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DEEP CORAL COMMUNITIES ALONG SARDINIAN SUBMARINE CANYONS

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Abstract

Abstract in English

Submarine canyons deeply incise the Mediterranean continental margins, becoming authentic conduits between the continental shelves and deep-sea basins. Enhanced habitat heterogeneity and organic inputs allow a noticeable benthic biodiversity development, creating authentic "hotspots" of biodiversity. However, if knowledge on distribution and composition of benthic assemblages dwelling in different canyon systems across the Mediterranean basin is considerably increasing; on the other hand, factors driving their distribution and biodiversity at multiple spatial scales are still difficult to discern and thus far to being well understood. Among these benthic communities, suspension feeders take considerable advantages from environmental features of canyons; because of this, they are often dominant showing dense patches of large Anthozoan. These organisms, along with other components of the benthos such as sponges, ascidians and bryozoans, enhance the three-dimensional development of the habitat, constituting the so called 'animal forests' which play a key ecological role in the benthic-pelagic coupling processes. Moreover, due to their slow growth rates, longevity and tridimensional structure, these species are particularly vulnerable towards mechanical injuries inflicted by fishing gears. In this regard, supporting knowledge on their distribution patterns and ecology are needed in order to develop sound conservation measures. Therefore, through a non-invasive protocol based on ROV footage coupled with multi-beam dataset, this thesis aims to document Sardinian deepwater coral forests communities dwelling along different canyons and other geological features of the Sardinian continental margin. The present work compares local, and regional biodiversity of coral communities inhabiting contrasting and similar geological features of the continental margin, considering different spatial scales and also exploring the role of a subset of environmental descriptors, obtained through the image analysis, in determining the observed patterns. Overall, multi-variate analyses emphasized a higher variability in the composition of coral communities at the smallest spatial scale investigated that overcomes the variability at regional/geographical scale. In particular, in the first study, patterns of observed diversity were significant different within the same geological feature rather than among contrasting geological setting, and the tested environmental variables explained the patterns of observed diversity. In the second study, results suggested that coral community composition differed across canyons within the same area, but not among the three geographical areas, and that variations patterns appeared to be mainly constrained by differences in the hydrodynamic conditions operating on local scales. The last part of the thesis aimed to describe the distribution and demographic features of scleractinian habitat-forming cold water coral Madrepora oculata encountered in the north eastern and southern western canyons of the island of Sardinia. These species are documented for the first time in the northeast Sardinian continental margin, extending the geographical framework of the recently discovered "Sardinian cold water coral province". Results revealed that, as for all the best developed CWC situations present in the Mediterranean Sea, the new Sardinian province is clearly dominated by patches of *M. oculata* occurring with small/medium size colonies in two different type of substrate (rocky wall and inclined silted bottom). Results from the present thesis increase knowledge on deep coral assemblages inhabiting Sardinian submarines canyons, providing new insights on the scale-dependent structure and dynamics of deep dwelling coral assemblages. These results will likely have considerable implications for the spatial development of forthcoming conservation strategies to preserve such biodiversity hotspots.

Riassunto in Italiano

I canyon sottomarini profondamente incisi nel margine continentale del Mediterraneo, sono considerati degli autentici canali in grado di collegare la piattaforma continentale con i bacini profondi. Aumentando l' eterogeneità dell'habitat e incrementando l'apporto di materia organica consentono lo sviluppo di una notevole biodiversità bentonica, creando autentici "hot-spot" di biodiversità. Tuttavia, se da un lato la conoscenza sulla distribuzione e la composizione dei popolamenti bentonici presenti in numerosi canyon di tutto il Mediterraneo è in considerevole aumento, dall'altro lato, i fattori che ne determinano la distribuzione e la biodiversità su diverse scale spaziali sono ancora difficili da discernere e perciò ancora lontani dall'essere annoverati. Tra le comunità bentoniche, gli organismi sospensivori sono quelli che maggiormente traggono vantaggio dalle caratteristiche presenti nei canyon, per tale motivo densi popolamenti di grandi Antozoi risultano spesso dominanti. Questi organismi, assieme ad altri componenti delle comunità bentoniche, come spugne, ascidie e briozoi, creano importanti strutture tridimensionali, chiamate anche "foreste animali", le quali svolgono un importante ruolo ecologico nei processi di connessione tra la componente bentonica e quella pelagica. Inoltre dato il loro lento accrescimento, la lunga longevità e la complessa struttura tridimensionale, queste specie risultano particolarmente vulnerabili ai danni meccanici causati delle attività di pesca. A questo riguardo, conoscenze di supporto sull'ecologia e sui modelli di distribuzione spaziale di questi importanti e fragili popolamenti sono necessarie al fine di sviluppare misure di conservazione adeguate. Pertanto, attraverso l'utilizzo di un protocollo basato sulla combinazione di filmati ROV e dati Multi-Beam, questa tesi ha come scopo quello di documentare le comunità di coralli profondi presenti in diversi canyon del margine continentale della Sardegna, confrontando la variabilità di queste comunità su scala locale e ragionale in rapporto a diverse strutture geologiche e diverse aree geografiche. Inoltre è stato investigato il ruolo di un insieme di caratteristiche ambientali coinvolte nella variabilità dei modelli osservati. In particolare, nel primo studio, i risultati hanno enfatizzato una maggiore variabilità all'interno di strutture geologiche simili, piuttosto che tra strutture diverse, e tali modelli di variabilità son stati spiegati dalle caratteristiche ambientali esaminate. Nel secondo studio, i risultati hanno mostrato che la composizione delle comunità di coralli profondi differiscono tra canyon appartenenti alla stessa area geografica, ma non tra le tre differenti aree investigate, e che tali variazioni

sembrano essere vincolate principalmente dalle differenze nelle condizioni idrodinamiche presenti su scala locale. Nell'ultimo capitolo della tesi è stata descritta la distribuzione e le caratteristiche demografiche della specie bio-costrutrice di corallo "d'acqua fredda" *Madrepora oculata* rinvenuta in differenti canyon localizzati nella parte nord orientale e sud occidentale della Sardegna. Questa è la prima documentazione di esemplari vivi di questa specie nel margine nord orientale della Sardegna, che và ad estendere il quadro geografico della neo-scoperta "provincia sarda dei coralli d'acqua fredda". I risultati hanno mostrato che, come per tutte le altre comunità di coralli d'acqua fredda ben sviluppate presenti nel Mar Mediterraneo, la nuova provincia sarda è chiaramente dominata da densi popolamenti di *M. oculata* presenti con colonie di dimensioni medio / piccole su due differenti tipologie di substrato (ripide pareti rocciose e fondali fangosi).

In sintesi i risultati ottenuti incrementano le conoscenze sui popolamenti di coralli profondi presenti in numerosi canyon sottomarini della Sardegna, e forniscono nuove informazioni utili per lo sviluppo di future strategie di conservazione atte a preservare questi importanti "hotspot" di biodiversità .

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Chapter 1

1.1 General Introduction

Until the end of the 20thcentury, oceanographic manuals communally agreed that no life was present below the continental shelf, believing in what became known as the "Azoic Theory" (Forbes., 1844). Luckily, this theory did not stop the interest of science for deep waters; on the contrary, it stimulated debates and investigations, slowly accumulating evidence of life in deep-sea systems worldwide, over the last century.

The deep-sea floor is formed by hundreds of millions of km² of abyssal plains and continental slopes. Even though this huge part of the oceans account for more than 95% of the habitable area for life, representing the largest ecosystem on Earth, only 5% of the deep sea has been explored and less than 0.01% of the deep-sea floor has been scientifically investigated.

Despite the common definition of "miniature Ocean" (0.82 % of world ocean's surface), the Mediterranean Sea hosts very high marine biodiversity and is globally regarded as a hot-spot for biodiversity, hosting between the 4 and 18 % of all marine species known (Bianchi and Morri., 2000). Within such biodiversity, benthic communities are possibly the most peculiar in terms of richness and originality in Mediterranean marine life (Gili et al., 2014). The distribution and structure of sessile benthic fauna is driven by environmental gradients that change with season, depth, type of substrate, but also by connectivity and inter/intra- specific interactions (Snikars et al., 2014). The combined action of these multiple factors results in a high variety of assemblages and communities. In this regard, the continental margin, the ribbon of sea floor between the continental shelf and the abyss from 150 m down to >4000 m depth, offer some of the most variable terrain in the ocean

(Levin et al., 2010). This relatively narrow zone (15% of worldwide seabed), defined as 'where the shallow meets the deep' (Levin and Dayton., 2009), connects the lower circalittoral and the upper bathyal zone, exhibiting extreme topographical and environmental heterogeneity that creates habitat for a vast assortment of biological communities (Buhl-Mortensen et al., 2010).

Submarine canyons are abundant and ubiquitous features that connect continental shelves to deep ocean basins (Shepard & Dill., 1966); their role in the interactions between these two habitats as well as in the functioning of the benthic and pelagic ecosystem has been object of numerous studies (Zúñiga et al., 2009; Pusceddu et al., 2010; Fabri et al., 2014). Mediterranean continental shelves are narrow, and are incised by numerous submarine canyons. As most of the Mediterranean coasts have deep-water bottoms near the shore, the Mediterranean basin stands out as a globally different region because its canyons are more closely spaced, more dendritic, shorter and steeper than those from other regions of the world (Harris and Whiteway., 2011). Such features allow an intense exchange between coastal areas, continental shelves, margins and deep basins (Canals et al., 2006). In addition, a great variability can occur for each single canyon (or canyon system) set along the continental margin of the Mediterranean basin.

The eastern part of Sardinia, the area under investigation in the present work, presents all previously cited features, being morphologically characterized by a narrow continental shelf incised by a complex submarine canyons system that locally alter water circulation, canalize organic matter particles, providing different physical substrates that significantly contribute to deep-water biodiversity.

Benthic suspension feeders are among the organisms that best take advantage of these habitats. Studies from the last 20-years emphasized the paramount role of suspension feeders in amplifying the pelagic–benthic transfer of energy in marine benthic food webs

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and the ecosystems overall complexity and functioning (Gili and Coma., 1998; Cerrano et al., 2010; Bianchelli et al., 2013). They also provide refugee, colonisable surface for other organisms and, nonetheless, significantly modifying biomass and species diversity; these features are even more emphasized along continental margins worldwide, as the depth gradient (i.e., fading light and primary production) make suspension feeders the main contributors to the benthic biomass (Rossi., 2013; Gili., et al. 2014). Among them, large Anthozoan gained considerable attention because of their conspicuous dimensions that enhance their habitat-structuring role, making them ecologically similar to terrestrial forests. This figurative and ecological similarity with their terrestrial counterparts had found increasing supporting scientific evidence over the last decades, consolidating the definition of these habitats as "Animal forests" (sensu Rossi et al., 2012). The development of advanced remote underwater technologies such as Remotely Operated Vehicles (ROVs) revealed how deep water coral forests represent keystone habitats of the Mediterranean continental margin (Bo et al., 2009,2012; Orejas et al., 2009; Madurell., 2009; Cerrano et al., 2010; Angeletti et al., 2010,2015; Morris., 2013; Topçu et al., 2015; Cau et al., 2015). From a conservation perspective, the collection of data on species composition and assemblage structure over a variety of spatial scales is of paramount importance for understanding local and regional variation of biodiversity (Levin., 1992; Casas-Güell et al., 2015). Although numerous scientific studies focused on coral forests, there are still considerable lack on the overall knowledge on distribution of these assemblages along continental margins and relative geomorphological features. This missing or scarce information on variations in *alpha* and *beta* diversity along multiple spatial scales considerably slows the development of sound conservation measures. Distribution of benthic community structure and productivity have been studied in a number of submarine canyons (e.g. Vetter., 1994; Vetter & Dayton., 1999; Hargrave et al., 2004; Canals et al.,

2006; Schlacher et al., 2007; Vetter et al., 2010; Würtz., 2012; Fabri et al., 2014). Several conclusion suggest that high habitat heterogeneity in canyons could enhance both benthic and pelagic productivity as well as biodiversity of benthic fauna (Schlacher et al., 2007; Vetter et al., 2010). Species abundance and biomass can be from 2 to 15 times higher compare to the surrounding areas at the same depths (Danovaro et al., 2010), highlighting the urgent need for increase knowledge on coral forests dwelling in these peculiar habitat.

1.2 Aim and objectives of the research project

Understanding how biodiversity varies at different spatial scales and the drivers behind these patterns is a crucial issue in ecology. This is particularly true for deeper waters, where there is little information available on the spatial variability of benthic biodiversity.

Indeed, the aim of this thesis is to document Sardinian deep-water coral communities inhabiting different canyons along Sardinian continental margin, testing both for the presence of a spatial patterns along Sardinian coasts and/or if intrinsic environmental features of canyons may lead to different coral communities. The goal behind this work is to possibly gaining new insights for define or maybe refine conservation strategies for these vulnerable ecosystems currently threatened by destructive human activities (e.g., fishery, pollution, etc. etc.).

This overarching objective has been achieved by performing experiments that combined ROV footage and Multi-beam datasets, a *non-invasive* tool that is particularly suited for investigating communities of such conservation interest.

In detail, the first part of the research is focused on a comparative analysis in *alpha* and *beta* diversity of eleven target species of Anthozoan inhabiting contrasting geological settings of the continental margin (canyons vs *roche du large* ecosystems); the goal is to

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test if substrate/geomorphology-based communities can be distinguished in Sardinian waters. In consideration of the results, the second part focuses exclusively on canyons, comparing the composition of coral assemblages dwelling canyons within and among three geographical areas located along the continental margin off the eastern coast of Sardinia. We also explored the role of a subset of environmental factors in determining the observed patterns in coral community composition over the areas under scrutiny. The third and last part of the thesis is a single-species based investigation, focused on the bathymetric distribution, density patterns and demographic features of Sardinian Cold Water Corals found in three Sardinian canyons, contributing to further extend knowledge on the recently discovered 'Sardinian Cold Water Coral province' (Taviani et al., 2016).

Chapter 2

Beta-diversity of deep water animal forests in submarine canyons and rocky outcrops along the south Sardinia margin (CW Mediterranean Sea): role of environmental drivers.

This study has been performed by myself and Alessandro Cau; both of us equally contributed to the data analysis and writing of the article titled "Beta-diversity of deep water animal forests in submarine canyons and rocky outcrops along the south Sardinia margin(CW Mediterranean Sea): role of environmental drivers" in preparation to be submitted to Marine Environmental research.

2.1 Introduction

The deep sea host rich and highly specialized benthic fauna that varies in composition and biodiversity across multiple spatial scales (Gambi et al., 2014). From a conservation perspective, the collection of data on species composition and assemblage structure over a variety of spatial scales is of paramount importance for understanding local and regional variation of biodiversity (Levin., 1992; Casas-Guell et al., 2015).Within temperate areas, benthic communities dominated by suspension feeders often represent the most common hard-bottom fauna of Mediterranean circa littoral zone, in which they playing a key ecological role enhancing environmental structural heterogeneity (Henry & Roberts, 2007; Buhl-Mortensen et al., 2010; Rossi, 2013). Such structural complexity influence the water flow at small spatial scale and thus increase the residence time of suspended particles (Gili & Coma, 1998; Gili et al., 2014). These complex and well-structured communities

constitute the so-called "animal forests," which accumulate one of the highest biomass within the world oceans because of their widespread distribution throughout different geomorphologies of the continental shelf/margin (*e.g.*, submarine canyons, seamounts, rocky outcrops; Ramirez-Llodra et al., 2010c; Misic et al., 2012; Rossi et al., 2012, 2013; Cau et al., 2015a).

Such high levels of biodiversity have been attributed to different equilibrium (linked with temporal stability, Casas-Guell et al., 2015) and non-equilibrium hypotheses (linked to disturbance, spatial heterogeneity and dynamic forces, (Cerrano et al., 2000, Garrabou et al., 2001). At the same time, variable combinations of biological and environmental factors could also affect the reproduction, larval settlement, growth, and death rates of deep-sea corals (Gori et al., 2011). Nevertheless, whether different geomorphological setting of substrates might have a role on deep-water corals' distribution is still largely an unexplored issue, with few insights arising from very recent studies performed in the Mediterranean basin (Casas-Guell et al., 2015, Cau et al., 2015). In fact, the spatial distribution and habitat selection by different Anthozoan taxa in the circa-littoral zone has been most often explained in terms of variations of the hydrodynamic regime and the type and availability of substrate for settlement (Bo et al., 2009, 2011, 2012); however, the turnover at the smallest spatial scale (*i.e.*, within tens/hundreds of meters) is still an almost unaddressed issue. Indeed, most studies on deep sea benthic communities have analyzed benthic biodiversity variations assuming each habitat to be operationally homogeneous, for instance by contrasting canyon vs. open slope sites but ignoring the spatial effect within sampling stations, almost avoiding to consider the role of bottom heterogeneity.

