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DEEP CORAL FORESTS FROM THE ISLAND OF SARDINIA

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1 Abstract

1.1 ABSTRACT IN ENGLISH

Forests are major ecological structures of the terrestrial environment that enhance biodiversity through different kinds of specialized relations that exist between the vegetal canopy and organisms from all animal reigns. The concept of forest has recently been transplanted in the marine environment; marine forests share all ecological features of terrestrial forests. What is actually peculiar in the marine environment is the shift from the ‘vegetal’ forest in shallow depths (within the photic zone) to the ‘animal’ forest (dwelling in the circalittoral zone) throughout the depth gradient. ‘Animal forest’ concept refers to forests entirely constituted by animals, that substitute to the vegetal canopy in the three dimensional development of the habitat. Benthic suspension feeders are, in fact, the main contributors to the habitat biomass and create important three-dimensional structures that enhance the ecosystem’s overall complexity. Among factors controlling the horizontal distribution of such ecosystems, the geomorphology of the sea bottom is thought to be a key factor in the distribution of deep-sea coral as many coral species require hard substrates, particular light and current conditions. Through the combination of non-invasive techniques such as ROV footage coupled with multi-beam surveys in contrasting geological settings, this thesis aims to test (both at species and community level) if and how distribution and composition of coral forests dwelling in the mesophotic environment (from 50 to 200m depth) could be influenced by contrasting geological settings. Uni- and multivariate as well as multiple regression analyses were used to test for differences in alpha and beta biodiversity in *roche du large* ecosystems and canyons, which are the most common geological features along continental margins worldwide. Results revealed that, in contrast to what was previously reported in scientific literature, red coral populations are not structured along a bathymetric gradient but other intraspecific, interspecific and environmental interactions may shape the population structure. Investigations on communities emphasized a high variability at mesoscale (10s of km) that overcomes the macroscale (100s of km). In particular, tested environmental variables explain patterns of observed diversity, that is higher within the same geological feature rather than among contrasting geological settings. Finally, a ROV survey conducted over a *roche du large* ecosystem off Carloforte (SW Sardinia) revealed the presence of a pristine millennial black coral forest of *Leiopathes glaberrima*. The investigated site is a millennial deep coral oases as well as a nursery area for the spotted catshark *Schyliorhinus canicula*, which deserves special protection.

Our results enhance knowledge on coral forests dwelling in the mesophotic environment and provide useful insights for recasting conservation strategies, both at species and community level.

1.1.1 Keywords

Animal forest; coral forest; red coral; black coral; mesophotic environment; nursery

1.2 RIASSUNTO IN ITALIANO

Le foreste sono le principali strutture ecologiche dell'ambiente terrestre che accrescono la biodiversità attraverso svariati tipi di relazioni specializzate tra il tessuto vegetale e gli organismi animali. Il concetto di foresta è stato recentemente traslato in ambiente marino; le foreste marine condividono tutte le caratteristiche ecologiche delle foreste terrestri. Quello che è veramente peculiare nell'ambiente marino è il cambiamento lungo il gradiente batimetrico dalla foresta 'vegetale' nelle acque superficiali (entro la zona fotica) alle foreste 'animali' (nel circalitorale). Le 'foreste animali' sono foreste costituite interamente da animali, che sostituiscono la componente vegetale nello sviluppo tridimensionale dell'habitat. Gli organismi bentonici sospensivori sono, infatti, i principali costituenti della biomassa dell'habitat; creano importanti strutture tridimensionali che aumentano la complessità complessiva dell'ecosistema. Tra i fattori che controllano la distribuzione orizzontale di questi ecosistemi, si ritiene che la geomorfologia del fondale marino sia un fattore chiave nel determinare la distribuzione dei coralli profondi dato che molte specie di coralli richiedono substrati duri e particolari condizioni di luce e corrente. Attraverso l'uso combinato di tecniche di campionamento non invasivo, come filmati ROV e campagne multi-beam in siti con diverse caratteristiche geologiche, questa tesi si propone come scopo di testare (sia a livello di singola specie che di comunità) se e come la distribuzione e composizione delle foreste di corallo presenti nell'ambiente mesofotico (tra 50 e 200 metri di profondità) possono essere influenzate dalle caratteristiche geologiche. Sono state usate analisi uni-, multivariate e di regressione multipla per testare differenze in alfa e beta diversità in diversi ecosistemi (*roche du large* e canyon), che sono le strutture geologiche più comuni lungo i margini continentali nel mondo. I risultati hanno mostrato che, in contrasto a quanto riportato finora dalla letteratura scientifica, le popolazioni di corallo rosso non sono strutturate seguendo un gradiente batimetrico ma che altre interazioni intraspecifiche, interspecifiche ed ambientali possono dare forma alla struttura di popolazione. Le indagini a livello di comunità hanno enfatizzato un'alta variabilità su scala geografica intermedia (10 di km) superiore a quello osservata su più larga scala (100 di km). In particolare, le variabili ambientali studiate spiegano la diversità osservata, che è risultata maggiore entro strutture geologiche simili piuttosto che tra strutture diverse. Infine, indagini con il ROV realizzate in un ecosistema tipo *roche du large* al largo di Carloforte (Sardegna SO) hanno rivelato la presenza di una foresta millenaria di corallo nero della specie *Leiopathes glaberrima*. Il sito merita special protezione in quanto rappresenta un oasi di corallo millenario e allo stesso tempo ospita una nursery area per una specie di elasmobranco, il gattuccio *Scyliorhinus canicula*.

