymetric range of 200-1560 m. A total of 46 vulnerable invertebrate taxa were identified, including several rare and new records. These findings provide a baseline for further surveys tailored towards more quantitative and qualitative assessments of VMEs in the EMED.

Key-words: biodiversity, sponges, corals, sessile invertebrates, Vulnerable Marine Ecosystems, bathyal zone

Introduction
Sessile benthic communities in the deep waters (>200 m) of the Eastern Mediterranean Sea (EMED) have attracted limited research effort compared with other Mediterranean basins. More specifically, in contrast to the Western and Central Mediterranean Sea, there is very little information available from underwater video material concerning deep-water biodiversity and Vulnerable Marine Ecosystems (VMEs) of the EMED. To this end, the aim of this work was to identify vulnerable sessile invertebrates in archive video material and highlight their presence in the deep waters of this understudied marine area.

Materials and methods
Over the last decades, the Hellenic Centre for Marine Research (HCMR) has coordinated and/or participated in numerous research projects and expeditions investigating deep-sea resources, seabed features and wrecks in the EMED, for scientific and operational purposes. Within the DEEPEASTMED project (coordinated by IUCN and HCMR) archive video material, recorded by remotely operated vehicles (ROVs), towed video systems and a manned submarine, was assessed for the presence and abundance of benthic fauna with a special focus on protected and indicator taxa for VMEs. The examined video material (250 hours of dive time) covered 36 sites from the Eastern Ionian to the Levantine Basin and a bathymetric range of 200-1560 m.

Results

A total of 46 sessile invertebrate taxa were identified, which belong to six major groups (16 Porifera, 24 Cnidaria, 1 Annelida, 2 Echinodermata, 1 Brachiopoda, and 2 Ascidiacea). Numerous rare and new findings were included among these records. The sponge *Phakellia* sp., the gorgonians *Acanthogorgia hirsuta* and *Viminella flagellum* were recorded for the first time across the EMED and the Greek waters. The glass sponges *Farrea* sp. and *Tretodictyum reiswigi* and the lithistid sponge *Leiodermatium* sp. were found for the second time in the EMED. Other notable records were the lithistid *Neophrissospongia* sp. and several vulnerable anthozoans with a restricted number of records in the EMED. These included the actiniarian *Amphianthus dohrnii*, the corallimorpharian *Sideractis glacialis*, the black corals *Antipathella subpinnata*, *Leiopathes glaberrima* and *Parantipathes larix*, the gorgonian *Callogorgia verticillata* and the seapen *Kophobelemnon stelliferum*. Furthermore, aggregations of siboglinid annelids (*Lamellibrachia* sp.) were found to be associated with cold seeps off the Nile Delta.

In addition to these records, interesting ecological habits were observed for several taxa. The scleractinian *Desmophyllum dianthus* formed dense aggregations on the sides or under-hangings of rocks (573-632 m, Antikythera, Eastern Ionian). The cold-water yellow coral *Dendrophyllia cornigera* was found building extensive frames, mainly consisting of coral rubble and few living colonies (540 m depth, Kimolos-Sifnos Strait, South Aegean). A facies of the yellow gorgonian *Eunicella cavolini* was recorded at 200 m, off the north coast of Crete (South Aegean), close to its deepest bathymetric edge. Abundant sponge assemblages, Cerianthidae fields and facies of ascidians, often mono-specific, were recorded in different volcanic areas (Paphsanias, Kolumbo and Santorini volcanos) of the South Aegean.

It should be noted that more than half (25 taxa - 54%) of the recorded taxa were identified only at higher taxonomic levels (genus, family, order or even class) or as morphological categories (e.g., lollipop and massive-tubular sponges), due to limitations in visual identification, suggesting a much higher hidden species diversity.

Discussion and conclusions

The finding of a rich and highly unknown invertebrate diversity in the examined archive video material, often in high abundances and including several VME indicator taxa, in agreement with previous studies (e.g., Gerovasileiou *et al*., 2019), is pointing to the deep EMED being far richer and more diverse than previously thought. This work, along with additional data from Otero & Mytilineou (2022), provides a baseline for future surveys tailored towards quantitative and qualitative sampling for accurate spatial and taxonomic characterization, as well as ecological state assessment of deep-water fauna, in the light of current and future anthropogenic impacts (e.g., offshore oil and gas exploration activities, various pipes and cables).

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ECOLOGICAL CHARACTERIZATION OF THE SEA ANEMONE *AMPHIANTHUS DOHRNII* IN THE NORTHERN SICILY CHANNEL

Abstract
The anemone Amphianthus dohrnii (Koch, 1878) is a small opportunistic epibiont known to colonize a large variety of host species. The species is widely distributed in the eastern Atlantic Ocean and in the Mediterranean Sea, from mesophotic habitats to bathyal depths. Despite being very common, information on its ecology is currently scarce and scattered. The dataset used in this study was obtained during a ROV survey carried out in a wide area of the northern Sicily Channel. In total, 1369 specimens of A. dohrnii were counted. 82.9% of the specimens were observed colonizing dead branches of both living and dead corals, particularly bamboo corals (Isididae), Callogorgia verticillata, Paramuricea hirsuta, Leiopathes glaberrima and Madrepora oculata. Wrecks appear to be also a suitable substrate for A. dohrnii (14.9% of the records). 75.8% of the individuals were observed growing on the dead skeletons of bamboo corals, whose high availability may be related to a poor health status of the population.