Submarine canyons dissect most of Europe's continental margins, with those from the Mediterranean basin opening their heads at short distance from the shoreline with steeper and more landward extensions compared to worldwide canyons (Canals et al., 2006;

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Pusceddu et al., 2010; Puig et al., 2014). Such particular feature allows benthic communities associated with submarine canyons to intercept organic-matter-rich-sediments being transported along the inner shelf zone (Harris &Whiteway., 2011). For these reasons submarine canyons are often inhabited by patches of deep-water coral forests among the most dense and diverse hard bottom communities along the continental margins worldwide (Danovaro et al., 2010; Ramirez-Llodra et al., 2010a).

Rocky elevations like pinnacle sand rocky shoals have been at times defined as deep coral oases because they can host very rich coral fauna with much higher levels of biodiversity than surrounding soft-bottom substrates (Bo et al., 2012; Bianchelli et al., 2013; Cau et al., 2015a).

Here, we analyzed if different deep water coral communities characterize the intrinsic geomorphological features of different hard substrates occurring on the top of canyons and on isolated rocky pinnacles emerging from the continental shelf; we then tested if the variability occurring within the smallest spatial scale could overcome the one observable among locations showing contrasting geological features. To test such hypothesis we compared the community composition of deep-water corals from two areas located along the south Sardinia continental margin, characterized by the presence of several canyon heads and isolated rocky pinnacles, respectively. In addition, as deep water coral communities are severely threatened by anthropogenic pressures which may cause mechanical injuries and sediment re-suspension (Ponti et al., 2014, Bo et al., 2014, Angiolillo et al., 2015), the presence of anthropogenic impact was documented in both areas.

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2.2 Materials and methods

2.2.1 Study area

We compared coral communities from two different areas, located off the south Sardinia continental margin (western Mediterranean Sea; Fig. 2.1), characterized by different geomorphological settings: i) the southwestern continental margin, characterized by a vast shelf-area where numerous volcanic outcrops arise from soft bottoms and ii) the southeastern continental margin, characterized by a narrow shelf area, dissected by numerous submarine canyons (Sulli., 2000; Mascle et al., 2001; Cau et al., 2015b).

A total of 8 different sites were chosen in the two locations: 4 rocky isolated pinnacles (henceforth W1, W2, W3, and W4) at depths varying from 100 to 160 meters and 4 canyon heads (henceforth E1, E2, E3, and E4) at similar depths (100 to 145 m). As comparative *a priori* analysis to discriminate geomorphological and environmental differences among locations and among sites, we performed a Canonical Analysis of Principal coordinates (CAP, Anderson & Willis., 2003) using a Euclidean distance based resemblance matrix on normalized geomorphological and environmental parameters (*i.e.*, depth, slope of the substrate, sediment accumulation, latitude, and longitude). The cross-validation from the CAP routine emphasized how all observations (100% for all 8 sites) followed the *a priori* grouping, enabling us to effectively test the proposed null hypothesis on differences in beta-diversity among and within locations.



Figure 2.1.Investigated area. Map of the investigated area showing sites within western (red dots) and eastern locations (blue dots).

2.2.2 Sampling

This study did not involve sampling or damage of any endangered or protected species and is actually based on direct observations with ROV footage and image analysis, a noninvasive approach that is particularly preferred in high conservation interest habitats like those under investigation. The video material was obtained during two surveys on board the R/V "Astrea" conducted along the south Sardinia continental margin in autumn 2011 and summer 2013 (Tab. 2.1). Each site was explored within the same day through a variable number of ROV dives (from a min. of 1 to a max. of 3), in order to collect enough video material for the further image analysis. The ROV "Pollux III" was equipped with a digital camera (Nikon D80, 10 megapixel), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), track-link system, a depth sensor, compass, and three parallel laser beams providing a constant 11-cm reference scale for the measurement of the frame area. Snapshot frames from the ROV footage were extracted at 30 seconds interval using the software DVDVIDEOSOFT. Overall, a total of more than 19 hours of ROV footage were analyzed and frames with non-clear visibility, compromised resolution/focus or not-suitable substrate were discarded for the purposes of the image analysis. A total of 669 video-frames were obtained, covering a total surface of 2267m²(Tab.2.1). For each frame, after the estimation of the frame area through image analysis (CPCe software, Kohler & Gill., 2006), the taxonomic identification (described below), along with abundance measures were performed. The coral abundance was estimated for total number of target corals per $m^2 \pm standard$ error (henceforth $m^{-2} \pm s.e.$) and for each target species. In addition, per each frame, the substrate sediment coverage was classified from 1 to 5, referring to 5 percentage ranges (*i.e.*, 1= 0-20%; 2= 20-40%; 3= 40-60%; 4= 60-80%; 5= 80-100%; Cau et al., 2015a).

	Site E1	Site E2	Site E3	Site E4
Coordinates	39° 25' 340" N	39° 04' 780"N	39° 06' 719"N	39° 05' 414"N
Coordinates	9° 47' 594" E	9°33'760" E	9°25' 970" E	9° 28' 327" E
N. of dives	2	2	4	1
Year	2011	2013	2011	2011
Depth range (m)	90-110	110-145	90-140	100-140
Time filming (h)	2h 08'	3h 02'	5h 47'	2h 30'
N. of frames	97	54	72	133
Frames area(m ²)	145.4	135.69	250.47	311.58
N. of coral colonies	553	165	308	2225
Coral abundance	86+15	3.0 ± 0.82	3.1 ± 1.14	128 ± 15
(colonies $m^{-2} \pm s.e.$)	0.0 ± 1.3	5.0 ± 0.82	3.4 ± 1.14	12.0 ± 1.3
Species richness	3	6	4	4
	CI4 11/1	CHA TTO	C*4 11/2	C114
	Site W1	Site W2	Site W3	Site W4
Coordinatos	39° 10' 122"N	Site W2 38° 44' 425" N	39° 05' 345" N	Site W4 38° 59' 064" N
Coordinates	39° 10' 122"N 8° 06' 133" E	Site W2 38° 44' 425" N 8° 29' 025" E	39° 05' 345" N 8° 10' 538" E	Site W4 38° 59' 064" N 8° 07' 270" E
Coordinates N. of dives	39° 10' 122"N 8° 06' 133" E 1	Site W2 38° 44' 425" N 8° 29' 025" E 1	39° 05' 345" N 8° 10' 538" E 1	Site W4 38° 59' 064" N 8° 07' 270" E 1
Coordinates N. of dives Year	39° 10' 122"N 8° 06' 133" E 1 2013	38° 44' 425" N 8° 29' 025" E 1 2013	39° 05' 345" N 8° 10' 538" E 1 2013	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013
Coordinates N. of dives Year Depth range (m)	39° 10' 122"N 8° 06' 133" E 1 2013 140-150	Site W2 38° 44' 425" N 8° 29' 025" E 1 2013 100-120	39° 05' 345" N 8° 10' 538" E 1 2013 80-100	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013 150-160
Coordinates N. of dives Year Depth range (m) Time filming (h)	Site W1 39° 10' 122"N 8° 06' 133" E 1 2013 140-150 1h 20'	Site W2 38° 44' 425" N 8° 29' 025" E 1 2013 100-120 1h 50'	39° 05' 345" N 8° 10' 538" E 1 2013 80-100 2h	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013 150-160 2h 57'
Coordinates N. of dives Year Depth range (m) Time filming (h) N. of frames	Site W1 39° 10' 122"N 8° 06' 133" E 1 2013 140-150 1h 20' 67	Site W2 38° 44' 425" N 8° 29' 025" E 1 2013 100-120 1h 50' 110	Site W3 39° 05' 345" N 8° 10' 538" E 1 2013 80-100 2h 49	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013 150-160 2h 57' 87
Coordinates N. of dives Year Depth range (m) Time filming (h) N. of frames Frames area(m ²)	Site W1 39° 10' 122"N 8° 06' 133" E 1 2013 140-150 1h 20' 67 284.8	Site W2 38° 44' 425" N 8° 29' 025" E 1 2013 100-120 1h 50' 110 648.74	Site W3 39° 05' 345" N 8° 10' 538" E 1 2013 80-100 2h 49 150.31	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013 150-160 2h 57' 87 330.15
Coordinates N. of dives Year Depth range (m) Time filming (h) N. of frames Frames area(m ²) N. of coral colonies	Site W1 39° 10' 122"N 8° 06' 133" E 1 2013 140-150 1h 20' 67 284.8 168	Site W2 38° 44' 425" N 8° 29' 025" E 1 2013 100-120 1h 50' 110 648.74 1287	Site W3 39° 05' 345" N 8° 10' 538" E 1 2013 80-100 2h 49 150.31 215	Site W4 38° 59' 064" N 8° 07' 270" E 1 2013 150-160 2h 57' 87 330.15 481
Coordinates N. of dives Year Depth range (m) Time filming (h) N. of frames Frames area(m²) N. of coral colonies Coral abundance (colonies m²² ± s.e.)	Site W1 39° 10' 122"N 8° 06' 133" E 1 2013 140-150 1h 20' 67 284.8 168 1.36 ± 0.34	Site W2 $38^{\circ} 44' 425"$ N $8^{\circ} 29' 025"$ E 1 2013 100-120 1h 50' 110 648.74 1287 4.98 ± 0.55	Site W3 39° 05' 345" N 8° 10' 538" E 1 2013 80-100 2h 49 150.31 215 5.53 ± 0.36	Site W4 $38^{\circ} 59' 064"$ N $8^{\circ} 07' 270"$ E 1 2013 150-160 2h 57' 87 330.15 481 1.8 ± 0.15

 Table 2.1. Geographical, technical details and coral community features of investigated sites.

2.2.3 Coral community composition and levels of anthropogenic pressure

We focused our investigation to the analysis of large Anthozoans as they represent the most conspicuous component of Mediterranean animal forests(Rossi., 2013, Cau et al. 2015); the relatively easy identification from photographic frames extracted from the ROV footage, their ecological relevance and their preference for specific environmental conditions including, among the others, the slope of the substrate or the current regime, make them a suitable descriptor of the megabenthic communities in the twilight zone. Moreover, the occurrence of certain species can be used as an indicator of good environmental status of the entire benthic community and their longevity may also provide information about the persistence of stable assemblages (Bo et al., 2009, 2012, 2015).

Alpha and *beta* biodiversity were estimated on counts of most representative species (Fig. 2.2) : four antipatharians belonging to four families [*Antipathella subpinnata* (Ellis and Solander., 1786) (fam. Myriopathidae), *Antipathes dichotoma* (Pallas, 1766) (fam. Antiphatidae), *Parantipathes larix* (Esper, 1790) (fam. Schizopathidae) and *Leiopathes glaberrima* (Esper, 1788) (fam. Leiopathidae)]; seven gorgonian species [*Callogorgia verticillata* (Pallas, 1766) (fam. Primnoidae), *Viminella flagellum* (Johnson, 1863) (fam. Ellisellidae), *Acanthogorgia hirsuta* (Gray, 1857) (fam. Acanthogorgiidae), *Corallium rubrum* (Linnaeus, 1758) (fam. Corallidae), *Eunicella cavolinii* (Koch, 1887) (fam. Gorgoniidae), *Bebryce mollis* (Philippi, 1842) (fam. Plexauridae), and *Villogorgia bebrycoides* (Koch, 1887) (fam.Plexauridae)]; and the soft coral *Chironephtya mediterranea*n. sp. (Fam. Alcyoniidae). *C. mediterranea*, though only occasional observed in our study, was added to the pool of the above mentioned twelve target species as this species has been only recently documented in the Mediterranean Sea (López-González et al., 2014), and our data represent a contribution to the comprehension of its distribution.

bebrycoides and *Bebryce mollis* on the sole basis of ROV images (Bo et al., 2012), the two species were pooled together and hereafter referred to as the *B. mollis-pool*. Most of the selected species are long-living, tall and highly branched corals, which are representative of a mature status of the animal forest (Fig. 2.2; Bo et al., 2012; Rossi., 2013). From the same frames used for the biodiversity analysis (i.e.,669), the level of anthropogenic pressure was also estimated. Litter lying on the bottom was counted and divided in two macro categories: recreational items (REi) and fishing gears (FGe).

2.2.4 Statistical analyses

Once the non-normal distribution of the dataset was verified through the software XLSTAT (function 'normality test'; P<0.0001), a 2-way non-parametric uni-variate analysis of variance was performed using the PERMANOVA routine (software PRIMER 6+, Plymouth Marine Laboratory), in order to analyze differences in i) total coral abundance (Bray-Curtis distance based similarity matrix of sqrt(x) transformed data) and ii) community composition (Bray-Curtis distance similarity matrix of sqrt(x) transformed data). The design used location (L, with two fixed levels) and site (with four random levels nested in L) as sources of variation.

The analysis was carried out with the PERMANOVA routine based on Bray-Curtis resemblance matrixes of either presence/absence and sqrt(x) transformed abundance data. Differences in the compositions of the coral communities between locations are illustrated using the bi-plot produced after canonical analysis of the principal coordinates (CAP; Anderson & Willis., 2003) using the same distance/ similarity matrices used for the PERMANOVA routine.

CAP was chosen as flexible method for constrained ordination on the basis of any distance or dissimilarity measure, which will display a cloud of multivariate points by reference to a specific *a priori* hypothesis, in our case, differences among coral communities dwelling in different locations and sites (previously discriminated using the same routine), showing different geomorphological features.

To identify the taxa explaining the largest fractions of variance between locations and sites, SIMPER analyses were carried out based on either square root-transformed abundance data with 90% cut-off or on a presence/absence data matrix. Variations in the coral community composition were, then, correlated with an array of environmental variables through constrained multivariate ordination analyses (Canonical Correspondence Analysis - CCA) of coral assemblages using the software XLSTAT. The environmental variables included in the CCA were slope of the substrate (covariate), water depth (covariate), silt (expressed as categorical percentage range, from 1 to 5).



Figure 2.2. Coral assemblages of investigated sites.A) Vertical wall colonized by small colonies of *C. rubrum*; B) Arborescent colonies of black coral *A. subpinnata*; C) Specimens of *A. dichotoma* and *P. larix*; D) Three big colonies of *A. hirsuta*; E) One big colony f red coral *C. rubrum* surrounded by colonies of *E. cavolinii* and *V. bebrycoides* (*B. mollis*-pool); F) A stretch of sea floor covered by *C. mediterranea*; G) Meadow of *V. flagellum* surrounded by colonies of the fan-like gorgonian *C. verticillata*; H) Several colonies of *E. cavolinii* and *C. verticillata* around a big colony of *C.rubrum*. In all pictures, a 10 cm scale (white bar) is provided.

2.3 Results

Within eastern location, E1 and E2 showed an average slope of the substrate of ca. 45° and 32° respectively, with a coverage of sediment comprised between 20 and 40%. A higher accumulated sediment was observed in E3 and E4 (*i.e.*, 40-60% for both sites), while the average slope was ca. 39° and 41° respectively. Within western locations,W1, W2 and W4 showed an overall low complexity of the habitat, with an average slope always lower than 5° (*i.e.*, ca. 5° , 1° and 3° , respectively) with a maximum of 13° observed in W3. W1, W3 and W4 showed a consistent accumulation of sediment, comprised between 40 and 60% for W1 and W3 and 80-100% for W4. On the contrary, W2 did not show any accumulated sediment (*i.e.*, 0% in all examined frames).

2.3.1 Anthozoans abundance and community composition

The area of each frame, estimated *a posteriori*, was on an average 3.37 ± 0.16 m², covering a total analyzed surface of ca. 2257 m². A total of 5391 colonies of target corals were cumulatively observed in the 8 sites. The highest total coral abundance (Fig. 2.3) was observed in E4 (12.8 ± 1.5 colonies m⁻²), whereas the lowest was observed in W1 (1.36 ± 0.4 colonies m⁻²) (Fig. 2.3). Since the PERMANOVA showed significant variations in the total coral abundance among sites within each location (P < 0.001), but not among locations (P = 0.347; Tab. 2.2), we analyzed separately the abundance of each single taxa separately for E and W sites.