In sintesi, i nostri risultati migliorano le conoscenze sulle foreste di coralli in ambiente mesofotico e forniscono importanti indicazioni per ridefinire le strategie di conservazione, sia a livello di specie sia di comunità.

1.2.1 Parole chiave

Foreste di animali; foreste di coralli; corallo rosso; corallo nero; ambiente mesofotico; nursery;

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Section one – an overview

5 General Introduction

5.1 THESIS STRUCTURE

The main body of this thesis is divided into four sections and an Appendix, as follows:

Section one - an overview

Two chapters (chapters 5 and 6) compose this section, and give an overview of the thesis:

- The **'General introduction'** (chapter 5) describes the structure of the thesis as well as the origins of the project, and the aims and objectives of the research project.
- The **'Literature review'** (chapter 6) provides an extended review of the literature background behind the experimental hypotheses, and, more specifically, provides the state of the art about the ecology of deep coral communities.

Section two – experiments and results

Five chapters (7- 11) compose this section, and illustrate the results of different studies. In particular:

- Chapter 7 contains the paper:

'Preliminary data on habitat characterization relevance for red coral conservation and management. Alessandro Cau, E. M. Paliaga, R. Cannas, G. Deiana, M. C. Follesa, F. Sacco, S. Todde and P. E. Orrù, *Italian Journal of Geosciences*, 2015, vol 134(1): 60-68.

Through a non-invasive and interdisciplinary approach, distribution and size/age structure of deep dwelling populations of the precious red coral *Corallium rubrum* (Linnaeus, 1758) were described in two different areas of the south Sardinia continental shelf. An extensive ROV survey, carried out in autumn 2011, provided useful information on occurrence, abundance, density and morphometric parameters of red coral colonies.

- Chapter 8 contains the paper:

'Deep dwelling populations of Mediterranean red coral along different geological settings: back to the future'. Alessandro Cau, L. Bramanti, M. Angiolillo, M. Bo, S. Canese, R. Cannas, D. Cuccu, M. C. Follesa, K. Guizien. *Scientific Reports* (in preparation).

ROV footage coupled with multi-beam surveys in contrasting geological settings enabled to test through multiple regression analyses the relationship between *C. rubrum* populations' features and some abiotic and biotic factors, within the mesophotic environment.

- Chapter 9 contains the paper:

'Drivers of deep-water coral beta diversity in different *roche du large* ecosystems along the south Sardinia continental margin (central western Mediterranean Sea)'. **Alessandro Cau,** M. C. Follesa, D. Moccia, A. Alvito, F. Sacco, M. Bo, E. M. Paliaga, S. Canese, P. E. Orrù, R. Cannas. *Marine Biology* (submitted)

Five rocky pinnacles from south Sardinian continental margin dwelling between 120 and 170 m depth, characterized by different geological setting, were studied to test differences in the associated coral communities. Eleven target hard-bottom coral species (four black corals and seven gorgonians) were used as valid surrogate descriptors of megabenthic biodiversity of the five pinnacles.