Key-words: ROV-imaging, Sicily Channel, *Amphianthus dohrnii*, epibiosis, ecology

Introduction
The sea anemone *Amphianthus dohrnii* is an epibiont suspension feeder, about 10 mm in diameter across the disk (Hiscock et al., 2011). It is widely distributed in North-eastern Atlantic Ocean and eastern Mediterranean Sea, from mesophotic habitats to bathyal depths. The species is known to colonize a large variety of substrates (Mastrototaro et al., 2016; Patzner, 2004; Pierdomenico et al., 2018; Taviani et al., 2019). The present study aims to collect information, currently scarce and scattered, about the ecology and habitat preferences of *A. dohrnii*, observed in a vast area hosting a great habitat diversity (e.g., muddy planes, coral mounds, animal forests, rocky cliffs).

Materials and methods
During an extensive oceanographic campaign conducted between September and November 2021 under the MedWind project, an area of 1651 km² in the northern Sicily Channel was explored through 140 ROV video transects, from 140 m to 950 m depth. The data collected during the video analysis included the number of individuals, with information about geographic position, depth and substrate, and the number of individuals on every hosts.

Results
A total of 1369 specimens of *Amphianthus dohrnii* were observed in 19 dives, at depths ranging from 142 m to 787 m. The anemone was mainly found as epibiont of habitat-forming anthozoans and bivalves (76% of the records) (Fig. 1), with two specimens observed on *Scyliorhinus* sp. eggs. The remaining specimens were found on rock (3 specimens), on the broadside of wrecks (204), and on a ghost net entangled on a wreck (25).

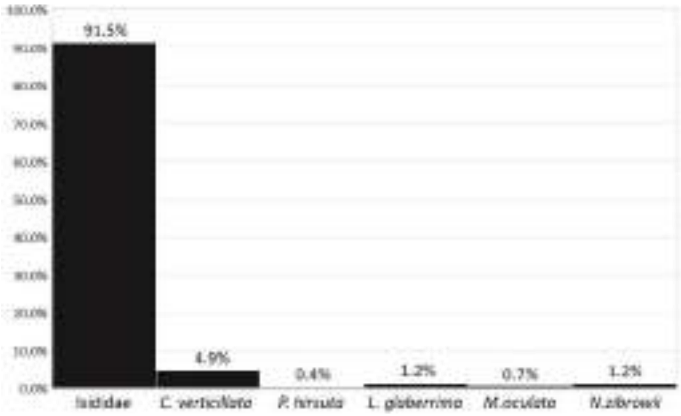

Fig. 1 Percentage of *Amphianthus dohrnii* on different biogenic substrates

1038 individuals were found on bamboo corals (Isididae), 56 on *Callogorgia verticillata*, 14 on *Leiopathes glaberrima*, 14 on the oyster *Neopycnodonte zibrowii*, 8 on *Madrepora oculata,* and 5 on *Paramuricea hirsuta*. The species was abundant on necrotic corals at times covering stems and branches entirely. It reached the abundance of 33 individuals on a single skeleton of an Isididae and 11 individuals on a necrotic colony of *C. verticillata*. Furthermore, bamboo corals hosted 75.8% of all the recorded specimens of *A. dohrnii* and represented 90.2% of all the counted hosts. The rate of epibiosis (colonized colonies/total of colonies) on Isididae ranged from 0% in healthy forests to 51.2% in forests with a poor health status.

Discussion and Conclusions

Amphianthus dohrnii turns out to be a strongly opportunist species able to colonize different substrates with a high exposure to nutrient-rich currents, with a marked preference for anthozoan hosts. The colonization of corals usually occurs on dead or damaged colonies, although it has been rarely observed on apparently healthy colonies. Concerning the dominance of bamboo corals as hosts, it may depend on several factors including the poor health conditions of the forests, here highly impacted by trawling and sedimentation. Overall, it must be pointed out that ROV video analysis carries the risk of underestimating small or cryptic species.

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A PHOTOGRAMMETRIC APPROACH TO EVALUATE THE ECOLOGICAL STATUS OF COLD-WATER CORALS: FIRST RESULTS FROM THE ITALIAN MONITORING WITHIN THE MARINE STRATEGY FRAMEWORK DIRECTIVE

Abstract
Cold-water Corals (CWCs) can form complex and extensive three-dimensional ecosystems, holding a key role in deep environments. Despite being identified as Vulnerable Marine Ecosystems (VMEs) because they are threatened *by numerous anthropogenic impacts, quantitative data on their conservation status is very limited. In this context, the aim of this study aimed to assess the potentiality offered by 3D photogrammetry as a monitoring tool for evaluating the ecological status of CWC habitats. Data were collected in the Dohrn Canyon, Gulf of Naples (Tyrrhenian Sea), during the first year of the CWC monitoring campaign within the Marine Strategy Framework Directive (MSFD) implementation in Italy. Results showed that this technology could be profitably used to evaluate habitat condition and integrity. However, an accurate 3D survey campaign is mandatory if fine-scale structural complexity data has to be collected.*

Key-words: 3D photogrammetry, Cold-Water Corals (CWCs), Marine Strategy Framework Directive (MSFD), Mediterranean Sea, ROV-imaging.

Introduction

Mediterranean Cold-Water Corals (CWCs) include a large variety of habitat-forming species thriving below 200 m and contributing to the formation of complex, vulnerable habitats, playing a significant ecological role (Chimienti *et al.*, 2019; Roberts *et al.*, 2006). In order to acquire quantitative data on their conservation status, CWCs are included in the Italian monitoring programs of the Marine Strategy Framework Directive (MSFD, 2008/56/EC). This research describes the first results obtained by applying a 3D photogrammetric approach (Rossi *et al.*, 2021) to evaluate the ecological status of CWC habitats. The aim was to assess the potentiality of this technology to be used as a monitoring tool in the context of the MSFD Italian monitoring context.