Total coral abundance



Figure 2.3.Total coral abundance. Boxplot showing differences of the total coral abundance (n.col $m^{-2} \pm st.dev$) among different sites from eastern and western locations

In E4, four over the twelve target species were observed: the precious red coral *Corallium rubrum* was the most abundant species $(14.5 \pm 2.1 \text{ col m}^{-2})$ followed by *E. cavolinii* and *C. verticillata* $(1.5 \pm 0.2 \text{ col m}^{-2} \text{ and } 0.05 \pm 0.02 \text{ col m}^{-2}$, respectively). Among the black coral species, which were consistently less abundant than the gorgonians, *A. subpinnata* was the most abundant one $(0.27 \pm 0.12 \text{ col m}^{-2})$. *C. rubrum* was the most abundant species $(4.08 \pm 0.7 \text{ col m}^{-2})$ also in E1, which was also characterized by the highest abundance of black corals among all investigated sites, with $1.58 \pm 0.32 \text{ col m}^{-2}$ of *A. subpinnata* (Fig. 2.4). Sites E3 and E2 were characterized by similar values of total coral abundance, but also by rather different community compositions. In more details, E3 was characterized by the large dominance of *C. rubrum* $(4.16 \pm 1.2 \text{ col m}^{-2})$ and the presence of only few and sparse colonies of *B. mollis-pool* and *E. cavolinii* (cumulative abundance < 0.1 col m^{-2}). *A.*

dichotoma, with < 0.1 col m⁻², was the unique black coral observed in E3. The coral community in E2 was dominated by *E. cavolinii* (1.38 \pm 0.27 col m⁻²)followed by *V. flagellum* (0.8 \pm 0.33 col m⁻²) and another four gorgonians including *C. rubrum* (0.51 \pm 0.26 col m⁻²), *A. hirsuta* (0.14 \pm 0.07 col m⁻²), *B. mollis-pool* (0.12 \pm 0.05 col m⁻²) and *C. verticillata* (0.11 \pm 0.07 col m⁻²).

Among western sites, W1 showed the presence of seven target species, with *A. hirsute* as the most dominant one $(1.15 \pm 0.17 \text{ col m}^{-2})$, followed by *E. cavolinii* $(0.68 \pm 0.15 \text{ colonies} \text{ col m}^{-2})$. The remaining few and sparse gorgonians showed very low abundance values (cumulatively < 0.1 col m⁻² of *B. mollis-pool* and *C. rubrum*). *P. larix* was the largely dominant black coral $(0.53 \pm 0.12 \text{ colonies m}^{-2})$, but also *A. subpinnata* and *A. dichotoma* were observed (cumulative abundance <0.1 col m⁻²).

W2 was characterized by the highest number of target species among all investigated sites the dominance of *E. cavolinii* and *C. verticillata* $(3.13 \pm 0.34 \text{ and } 3.04 \pm 0.399 \text{ col m}^{-2}$, respectively), and a very high density of *C. mediterranea* $(1.80 \pm 0.30 \text{ col m}^{-2})$ and *V. flagellum* $(1.63\pm0.52 \text{ col m}^{-2})$. *C. rubrum* $(0.57 \pm 0.10 \text{ col m}^{-2})$, *A. hirsuta* $(0.2 \pm 0.04 \text{ col m}^{-2})$ and *B.mollis-pool* $(0.70 \pm 0.16 \text{ col m}^{-2})$ were also present in this site. Only few and sparse specimens of the black corals *A. subpinnata* and *P. larix* were found with a cumulative abundance < 0.1 coloniesm⁻².

W3 was characterized by the highest value of total coral abundance among the western sites, showing the presence of 6 target species. *E. cavolinii* was the dominant species (3.11 \pm 0.76 col m⁻²), followed by *C. rubrum* and *P. clavata* (0.16 \pm 0.03 and 0.31 \pm 0.02 col m⁻², respectively); also *A. hirsuta, C. mediterranea* and *A. dichotoma* were observed, all showing cumulative abundances lower than 0.1 col m⁻².

Five out of the thirteen target species were found over W4. However, apart for *C*. *verticillata*, which was the dominant species $(5.25 \pm 0.36 \text{ col m}^{-2})$ with more than 457

counted colonies; remaining species were few and sparse gorgonians (*C. rubrum, B. mollis-pool*) and black corals (*A. subpinnata, P. larix*) with abundance values lower than 0.1 col m⁻²(Fig. 2.4).Though not considered in the biodiversity analysis, we notice here the presence of the bamboo coral *Isidella elongata*, which occurred in few sparse colonies over the gently sloping walls of this site, characterized by the presence of large silt deposits.





Figure 2.4. Community composition. Percentage histogram showing community composition of different sites.

2.3.2 Deepwater corals beta diversity

The PERMANOVA tests ran using either the coral abundance or the presence/absence matrix reveal that the factor site is the unique significant source of variance in the composition of the target coral communities (Tab. 2.2-2.3), while the bi-plot produced after the CAP analysis, did not shows any segregation of eastern sites from western (Fig. 2.5). Indeed, the cross validation test obtained after the CAP emphasized how only near 60% of sites followed the *a priori* assignation.

Table 2.2.Results of the PERMANOVA testing for the effect of locations and sites on the total coral abundance; df = degrees of freedom; MS = mean square; Pseudo-F = Permutational F (*** = ≤ 0.001 ; * = ≤ 0.05 ; ns= not significant).

PERMANOVA				
Source	df	MS	Pseudo-F	Р
Location	1	4011.3	1.1855	ns
Site(Location)	6	11577	15.448	***
Res	491	749.44		
Tot	498			

Table 2.3.Results of the PERMANOVA testing for the effect of location and site on composition of the coral communities based on abundance and presence/absence matrixes; df = degrees of freedom; MS = mean square; Pseudo-F = Permutational F; ***=P<0.001; N.S.= not significant).

Abundance matrix				
Source	df	MS	Pseudo-F	Р
Location	1	$1.860 \cdot 10^{5}$	2.306	ns
Site(Location)	6	94836	52.246	***
Residual	491	1815.2		
Total	498			
	Presen	ce/absence mat	rix	
Source	df	MS	Pseudo-F	Р
Location	1	$1.590 \cdot 10^{5}$	2.142	ns
Site (Location)	6	87182	37.535	***
Residual	491	2322		
Total	498			



Figure 2.5. Canonical analysis of the principal coordinates. Bi-plot produced after the multivariate analysis showing segregation of sites from different locations according to: A) environmental parameters (i.e., slope of the substrate, depth, latitude, long and B) variations in abundance of target coral species

These results plausibly explain the lack of significant differences among locations obtained after the PERMANOVA test. The SIMPER analysis carried out on the presence/absence data shows that *C. verticillata, C. rubrum* and *E. cavolinii* contribute (altogether) to 67% of the dissimilarity (Tab. 2.4) between locations. The same analysis performed on the abundance data matrix shows the same result: *C. rubrum*, (explaining 29.9 % of the dissimilarity), *E. cavolinii* (21.6 %) and *C. verticillata* (21.4%; Tab. 2.4).

Table 2.4.Results of the SIMPER analyses from abundance data matrix and presence/absence data matrix showing dissimilarities between the two investigated locations and the contribution of the different species to the observed differences.

Abundance matrix				
Contrast	Dissimilarity (%)	Species	Contribution (%)	Cumulative (%)
East vs. West	92.94	C. rubrum	29.9	29.9
		E. cavolinii	21.6	51.5
		C. verticillata	21.4	72.9
		P. larix	5.9	78.8
		A. subpinnata	5.5	84.3
		A. hirsuta	4.7	89.0
		C. mediterranea	3.9	92.9
		B. mollis	2.7	95.6
		V. flagellum	2.5	98.1
		P. clavata	1.5	99.6
		A. dichotoma	0.4	100.0
		L. glaberrima	0.0	100.0

Presence / absence matrix				
Contrast	Dissimilarity	Species	Contributio	Cumulative
	(%)	-	n (%)	(%)
East vs. West	85.21	C. verticillata	23.8	23.8
		C. rubrum	22.3	46.8
		E. cavolinii	20.2	67
		A. hirsuta	8.2	75.2
		A. subpinnata	6.4	81.5
		C. mediterranea	4.7	86.2
		B. mollis	4.7	90.9

Among the four eastern sites, differences in the benthic coral assemblages were mostly explained by variations in the abundance of the species *C.rubrum*, which was up to 3 times higher in E3 than in E2, and more than 25% more abundant compared to other eastern. Among the four western sites, differences in the composition of coral assemblages were mostly explained by variations in the abundance of *C.verticillata* (overall contribution of dissimilarity of 32.11%), which was almost totally dominant (>95%) in W4.

Table 2.5.Results of the SIMPER analyses from abundance data matrix showing dissimilarities between the eastern sites and the contribution of the different species to the observed differences.

	Dissimilarity		Contribution	Cumulativa
Contrast	(%)	Species	(%)	(%)
El vs E2	89.69	C rubrum	39.33	39.33
L1 V3. L2	07.07	E. cavolinii	25.62	6/ 95
		A subninnata	20.13	85.08
		V flagellum	7 268	02.35
		C verticillata	3 159	95 51
		R mollis	2 623	08.13
E1 vc E3	83.03	C rubrum	62.0	62 0
E1 VS. E5	85.05	C. Tubrum E. aquolinii	18.1	80.1
		L. cuvolimi A subpinnata	17.7	07.8
		A. subplitudia C. verticillata	1 00	00.8
		C. veriiciiiaia	0	100
		D. MOULS	0	100
El va E4	60.2	P. ciavaia	64.4	100 64 4
E1 VS. E4	09.2	C. <i>Tubrum</i>	04.4	04.4
		A. subpinnaia	29.5	95.9
		E.cavolinii	2.9	90.8
		A. alcholoma	1.8	98.0
		B_mollis	1.0	1.4
F2 F2	05 54	P. clavata	0	100
E2 vs. E3	85.54	C. rubrum	49.4	49.4
		E. cavolinii	33./	83.1
		V. flagellum	6.1	89.2
		C. verticillata	4.1	93.3
		A. subpinnata	3.2	96.4
50 54	00.40	B. mollis	2.1	98.6
E2 vs. E4	80.18	E. cavolinii	39.6	39.6
		C. rubrum	36.2	75.8
		V. flagellum	10.6	86.4
		C. verticillata	5.1	91.5
		B. mollis	4.8	96.4
		A. hirsuta	2.3	98.7
E3 Vs. E4	79.88	C. rubrum	64.0	64.1
		E. cavolinii	26.8	90.9
		A. subpinnata	4.0	95.0
		C. verticillata	3.1	98.1

	Dissimilarity		Contribution	Cumulative
Contrast	(%)	Species	(%)	(%)
W1 vs. W2	93.7	E. cavolinii	23.5	23.4
		C. rubrum	18.9	42.4
		C. verticillata	17.5	59.9
		C. mediterranea	10.7	70.7
		A. hirsuta	10.2	80.9
		P. larix	7.1	88.0
W1 vs. W3	88.84	E. cavolinii	48.3	48.3
		C. rubrum	24	72.3
		A. hirsuta	18.9	91.2
		P. larix	3.6	94.9
		P.clavata	3.4	98.3
		A. dichotoma	1.1	99.3
W1 vs. W4	98.5	C. verticillata	50.3	50.3
		C. rubrum	22.0	72.3
		A. hirsuta	11.3	83.7
		E. cavolinii	12.4	92.5
		P. larix	1.6	97.9
		B. mollis	1.1	99.0
W2 vs. W3	86.4	E. cavolinii	40.0	40.0
		C. verticillata	20.1	60.1
		C. mediterranea	12.4	72.6
		C. rubrum	8.0	80.6
		B. mollis	6.6	87.3
		V. flagellum	5.5	92.9
W2 vs. W4	82.8	C. verticillata	39.5	39.5
		E. cavolinii	20.8	60.3
		C. mediterranea	10.1	70.4
		B. mollis	6.9	77.4
		C. rubrum	6.9	84.3
		V. flagellum	5.5	89.9
W3 vs. W4	99.4	C. verticillata	52.5	52.5
		E. cavolinii	38.0	86.9
		C. rubrum	3.0	93.7
		P. clavata	2.8	96.6
		P.larix	1.9	98.6
		B.mollis	1.0	99.7

Table 2.6.Results of the SIMPER analyses from abundance data matrix showing dissimilarities between the western sites and the contribution of the different species to the observed differences.

The CCA revealed that the combination of all included environmental factors explains about 82.58% of the total variations in the composition of target coral assemblages of the eight sites. The bi-plot (Fig. 2.6) generally discriminate eastern sites from western sites, apart from E2. In detail, E1, E4 and E3 are segregated from other sites along the slope factor axis, which led to a higher abundance of *C. rubrum* and *A. subpinnata*, and also along the factor impact (E3, particularly). In the same way, W1 and W4 can be found along the depth axis and are characterized by low current species such as *P. larix, C. verticillata* and *A. hirsuta*. On the other hand, E2 can be found along the distance from the coast axis, while W2 and W3 did not follow any of the axes produced by the by-plot. However, all three sites are displayed in a separated quadrant from other sites and are characterized by the abundance of *B. mollis-pool, C. mediterranea* and *E.cavolinii*.



Figure 2.6. Canonical Correspondence analysis. Bi-plot produced after the multivariate analysis showing segregation of sites according to environmental factors.

2.3.3 Anthropogenic pressure

Regarding anthropogenic pressure, the eastern sites were considerably more impacted (cumulatively 69 items) compared to the western ones (10 items; Tab. 2.7). W3 is the only litter-free site for both REi and FGe. Notably, all other sites were characterized by the presence of FGe. The most impacted site was E3 (n. 33 FGe and n. 4 REi). About 73% of FGe items were lost trammel nets (n=24). E2 is the second most impacted site (with 3 Rei and 16FGe). Despite the high number of litter, only two nylon lines have been found rolled on two coral gorgonian colonies but without evident signs of damage. Overall only a few colonies (<0.1% of the observed specimens) were visually damaged.

Table 2.7. Anthropogenic pressure analyses. Number of items (n) for the two categories of litter: recreational items (REi) and fishing gear (FGe).

Sites	REi (n)	FGe (n)	Tot
W1	1	4	5
W2	1	3	4
W3	0	0	0
W4	0	1	1
E1	0	7	7
E2	3	16	19
E3	4	33	37
E4	0	6	6

2.4 Discussion

Our investigation provides comparative analysis among deep coral forests dwelling along contrasting geological features of the continental shelf, addressing patterns of biodiversity through the use of different spatial scales, which is an urgently needed tool for assessing sound *in situ* conservation strategies (Casas-Güell et al., 2015).

Because of their habitat heterogeneity, hydrodynamic and accumulation of organic matter, canyons are predicted to support a higher diversity and abundance than the adjacent slope (Ramirez-Llodra et al., 2010b). However, this statement is not always true, as no significant differences in biomass and abundance of benthic fauna between the slope and canyon habitat (Houston & Haedrich., 1984) or lower biomass and abundance in the canyon have been reported (Maurer et al., 1994). The modified currents within the canyon can shape faunal distribution patterns by modulating availability of resources or disturbance; in particular, the canyon head and walls can present rocky suitable surfaces for sessile suspension feeders such as cnidarians and sponges, while the axis of the canyon can accumulate soft sediment and have a fauna dominated by deposit feeders (Zúñiga et al., 2009; Ramirez-Llodra et al., 2010b).

On the other hand, if the awareness of seamounts and canyons as biodiversity hot-spot is consolidated (Hamilton., 2009; Misic et al., 2012), only recent studies have proved how even way smaller rocky outcrops could become oases for benthic biodiversity, enhancing ecosystems' overall complexity (Bo et al., 2012; Cau et al., 2015a). Also in these habitats, along with physical protection against the most destructive human practices (e.g., trawling fishery), hydrodynamic and other environmental factors may alter deep coral forest communities(Cau et al., 2015a).

Within eastern location, a noticeable environment was observed in E2 (see also plots from CAP and CCA analysis, Fig. 2.5-2.6). This site showed the presence of large amounts of accumulated dead *Posidonia oceanic* (Fig. 2.7 C, D, E), transported from neighboring shallow waters, along with thousands of specimens of *Plesionika spp* shrimps (Fig. 2.7 A, B). Submarine canyons support high biodiversity, prodigious biological productivity and conduits for transport of particulated organic carbon from the coast or shelf to bathyal or abyssal depths (Sardà et al., 2009; Zúñiga et al., 2009). Our observations could possibly emphasize a strong 'flushing effect' (Canals et al., 2006; Pusceddu et al., 2010) of this canyon E2 that possibly shaped the coral community of this site and surely deserves further
investigations. The reason for this particularly consistent transport might be the closer position to the coastline (see CCA analysis), compared to other investigated sites. The different species composition in this site may reflect the high variability that can be found among canyons.