- Chapter 10 contains the paper:

'Beta-diversity of deep-water animal forests along the south Sardinia margin (Mediterranean Sea): role of contrasting environmental drivers'. **Alessandro Cau**, D. Moccia, A. Alvito, M. Angiolillo, M. Bo, S. Canese, M. C. Follesa, D. Cuccu, R. Cannas, *Plos One,* in preparation.

Four *roche du large* ecosystems and four canyons from south Sardinia continental margin where investigated through eleven target species of gorgonians and black corals, in order to test for differences in alpha and beta biodiversity in contrasting geological setting.

- Chapter 11 contains the paper:

‘Nursery area for the small spotted catshark *Scyliorhinus canicula* in a millenary forest of *Leiopathes glaberrima* in South-western Sardinia’.

Alessandro Cau, Maria Cristina Follesa, Marzia Bo, Simonepietro Canese, Andrea Bellodi, Danila Cuccu & Rita Cannas. Biodiversity and conservation (in preparation).

It describes a nursery area for the lesser spotted catshark *Scyliorhinus canicula* within a *Leiopathes glaberrima* forests in South West Sardinia. The forest dwells on a roche du large ecosystem surrounded by miles of highly trawled muddy bottoms. The ecological role as nursery area for elasmobranchs of this millennial forest as well as the reasons and urgency for its conservation are discussed in detail.

Section three – general discussion and final conclusions

Two chapters (chapters 12 and 13) compose this section. In particular:

a. In the '**General discussion**' (chapter 12) the main findings of the previous papers are integrated and discussed on a broad perspective.
b. The '**Final conclusions**' (chapter 13) briefly resume the bullet points of the present research and how these results could improve conservation strategies both at species level (red coral *Corallium rubrum*) and at the community level.

Section four – references and appendix

Finally, on section four the final two chapters:

- Chapter 13 includes the '**References**' for all the previous sections and chapters.
- The '**Appendix**' contains the 'bibliography of the candidate', that is the list of the other papers co-authored by myself. The abstract of the articles focused on topics pertinent to the coral communities studies are included.

5.2 ORIGINS OF THE PROJECT

The *Mare medi terraneum* (''sea in the middle of the land'', in Latin) is one of the most important biodiversity hotspots of the world, hosting many different species and natural resources (Bianchi and Morri 2000; Coll et al 2010). Among Mediterranean marine life, benthic communities are possibly the most peculiar in terms of richness and originality (Gili et al 2014). Within Mediterranean Sea, coral communities play an important role and have been widely investigated in the last several decades. Numerous studies have been published dealing with the biology, ecology and distribution of the most common coral species dwelling in the photic zone of the Mediterranean basin (Gili and Coma 1998; Coma et al 2000) or in the deep sea (Bongiorni et al 2010; Danovaro et al 2010). On the contrary, the mesophotic zone of the Mediterranean basin, that extend from 50 to 200m depth, remains among the less explored environments of this basin (Cerrano et al 2010; Bo et al 2012b).

Along the Sardinian continental shelf (central western Mediterranean) investigations on coral assemblages are limited to the target species *Corallium rubrum,* which has been studied across the last decade mainly for management purposes (Follesa et al 2013a; Cannas et al 2014), whereas the ecology and distribution is far less known.

5.3 AIMS AND OBJECTIVES OF THE RESEARCH PROJECT

The aim of this thesis is to document the deep-water coral communities of Sardinia (their species richness and distribution), and to test if intrinsic geomorphological features of different hard substrates occurring along the continental margin lead to different deep-water coral communities.

The ultimate goal of this work is to highlight new insights for refining conservation strategies, both at species and community level.

This overarching objective has been achieved by performing experiments that combined ROV footage and Multi-beam datasets; in detail: a) two single-species based investigations focused on the Mediterranean red coral *Corallium rubrum* (Linnaeus, 1758), along different geological settings; b) two community based studies focused on ten/twelve target species along contrasting geological settings (rocky outcrops and canyons) and c) an investigation on a black coral forest of *Leiopathes glaberrima* (Esper, 1788) and its role as nursery area for elasmobranchs.