Materials and methods

The experimentation was carried out in August-September 2020, during a monitoring campaign performed using a Perseo (L3 Calzoni) Remotely Operated Vehicle (ROV) in the Dohrn Canyon, Gulf of Naples (Tyrrhenian Sea). Videos and photos collected during two dives were used to reconstruct, in 3-dimensions, two selected areas in the canyon where two target species (*Madrepora oculata* and *Lophelia pertusa*) were settled. 3D models were processed to measure specific parameters useful to be integrated into the MSFD evaluation criteria, specifically “Colony heights” (size structures) and “Density” of target species.

Results

A total area of 45 m^2 was reconstructed. A total number of 92 colonies were counted and measured. The density was 2.53 col. m^{-2} and 0.3 col. m^{-2} in Dive 1 and 2, respectively, whereas the average size (height) was 9.7 cm and 6.4 cm (Fig. 1). No significant difference was revelated with respect to the imaging analysis.

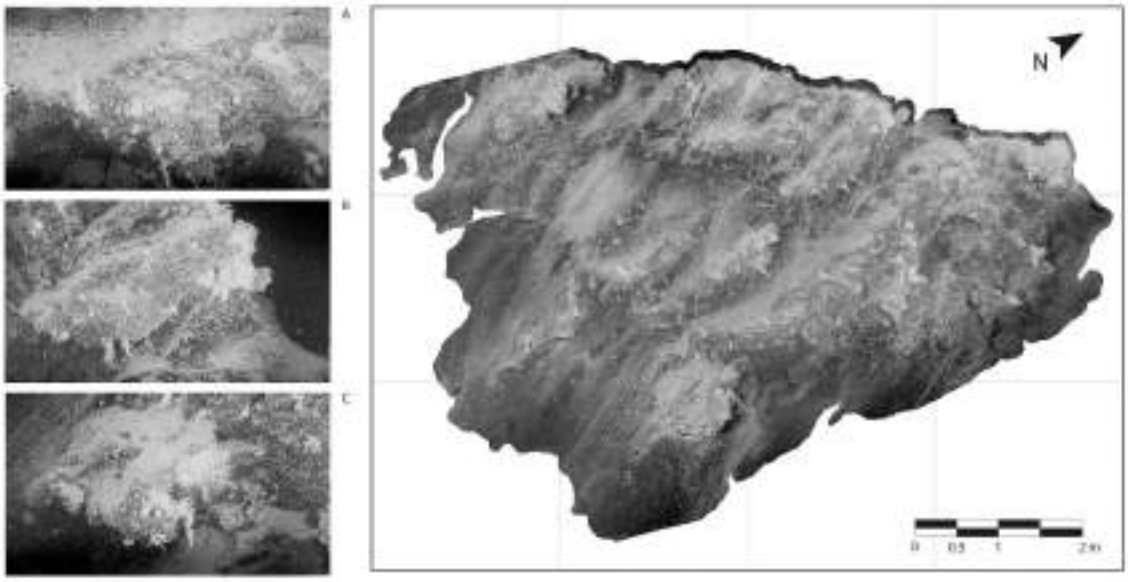

Fig. 1 Example of the 3D model created in this study. (A-C): close-up images extracted from the photogrammetric dataset.

Discussion and conclusions

The preliminary results demonstrate that photogrammetry could be profitably used to evaluate habitat condition and integrity, providing quantitative, replicable parameters useful for long-term monitoring. This technological approach can be especially applied to vulnerable benthic habitats settled on rocky outcrops with complex topography. However, an accurate 3D survey campaign has to be planned to solve some issues in data acquisition and obtain 3D reconstructions with a higher level of detail (with a centimetric accuracy), which is essential for measuring structural complexity and increasing data comparability.

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3D MAPPING OF A MARINE CAVE, THE IMPORTANCE OF ESTABLISHING A BASELINE

Abstract
Marine caves are only a small fraction of Mediterranean marine environments, where they represent fundamental biodiversity hotspots. The rise of new technologies, i.e., Structure from Motion (SfM) photogrammetry, gives the opportunity to overcome the equipment limitation issues in sampling these peculiar ecosystems. In this context, we investigated the application of SfM to assess the benthic communities of Grotta Azzurra (Ancona, Adriatic Sea), focussing on the characterization of its assemblage. A photographic sampling was performed from which high-resolution orthomosaics were produced, and 5 phyla were accounted for the walls' segmentation, with Porifera being the most diverse and dominant taxa. SfM allowed the characterization of cave's benthic assemblages, setting a baseline to identify possible community shifts over time due to climatic or anthropic pressures.

Key-words: Habitat mapping, marine cave, sponge community, conservation, photogrammetry.

Introduction
Although marine caves are only a small fraction of Mediterranean marine environments, they are considered biodiversity hotspots, acting as a reservoir for many endemic species, especially sponges (Gerovasileiou & Bianchi, 2021). Traditional approaches to survey underwater caves are still employed due to the equipment limitations and strict safety protocols of cave diving (Iliffe & Bowen, 2001). The rise of new technologies, such as Structure from Motion (SfM) photogrammetry, gives now the opportunity to obtain a detailed three-dimensional digital reconstruction from a series of overlapping images. In this context, the current study investigates the application of this technique to assess the assemblages inside a small and shallow marine cave, obtaining a baseline to document possible shifts in the community composition due to climate warming and anthropic pressures.