Within the western location, 3 out of 4 western sites were covered by silt (>50 % of coverage on average) and this might explain the low abundance of species like *C. rubrum* or *A. subpinnata* that, on the other hand, dominate the eastern location. These sites showed a higher abundance of the fan like gorgonian *C. verticillata* (W4) and *E. cavolinii* (W3).*E. cavolinii* showed a wide range of abundance (up to 43 col m⁻²), similarly to *C. rubrum* in eastern site (up to 100 col m⁻²); which is usually related to reproduction and recruitment success, on the basis of substrate availability, local turbulence, intra-specific competition or anthropogenic disturbances, shaping population structure accordingly (Sini et al., 2015). In the present study, the co-occurrence of these two species was observed in 37 frames out of >600, contrarily to other observation from NW Mediterranean Sea, where the two species occurred very often, sharing the same habitat (Angiolillo et al., 2015b). As showed by the SIMPER analysis, variations in presence and abundance of these two species, along with the fan-like gorgonian *C. verticillata*, are the main responsible for dissimilarities observed among locations and sites (Tab. 2.4-2.5-2.6).

A remarkable finding was the presence of the bamboo coral *Isidella elongate* over heavily silted rocky surfaces of the site W4; this species is an important component of the soft bottom animal forests of the circa-littoral zone of the Mediterranean Sea (Cartes et al., 2013). W2 is the only western site that is not covered by silt (*i.e.*, 0%) and shows a higher heterogeneity in the species composition compare to other sites. The complex hydrography and current system of the zone, which is interested by transit and formation of anticyclonic eddies and gyres (Sorgente et al., 2011; Olita et al., 2013; Martin et al., 2014), could be

invoked as explanation. Another important finding in W2 and W3 is the presence of the recently documented species *C. mediterranea*, which was recently found in Balearic Island as the only known site where this soft coral dwells (López-González et al., 2014).

Overall, while no difference can be observed in the coral abundance, significantly different communities characterize the two locations. Indeed, what is actually of particular interest is the fact that most of the variability observed occurred at the smallest spatial scale investigated: within each location, rather than among putatively different geomorphologies of the continental margin, separated by considerable spatial distance (*i.e.*,>100 km). The 2-level PERMANOVA with nested design, allowed us to emphasize that most of the variability occurs, interestingly, among sites. This was observed for all investigated features of the animal forest: the total coral abundance and the community composition. A similar spatial pattern was observed within shallower coralligenous communities (Casas-Güell et al., 2015), where the highest source of variability was found indeed among sites or even at the replicate level, which refers to spatial scale comprised within tens of meters.

Overall, we document the same interaction of different biological processes shaping the species composition and abundance at each site (or even patches within sites) as reported for shallower areas in NW Mediterranean Sea (Casas-Güell et al., 2015).From a conservation perspective, such spatial variability could deeply alter our capability to detect the effect of natural or anthropogenic perturbations and to predict ecological processes involved at regional scale (Ponti et al., 2011).

The investigation on the human impact revealed how eastern locations were much more impacted compared to western ones. Artisanal fishing, which is very popular in Sardinia, is often associated with the notion of 'coastal fishing': which refers to a fishing effort essentially located on the continental shelf (0–200 m) that exploit areas that can be reached in a few hours from fishermen bases (Follesa et al., 2011; Angiolillo et al., 2015a). In our

case, we can justify the higher impact found along the eastern location with the lower distance from local fishery ports but also with the higher complexity of the seabed, which leads to a higher rate of accidental lost of the fishing gear.

Comparing our results with other areas and/or other investigations conducted in Sardinian waters, we confirm that investigated deep waters appears, overall, less impacted than other realities of the Tyrrhenian Sea(Bo et al., 2014; Angiolillo et al., 2015a) and of North western Mediterranean basin (*i.e.*, Cap de Creus; Purroy et al., 2014).

In conclusion, we show that processes occurring at smaller spatial scales control variability of abundance and biodiversity among locations. If we focus on the applicability of our results for conservation measures, the micro scale variability among sites overcoming the macroscale diversity of locations highlights that a substrate-based policy (where particular topographies are object of conservation rather than others) could not be enough for preserving ecologically or biologically significant areas (EBSA) for benthic biodiversity; indeed, local scale plans should be more appropriate to preserve particular oases of biodiversity. Along with these insights regarding conservation it appears clear the necessity of more detailed and sparse ROV investigations coupled with geomorphological datasets within mesophotic habitats of the Mediterranean basin.



Figure 2.7.Site E2 snapshots. A and B) thousands of specimens of *Plesionika spp* shrimps moving along the canyon walls; C, D and E) large amounts of accumulated dead *Posidonia oceanic* within the canyon head; F) ghost fishing net.

Chapter 3

Spatial distribution of deep-water coral forests in submarine canyons (eastern Sardinia, Western Mediterranean Sea)

3.1 Introduction

Clarify the spatial scales at which biodiversity varies and what are the actual drivers of such variations are crucial issues in ecology, in particular for conservation purposes. These issues are of particular relevance when dealing with spatially heterogeneous environments, including the continental margins of world oceans.

Continental margins, where land becomes ocean and plunges to the deep sea (i.e., below 200 m depth), provide valuable food and energy resources, and perform essential functions such as carbon burial and nutrient cycling (Levin and Dayton., 2009).Continental margins are characterized by a number of topographic distinctive features including open slopes, landslide-affected areas, and submarine canyons (Weaver et al., 2004).

Submarine canyons, in particular, are valleys deeply cut in the continental slope that may extend to the continental rise downwards and to the continental shelf upwards. They are present in all continental margins worldwide (Harris and Whiteway, 2011). Submarine canyons dissect most of Europe's continental margins, with some of them opening their heads at short distance from the shoreline (Canals et al., 2006), and play a fundamental ecological role as they act as conduits for organic matter from the continental shelf to the deep basin (Vetter and Dayton 1998). The surrounding topography and the oceanographic conditions surrounding and inside canyons affect the water currents, generating occasionally dense shelf water cascading phenomena. These, in turn, can at times generate turbulence and sediment resuspension events able organically enrich canyon sediments

and the adjacent bathyal plain (Tews et al., 2004; Puscedduet al., 2010). Furthermore, recent studies suggest that increased habitat heterogeneity associated with canyons is responsible for enhancing both pelagic and benthic productivity, as well as benthic biodiversity (Schlacher*et al.*, 2007; Buhl-Mortensen et al., 2010; Vetter *et al.*, 2010), with faunal abundance and biomass values from 2 to 15 times higher than those encountered in the surrounding areas at the same depths (Danovaro et al., 2010). These characteristics make submarine canyons "hotspots" of biodiversity (Schlacher et al., 2007, 2010; Levin et al., 2010; De Mol et al., 2010;Danovaro et al., 2014).

Communities of benthic suspension feeders, that more take advantage of the environmental characteristics of canyons are dominated by large arborescent Anthozoans. These organisms create important three-dimensional 'animal forests' (*sensu* Rossi et al., 2013) and, through the enhancement of habitat structural heterogeneity, play a key ecological role in the benthic-pelagic coupling processes (Henry & Roberts., 2007; Buhl-Mortensen et al., 2010; Rossi., 2013; Gili and Coma., 1998). Due to their slow growth rates, longevity and tridimensional structure, these species are particularly vulnerable towards mechanical injuries inflicted by fishing gears (Cau et al., 2015; Angiolillo et al., 2015).

Therefore, in highly overexploited oceans, untouched deep-water assemblages are no longer expected to prosper: instead, a great reduction in the original abundance of animal forests as well as in their geographic and bathymetric ranges is expected (Bo et al., 2014, 2015). Such concerns are even more stringent for the Mediterranean Sea (Bianchi et al., 2012). In fact, the Mediterranean Sea canyons owns peculiar features which make them highly vulnerable to human impacts: closely spaced, more dendritic, shorter and steeper than those from other regions of the world (Harris and Whiteway., 2011), with a great variability that can occur for each single canyon (or canyon system) set along the continental margin. In this regard, the eastern coast of Sardinia (Western Mediterranean

Sea), shows all of the above-mentioned features: a narrow continental shelf, deeply incised by a complex submarine canyons system (Würtz et al., 2012).

In the past, the number of studies on submarine canyons was limited as investigations were extremely challenging because of their inaccessibility due to their rough topography. Recent advances in technology such as Remotely Operated Vehicles (ROVs), swath bathymetry, side-scan sonar and definitive position-fixing systems allowed substantial advance in their study through non-invasive procedure based on underwater footage.

Based on the high structural heterogeneity of submarine canyons at multiple spatial scales, we would expect that distribution and biodiversity of coral communities can vary among different submarine canyons as a result of variations in the environmental characteristics that span from punctual (i.e., within each canyon), local (i.e. among canyons at similar latitude) and regional (i.e. among areas at different latitude) scales.

Here, we tested the null hypothesis that the composition of coral assemblages do not differ among canyons within each of three geographical areas (namely northern, central and southern area) and among the three areas located along the continental margin off the eastern coast of Sardinia (Western Mediterranean Sea). We also explored the role of a subset of environmental factors (distance from the coast, water depth, silt coverage and slope of the substrate) in determining the observed patterns in coral community composition over the areas under scrutiny.

3.2 Materials and Methods

3.2.1 Study area

Sardinia is one of the most interesting areas of the Western Mediterranean basin, not only for its geographical location but also for its geomorphological characteristics. The sea floor along the coasts is not homogeneous both for its extension and geomorphologic characteristic. The southern and western coasts are characterized by a wide continental shelf with a gradual decline that ends 200m depth. The eastern coast on the other hand is characterized by a limited continental shelf that terminates at about 60-100m depth, that is connected to the peculiar inland orographic structure and river basin: narrow inlets, interspaced by high and steep mountains, correspond to a very narrow continental shelf with irregular bottoms, with a slope interspaced by very deep canyons (Ulzega and Fais., 1980; Lecca., 2000; Harris and Whiteway., 2011; Würtz et al., 2012) (Fig. 3.1).



Figure 3.1.Marine and Continental geomorphology map of Sardinia island (modified from Ulzega, 1988)

The study area covers the shelf break of the eastern Sardinian continental margin, which represents the passive margin of the Tyrrhenian basin, delimited to the north by the Etruschi seamount, and to the south by the Ichnusa Seamount. Within this area we selected and investigated nine canyons which were *a priori* allocated to three geographical locations: namely Northern, including Caprera, Mortorio, Tavolara canyons (hereafter NC,NM and NT), Central, including Orosei, Gonone, Arbatax canyons (hereafter CO,CG,CA.) and Southern, including Pelau Porto Corallo, Simius canyons (hereafter SP,SC,SS,).

The three northern canyons dissect the considerable width portion of the northern continental shelf (around 20 km) and they are located respectively at ca. 9.27, 7.54 and 7.24 nautical miles from the nearest coast. The mean depth of the three dives conducted in NC was 136 m, with 74% of the analyzed frames for this canyon showing incline and vertical rocky substrate, with value of silt coverage comprise between 20 and 40 %. In NM canyon the average depth of the 3 ROV conducted dives was 127 m, with most of the footages showing an inclined seafloor (68 % of the total frames), with an average of silt coverage again comprise between 20-40%. NM was one of the deepest canyon investigated (max depth 272 m), with most of the transects showing rocky substrate with low percentage of silt coverage (0-20%).

In the central part of the Sardinia margin seven ROV dives were carried out (three in CO, two in CG and two in CA) in order to investigate the large Orosei-Gonone canyon system, characterized by the absent of large rivers, and the narrow Arbatax canyon. CO was the central farthest canyon from the coast as it was located at 6.16 nautical miles from the Orosei bay, while CG and CA were distant respectively at 0.4 and 1.9 nautical from the nearest coastline. The investigated depth range for this area was comprise between 90 and 186 meters (98-186 m in CO, 90-120 in CG and 108-180 in CA). CO was characterized by low level of silt coverage with (87 % of the frames with percentage of silt coverage of 0-20%) and with an high occurrence of slightly inclined substrate (30°-60°). While both CG

and CA were characterized by long and sloping walls with a high presence of small size rocks surrounded by a bottom floor covered by a thick silt deposits.

In the southern canyon group the Pelau, Porto Corallo and Simius canyons were investigated by means of seven ROV dives (two in SP, three in SC and two in SS) at depth ranging from 95 to 145 meter for SP, .from 105 to 280 meter for SC and from 111 to190 meters for SS. Their distance from the coast varied from the closest SP and SS canyons located at ca. 1.1 and 1.4 nautical miles respectively, to the farthest SC canyon located at ca 4.61 nautical miles from the nearest coastline (Fig. 3.2).



Figure 3.2. Map of the investigated canyons along the eastern coast of Sardinian

3.2.2 Sampling

The video material was obtained during two ROV missions conducted along the east and south Sardinia continental margin in October 2011 and August 2013 on board of the Research Vessel "*Astrea*" .ROV campaigns were funded by the department of Environmental and life Science of the University of Cagliari, with the main focus on the investigations of the status of *Corallium rubrum* populations. The remote operate vehicle "*Pollux III*" was used in both campaigns; it was equipped with a digital camera (Nikon D80, 10megapixel), a strobe light (Nikon SB 400), a high definition video camera (Sony HDR-HC7), track-link system, depth sensor, compass, and two parallel laser beams providing a constant 11-cm reference scale in the video frame, for the measurement of the frame area. Each of the nine submarines canyon was investigated through a different number of ROV dives (from a min. of 2 to a max. of 3), to collect enough video material for the further image analysis (Tab 3.1).

The software DVDVIDEOSOFT was used to extract video frames every 30 seconds; overall, a total of 46 hours of ROV footage were analyzed. Frames with non-clear visibility, compromised resolution/focus were discarded in the initial stage of the image analysis.

The analysis was performed with the CPCe software (Kohler and Gill 2006) to obtain coral abundance (number of colonies per col m⁻² \pm standard error, henceforth col m⁻² \pm s.e.) and the species composition of the coral community per each frame. In order to give a better geomorphological characterization of the investigated canyons for each frame was also registered: 1) latitudinal gradient 2) distance from the coast 3) depth, as covariate 4) sediment coverage of the substrate, as covariate, classified using a scale from 1 to 5, referring to 5 percentage ranges (1= 0-20%; 2=20-40%; 3=40-60%; 4=60-80%; 5= 80-

100%); 5) slope of the substrate, as covariate, estimated using a scale from 1 to 3 where 1 was considered as flat (0°-30°), 2 as inclined (30°-60°) and 3 as vertical (>60°).

 Table 3.1. Details of the investigated areas.