6 Literature review

6.1 THE 'ANIMAL FOREST' CONCEPT

The concept of forest is widely used in the terrestrial environment, being defined as complex ecosystems representing important three-dimensional habitat supporting high levels of biodiversity both within and in its surrounding areas (FAO 2006). Forests supply food, protection, and support to a great variety of organisms, which may establish occasional or highly specialized relationships both with the dead and living portions of the trees (FAO 2006). The forest concept has recently been shifted to the marine environment (Cerrano et al 2010; Rossi 2013; Bo et al 2014b) as, similarly to terrestrial ones, oceans host extraordinary examples of forests, comparable in complexity, biodiversity, and structuring role to the terrestrial ones (Bo et al 2015). What is peculiar and, actually, of great interest in the marine environment, is the ecological shift along the water depth gradient from 'vegetal' (*sensu lato,* e.g., seagrass meadows or encrusting algae) forests to the so-called 'animal forests' (Rossi et al 2012; Rossi 2013; Gili et al 2014). Especially in the deep circalittoral zone, where light is feeble or almost absent and the level of environmental stress are larger due to high sedimentation and the scarce food supply, macro-benthic communities are almost entirely composed of sessile animals, mainly k-strategists with far-reaching specializations (Bo et al 2009; Cerrano et al 2010; Gili et al 2014). Engineering species of these typical of these communities include various anthozoans such as gorgonians, black corals and soft corals which typically show an arborescent vertical development and may inhabit vast areas of rocky bottoms (Bo et al 2009; Cerrano et al 2010; Gori et al 2011b). Corals play a paramount role in marine benthic food webs, amplifying the pelagic–benthic transfer of energy and ecosystem's overall complexity, by providing colonisable surface for other organisms of the benthos (Gili and Coma 1998; Buhl-Mortensen et al 2010; Cerrano et al 2010).

6.2 MESOPHOTIC BENTHIC COMMUNITIES

Benthic communities dwelling in the lower circalittoral zone and/or the upper bathyal zone are still poorly known, although the awareness of their ecological relevance is progressively increasing (Bo et al 2012b; Bianchelli et al 2013). In fact, forests of these deep-sea corals along with other benthic organisms becomes the most important engineering species along the continental margin (Rossi et al 2012; Rossi 2013), which creates hot-spots for biodiversity, providing refuges for numerous specimens (Bo et al 2011b; Cau et al 2013b) and represent a nursery area for several commercial fish (Ballesteros 2006; Etnoyer and Warrenchuk 2007; Baillon et al 2012; Baillon et al 2014). The three-dimensional complexity originated by their populations may influence the water flow at a local scale and increase the residence time of suspended particles (Gili and Coma 1998; Bianchelli et al 2013; Gili et al 2014). These complex and well-structured communities accumulate one of the highest biomasses within world seas and oceans because of their widespread distribution over the continental shelf, canyons and seamounts worldwide (Gili et al 2014).

The main justification for the ‘delayed’ interest of scientists in mesophotic coral communities compared to their coastal counterpart, is imputable to the technical difficulties of sampling; in fact, because of the poor reliability and spatial resolution of traditional destructive benthic sampling techniques (e.g., trawls and dredges) quantitative and qualitative information on sessile mesophotic fauna were hard to obtain. The fast development of underwater technologies such as remotely operated vehicles (ROVs) broke the previous limitations of traditional (and often blind) sampling techniques as well as limits of the human diving such as depth, diving time and temperature. It basically provided the opportunity of controlled sampling and detailed observation of specific habitats, including mesophotic (Gori et al 2011a; Bo et al 2012a; Bo et al 2012b). In addition, ROVs have proven to be particularly useful for aquiring high definition footage for image analysis, a non-invasive tool that is particularly relevant for such communities of high conservation interest.

6.3 DISTRIBUTION OF BENTHIC COMMUNITIES

The main variables delimitating anthozoan communities are changes in the relief, slope of the substrate, grain size of the sediments, inter-specific competition and the alternation between hard and soft bottoms; these factors can affect the birth, larval settling, growth, and death rates of individuals (Lesser et al 2009; Edinger et al 2011; Gili et al 2014).

Superficial complexity of rocky outcrops are likely to have considerable influence on their distribution (Bo et al 2009; Edinger et al 2011) as they modulate the accumulation of sediments and contribute to current flow modifications, which altogether have ultimate consequences on the supply of food to the benthic fauna (Wilson et al 2007).