Material and methods
Grotta Azzurra is a semi-submerged, tunnel-shaped cave, located at Ancona (Adriatic Sea). This cave is 15 m in length and has a maximum depth of 4.5 m at the entrance, with the floor mainly made of gravel and small rocks. A photographic sampling of the cave was performed during September 2021, using a GoPro HERO8 Black equipped with two AKKIN 5000 underwater lights. From the series of 3,600 overlapping images, a high-resolution orthomosaic of each wall was produced using Agisoft Photoscan software (Agisoft LLC., St. Petersburg, Russia). The area covered by the various taxa was quantified from the orthomosaics using the QGIS software (QGIS.org, 2022). Two ecological zones, the entrance and the semi-dark zone, were considered for the cover quantification.

Results
A total of 19 benthic taxa were identified, including Rhodophyta (2), Porifera (13), Cnidaria (1), Echinodermata (1) and Tunicata (2). With the exemption of Rhodophyta, all

taxa were present along the entire cave, even though a gradual change in their coverage could be recorded through the walls (Fig. 1). As expected, the sponge community was predominant through the cave, followed by the red algae in the entrance and by hydroid assemblages in the semi-dark zone (Fig. 1b,c). A clear increase in the cover of the bare substrate is also evident through the deeper dark part of the cave (Fig. 1b,c).

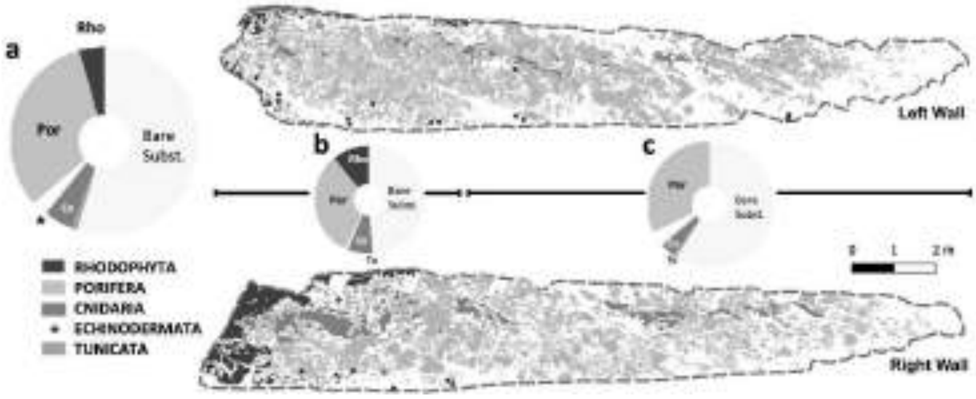

Fig. 1: Schema of the benthic taxa coverage of Grotta Azzurra's walls. a) Total cover of the whole cave; b) benthic coverage along the entrance of the cave; c) sessile coverage along the semi-dark zone of the cave.

Discussion and conclusions

The establishment of a first baseline is crucial to identify community changes during the current climate crisis (Garrabou et al., 2019). SfM-Photogrammetry demonstrated to be a suitable non-invasive technique to record the benthic communities covering the 60 m^2 of substrate present in Grotta Azzurra's walls. Nevertheless, it showed a few limitations: i) for some taxa, invasive sampling approaches must be coupled to arrive at the species-level identification; ii) in the darker parts of the cave, a good illumination system is crucial to obtain quality images. In fact, in this study case, the areas not-clear enough to be segmented represented only the 2.87% of the orthomosaics and were mainly located in the inner part of the semi-dark zone.

Acknowledgments

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MONITORING THE COMPLEX BENTHIC HABITAT IN SEMI-DARK UNDERWATER MARINE CAVES USING PHOTOGRAMMETRY-BASED 3D RECONSTRUCTIONS

Abstract
In the present study, we aim to build a monitoring framework to evaluate changes in cave community assemblages using a novel, non-invasive technique named Structure-from-Motion (SfM) photogrammetry. This method relies on images acquired by video footage to build fine-scaled 3D digital models of the substrate using overlapping imagery. We carried out two surveys by scuba diving in June 2019 and November 2021 in a marine cave highly frequented by divers, located in Illa de l'Aire (Balearic Islands, Spain) to evaluate the effectiveness of this methodology. As a result, we found a loss of 12 colonies of bryozoans with erect skeletons and 5 individual sponges with globose morphotypes. We could also observe the appearance of 7 new colonies and the increase in the size of 30 colonies of the bryozoan Schizoretepora serratimargo (Hincks, 1886). Our results indicate that this methodology enables accurate and efficient monitoring of benthic communities in underwater caves, allowing us to better understand their dynamics and, therefore, to develop the necessary management measures.

Key-words: 3D digital models, marine caves, structure-from-motion, monitoring

Introduction

Marine caves are dark environments considered a priority habitat for conservation included in the EU Habitats Directive (H8330). They harbor fragile benthic communities and represent a major reservoir of marine biodiversity (Gerovasileiou & Voultsiadou, 2012). However, there is a lack of knowledge of these habitats due to the difficulties of creating detailed benthic maps and characterizing the biodiversity, structure, and dynamics of their communities. This study aims to build a monitoring framework to characterize the structure and temporal dynamics of marine caves using SfM photogrammetry. SfM is a novel, non-invasive technique that relies on images acquired by video footage to build fine-scaled 3D digital models of the substrate using overlapping imagery (Fonstad *et al.*, 2013).

Materials and methods

Sampling took place in a semidark underwater marine cave highly frequented by divers in Illa de l'Aire (Balearic Islands, Spain). We installed a permanent longitudinal transect of 9 meters in length delimited by polyamide screws fixed with water-resistant polyvalent putty on the surface of the cave and recorded it by scuba diving in June 2019 and November 2021. Photogrammetry reconstructions were built using Pix4D MapperPro software and compared with CloudCompare software.