	NC	NM	NT
Coordinates	41° 20' 433" N	41°04'152 "N	40° 54' 860"N
Coordinates	9° 38' 121" E	9°47'525" E	9°54' 041" E
N. of dives	2	3	3
Year	2013	2013	2013
Depth range (m)	112-205	100-160	106-272
N. of frames	287	211	339
Frames area(m ⁻²)	797.1	540.9	967.3
N. of coralcolonies	2314	705	1422
Coral abundance	3.2 ± 1.5	1.2 ± 0.1	1.5 ± 0.1
(colonies $m^{-2} \pm s.e.$)	5.2 ± 1.3	1.3 ± 0.1	1.3 ± 0.1
Species richness	9	10	11
	CO	CG	CA
Coordinatas	40° 21' 767"N	40° 21' 825" N	39° 58' 136" N
Coordinates	9° 53' 657" E	9° 53' 670" E	9° 43' 734" E
N. of dives	3	2	2
Year	2013	2013	2013
Depth range (m)	95-188	48-134	108-183
N. of frames	278	110	102
Frames area(m ⁻²)	780.8	219.74	281.31
N. of coralcolonies	1438	79	215
Coral abundance	2.3 ± 0.1	0.4 ± 0.12	0.4 ± 0.00
(colonies $m^{-2} \pm s.e.$)	2.3 ± 0.1	0.4 ± 0.12	0.4± 0.09
Species richness	6	5	6
	SP	SC	SS
Coordinatas	39° 10' 122"N	38° 44' 425" N	39° 05' 345" N
Coordinates	8° 06' 133" E	8° 29' 025" E	8° 10' 538" E
N. of dives	2	2	2
Year	2011	2011	2013
Depth range (m)	110-198	105-275	93-144
N. of frames	119	201	115
Frames area(m ⁻²)	242.5	385.8	140.9
N. of coralcolonies	168	1287	215
Coral abundance	27 ± 0.24	25 ± 0.55	1.4 ± 0.26
(colonies $m^{-2} \pm s.e.$)	2.7 ± 0.34	2.3 ± 0.33	1.4 ± 0.30
Species richness	4	10	7

3.2.3 Biodiversity analyses and human pressure

To characterize the local and regional biodiversity of coral fauna present along the submarine canyons eleven target species were chosen: seven gorgonian species [*Callogorgia verticillata* (Pallas, 1766) (fam. Primnoidae), *Paramuricea clavata* (Risso 1826) (fam.Plexauridae), *Corallium rubrum* (Linnaeus, 1758) (fam. Coralliidae), *Viminella flagellum* (Johnson, 1863) (fam. Ellisellidae), *Acanthogorgia hirsuta* (Gray, 1857) (fam. Acanthogorgiidae), *Eunicella cavolinii* (Koch, 1887) (fam. Gorgoniidae), *Bebryce mollis* (Philippi, 1842) (fam. Plexauridae), and four antipatharians [*Antipathella subpinnata* (Ellis and Solander, 1786) (fam. Myriopathidae), *Antipathes dichotoma* (Pallas, 1766) (fam. Antiphatidae), *Parantipathes larix* (Esper, 1790) (fam. Schizopathidae) and *Leiopathes glaberrima* (Esper, 1788) (fam. Leiopathidae)].

Even though we recognize that the selected Anthozoan species represents a portion of the complex hard-bottom community inhabiting the investigated rocky canyons, we chose them as the target of our investigation because their presence is either supportive of high levels of megabenthic biodiversity and, based on their longevity, indicative of the persistence of stable assemblages (Bo et al., 2012). All of the selected species are long-living organisms representative of animal forests, which are composed highly branched and tall corals that can influence major current flows and particle retaining, thus retaining more zooplankton, eggs, larvae, juveniles and adults of vagile species than in their surroundings (Baillon et al., 2012; Rossi., 2013; Fig. 3.3).

3.2.4 Statistical analyses

To asses differences in the composition of coral community between and different geographic areas 2-way multivariate analysis of variance was performed using the PERMANOVA routine with nested design (software PRIMER 6+, Plymouth Marine Laboratory), using areas (A, with three fixed levels North: Central and South) and canyons (with three random levels nested in A) as sources of variation with n=100 for the combination of factor. Since the PERMANOVA showed significant variations in the composition of coral community among canyons within each area, but not among areas, we then run 1-way PERMANOVA analyses using canyons as only source of variation, separately for the three areas. These analyses were based on resemblance matrix using the Gower exc 0-0 similarity index after square-root transformation of the abundance data. Gower coefficient is well-suited for quantitative abundance data excluding double-zeros from comparison (Legendre and Legendre., 1998).

Within each investigated area Distance–based Linear Model (DistLM, Legendre., 1999) procedure was performed to analyze the relationship between the abundance of the eleven target species and environmental variables (*i.e.* depth, latitude, distance from the coast, percentage of sediment coverage and slope). DistLM is a regression analysis that models the relationship between a resemblance matrix (*e.g.*, Gower similarity matrix) and a set of variables, which in this study were a range of environmental variables.

Furthermore canonical analysis of the principal coordinates (CAP; Anderson& Willis, 2003) was carried out, using the same distance/ similarity matrices used for the PERMANOVA routine, in order to graphically represent differences in the compositions of the coral communities between canyon in each investigated area.

A similarity percentage analysis (SIMPER) was subsequently employed to reveal which species contributed the most to the dissimilarity within and among the assemblages identified by the PERMANOVA analysis to be significantly different. Simper analyses were carried out based on the square root-transformed abundance data with 90% cut-off.

Multivariate analysis of variance, PERMANOVA, CAP, DISTLM, and SIMPER analyses were carried out using routines included in thePRIMER6 + software package (Plymouth Marine Laboratory).



Figure 3.3. Coral assemblages of investigated canyons. A) Vertical wall colonized by big fan-like specimens of *P. clavata;* B) Arborescent colony of black coral *A. subpinnata* with few colonies of *E. cavolinii* and *C. rubrum;* C) Inclined wall with several colonies of *E. cavolinii;* D) Meadow of *V. flagellum* with few specimens of porifera *C. compressa* and *C. monolifera* on the background; *E)* Two colonies of the bottle-brush black coral *P. larix* together with few sponges *P. compressa* F) Dense patches of red coral *C. rubrum* with a specimen of *P. elephas* at the base of the wall. G) Dense assemblage of two phenotype of black coral *L. glaberrima;* H) Isolated colony of black coral *A. dicothoma* and *L. glaberrima.* A 10 cm scale (white bar) is provided in each picture.

3.3 Results

3.3.1 Variations among areas and canyons

The results of the PERMANOVA test show that the composition of coral communities did not differ among the north, central and south areas, but reveal the presence of significant differences among canyons within each area (Tab 3.2).

Table 3.2. Results of the PERMANOVA testing for differences in the composition of coral assemblages among areas and canyons within areas Gower exc 0-0 similarity matrix; df = degrees of freedom; MS = mean square; Pseudo-F = Permutational F; ***=P<0.001; ns = not significant.

Abundance matrix								
Source	df	MS	Pseudo-F	Р				
Area	2	6284,2	1,2275	ns				
Canyon (Area)	6	7321,8	10,624	***				
Residual	519	687,99						
Total	527							
	Presence	/absence ma	trix					
Source	df	MS	Pseudo-F	Р				
Area	1	58753	1,1471	ns				
Canyon (Area)	6	58188	18,09	***				
Residual	519	3216,6						
Total	527							

The results of the 1-way PERMANOVA tests carried out separately for each area reveal the presence of significant differences in the composition of coral communities among different canyons (Tab 3.3).

Table 3.3. Results of the PERMANOVA testing for differences in the composition of coral assemblages among canyons within each area; df = degrees of freedom; MS = mean square; Pseudo-F = Permutational F; ***=P<0.001; ns = not significant.

Northernarea								
Source	df	MS	Pseudo-F	Р				
Canyon	2	13202	16,833	***				
Residual	224	784,3						
Total	226							
Central area								
Source	df	MS	Pseudo-F	P(perm)				
Canyon	2	10812	14,435	***				
Residual	282	748,97						
Total	284							
Southern area								
Source	df	MS	Pseudo-F	P(perm)				
Canyon	2	3334,1	2,8569	***				
Residual	210	1167						
Total	212							

The CAP outputs show a generally weak segregation pattern among the three northern canyons, with a pronounced level of overlapping and a high dispersion of the data. The strongest segregation can be observed between NT and NM (Fig. 3.4A). The SIMPER analysis reveal that variations within each northern canyon are mostly due to changes in the abundance of *Eunicella cavolinii, Corallium rubrum* and *Antipathes subpinnata,* whereas differences in the composition of the coral communities among the three northern canyons are mostly explained by variations in the abundance of *C. rubrum* (NM vs. NC and NM vs. NT), and *E. cavolinii* (NC vs. NT) (Tab 3.5A). The SIMPER analysis also show that the range of dissimilarity in the composition of coral assemblages at the scale of area (84%-90%) is slightly higher than that at the scale of each single northern canyon (80 %- 87 %; Fig. 3.5A).

In the central area, CAP analysis show a more evident segregation pattern among the three canyons, with a relatively low level of overlapping between CO and CG, and a clear segregation between CG and CA (Fig. 3.4B). The SIMPER analyses reveal that variations within each central canyon are mostly due to changes in the abundance of *E. cavolinii, C. rubrum* and *Parantipathes larix*, while differences in the compositions of the coral communities are mostly explained by variations in the abundance of the gorgonian *E. cavolinii* among all the three central canyons(Tab. 3.5B). From the SIMPER analyses also emerge that the range of dissimilarity of coral assemblages at the scale of area (61%-93%) is higher than that at the scale of each single central canyon (66%-80%) (Fig. 3.5B).

In the southern area, segregation among the three canyons, in spite of the significant effects identified by the formal PERMANOVA, were very weak, with high levels of overlapping between all of the three canyons (Fig. 3.4C). The results of the SIMPER analyses show that variations within each southern canyon are mostly due to changes in the abundance of *E. cavolinii*(SP and SS) and *V. flagellum* (SC), whereas differences in the composition of

the coral communities among the three southern canyons are mostly explained by the variation in the abundance of *E. cavolinii* colonies (Tab. 3.5C). The SIMPER analysis also show that the range of dissimilarity in the composition of coral assemblages at the scale of area (72%-79%) is slightly higher than that at the scale of each single southern canyon (70 %- 75 %; Fig. 3.5C).



Figure 3.4. Canonical analysis of the principal coordinates illustrating variations in the composition of coral communities among canyons from north (A), central (B) and southern (C) areas.

Table 3.5. Results of the SIMPER analyses showing dissimilarities within and between central canyons of the target-coral species mostly responsible for the observed differences in the northern (A), central (B) and southern (C) areas. Contr. (%) percentage of explained dissimilarity attributable to the i species. Cum. (%) cumulative percentage of explained variance.

A. Northern Area

Within canyons				Among canyons					
Canyon	Diss. (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)	Contrast	Diss. (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)
NC	79.7	E. cavolinii	48,2	48,2	NC vs. NM	84.5	C. rubrum	23,2	23,2
		V. fagellum	16,2	64,5			E. cavolinii	21,0	44,2
		C. verticillata	14,6	79,1			V. flagellum	14,4	58,7
NM	65.3	C. rubrum	69,2	69,2	NC vs. NT	89.8	E. cavolinii	18,4	18,4
		E. cavolinii	15,1	84,3			C .rubrum	12,4	30,9
		A. subpinnata	6,4	90,7			A.subpinnata	12,4	43,4
NT	87.2	A. subpinnata	32,7	32,7	NM vs. NT	87.9	C. rubrum	23,5	23,5
		C. rubrum	13,6	46,3			A. subpinnata	15,4	38,9
		L.glaberrima	11,9	58,3			E. cavolinii	14,6	53,6

B. Central Area

Within canyons					Among canyons				
Canyon	Diss (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)	Contrast	Diss. (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)
СО	65.9	E. cavolinii	62,2	62,2	CO vs. CG	70.5	E. cavolinii	29,9	29,9
		C. rubrum	24,4	86,7			C. rubrum	25,9	55,8
		A. subpinnata	13,2	99,9			A. subpinnata	20,3	76,2
CG	61.9	E. cavolinii	88,2	88,2	CO vs. CA	93.5	E. cavolinii	27,3	27,3
		P. clavata	11,7	100			C. rubrum	18,2	45,5
							A. subpinnata	16,0	61,5
CA	80.5	P. larix	44,1	44,1	CG vs. CA	96.8	E. cavolinii	31,4	31,4
		C. verticillata	24,1	68,2			P. clavata	15,2	46,6
		A. dichotoma	20,1	88,3			P. larix	12,2	58,9

C. Southern Area

	W	ithin canyons					Among canyons		
Canyon	Diss. (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)	Contrast	Diss (%)	Species <i>i</i> responsible	Contr. (%)	Cum. (%)
SP	69.3	E. cavolinii	66,8	66,8	SP vs. SC	75.3	E. cavolinii	31,2	31,2
		V. flagellum	24,4	91,2			C. rubrum	27,8	59,1
							A. subpinnata	12,6	71,7
SC	75	V. flagellum	57,2	57,2	SP vs. SS	72	E. cavolinii	31,7	31,7
		E. cavolinii	33,1	90,4			C. rubrum	26,1	57,8
							A. subpinnata	14,9	72,7
SS	73	E. cavolinii	84,0	84,0	SC vs. SS	78.9	E. cavolinii	30,6	30,6
		V. flagellum	8,0	92,1			P. larix	24,8	55,4
							P. clavata	10,6	66,1







Figure 3.5. Dissimilarity in the composition of coral assemblages within each canyon and among canyons in the northern (A), central (B) and southern (C) areas

3.3.2 Relationships between coral assemblages and environmental setting

The results of the distance-based linear model (DistLM) reveal that, in all of the three areas distance from the coast and water depth explain significant proportions of variation in the coral community composition among canyons wheareas variables that contributed least were silt and slope of the substrate (always <2% of the total variation) (Tab.3.5).

Table 3.5. Results from the Distance based multivariate analysis for a linear model (DISTLM). The following abbreviations are used: Pseudo-F = Permutational F; ***=P<0.001; ns = not significant; Prop. (%) percentage of explained variation; Cumul. (%) cumulative percentage of total variation.

Northern area				
SEQUENTIAL TESTS				
Variable	Pseudo-F	Р	Prop. (%)	Cumul. (%)
Distance from the coast	25,94	***	10%	10%
Water Depth	10,66	***	9%	19%
Silt coverage	4,304	*	1,6%	20,6%
Slope of the substrate	2,567	*	1%	21%
Central area				
SEQUENTIAL TESTS				
Variable	Pseudo-F	Р	Prop.	Cumul (%).
Water Depth	26,46	***	9%	9%
Distance from the coast	30,33	***	8%	18%
Silt coverage	5,41	*	1,5%	19%
Southern area				
SEQUENTIAL TESTS				
Variable	Pseudo-F	Р	Prop.	Cumul.(%)
Water Depth	7,99	***	9%	9%
Distance from the coast	4,77	***	8%	18%
Silt coverage	2,13	*	1%	19%

3.3.3 Coral assemblage composition in the investigated canyons

The percentage contribution of the eleven taxa considered in this study in all of the canyons under scrutiny are illustrated in Fig. 3.6A-C.

Among the three northern canyons, NC was the farthest from the coastline (9.27 Nm). NC is characterized by the highest mean total coral abundance among all the nine investigated

canyons $(3.21 \pm 0.2 \text{ col m}^{-2})$, and the presence of nine over the eleven target species observed (Fig. 3.5A). In NC, *facies* of the gorgonian *E. cavolinii* $(1.11 \pm 0.2 \text{ col m}^{-2})$ along with numerous patches of the red coral *C. rubrum* $(1.04 \pm 0.7 \text{ col m}^{-2})$ were documented dwelling several slightly inclined rocky wall. Patches of *Viminella flagellum* $(0.55 \pm 0.3 \text{ col} \text{ m}^{-2})$ together with the black coral *P. larix* $(0.17 \pm 0.05 \text{ col m}^{-2})$ occurred over the most silted surfaces of NC. Among the remaining target species found, the two black corals *A. subpinnata, A. dichotoma* and the gorgonian *C. verticillata, A.* hirsuta and *B. mollis* showed a scattered and isolated distribution, each with mean abundances< 0.1 col m⁻².

In NM canyon the mean of the total coral abundance was of 1.38 ± 0.09 col m⁻² with the presence of ten out of the eleven species documented (Fig. 3.5A). Discontinuous and numerous patches of the whip-like coral *V. flagellum* (0.15 ± 0.09 col m⁻²) associated with few colonies of the black coral *P* .*larix* (<0.1 col m⁻²) occupied the silted top above the steep rocky walls in which small caves and crevices hosted numerous patches of *C. rubrum* and *E. cavolinii* (0.6 ± 0.09 and 0.18± 0.03 col m⁻², respectively). Aggregate of several colonies of black coral *A. subpinnata* (0.16 ± 0.04 col m⁻²) and only occasional colonies of *C. verticillata, A. hirsuta* and *A. dichotoma* were also present over large to medium size rocks occurring along NM.

NT canyon is characterized by a rectilinear path with gullies and arcuate slide scars along the canyon walls with small slump silt deposit at the bottom of the canyon. It hosted a mixed and a richer assemblage of gorgonians and antipatharians compared to the other two northern canyons, the presence of all the eleven target species were observed (Fig. 3.5A) with a mean total coral abundance of 1.5 ± 0.03 col m⁻². Two big meadow of *V. flagellum* were documented (0.26 ± 0.07 col m⁻²), along with numerous colonies of *C. rubrum* and *E. cavolinii* (0.22 ± 0.05 and 0.16 ± 0.03 col m⁻²). A long rocky ridge that gently sinks towards the base of the canyon was characterized by big boulders, dominated by a dense assemblages of the black coral *A. subpinnata* and *L. glaberrima* $(0.34 \pm 0.04$ and 0.31 ± 0.03 col m⁻²). The other components (between <0.1. and 0.17 col m⁻²) were the fan shape *C. verticillata, P. clavata, B. mollis, P. larix and A. dichotoma*. It is worth noting that the presence of the rare and ecological valuable black coral *L. glaberrima* was observed only in this canyon.