The distribution of benthic communities is ruled by several environmental gradients that tend to be more sharp along the vertical axis rather than the horizontal plane: light spectrum, temperature, water movement, grain size of soft sediment (all factors decreasing with depth) and also the amount of hard substrates (higher in shallow depth and in the continental shelf zones) plays an important role influencing the *biota* distribution. Along with the vertical axis gradient, the horizontal plane heterogeneity is the main driver of distribution and diversity of benthic organisms, which is caused mainly by the sea-bottom inclination, exposure to light, texture, type of substrate, orientation to predominant currents.

6.4 CANYONS, SEAMOUNTS, AND ROCKY OUTCROPS

Recent interest has focused on the variability of coral communities associated with several geological features (e.g., canyons, seamounts, and small rocky outcrops arising from surrounding muddy bottoms), which are widely distributed throughout continental shelf, along the Mediterranean basin. Concepts driving current methods for the characterization of marine benthic habitats are presently leading to a strict conjunction of geophysical methods with biological perspectives, which together are necessary for the ecologically sustainable management of living resources (Diaz et al 2004).

Rocky elevations like *roche du large* ecosystems or seamounts, depending on their dimensions and elevation across the water column provide dramatic contrast to the surrounding flat abyssal plains and edges of continental margins, having the potential role to work as an aggregating structures that, most typically, hosts abundant benthic communities dominated by suspension feeders (Ramirez-Llodra et al 2010). In particular, they have proved to host rich coral fauna, greatly enhancing biodiversity compared to surrounding substrates (e.g., gamma diversity at the basin scale); because of this, the awareness of their ecological role as biodiversity oases has considerably increased in the last few years (Bo et al 2011b; Misic et al 2012; Bo et al 2012b; Bianchelli et al 2013).

Because of their rugged topography and difficult access for sampling, detailed multidisciplinary (geological, physical and biological) studies of the ecology of submarine canyons have developed only in the last three decades, in parallel with advances in marine technology: previous sampling gears were indeed not adapt for investigating either the complex topography of these geological features or the hosted communities. In spite of this difficulties, recent studies have repeatedly shown that submarine canyons, which dissect most of Europe’s continental margins, enhance the habitat heterogeneity of the continental margins (Levin and Dayton 2009) and act as hot-spot for biodiversity are submarine canyons, which dissect most of Europe’s continental margins

(Canals et al 2006; Danovaro et al 2010; Pusceddu et al 2010; Ramirez-Llodra et al 2010). Mediterranean canyons that extend across the continental shelf are known to be particularly steep and landward extended compared to worldwide canyons; this particular characteristics let benthic communities associated with these geological features intercept organic-matter-rich-sediments being transported along inner shelf zone (Pusceddu et al 2010). Furthermore, canyons are heterogeneous habitats; canyon head and walls can present rocky outcrops suitable for sessile filter feeders such as anthozoans, while the axis of the canyon can accumulate soft sediment and have a fauna dominated by deposit feeders, scavengers and predators such as echinoderms, crustaceans and fish. Because of their higher habitat heterogeneity and accumulation of organic matter, canyons are predicted to support a higher diversity and biomass than the adjacent slope (Canals et al 2006; Sardà et al 2009; Pusceddu et al 2010; Ramirez-Llodra et al 2010).

Such studies have defined canyons and also *roche du large* ecosystems as essential fishing habitats (EFH) (Sardà et al 2009), which can be defined as "ecosystems used by the fauna for a critical stage of their life cycle": thus, such ecosystems showing a particular combination of abiotic and biological characteristics, with a highly complex structure and/or those that favour critical aspects of life such as reproduction, feeding and refuge" (Rosenberg et al 2000; Ramirez-Llodra et al 2010; Baillon et al 2012; Baillon et al 2014). Furthermore, as these habitats are potential fishing commercial grounds, they can be subject to fishing activities that directly damage these fragile corals communities (Bo et al 2014a).

Section two – experiments and results

7 Preliminary data on habitat characterization relevance for red coral conservation and management

Alessandro Cau, E. M. Paliaga, R. Cannas, G. Deiana, M. C. Follesa, F. Sacco, S. Todde and P. E. Orrù, *Italian Journal of Geosciences,* 2015, vol 134 (1): 60-68.