Results

We identify a total of 27 species. In order to analyze the temporal dynamics, we focus on the bryozoa and some sponges. We found a loss of 12 colonies of erect bryozoans with fragile skeletons and 5 individual sponges with globose morphotypes. In addition, we were also able to observe the growth of 7 new colonies and the increase in the size of 30 colonies of the bryozoan *S. serratimargo* (Fig. 1).

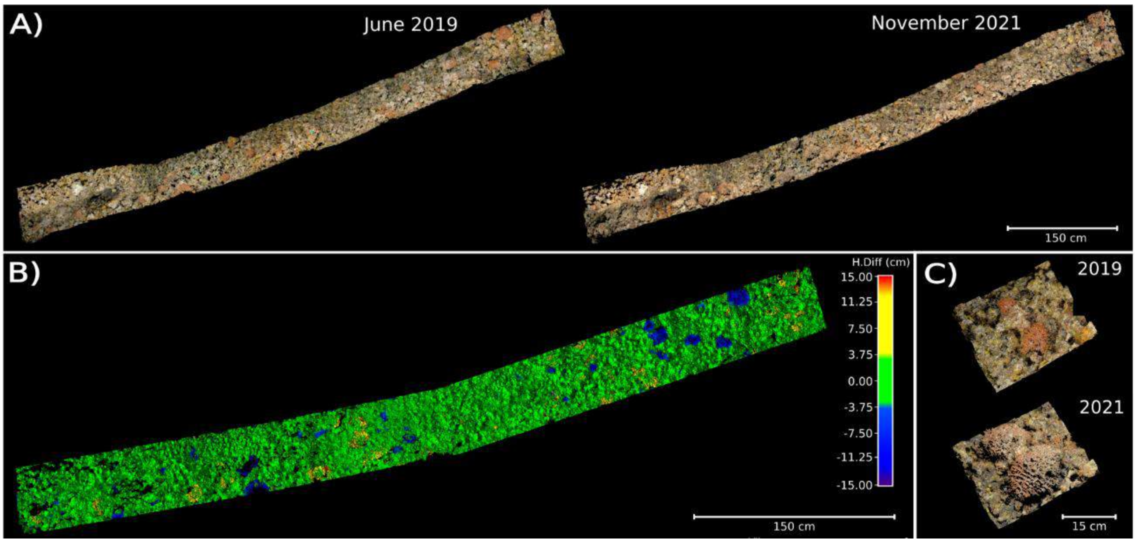

Fig. 1: A) 3D point cloud from June 2019 and November 2021. B) Map of estimated height difference between the two point clouds (values between ± 15 cm) using Multiscale Model to Model Cloud Comparison (M3C2) plug-in included in CloudCompare software (red colours indicates growth and blue colour indicates losses). C) Detail of two specimens of *S. serratimargo* in the 2019 and 2021 3D point clouds.

Discussion and conclusions

Our results indicate that this methodology produces a detailed 3D reconstruction of the marine cave surface which allows us to easily visualize and identify changes in benthic organisms over time. Thus, this technique enables accurate and efficient monitoring of benthic communities in underwater caves that lead to a better understanding of their dynamics and, therefore, to the development of the necessary management measures.

Acknowledgments

This work was carried out in the framework of the LIFE IP INTEMARES project (LIFE15 IPE/ES/000012), and the support of the Estació d'Investigació Jaume Ferrer – La Mola.

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BATHYAL MEGABENTHIC ASSEMBLAGES IN THE SE IBERIAN PENINSULA (WESTERN MEDITERRANEAN SEA)

Abstract
Nineteen megabenthic assemblages have been identified in the SE Iberian area by analysing ROV transects (183-1735m depth) during the LIFE IP Intemares project, based on habitat-forming species and their abundance, substratum type (hard, soft), sediment cover and depth.

Key-words: Continental slope, benthic community, facies, hard and soft bottom, vulnerable ecosystems.

Introduction
The Iberian SE is an interesting transition and connectivity zone between the Alboran Sea and the Algerian-Balearic basin. It hosts important deep water fisheries targeting mostly red shrimp (*Aristeus antennatus*). The area comprises a complex system of tectonic canyons (Mazarron Escarpment), seamounts, knolls and hills (Palos, Planazo, Plis-Plas), and pockmark fields (Acosta *et al.*, 2013). Few studies have been conducted on the bathyal megabenthos, unlike in the neighbouring Chella Bank (De la Torriente *et al.* 2018) and Balearic Islands (Massuti *et al.* 2022). The LIFE IP Intemares project is filling this gap.

Material and methods
The study area is in the Iberian SE region, off the Alicante and Murcia coasts. Based on detailed cartography obtained with a multibeam echo sounder and prior faunal sampling by diverse methods, a selection of sites was prospected in 2020 with IEO ROV Liropus-2000 between 183 and 1735m depth.