Among the central canyons CO showed the highest total coral abundance (2.3 ±0.1 col m⁻²), with four target species found (Fig. 3.5B). The low level of silt coverage registered CO (more than 87 % of the frames with 0-20% of silt coverage) favored the settlement of more than 1000 colonies of both *C. rubrum* and *E. cavolinii* (0.98 ± 0.1 and 0.94± 0.09 col m⁻²). Large size rocks exhibited also extensive patches of black coral *A. subpinnata* (0.31 ± 0.04 col m⁻²), while only few and sparse specimens of black corals *A. dichotoma* occurred (<0.1col m⁻²).

CG and CA present the lowest mean total abundance values among all canyons $(0.4 \pm 0.1 \text{ and } 0.4 \pm 0.09 \text{ col m}^{-2}$, respectively). *E. cavolinii* was dominant in CG canyon $(0.22 \pm 0.07 \text{ col m}^{-2})$ followed by *P. clavata* ($0.12 \pm 0.07 \text{ col m}^{-2}$). The remaining few coral specimens showed very low abundance values (cumulatively < 0.1 col m^{-2} for *B. mollis and C. rubrum*) (Fig. 3.5B).

Six out of the eleven target species were found in CA (Fig. 3.5B) and only the red coral *C*. *rubrum* reached a mean density >0,1 colonies m^{-2} ; the rest of the target species, *C*. *verticillata, E. cavolinii, P. larix ,A. dichotoma* and *A. subpinnata* occurred with few and isolated colonies (<0,1 col m⁻²).

Among the southern canyons SP was the canyon presenting the highest mean total abundance value $(2.7 \pm 0.4 \text{ col m}^{-2})$ among the three investigated canyons, but it was the poorest canyon in terms of target species richness with only four targets found (Fig. 3.5C). None Anthipatarians were found in this canyons, whereas *E. Cavolinii* was most abundant



followed by *V. flagellum* (1.12 \pm 0.02 col m⁻²). *C. verticillata* and *C. rubrum* were also documented with low abundances (<0.1 col m⁻²).

The presence of ten target species were found in SC (Fig. 3.5C). This canyon was characterized by several rocky wall with high percentage of silt coverage registered and with the bottom composed of medium size rocks with a slope of approximately 40°. *V. flagellum* was the largely dominant species $(1.2 \pm 0.39 \text{ col m}^{-2})$, followed by *C. ruburm* and *C. verticillata* present mostly on the rocky wall of the large size rocks. *P. clavata* also occurred in one single dense patch $(0.1 \pm 0.03 \text{ col m}^{-2})$ together with several specimens of *B. mollis* and *A. hirsuta* (<0.1 colm⁻²). Few and sparse black corals colonies belonging : *P. larix*, *A. dichotoma* and *A. subpinnata* were found all with abundance <0.1 col m⁻².

SS, the southernmost investigated canyon, showed the presence of seven target species (Fig. 3.5A). *E. cavolinii* was the most abundant species $(0.6 \pm 0.16 \text{ col m}^{-2})$, followed by V. flagellum $(0.33 \pm 0.24 \text{ col m}^{-2})$ and C. rubrum $(0.20 \pm 0.05 \text{ col m}^{-2})$. The black coral *P. larix* was the only black coral encountered in this canyon with very low abundances (<0.1 col m⁻²).

Α

В



Figure 3.6. Species composition (percentage) of coral assemblages within each canyon and among canyons in the northern (A), central (B) and southern (C) areas

3.4 Discussion

Deep-sea habitats worldwide are characterized by high spatial heterogeneity at all spatial scales: from the macro-scale (different continental margins across longitude and latitude), to the meso-scale (e.g., open slopes, rocky pinnacles, canyons), to the small scale (i.e., within the same habitat; (Fabri et al., 2014, Cau et al., 2015). The role of medium to largescale setting variability in influencing biological communities has been investigated for terrestrial ecosystems, but is rarely addressed in deep-sea biodiversity studies (Del Leo., 2010). Addressing patterns of biodiversity through the use of different spatial scales is a urgently needed tool for assessing sound in situ conservation strategies (Danovaro et al., 2014, Casas-Güell et al., 2015). Canyons habitats characterized by coralligenous assemblages can be defined as highly heterogeneous systems, where the environmental variables together with distribution and abundances of taxa can differ greatly on both a geographical and a local scale (Molina., 2016). In this regard, our investigation provides comparative analysis among deep coral forests dwelling in different canyon systems at both local and regional scale along the continental margin off the eastern coasts of Sardinia.

Our results emphasized how most of the observed variations in the composition of coral communities occurred at the smallest spatial scale investigated. The PERMANOVA analysis revealed that coral community composition differed at local spatial scale (across canyons within the same area), but not among regional spatial scale (among the three geographical areas) (Tables 3.2-3.3), with the largest degree of community dissimilarity occurring among northern canyons. This result is in accordance with the results reported in the previous chapter (Chapter 3), where differences in the composition of coral communities among submarine canyons and pinnacles in the same area were found to be much larger than those observed among areas. Similar results for the Northwestern

Mediterranean Sea, though for shallower communities, have been also reported by Casas-Güell et al. (2015) who found that the composition of coralligenous communities exhibited the highest variations among sites or, even, among replicates in the same site.

Different factors may be mentioned to explain a small scale diversity in deep-sea benthic biodiversity (Levin et al., 2003). Generally, the spatial patterns in faunal composition may be seen as the results of interactions between different biological and environmental factors acting differently or in concert to shape benthic populations (Rex., 1981). For instance, an increasing number of studies seem to identify biological factors such as recruitment, larval dispersal, competition for food and space as crucial drivers of these biodiversity patterns in several Mediterranean marine habitats (*e.g.*, in mesophotic coralligenous assemblages; Casas-Güell et al., 2015). This holds true when dealing with suspension feeders like those used as target species in the present study, which show a low dispersal capability and a long life span that, potentially, could shape the community composition for long periods after a successful reproductive pulse (Garrabou & Harmelin., 2002). In this regard, as highlighted by the SIMPER analysis, the largest variations in benthic community composition within each canyon and among canyons investigated here were related to changes in the relative of colonial species (*e.g.*, *C. rubrum* and *E. cavolini*).

At the same time, numerous studies dealing mostly with macro- or meiobenthic communities in submarine canyons have focused on spatial scales ranging from 0.1 to 10 km, hightailing the major role of environmental variables on alpha- and beta-diversity, and therefore potentially indicating the larger relevance of abiotic vs. biotic drivers on local biodiversity patterns (Rowe et al., 1982; Vetter and Dayton., 1999; Cunha et al., 2011; Ingels et al., 2011; Gambi et al., 2014).

In the present study, water depth and distance from the coast, as surrogate indications of hydrodynamic forcing acting at the scale of each canyon, were extracted by the DISTLM

models as the environmental variables that explain the highest proportion of the variations in coral communities structure among canyons. This result finds support from previous studies which showed that, in shallow habitats at the head of submarine canyons, particularly those with steep-walled V-shaped profiles, the intensity and frequency of physical disturbance from accelerated bottom currents, disrupting surface sediments and 'washing-out' benthic fauna, could provide a too harsh condition for coral settlement (Bosley et al., 2004; Hargrave et al., 2004). This, in principle, would lead to high variations in the abundance of corals among the head of the canyons and its deeper depths.

Our results pinpoint that the bottom geomorphological features of the canyons' (here estimated in terms of silt coverage and slope of the substrate), did not explain significant proportions of coral community composition variations at all of the investigated spatial scales (consistently <2% of the total variation). This result is in contrast with previous findings that reported those variables to be crucially involved in shaping coral communities (Cau et al., 2015). Such a discrepancy can be attributable to the very different geomorphological assets target of our study (continuous submarine canyons) and those of the area under scrutiny by Cau et al., (2015). The latter indeed included *roche du large* ecosystems, which represent abrupt rocky bottom discontinuities emerging from a continuous soft bottom, which could ecologically act like a deep oasis for either both macro- and megafauna (Bo et al., 2009) and meiofauna (Bianchelli et al., 2013).

A remarkable finding in the NT canyon was the presence of the long living black coral *L. glaberrima*, which, up to date, was documented only in the southwestern margin off Sardinia (Bo et al., 2015). The extreme longevity of these black corals has triggered the use of this species as paleo-climatic archive (*e.g.*, superficial and deep water temperatures; Williams et al. 2006), and bioindicator for anthropogenic carbon and trace elements in the environment (Williams et al. 2007) and, nonetheless, as a tool to determine possible

unperturbed ecosystems (Bo et al., 2015). The survey conducted in NT canyon allowed to document dense patches of this species, distributed in the lower part of the canyon head, showing lower densities compared to other areas of the Mediterranean basin (e.g., Malta, NW Sicily, SW Sardinia; Bo et al., 2013, 2015, Deidun et al. 2015).

Our results, though based on a limited number of different canyons and a limited set of benthic megafaunal organisms, suggest that the area under scrutiny is relatively homogeneous (at least for coral communities), and that largest variations in the coral community composition appear to be constrained by variations in the hydrodynamic conditions operating at the local scale. Though much work needs to be carried out to ascertain scales of variation in benthic biodiversity in submarine canyons, our results provided new insights on the scale-dependent structure and dynamics of deep dwelling coral assemblages.

Chapter 4

The "Sardinian cold water coral province": new spatial extension, demographic traits and coral biodiversity

4.1 Introduction

Cold-water corals (CWCs) form one of the most complex biological habitats of continental slopes, indeed considered of great concern by the scientific community (Roberts et al., 2006). These habitats are regarded as ecosystems of ecological and economic value, raising concern over their rapid destruction since they resulted threatened more and more often by commercial fishing and other human activities (deep sea trawl-fishing, cable laying, oil and gas drilling) (Mortensen., et al., 1995, 2008; Fossa et al., 2002; Roberts et al., 2006, 2009: Hovland., 2008; Freiwald et al., 2009).

CWCs are framework-building organisms that produce calcium carbonate (aragonite or calcite) skeletal structures generating spatial heterogeneity which interact with the local hydro-dynamic flow (White., 2007) providing enhanced feeding opportunities and protection against predators, often acting as nursery area for different associated species of fish and other mobile invertebrate fauna (Watling and Auster., 2005; Costello et al., 2005,2009; Henry and Roberts., 2007; O'Hara et al., 2008; Lessard-Pilon et al., 2010; Buhl-Mortensen et al., 2010; Clark and Tittensor., 2010; Soffker et al., 2011; Baker et al., 2012).

Furthermore, structures formed by scleractinian corals are perennial after their death (i.e., carbonate mound) and support biodiversity for centuries to millennial scales, providing suitable substrate for new colonies settlement along with a variety of other sessile

organisms (Mortensen et al., 1995; Metaxas and Davis, 2005; Roberts et al., 2006; Henry., 2007; Orejas et al., 2008; Purser et al., 2010)

CWCs are widely distributed in every ocean of the world and can be found from polar to tropical regions at wide range of depths from 200 to 2000 m (Freiwald et al., 2004; Roberts et al.,2009; Baker et al., 2012), however their presence has been greatly underestimated in the Mediterranean Sea (Vertino et al., 2010). Indeed, taking advantage of the enormous technological development occurred in the last decades, recent studies performed with underwater vehicles have demonstrated how, in the Mediterranean Deep Sea, CWCs coral assemblages appear to be much more extensively distributed (Tursi et al., 2004;Taviani et al.,2005;Freiwald et al., 2009; Mastrototaro et al., 2010; McCulloch et al.,2010; Gori et al., 2013, Fabri et al., 2013, Taviani et al., 2016) and even more prosperous than expected (Vertino et al., 2010, Gori et al., 2013 and references therein). Nowadays, vast areas of the basin are still unexplored and lot of work needs to be done in order to improve the actual fragmentary knowledge on the distribution of CWC provinces in the Mediterranean basin. Remotely Operated Vehicles (ROVs) has not only revealed the presence of more numerous CWCs provinces than previously thought, but it also proved to be a good and non-invasive tool for understanding of the distribution and abundance of cold-water coral reefs in relation with biotic and abiotic factors, through the use of video transects.

Submarine canyons in the Mediterranean Sea are known as one of the seafloor morphological features where living CWC communities develop and grow in big frameworks (Gori et al., 2013) and in scattered patches (Freiwald et al., 2009). The occurrence of cold-water coral in submarine canyons is related to the energetic current flows that periodically carry nutritive suspended particles (i.e., river and atmospheric inputs, phytoplankton biomass and sediment re-suspension), from the shelf to the deep-sea environment areas (Canals et al., 2006). Many physical factors (i.e., temperature, salinity,

hydrodynamic and habitat complexity) have been proposed to be important in influencing the spatial distribution of cold-water corals (Mortensen et al.,2001; Freiwald et al., 2002; Masson et al., 2003; Kiriakoulakis et al.,2004; Davies et al., 2008; Dullo et al., 2008; White, 2007;) with their relative importance varying between regions and taxonomic groups (Mortensen et al., 2001, 2006). Unlike the northeastern Atlantic Ocean, Mediterranean deep water coral reefs are mainly constructed by the scleractinian *Madrepora oculata* (Linnaeus., 1758) (fam. Oculinidae) and in a smaller part by *Lophelia pertusa* (Linnaeus., 1758) (fam. Caryophylliidae), along with solitary species such as *Desmophyllum dianthus* (Esper 1794) (fam. Caryophylliidae) and *Caryophyllia sp.*, which often contribute to the reef construction framework (Taviani et al., 2005, 2011;Freiwald et al., 2009).

In Sardinian waters the presence of *M. oculata* and *L. pertusa* was first documented by Zibrowius (1980) in his monograph "Les scléractiniaires de la Méditerranée et de l'Atlantique nord-oriental". Actually, Zibrowius cites Cecchini (1917) to report the occurrence of both species in Sardinian waters.

The present work, reports the observation of new sites from the northeast Sardinian coasts colonized by living cold-water coral populations (i.e., *M. oculata* and *L. pertusa*) belonging to the "Sardinian cold water coral province" (Taviani et al., 2016). Both the environmental suitability and the stability of a habitat may be reflected in the distribution patterns of coral colonies, as well as in the size structure of coral populations, because the size structure reflects the factors affecting recruitment, growth, and mortality rates in a particular habitat for a period of time equal to the longevity of the population (Gori et al., 2012). Because of this, through the use of non-invasive protocols based on ROV footage and image analysis, we compared the bathymetric distribution, density patterns and demographic features of CWC populations dwelling in 3 canyons located in the north-east
and in the south-west Sardinia continental margin. These preliminary data will be used to plan further, more detailed research on white coral distribution along Sardinian coasts. In addition, the abundance of the most conspicuous species of anthozoans coexisting in the CWC is reported, along with the presence of anthropogenic litter (e.g. lost fishing gears) in order to evaluate the anthropic impacts over these delicate ecosystems.

4.2 Materials and methods

4.2.1 Study area

Among the complex system of 29 canyons that deeply incise both the western and eastern slopes of Sardinian Island (Würtz et al., 2012) we investigated three sites: two along the Sardinian northeastern margin (i.e., Tavolara, and Capo Coda Cavallo, hereafter TVc and CCc, respectively) and one located in the southeastern continental margin, the Nora canyon (hereafter NOc) (Tab 4.1), where it has been firstly described the "Sardinian CWC province" (Taviani et al. 2016).