7.1 ABSTRACT

Through a non-invasive and interdisciplinary approach, distribution and size/age structure of deep dwelling populations of the precious red coral *Corallium rubrum* (Linnaeus, 1758), were described in two different areas of the south Sardinia continental shelf: San Pietro Island and Capo Carbonara. Although its presence in south Sardinian waters is known, data on coral populations dwelling in deeper waters (below 50 meters of depth) are still represented by few studies. Data collected in the framework of the project "MaGIC" (Marine Geohazard along Italian Coasts), carried out in summer 2009 and spring 2010, furnished the guideline to map and identify rocky habitats (e.g., submarine canyons and outcrops) along the continental margin, which are suitable for red coral settlement. An extensive ROV survey, carried out in autumn 2011, provided useful information on occurrence, abundance, density and morphometric parameters of coral colonies. Red coral was found in both sites, distributed in patches, in different scenarios characterized by different habitat complexity. Over 1400 colonies were found in Capo

Carbonara along the steep walls of a secondary channel of the Carbonara Canyon, while hundreds of specimens were found west of San Pietro Island along the outcrops ridges and on small boulders. Both populations were mainly composed by small and young colonies, while densities were different among sites, possibly affected by the role that habitat complexity plays in hydrodynamic and, consequently, on food supply, sedimentation, and settlement of colonies. These preliminary results contribute to advance knowledge on distribution and demography of deep dwelling populations of *C. rubrum*, which gain considerable importance in conservation and management of this precious resource. We strongly stress that interdisciplinary approaches that combines different scales of multibeam and ROV investigation are today fundamental to better understand distribution patterns within habitats, at species or community level.

7.2 INTRODUCTION

Concepts guiding methods for characterizing the benthic habitats are leading to a strict conjunction of geophysical methods with biological perspective, required for living resource management (Diaz et al 2004).

The precious red coral *Corallium rubrum* (Linnaeus, 1758) is a long living gorgonian with high commercial value, and is a component of the circalittoral and semi-dark cave community dwelling on rocky bottoms of the Mediterranean Sea (Costantini et al 2011; Priori et al 2013; Costantini et al 2013). This species can be found at depths ranging from 5 to 800 m

(Taviani et al 2010), although more commonly at 30-200 m (Follesa et al 2013a; Cerrano et al 2013). The populations occupying the shallower part of the distribution range (above 50 m depth) are characterised by small and dense colonies (Santangelo and Abbiati 2001) while deep dwelling populations, living below 50 meters of depth, are generally composed of larger, sparse and highly valuable colonies, which are the main target of professional divers (Rossi et al 2008; Priori et al 2013).

Corallium rubrum, like many species of corals, requires hard substrates, and particular light and/or current conditions, then the geological setting of the substrate has considerable influence on its distribution (Edinger et al 2011). In particular, deep populations of red coral form small aggregates (patches) on hard ground areas, where colonies are concentrated on the exposed surface facing into high-current areas (Rossi et al 2008; Cannas et al 2011).

For centuries, this precious coral has been of paramount importance for the island of Sardinia (central western Mediterranean Sea), which is still regarded as a worldwide pioneer in the management of red coral harvesting through an aware regulation (Cau et al 2013a). Nowadays, its fishery is allowed only to populations dwelling below 80 meters of depth within the mesophotic biogenic reefs (80–150 m depth), in an effort to protect the shallower populations, which had been overharvested with non-selective gears for centuries (Follesa et al 2013a).

Providing scientific knowledge on deep dwelling populations of red coral has become a priority for the implementation of effective management and conservation policies, as strategies to ensure conservation and rational harvesting need to be based on a sound background of population distribution and demographics traits, particularly for long living species like *C. rubrum* (Bramanti et al 2007; Rossi et al 2008; Tsounis et al 2013; Costantini et al 2013; Bramanti et al 2014).

So far, very few ROV investigations were carried out in Sardinia, in the north and north-western coast and in the San Pietro Island area (Cannas et al 2011). As consequence, deep dwelling populations of red coral are still poorly known, and no data are available from the southeastern part of Sardinia.

Aim of this work is to present new preliminary data regarding:

- Occurrence, abundance, size and age structure of deep dwelling populations of *Corallium rubrum*, in a poorly known area in the heart of Mediterranean Sea.
- Geomorphologies supporting red coral distribution, at different scales, along south Sardinian continental margin.