Results and discussion
Hard bottoms: i) upper bathyal horizon (180-350m depth) with *Neopycnodonte cochlear*-*Megerlia truncata*, *Ellisella flagellum*, *Antipathes dichotoma*; ii) middle bathyal horizon (350-650m) with Demospongiae (*Pachastrella, Phakellia, Aaptos*), small gorgonians (*Brebyce, Swiftia, Nicella, Dendrobranchia*) and large gorgonians (*Acanthogorgia, Placogorgia, Villogorgia, Paramuricea*), *Leptometra phalangium*, cold-water corals (*Desmophyllum, Madrepora*), Hexactinellida (*Farrea, Tretodyction*), Antipatharia (*Antipathella, Leiopathes*, *Parantipathes*); lower bathyal horizon (> 600m) with *Dicopia antirrhinum* facies, 645-1167m. The *Chironephthya mediterranea* facies can reach 614m depth, and there are live individuals of *Neopycnodonte zibrowii* between 422 and 602m.
Soft bottoms (mud, sandy-mud): i) upper bathyal horizon (180-350m) with Ceriantharia and *Gryphus vitreus*; middle bathyal horizon (350-600m) with Pennatulacea (*Funiculina, Kophobelemnon*), *Thenea muricata* and *Isidella elongata*. Ubiquitous *Pelosina* cf. *arborescens* facies throughout the middle and lower bathyal (388-1322m).

The bathyal zone of the SE Iberian Peninsula has been little studied (Massutí *et al*. 1975) and harbours diverse benthic assemblages, similar to neighbouring areas (De la Torriente *et al.*, 2018; Massutí *et al.*, 2022) but with certain peculiarities. *Asconema setubalense* (present in Chella Bank) has not been detected. The assemblages including cold-water corals, antipatharians, *Leptometra*, *Funiculina* and *Isidella* fall within the category of Natura 2000 priority habitats (1170, 1180) and Vulnerable Marine Ecosystems (FAO, 2016). Other species are listed as threatened or endangered in Annex II of UNEP/MAP-SPA/RAC (2018).

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MONITORING TOURISTIC, SEMI-SUBMERGED CAVES AT TREMITI ISLANDS MPA (ADRIATIC SEA)

Abstract
Semi-submerged marine caves are habitats of great aesthetic and conservation value that are often threatened by numerous touristic activities. Through a non-invasive approach, the potential impact of tourism on benthic community in two semi-submerged marine caves at Tremiti Islands Marine Protected Area was quantified, before and after the touristic season, comparing benthos coverages. The touristic flow and tourists' activities inside the most accessible of the two caves were also evaluated. Our analyses did not reveal any short-term impact in terms of direct damage to the habitat and species. However, we observed some activities, which could affect benthic communities over time. A management plan is needed in order to encourage a more ecological use of caves, counteracting negative human behaviour for benthic communities.

Key-words: Benthic communities, tourism, recreational boating, management, conservation

Introduction

Semi-submerged marine caves are important hotspots of biodiversity among coastal-habitats, present in most marine protected areas (MPAs) throughout the Mediterranean basin (Abdulla *et al*., 2008). Due to their accessibility, they represent an important touristic attraction, but certain toruistic activities can alter the peculiar benthic communities that inhabit the caves (Gerovasileiou & Bianchi, 2021). Albeit these habitats are protected, specific regulations and/or management plans are often missing. In this study we assess the short-term effects of anthropogenic impacts during 2021 touristic season on the benthic communities of the two most visited semi-submerged caves of Tremiti Islands MPA (Adriatic Sea), where 29 marine caves have been registered thus far (Cicogna *et al*., 2003). To assess the extent of anthropogenic pressures, a monitoring of the touristic frequentation during the season (June to September) was also carried out.

Materials and methods

Surveys were carried out on May and November 2021 through horizontal video transects carried out with SCUBA diving along the right and left walls of each cave using a 4k video camera equipped with suitable lighting system and two lasers for metric reference.
Due to the different depths of the seabed, different replicate transects were performed in the two caves, three in Viole cave (at 1.5 m, 4 m and 8 m) and two in Rondinelle cave (at 3.5m and 6 m). About 20 frames were captured from each video transect and analysed using photoQuad software. A standard area of 400 cm^2 was extracted in the centre of each image, then the area occupied by each taxon, identified at the lowest possible taxonomic level, was estimated as area (cm^2) and percent coverage. The results were validated through Shapiro-Wilk and Kruskal-Wallis tests, using Past 4.03 software. Touristic frequentation for the summer season 2021 (June to September) was estimated only at Viole cave, the most accessible for boats and the most visited one. It involved two surveys

during weekdays and two during weekend/holidays, carried out every two weeks. Information about the number, size and type of boats entering the cave were collected. For each month, the daily data were averaged to obtain the average values for both weekdays and weekend. These values were then multiplied by the total number of days with marine weather conditions that allowed visits to the cave.

Results

The benthic community at the entrance of both caves was generally dominated by green algae (e.g. *Cladophora* spp.), brown algae (e.g. *Dictyota* spp. and *Padina* spp.) and the erect red algae *Ellisolandia elongata*. A similar benthic composition occurred at the end of both caves, due to the collapse of their ceilings, which exposes to sunlight their terminal portions. The low light zones inside the caves were mostly dominated by encrusting red algae (e.g. *Peyssonnelia* and *Lithophyllum* genera), the sciaphilic green algae *Pseudochlorodesmis furcellata* and *Palmophyllum crassum*, the patchy presence of the zoanthid *Parazoanthus axinellae* and several unidentified sponges. Dark zones, particularly present in cervices and small fractures in the central area of the caves, were uniquely dominated by sponges. The total area covered by the benthos in each sampling unit was 98 ± 1 % (mean ± standard deviation). The Kruskal-Wallis test showed no significant differences between the average covers of each transect in May and November and in the comparison among the average coverage of the observed macro-categories and the total cover percentages performed for the single sampling units in the two survey periods. Considering the touristic frequentation of Viole cave, more than 5,100 boats were estimated to visit the cave during summer 2021, with more than 75,800 visitors.