The north-eastern coast of Sardinia is characterized by a narrow continental shelf deeply incised by canyons originating from small rivers (mostly ephemeral) which can be followed down to the abyssal plain (Dalla Valle and Gamberi 2010; Giresse et al. 2014). TVc and CCc are two submarine canyons both located out of the northeastern coast of Sardinia. TVc is located 8 nautical miles (nm) from the coast (40°54'46.08"N, 9°54'54.48"E; Fig. 4.1). The upper TVc fan consists of an erosional V-shaped valley bounded by low relief longitudinal ridges that gradually taper in width and relief to their down slope terminations. TVc is less incised on the continental slope due to its disconnection from Sardinia river systems and faces a wider shelf sector. The second investigated canyon is CCc which is located 8 nm south from TVc and 8.35 nm from the nearest coastline (40° 47' 340"N, 9° 51' 615"E; Tab. 4.1). The last investigated canyon

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(NOc) is located 11 nm south of Capo Spartivento, on the southwestern coast of Sardinia (38°42'31.83"N, 8°54'38.52"E; Tab. 4.1) and it is one of the two coalescent branches of the Capo Spartivento canyon system, which entails the continental shelf down to over 2000 m water depth in the bathyal plain. The canyon is asymmetrical showing the right side smoothed than the left side. NOc is right above the Sardinian channel, which is strongly influenced by the general mesoscale circulation of the Levantine Intermediate Water (LIW) and episodically influenced by wind-induced advection of coastal water (Bouzinac et. al., 1999). The Levantine flow is actually composed of two water types: the one known as LIW that is characterized by a high temperature and high salinity (T= 13.85° S=38.75) and another one colder and denser which always flows close to the bottom of the Tunisian side of the Strait. These waters flow into the Algerian basin south of Sardinia and turn to the North following the Sardinian slope (Testor et al., 2005)

	TVc	CCc	NOc
Coordinates	40° 54' 45.6"N	40° 47 <i>´</i> 20.6"N	38° 42' 30.2"N
Coordinates	9° 54' 54.5"E	9° 51´ 36.8" E	8° 54' 34.6" E
N. of dives	1	1	2
Year	2013	2013	2013
Depth range (m)	175-283	428-397	200-470
N. of frames	127	72	179
Frames area (m ⁻²)	203	101	150.31
N. of <i>M.oculata</i> coral colonies	30	33	80
N. of <i>L.pertusa</i> coral colonies	0	0	2
Mean <i>M.oculata</i> Abondance	0.29 ± 0.1	0.54 ± 0.2	0.8 ± 0.36
(colonies m $^{2}\pm$ s.e.)			

Table 4.1. Location and characteristics of each transect in all three investigated canyons



Figure 4.1. Investigated area. Map of the investigated canyons showing ROV dive transects (grey square) with respect to the bathymetry lines

4.2.2 Video surveys and analyses

The video material was gathered by the ROV "*Pollux III*" during a ROV investigation conducted along Sardinian coasts in September 2013 on board of the r/v "*Astrea*". The ROV was equipped with a digital camera (Nikon D80, 10 megapixel), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7), track-link system, depth sensor, compass, and three parallel laser beams providing a constant 11-cm reference scale in the video frame, for the measurement of the frame area. The ROV was also equipped with an underwater acoustic tracking position system (Tracklink 1500 MA, LinkQuest Inc.) providing detailed records of the tracks along the seabed; transects could not be linear as the survey was focused on the target species *Corallium rubrum*, which is distributed in patches along the seabed (Cau et al., 2015b). A total of 4 transects showing the presence of CWCs were analyzed: one in TVc canyon, one in CCc and two in NOc. Overall, 7 h and 16' of ROV footage were processed through the software 'DVDVideoSoft'.

The image analysis was performed with CPCe Software (Coral Point Count with Excel extensions; Kohler and Gill, 2006) and recorded in order to calculate the mean density of each target species (N° colonies $m^{-2} \pm$ Standard Error; hereafter col $m^{-2} \pm$ s.e.). All living colonies of the most conspicuous anthozoan species encountered and their depth recorded in order to estimate density patterns. In addition, morphometric characteristics, size/structure, and position (orientation) with respect to the substrate of the white coral colonies *L. pertusa* and *M. oculata* were retrieved.

Measurements were performed using two parallel laser beams as 11 cm-scale and classified into four categories: small (colonies with one or a few branches, height between 0.1 to 20 cm), medium (colonies presenting a height between 20 and 40 cm), large (height between 40 and 60 cm), or very large (height higher than 60 cm). The video analysis

method allowed extensive sampling over the species distribution but could have entailed a loss of accuracy in the measurements of morphometric parameters.

Colony position with respect to the substrate was classified into four categories according to their location and orientation following Gori et al., (2013): 0° (facing straight up); 90° (perpendicular to vertical rocky walls); 135°(the edge or rocky outcrops, facing downwards); 180°(below rocky outcrops, facing downwards).

In order to give a better geomorphological characterization of the investigated sites, for each frame were also registered: 1) sediment coverage of the substrate classified using a scale from 1 to 5, referring to 5 percentage ranges (1=0-20%; 2=20-40%; 3=40-60%; 4=60-80%; 5=80-100%); 2) slope of the substrate classified as flat ($0^{\circ}-10^{\circ}$), inclined ($10^{\circ}-70^{\circ}$), and vertical (>70°).

The recognizable mega-benthic and ichthyic species observed in the examined photographic archive were registered (Tab. 4.2). Every specimen was identified at the lowest possible taxonomic level. However, identification at species level for some organisms from video footage was sometimes hampered due to poor video quality and limited resolution for detecting morphological characteristics.

In addition, to quantify human impact on investigated sites, through the same image analysis performed for investigations on biodiversity, marine debris was quantified and classified as recreational item or lost fishing gear.

4.2.3 Data analysis

The bathymetrical distribution of the coral species was determined based on the recorded depth of each of the observed coral colonies. The size/frequency distributions of *M*. *oculata* was analyzed in terms in terms of descriptive statistics using the skewness of the distribution calculated with the R language function agostino.test, which is available in the

moments library (Komsta and Novomestky., 2012). Skewness is a measure of the symmetry of a distribution using its mean; if skewness is significant, the distribution is asymmetric. Compared to the mean of the population, positive skewness indicates the prevalence of smaller size colonies in relation with the mean height of the population, whereas negative skewness indicates an higher presence of larger size classes (Bramanti et al., 2014; Gori et al., 2013; Linares et al., 2008). Test for normality was performed using the software PAST (function 'normality tests'), performing both Shapiro-Wilks and Anderson-Darling tests. Once non-normality was verified, a non-parametric univariate analysis of variance (Kruskal-Wallis procedure, software PAST 2.17Hammer et al., 2001) was run to test for differences in the *M. oculata* mean density among the three investigated canyons (TVc, CCc, NOc) and among the three investigated depth-range in which *Madrepora* colonies occurred (170-270,300-400, >400).



Figure 4.2..Selected ROV images of several colonies of *M. oculata* observed in the three investigated canyons. A 10 cm scale (white bar) is provided in each picture.

4.3 Results

The area of each frame was estimated *a posteriori*, resulting in an average of $3.5 \pm 1.3 \text{ m}^2$, covering a total 554.31 m² of investigated surface among the three canyons. A total of 140 colonies of *M. oculata* and 2 colonies of *L. pertusa* were recorded in all explored sites (Tab. 4.1). *M. oculata* was the most frequent and abundant species, representing 98.5 % of the total number of colonies observed, occurring in 48.1 % of investigated frames, with a maximum density of 6.4 col m² in NOc.

Several mega-benthic and pelagic species belonging to different Phyla (Cnidaria, Porifera, Crustacea, Echinodermata, Chordata) occurred and were classified within the cold water corals framework in the three studied canyons (Tab. 4.2). Additionally, encrusting algae, encrusting sponges, ascidians, barnacles, bryozoans were frequently found hanging on the branches of the CWC dead colonies, but were not put in the list since it was not possible classified them (for a more detailed list of species present in Nora CWC framework see Taviani et al.. 2016). In TVc site 32 mega-benthic and pelagic species were registered followed by 30 for NOc and only 15 for CCc.

Species	Taxa	TVc	CCc	NOc
Characteristic species				
Madrepora oculata (Linnaeus, 1758)	Cn	+	+	+
Lophelia pertusa (Linnaeus, 1758)	Cn			+
Dendrophyllia cornigera (Lamarck, 1816)	Cn	+		+
Desmophyllum dianthus (Milne-Edwards & Haime, 1848)	Cn	+	+	+
Associated species				
Pachastrella monilifera (Schmidt, 1868)	Pr	+		+
Poecillastra compressa (Bowerbank, 1866)	Pr	+	+	+
<i>Rhizaxinella pyrifera</i> (Delle Chiaie, 1828)	Pr	+		·
Hexadella dedritifera (Topsent, 1913)	Pr	+	+	
Javania cf. cailleti (Duchassaing and Michelotti, 1864)	Pr	+	+	
Leiopathes glaberrima (Esper 1792)	Cn	+	ŗ	
Filograna-Salmacina complex (Berkeley 1828)	Cn	+		
Callosoroja verticillata (Pallas 1766)	Cn	+		
Acanthogorgia hirsuta (Gray 1857)	Cn	' +		+
Antinathella subninnata (Ellis & Solander, 1786)	Cn	, Т		I
Antipathes dichotoma (Pallas, 1766)	Cn	т 1		
Parantingthes larix (Espor 1788)	Cn	+		
Vininglig flagglium (Lehnson, 1962)	Cn	+		
Vimineila jiageilum (Johnson, 1805)	Cn Cn	+		
Euniceua cavoinni (Kocn, 1887)	Cn C	+		
Bebryce mollis (Philippi, 1842)	Cn	+	+	+
Corallium rubrum (Lamarck, 1816)	Cn	+		+
Axinella sp.	Cn	+		
Munida tenuimana(Edwards and Bouvier, 1899)	Cr	+	+	+
Anamathia rissoana (Roux, 1828)	Cr	+		+
Bathynectes maravigna (Prestandrea, 1839)	Cr		+	+
Plesionika sp.	Cr	+	+	+
Plesionika gigliolii (Senna, 1902)	Cr			
Homola barbata (Fabricius, 1793)	Cr			+
Anamathia rissoana (Roux, 1828)	Cr			+
Paromola cuvieri (Risso, 1816)	Cr		+	+
Phycis blennoides (Bru ⁻ nnich, 1768)	Os			+
Hymenocephalus italicus (Giglioli, 1884)	Os			+
Nezumia sclerorhynchus (Valenciennes, 1838)	Os			+
Coelorinchus caelorhincus (Risso, 1810)	Os			+
Lepidorhombus whiffiagonis (Walbaum, 1792)	Os	+		+
<i>Epigonus constanciae</i> (Giglioli, 1880)	Os			+
Benthocometes robustus (Goode & Bean, 1886)	Os			+
Helicolenus dactylopterus (Delaroche, 1809)	Os	+	+	+
Anthias anthias (Linnaeus, 1758)	Os	+		
Polyprion americanus (Bloch & Schneider 1801)	Os .	+	+	+
Pagellus hogaraveo (Brijnnich 1768)	Os	+	,	+
Synchiropus phaeton (Günther 1861)	05	+		
Scorpana alongata (Cadenat, 1001)	05	I		1
Labrus mixtus (Linnaeus 1758)	0.	+	+	1
Lanidonus caudatus (Euphresen 1788)	Ω_{s}	1-	I	Т
Calaus malastomus (Definescue, 1910)	Ch		I.	т ,
Cidaris aidaris (Linnecus, 1759)	Ec		Ŧ	Ŧ
Caramastar placenta (Müller & Treschel 1942)	Ec	Ŧ	1	
Cerumusier piacenia (Munch & Hoschel, 1042)			+	

Table 4.2. Absence and presence of benthic and ichthyic species identified by images at the study sites in the eastern and southern coast of Sardinia (Cn= Cnidaria, Pr=Porifera, Cr= Crustacea, Os=Osteichthyes, Ch= Chondrichthyes, Ec= Echinodermata)

In TVc, a 1139m transect at depth ranging from 182 m to 270 m was conducted. The first part of the dive was characterized by a ridge that gently sinks towards the base of the canyon, characterized by big boulders, dominated by a dense meadow of the black coral *Leiopathes glaberrima* [(Esper, 1788) (fam. Leiopathidae)] $(0.3 \pm 0.04 \text{ col m}^2)$ together with numerous colonies of Antipathella subpinnata [(Ellis & Solander, 1786) (fam. Myriopathidae)] $(0.3 \pm 0.003 \text{ col } \text{m}^2)$, Antipathes dichotoma [(Pallas, 1766) (fam. Antipathidae)] $(0.02 \pm 0.03 \text{ col m}^2)$ and *Parantipathes larix* [(Esper, 1788) (fam. Schizopathidae)] $(0.025 \pm 0.07 \text{ col m}^2)$. The canyon's walls also hosted specimens of red coral Corallium rubrum (0.16± 0.03 col m⁻²), and gorgonians such as Eunicella cavolinii [(Linnaeus, 1758) (fam. Gorgoniidae)] (0.22 col $m^2 \pm 0.3$), and Acanthogorgia hirsuta [(Gray,1857) (fam. Acanthogorgiidae)] (0.01col $m^2 \pm 0.01$). Towards the bottom of the canyon, the rocky substrate becomes gradually covered by a silt layer dominated by species such as Viminella flagellum [(Johnson, 1863) (fam. Ellisellidae)] (0.3 col m⁻²± 0.08), and *Callogorgia verticillata* [(Pallas, 1766) (fam. Primnoidae)] (0.03 col m⁻² \pm 0.01). The first observations of cold-water corals occurred at a depth of 250 m over a steep rocky wall covered by patches of numerous fan-shape colonies of M. oculata. A total of 30 colonies of *M. oculata* occurred with a mean density of 0.29 col m⁻² \pm 0.1within a depth range of 250 to 263m (Fig. 4.4). Most of the colonies were directly attached to a vertical rocky substrate, while few others living colonies were developed over the skeleton of others dead *M. oculata* and *Dendrophyllia cornigera* (0.006 col $m^2 \pm 0.05$ SE) [(Blainville, 1830) (fam. Dendrophylliidae)] colonies.

In CCc, a 560m transect was carried out, and a living populations of *M. oculata* composed by 34 colonies occurred at a depth ranging from 400 to 430 m (with a mean density of 0.54 col m⁻² \pm 0.2; Fig. 4.4). The sea bottom here is characterized by strongly bioturbated soft sediments, interrupted by black-coated boulders at places colonized by epifaunal organisms. Most of the colonies developed on a muddy, slightly inclined seabed, probably an old Messinian delta, where they grew on the fossil framework built by the skeleton of dead *M. oculata* individuals. The white coral community in CCc was associated with numerous specimens of the scleractinian *D. dianthus*.

Along NOc two ROV dives at depth ranging from 200 m to 460 m were carried out and 80 colonies of *Madrepora oculata* were counted together with 2 colonies of *L. pertusa*. As for the other two canyons, the white coral community was dominated by colonies of *M. oculata*, which occurred from 380 to 390 m depth and from 420to 460 m depth (Fig. 4.4). The shallower colonies were found in the northern part of the canyon, on an inclined seafloor covered by pelagic mud, in which over 65% of *M. oculata* living colonies grew upon dead colonies skeleton. Upright-growing colonies were predominantly fan-shaped, flattened and with apical live portions. The ROV dive performed in the deepest depth range inspected the southern part of the Nora's canyon from 420-460 m depth. The general seafloor morphology is very similar to the northern part, with an inclined muddy covered seafloor with a dead coral framework functioning as substrate where a patch of living white corals developed. The CWC community in the southern part of the canyon seemed to be more developed and more abundant (0.74 col m⁻² ± 0.11) than the shallower population (0.1 col m⁻² ± 0.12).







Figure 4.3. Bathymetrical distribution of *Madrepora oculata* colonies in TVc, CCc and NOc canyon: black line indicates the number of colonies; grey-scale histograms represent the explored sea bottom (m²) of each frames.

Furthermore, 2 isolated live colonies of *L. pertusa* growing upon dead colonies of *M. oculata* occur in this part of Nora canyon from 427 to 452m depth. *L. pertusa* was observed only in one out of three canyons. Due to the low presence of *L. pertusa*, statistical analysis were not performed for this species.

In all three investigated sites *M. oculata* was the most abundant species, varying from 0.78 $\operatorname{colm}^2 \pm 0.1 \operatorname{colm}^2$ for NOc population, to 0.54 col $\operatorname{m}^2 \pm 0.2$ for CCc, with the lowest mean density of 0.29 $\operatorname{colm}^2 \pm 0.19$ calculated for TVc population (Fig.4.4).



Figure 4.4.Density (colonies m⁻²) of *Madrepora oculata* colonies in the three investigated sites

Data significantly deviated from normality (*i.e.*, p-value<0.001 of the Shapiro-Wilks and Anderson-Darling tests), therefore a non parametric univariate test (Kruskal-Wallis) was performed to test for differences in mean density of *M. oculata* colonies among the three investigated locations. No significant differences were showed between all three populations TVc, CCc and NOc (Tab. 4.3; P> 0.001). Kruskal-Wallis test was also performed to test for statistical differences in mean density along 3 different depth range in which Sardinian *M. oculata* population occurred (170-270 m; 300-400 m; >400 m) showing significant difference between the shallowest population and the deepest population (Tab. 4.4).