Discussion and Conclusions

The touristic frequentation during summer did not alter significantly the composition of the percent coverage at macro-taxa level. Considering that benthic communities in semi-submerged caves are quite stable in terms of composition, we can infer that no short-time changes have been observed. Some human activities in the caves, including anchoring, could affect the marine community over time. To ensure the conservation of semi-submerged caves and a sustainable use for touristic purposes of such a resource for the local population, a management plan is needed. It should include the installation of a mooring buoy system right outside the entrance, in order to foster a non-motored visit inside the cave (e.g. swimming, kayaking, SUP), which is now discouraged by the high number of boats visiting the caves. On the contrary, the access of local touristic boats, driven by professional boat pilots, allows the visit of 30-50 persons per time with short stops, limiting the number of private boats and the risks due to improper manoeuvres conducted by inexperienced boaters.

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LARGE-SCALE CHARACTERIZATION OF DEEP MEGAFAUNA: FIVE CASE STUDIES

Abstract
Large faunal datasets, derived from the extensive employment of Remotely Operated Vehicles (ROVs), represent essential tools to evaluate basin-scale biodiversity and distribution patterns of mesophotic and bathyal megabenthic and demersal taxa. In turn, these patterns provide fundamental information to depict the best conservation and management actions. The ROV archive considered in the present study accounts for 654 dives, over 660 hours of video, 29,859 high-resolution pictures and over 700,000 m^2 of explored seafloor, mainly targeting hardgrounds and nearby soft bottoms along the Italian coasts. Videos and photos were used to record the presence of species in each considered site, their depth of occurrence, the habitat characteristics (e.g., substrate and slope), and any other useful ecological feature. Five different large-scale studies targeting charismatic taxa of megafauna are presented here as examples of the potentialities derived from the analysis of such archive.

Key-words: ROV-imaging, Vulnerable Marine Ecosystems (VMEs), megabenthos, distribution, Mediterranean Sea

Introduction

Remotely Operated Vehicles (ROVs) have been widely employed in the past 15 years along the Mediterranean coasts, mainly between 40 and 800 m, supporting numerous studies on deep biocoenoses, with specific attention to coralligenous concretions, mesophotic animal forests and bathyal habitats dominated by cold-water corals (e.g., Rossi *et al*., 2017; Orejas *et al*., 2019). This large effort contributed to enhancing the knowledge about dark Mediterranean habitats, whose classification has been recently revised and updated in the SPA/RAC manual (Montefalcone *et al*., 2021). The Italian ROV archive used here, obtained from over 45 surveys carried out from 2006 to 2021 between 40 and 1820 m, provided the unique opportunity to explore a large faunal dataset with the aim to depict basin-scale biodiversity and distribution patterns.

Materials and methods

In total, 654 dives, for over 660 hours of video, and 29,859 high-resolution pictures were considered, covering over 700,000 m^2 of seafloor, mainly hardgrounds and nearby soft bottoms, over a latitudinal gradient of 600 NM along the Ligurian and Tyrrhenian coasts and the Sicily Channel. Through the video and photo analysis, the presence ofm egabenthic and megabentho-nektonic taxa was noted for each site, including depth of occurrence, habitat characteristics (e.g., substrate and slope), and any other useful ecological feature, including morphometry of the habitat-forming species and evidence of anthropic impact as well as feeding habits.

Results

The five case studies selected to represent the potentialities of the large Italian ROV archive are the following:

i) A total of 565 species have been detected in the entire dataset. Echinoderms represent a particularly rich taxon (40 species), with eight rare species, two recorded for the first time. ii) The large-scale geographical distribution of the precious coral *Corallium rubrum* highlighted the occurrence of a Tyrrhenian hotspot, particularly along the NW Sardinian coasts, and a marked preference for biogenic sub-vertical habitats (Toma *et al*., 2022). iii) The large-scale bathymetric distribution of the brachiopod populations showed a peak of occurrence in the depth range 150-250 m, also for species mainly considered restricted to shallow water caves. iv) The morphometric analysis of 100 populations of the structuring species *Callogorgia verticillata* highlighted a peak of occurrence in SW Sardinia, with densities up to 8.8 individuals m^{-2} and colonies reaching 150 cm in height, with no evidence of human impact. v) The distribution of 7 heterobranchs species generally declines with depth, in accordance with the depletion of the putative preys (Toma *et al*., 2022).

Discussion

Point-like biocoenotic characterizations are fundamental to describe deep assemblages, however large datasets allow giving more complete pictures of basin-scale patterns. Species rarity, for example, a parameter used to define the degree of vulnerability of deep ecosystems, is strongly influenced by the sampling effort. The identification of geographical hotspots and bathymetrical belts of occurrence of sensitive species, together with the definition of their population structure, are fundamental to implement targeted conservation measures such as the definition of protected areas, as required by the European Biodiversity Strategy 2030. Ecological data are useful to refine and verify habitat prediction models, but may also contribute in shedding light on the mechanisms limiting or supporting the colonization of the deep sea for certain taxa.

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DEEP-SEA ECHINODERMS OF THE SICILY CHANNEL: TWO CASE STUDIES

Abstract
Mediterranean echinoderms currently account for 154 species, many of which have been widely studied in coastal ecosystems. Investigations on deep-sea echinoderms, on the contrary, have been challenging in many ways, and very few targeted in situ studies have been carried out so far. Here we took advantage of an extensive ROV survey carried out in the Sicily Channel in 2021 to define the abundance, distribution, ecology and habitat preferences of two highly abundant yet poorly known deep echinoderms found in the explored area, namely the sea star Hymenodiscus coronata (Sars, 1871) and the holothurian Holothuria (Vaneyothuria) lentiginosa lentiginosa Marenzeller, 1892. About 2400 specimens of the brisingid sea star have been counted during the explorations between 150 and 950 m depth supporting the existence of dense bathyal aggregations on muddy planes. Several specimens of the dotted sea cucumber, an Atlantic species recently reported in the basin, were recorded on different substrates between 140 and 356 m, expanding the knowledge on the ecological preferences and bathymetric distribution of this species.

Key-words: ROV-Imaging, Sicily Channel, echinoderms, ecology, habitat preferences

Introduction

Mediterranean echinoderms represent about the 2% of the global echinoderm diversity, with 154 species recorded until 2010 (Coll *et al*., 2010), mostly studied in coastal environments. On the contrary, very few targeted *in situ* investigations have been carried out so far in deep ecosystems (e.g., Leonard *et al*., 2020).
Investigations on deep-sea echinoderms traditionally focused on soft bottoms, sampled through destructive methods (e.g., Mecho *et al*., 2014). However, with few exceptions (e.g., cidarids and crinoids), deep-sea echinoderms are often scattered on the seafloor, especially on hardgrounds, limiting the chances of being collected. In addition, the analysis of the collected specimens solely does not provide information regarding the species' ecological preferences. In the present study, we took advantage of an extensive Remotely Operated Vehicle (ROV) survey to better describe the abundance, distribution, ecology and habitat preferences of two highly abundant yet poorly known deep echinoderms found in the Sicily Channel.

Materials and methods

The ROV survey was carried out in the northern Sicily Channel, between September and November 2021. The region is characterized by a complex topography and water circulation, providing various habitat to the benthic fauna and making this area an important hotspot of biodiversity (e.g., Consoli *et al*., 2021).
Overall, the 140 ROV tracks covered about 260,000 $m^2$of seafloor between 150 and 950 m, for over 406 hours of video footage, analysed using OFOP software. In order to define the abundance, distribution and ecology of the two target species (*Hymenodiscus coronata* (Sars, 1871) and *Holothuria (Vaneyothuria) lentiginosa lentiginosa* Marenzeller, 1892), the number of individuals, geographic and bathymetric position,

substrate and additional features were noted. The analysis allowed recording specimens larger than two cm, identified by external morphological features. Specimens collected during the explorations were used to verify the video identifications.

Results

About 2400 specimens of the *Hymenodiscus coronata* were counted, with a population density of 0.19 individuals m^{-2}over the 1km transect considered. The brisingid sea star was observed on horizontal muddy bottoms, between 310 and 714 m, often with its arms lifted in the water current direction. Aggregations of adult and juveniles were observed. Ten specimens of the dotted sea cucumber *Holothuria (Vaneyothuria) lentiginosa lentiginosa* (up to 28 cm long) were recorded on mud, fine and coarse detritic substrates, also dominated by coral forests, between 140 m and 356 m depth.

Discussion

Hymenodiscus coronata is a frequent species in the explored area, thriving on bathyal muddy planes. It creates aggregations with density values higher than those reported in literature for the Mediterranean Sea (Leonard *et al*., 2020). This species is subjected to high impact in areas characterized by high trawling effort (Massi & Titone, 2017), suggesting that the explored sites are relatively pristine. The nature of the aggregations is
unclear; however, it is probable that, being suspension feeders (Mecho *et al*, 2014), they exploit favorable current conditions.

Holothuria (Vaneyothuria) lentiginosa lentiginosa was recently reported in the Mediterranean Sea and along the Italian coasts (Toma & Giova, in press). The observations in the present study expand eastward the known distribution of this rare Atlantic species and extend its bathymetric distribution down to 355 m.

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CONCLUSIONS AND RECOMMENDATIONS OF THE 3RD MEDITERRANEAN SYMPOSIUM ON THE CONSERVATION OF DARK HABITATS

1. The meeting highlighted the need to consider historical data and couple the large-scale characterisation of deep benthic biocenoses to large scale monitoring to identify the most sensitive areas for conservation.

2. The meeting addressed the need to further develop monitoring and assessment activities on caves at regional level as well as the importance of building capacities on species identification and raising knowledge on benthic communities. This is also valid for deep-sea.

3. Dark habitats are facing pressures and threats including among others: marine litter and fishing activities (e.g. entanglement with fishing gear) for deep-sea vulnerable habitats, diving activities and non-indigenous species for caves. There is a need to evaluate and quantify these threats in both habitats.

4. The meeting underlined the importance of baseline studies which are still absent from many areas and especially from the southern Mediterranean Sea.

5. Further efforts are needed for the enforcement of regulations and protection (even within MPAs and no-go area) and collaborate with the different stakeholders (fishing sector, the general public, etc.) to protect the dark habitats.

6. Information about dark habitats has significantly advanced during the last years, although there are still important knowledge gaps. Recent findings have expanded the distribution range of several species and revealed previously unknown species. Gaps regarding ecological preferences and life history traits should be addressed to better target conservation measures.

7. Implement effective management and conservation measures to reduce the bycatch: i.e.: reduce soaking time of fishing nets, avoid specific locations where threatened species are located, improve fishing nets to reduce bycatch, and implement active restoration technics, which appear in some cases promising.

8. Given the costs of deep benthic explorations, inter-discipline and international cooperation must be encouraged.

9. Progress towards common monitoring methods could be envisaged for caves including modern technologies e.g. photogrammetry modelling as well as in deep sea habitats.

10. Progress could be done for caves and deep-sea to go towards a more holistic approach bringing together knowledge on different compartments and quantified pressures.

11. Use communication and sensibilisation actions with the public, including youth, to help raise awareness on threatened species and dark habitats in particular.

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