Table 4.3.Results of the non-parametric univariate analysis of variance(Kruskal-Wallis procedure) testing for differences in the mean density of *Madrepora oculata* in the three investigated sites

Kruskal-Wallis Test			
Sites	TVc	CCc	NOc
TVc		0,208	0,590
CCc	ns		0,367
NOc	ns	ns	

Table 4.4. Results of the non-parametric univariate analysis of variance (Kruskal-Wallis procedure) testing for differences in the mean density of *Madrepora oculata* along the investigated depth ranges.

Kruskal-Wallis Test			
Sites	170-270	300-400	>400
170-270		< 0,0001	< 0,0001
300-400	***		0,246
>400	***	ns	

In all three canyons where *M. oculata* was documented, the size/class frequency distribution highlights the dominance of small size colonies (0.1 to 20 cm), except for NOc

where the medium size class (20 to 40 cm) resulted to be the more abundant class (40 % of the colonies; Fig. 4.5). Small colonies dominated in TVc canyon where they accounted for 50%, while in , CCc and NOc there were only 42%, and 32% of the colonies, respectively. The percentage of large colonies (40 to 60 cm) was almost the same for all the sites (13%, 12%,15% in TVc, NOc), while the highest percentage of very large colonies was recorded in CCc with 18% of the colonies higher than 60 cm (7% TVc, 13% NOc) (Fig.4.3). Mean height value in TVc was 29 \pm 0.4 cm, with a maximum height value of 70 cm; in CCc the mean height was 34 \pm 0.05 cm, with a maximum height of 107 cm; while in NOc the mean height value was 34 \pm 0.2 cm, with a maximum height value of 1.2 m.







Figure 4.5.Size-frequency distribution of *Madrepora oculata* populations in TV, CCc and NOc

This size frequency analysis emphasizes a positively skewed distribution for TVc and CCc populations (Tab.4.5.Skewness: TVc =1.57, CCc = 1.1501; *p*-value< 0.01), which indicates an higher number of smaller colonies respect to the mean height value of the population. While NOc present a lower skewness value (skewness = 0.7845, *p*-value > 0.01) due to the higher presence of larger sized colonies which skewed less the size class distribution.

Table 4.5. Values of skewness for the size/class distribution (Height) of *M. oculata* populations in TVc canyon, CCC canyon and NOc canyon, significant p values are indicated with one (p value<0.05), two (p value<0.01), or three asterisks (p value<0.001).

Skewness			
	TVc	CCC	NOc
Values	15.790	11.501	0.7845
p-Value	1.518e ⁻ 09 (***)	0.00642 (**)	0.06 (ns)

In all three canyons, the *M. oculata* colonies were mostly vertically oriented : 94% were oriented at 0° and 90 ° all together. However, in TVc 17% of colonies were oriented at 135° and 3% oriented at 180°. In CCc and NOc,6% of colonies were orientated 135°, and no colonies was found in overhanging position (*i.e.*, 180°; Fig. 4.6). Colonies of *L. pertusa* were primarily orientated at 90°.







Figure 4.6. Percentage of *Madrepora oculata* colonies with different orientation with respect to the substratum $(0^{\circ}, 45^{\circ}, 90^{\circ}, 135^{\circ}, 180^{\circ})$ in TVc, CCc and NOc.

Regarding the investigation of anthropogenic impact, overall, all three investigated sites showed the presence of anthropic debris (Fig 4.7). In TVc 10% of the analyzed frames showed the presence of lost fishing gear (7 fishing lines an 2 pieces of fishing net). In CCc 14 % of the frames presented anthropogenic recreational debris (3 plastic bags). In NOc was registered the highest number of debris items among the three canyons belonging to both categories (marine litter and fishing gears): 16 % of the frames showed the presence of anthropogenic impact (3 plastic bags along with other 3 non recognizable plastic items, 2 oil barrels and 1 fishing net). Several CWCs colonies were littered with plastic bags and discarded fishing gear, which are harmful to the corals as can rip off portions of the coral as they are dragged away by currents (Fig 4.7).



Figure 4.7.. Anthropogenic impact in the investigated canyons: A) plastic net lying on muddy floor among *M. oculata* coral frame; B) lost fishing nets on the muddy bottom above dead and living coral colonies of *M. olulata* C) D) Large plastic bags entrapped on live and dead *M. oculata* coral ground; E) plastic litter around a live *M. oculata* bush; F) abandoned barrel serving as substrate for elongated specimens of *D. dianthus*, note a specimens of *P. cuvieri* on the left side. A 10 cm scale (white bar) is provided in each picture. A 10 cm scale (white bar) is provided

4.4 Discussion

Recent ROV explorations of the Sardinian continental shelf has provided significant new geographic and bathymetric documentation of rich and diverse cnidarians-dominated communities (Bo et al., 2015; Cau et al., 2015a). Several species of scleractinian habitat-forming (deep) cold water corals, *M. oculata, D. cornigera,* and *D. dianthus*, were documented for the first time in Sardinia canyons from the northeast Sardinian continental margin, extending the geographical framework of the recently discovered "Sardinian cold water coral province" (Taviani et al., 2016).

All three investigated canyons showed M. oculata as the most abundant CWC species, while for L. pertusa, D. cornigera and D. dianthus abundances were significantly lower. The dominance of *M. oculata* populations in this study is in agreement with the results from previous investigations on other CWCs provinces throughout the Mediterranean basin (Taviani et al., 2005; Freiwald etal., 2009; Orejas et al., 2009; Vertino et al., 2010, Gori et al., 2013;) and especially those dealing with temperature as one significant environmental constraining factor for these species (Freiwald., 2002; Davies et al., 2008, Flogel et al., 2014). To date, the diversity of CWC and their associated fauna has decreased considerably since the end of the last glacial period due to the major changes in oceanographic and geologic conditions (Pérès., 1982, Delibrias & Taviani 1985).During the Holocene period as sea level rise, changes in climatic conditions produced a strong increase in Mediterranean water temperatures, modifying the environmental conditions that were present and favorable to CWCs during the last glacial maximum (Delibrias & Taviani 1985). Currie et al. (2004) in his physiological tolerance hypothesis suggested that richness and climate may covary simply because fewer species can physiologically tolerate conditions in colder places than in warmer places. M. oculata has a wide temperature

tolerance being able to function at temperatures up to 20°C (Keller & Os'kina 2008) making this CWC species the most dominant in Sardinian waters.

L. pertusa in contrast, according to Freiwald (2002),has a temperature tolerance limits which ranges from 4 to 14°C. This would explain the scarcity of this species within our study since Mediterranean waters would be close to its upper temperature limit. However, even if restricted to certain locations such as the canyon flanks of the Lacaze-Duthiers Canyon (Cap the Creus), CWC provinces mostly composed of living specimens of *L. pertusa* may occur also in the Mediterranean basin (Gori et al., 2013).

Comparing the results from the present study with other data sets from available literature focused on *M. oculata* dominated CWC banks (Tursi et al., 2004, , Freiwald et al., 2009; Taviani et al., 2011; Fabri et al., 2013), we found that Sardinian populations seem to be less abundant and developed. While in the well-known Santa Maria di Leuca coral province (Taviani et al., 2011a) and in the French Mediterranean submarine canyons (Fabri et al., 2014) extensive reefs and mounds are documented, Sardinian populations of *M.oculata* show a patchy distribution pattern, mainly composed by small and isolated colonies. A similar patchy distribution was found within Montenegrin canyons (Angeletti et al. 2014) and along the south-western coast of Malta (Schembri et al. 2008). The differences in the spatial distribution patterns of *M. oculata* reflect its response not only to changes in environmental variables (temperature, substratum, currents and food availability) between different geographical sites but also the connectedness of suitable habitat for adults and larvae i.e. their possibility to colonize.

Overall no statistical differences were observed in mean density between TVc, CCc and NOc, however values of the total coral abundance were clearly different between northeastern and south-western canyons, probably due to the main in terms of geographical position and topography of the substrate. A higher mean density value was calculated for

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NOc populations where the higher sedimentation rates (82% of the analyzed frames showing an average value comprised between 60 and 100 % of silt coverage) could act as a factor for the development of a denser and more isolated CWC communities. South-western populations of *M. oculata* developed in patches, on a slightly inclined sea bed, surrounded by a thick layer of mud making the CWC frameworks the only hard substrate colonizable nearby. On the other hand in TVc and CCc a lower density value was documented together with a more heterogeneous substrate, characterized by rocky ridges and steep rocky walls in which the level of silt coverage sediment was comprised between 0 and 40% along the entire transects. In general, the different distribution and density of (deep) cold-water corals in the three Sardinian canyons could derive from the different influence of current systems and substrate availability of these sites.

Furthermore since, in a variety of aquatic settings, habitat complexity and heterogeneity has been shown to have a positive relationship with species diversity and abundance (Hixon and Menge, 1991), also the highest number of benthic and pelagic species recorded in all three canyons emphasize the key role of these important in enhancing these environmental characteristics.

M. oculata populations in all three sites show similar demographic patterns with higher percentage of young and small colonies contrasted with low percentage of medium, large and very large colonies. Even though there is a lack of information on CWC reproductive ecology in Mediterranean Sea, the observed abundance of small- and medium-size colonies that characterized all three canyons could possibly be considered as the result of an active recruitment (Grigg, 1977; Lasker, 1991) in the Sardinian CWC population, as was suggested also for Cap de Creus and Lacaze-Duthiers populations by Gori et al 2013.

The preferential orientation at 90° of *M. oculata* colonies in TVc is also probably associated with the geomorphology of the seabed, characterized by vertical walls, used as substrate in

which most of the colonies develop. On the other hand, *M. oculata* populations dwelling in CCc and NOc are mostly oriented in the upright position at 0° orientation as they grow on a sub-horizontal slightly inclined seafloor in which, when they reach a certain height, they tend to tip over and continue to grow upright on upper parts of the structure. These orientations of corals could represent on one hand a compromise between protection from the sediment, and exposure to the water flow to ensure feeding (Gori et. al. 2013), on the other hand it could be a consequence of anthropic impact caused by fishing activities.

In order to define what drives the variation of biodiversity in deep sea benthic communities, which host very rich and specialized species, various explanatory factors have been attributed to different equilibrium (linked with temporal stability; Casas-Güell et al., 2015) and non-equilibrium hypotheses (linked to disturbance, spatial heterogeneity and dynamic forces; Angiolillo et al., 2015; Cau et al., 2015). In the case of non-equilibrium hypotheses, it has been widely documented that human pressure has to be considered as one of the main threats for deep sea benthic communities (Pusceddu et al., 2014), especially for conspicuous long-living corals (Bo et al., 2014).

In TVc the high number of lost long-line fishing gear found entangled in coral colonies suggest that this canyon could be more affected by fisheries impact than the other two canyons. On the other hand in Nora canyon we found an higher percentage of anthropogenic debris such as plastic bags and oil barrels, together with a lower percentage of discharged fishing gears, which may indicate a stronger influence of anthropic recreational activities. In general all three study areas are characterized by the presence of an intense anthropogenic impact derives by discarded/lost fishing gears and litter object, which represent the most potentially harmful damage on CWC grounds (Taviani et al 2016). These data also confirm and expand the situation of the heavily impacted

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anthropogenic action occurring in the southern Sardinian area as documented also by Angiolillo et at., 2015.

Our study shows evidence of new live assemblages of habitat forming cold-water corals dominated by *M. oculata* developed close to the shallowest limit depths (200 m) documented for these species in the Mediterranean sea (Fabri et al 2014). Furthermore, this new occurrence increase the extension of the "Sardinian Cold Water Coral Province" described by Taviani et al. (2016). These new occurrences are central in the understanding the connectivity, distribution patterns and population structure among discontinuous deepwater coral grounds within the Mediterranean Sea. Its unique geographical location in the Mediterranean CWCs provinces framework prompt the need to increase the effort in ROV investigations along Sardinian waters. In the context of a growing concern by the scientific community about the importance for deep-sea marine resources, the precious white corals community certainly deserves a higher attention and a better management.

In conclusion, concerning the increasing needs of usefull knowledge on the distribution and composition of benthic assemblages dwelling in different canyon systems across the Mediterranean basin is considerably increasing; on the other hand, factors driving their distribution and biodiversity at multiple spatial scales are still difficult to discern and thus far to being well understood

Final discussion

The improvement of a strong and valid way to spatially represent the world's marine biodiversity is one of the greatest challenges that marine scientists are facing (Heap., 2011). In the last decades new scientific questions have raised about the relation between benthic diversity and various forms and scales of margin heterogeneity (Menot et al., 2010). The Sardinian continental margin (western Mediterranean), is composed by a complex collection of markedly topographically complex features, including sedimentary slopes, submarine canyons and rocky outcrops. The particular geomorphologic structures of these habitats and the associated abiotic processes result in increased habitat heterogeneity of the continental shelf, thus influencing the distribution and abundance of deep-sea benthic fauna and increasing both local and regional scale diversity (Company J.B., Bo et al., 2012; Cau et al., 2015). Recent findings suggest that increased habitat heterogeneity in canyons is responsible for enhancing benthic biodiversity and creating biomass hotspots (Vetter 1994; Vetter et al. 2010). Since a variety of environmental and physical characteristics interact often canyon habitats, predictions concerning the effects of canyons on local (alpha) and regional (gamma) diversity are still not clear. Very few studies have focused on comparing canyon effects on biodiversity at several spatial scales: i.e., at local or within habitat (alpha diversity), across different habitat types (beta diversity), and regional scales (gamma diversity). Furthermore in consideration of possible conservation plans, Beta diversity is thought to be a particularly important component of biodiversity as it can be used to identify heterogeneity in species assemblages along environmental gradients as well as biodiversity hotspots (Magurran, 2005).

In this concern, two community-studies were conducted and the following hypotheses were tested: (1) deep coral community differ among locations showing contrasting geological

features; (2) deep coral community vary across spatial scales of observation and environmental settings. Based on the results of each study, the following conclusions emerged.

The first chapter of the thesis was dedicated to a comparative analysis in *alpha* and *beta* diversity of coral community composition inhabiting Sardinia continental margin characterized by highly contrasting geological settings (canyons vs *roche du large* ecosystem). Through a nested experimental design, results showed how contrasting geological setting actually do not influence the animal forests, both quantitatively and qualitatively. Therefore, it was not possible to discriminate "pinnacle communities" from "canyon communities" as the high heterogeneity observed among sites overcame the variability observed among locations. The role of the tested environmental factors within each different morphological features, which are crucially involved in current flow modifications and consequently in the distribution of benthic fauna, seem to play a primary role driving diversity in these deep-coral communities.

In chapter 3, considering the results emerged from the first study, we than decide to further extended the investigation on coral community composition along a wider spatial scale (from the northeastern to the southeastern of Sardinia continental margin), though comparing similar geomorphological setting (submarine canyons). Our results, based on a limited number of different canyons and a limited set of benthic megafaunal organisms, suggest that coral community composition differed at local spatial scale (across canyons within the same area), but not among regional spatial scale (among the three geographical areas). The main environmental factors such as the bottom geomorphological features of the canyons' (estimated in terms of silt coverage and slope of the substrate), have not show any relevant explanatory role in the coral communities variations at all of the investigated spatial scales, whereas, that largest variations in the coral community composition appear

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to be constrained by variations in the hydrodynamic conditions (depth and distance from the coast) operating at the local scale.

In the last chapter to corroborate the show evidence of new live assemblages of habitat forming cold-water corals dwelling along Sardinia submarine canyons. We focus our study on a single species investigation as we described the distribution and demographic features of scleractinian habitat-forming cold water coral M. oculata documented for the first time in the northeast Sardinian continental margin, extending the geographical framework of the recently discovered "Sardinian cold water coral province" documented by Taviani et al., (2016). Results showed how the Sardinian CWC province is a relevant biodiversity area in the Mediterranean deep-sea characterized by a dense growth of M. oculata colonies, abundance of D. dianthus, presence of living L. pertusa. These new occurrences are central in the understanding the connectivity, distribution patterns and population structure among discontinuous deep-water coral grounds within the Mediterranean Sea. Its unique geographical location in the Mediterranean CWCs provinces framework prompt the need to increase the effort in ROV investigations along Sardinian waters. In the context of a growing concern by the scientific community about the importance for deep-sea marine resources, the precious white corals community certainly deserves a higher attention and a better management.

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