These preliminary data will be used to plan further, more detailed researches on red coral distribution along Sardinian coasts. The study areas have been selected in order to document different geological morphotypes, representing the different geological setting of the south western and the southeastern margin of Sardinian continental shelf.

7.3 MATERIALS AND METHODS

The dataset discussed here was collected during two cruises carried out in the framework of the “MaGIC” project (Marine geohazard along Italian coasts) in 2009 and 2010 by CoNISMA’s R/V Universitatis, using two acoustic systems: I) RESON SEABAT 8160 50 kHz multiBeam Echo-Sounder (MBES) and II) GEOACOUSTIC CHIRP II Sub-Bottom Profiler (SBP).

Onboard the R/V Universitatis, the integrated system used an IXSEA OCTANS motion sensor and gyro and a Satellite Differential GPS (SDGPS). The datum was WGS84 and the UTM projection was chosen for navigation and display, fuse 32 N, providing a detailed data coverage of South Sardinia geomorphology through high-resolution morpho-bathymetric maps. Data collected during the survey were integrated with CARG project (Official National Italian Geological Cartography). This Geomorphological analysis provided a useful guideline to plan the further ROV survey, as data obtained through Multibeam echosounder were used to create 3 different maps where ROV transects are reported in respect to:

i) Three-dimensional georeferenciated shaded relief maps of investigated sites;

ii) Profile curvature, which describes the rate of change of slope along a profile in the surface and may be useful to highlight convex and concave slopes across the Digital Terrain Model (Wilson et al 2007);

iii) Slope of the sea bottom.

Biological data on red coral were collected during a Remotely Operated Vehicle survey (using the underwater ROV "Pollux") carried out on board the R/V Astrea during autumn 2011. The ROV was equipped with a digital camera (Nikon D80, 10 megapixel), a strobe (Nikon SB 400), and a high definition video camera (Sony HDR-HC7). The ROV hosted also a depth sensor, a compass, and three laser beams providing a continuous 10-cm scale. Surveys have been conducted at depths ranging from 80 m to 115 m in two areas: west San Pietro Island and in the Capo Carbonara canyon (henceforth SPi and CCc, respectively) (Figure 7.1, Figure 7.2). The resolution of the camera allowed very precise measurements (i.e., down to 1 mm). 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 a target species, *Corallium rubrum*, which is distributed in patches in semi dark caves, steep walls, boulders. The precise measure of the length of each track was obtained, assuming a constant speed of the ROV; soft bottoms and low visibility frames were discarded in the initial stage of video analysis. When patches of red coral were found (a patch is defined as per Follesa et al 2013 a group of >2 colonies), a representative number of frames were taken. Within each frame, randomly positioned 50x50 cm squares (used as Useful Sampling

Unit) were obtained with CPCe Software (Coral Point Count with Excel extensions) (Kohler and Gill 2006). Through the ROV laser beams, a scale for CPCe software calibration was provided. The number of Useful Sampling Units (henceforth called USU) was proportional to the patch extension, in order to better represent it; a minimum of 4 USU was taken per each patch, always covering a minimum surface of 1 m^2 (i.e., four 50x50cm squares). Although considerable distances separated patches, a minimum of 10 meters was used as a reference to define two patches distinct (Rossi et al 2008).

7.3.1 Geological setting

Study areas are located along the south Sardinian continental margin (Figure 7.1), whose structural setting is the result of the superimposition of two successive deformational regimes. The first deformation is related to a compressive geodynamic phase of crustal thickening occurred during the Oligocene - Miocene, contemporary to the Sardinia – Corsica microplate rotation and the opening of the Algero - Provençal basin. The second deformation, more recent, is associated with the Tyrrhenian spreading stage, characterized by extensional tectonic and a slight crustal thinning before the Messinian crisis (Mascle et al 2001). Along the southern continental margin, two different tectonic styles can be recognized:

i) To the west, a direct fault system produced a series of intraslope basins within continental shelf and upper slope. These basins probably evolved during early-middle Miocene. The outer part of the continental margin is also affected by the same faults system along which lower margin blocks are lowered (Lecca 2000).

ii) In the Cagliari gulf area and its eastern part, the morpho-structural setting of the southern margin is articulated in valleys and isolated seamounts. The southern part of the Sardinian rift, with the superimposed Campidano graben structure continues at sea within the Cagliari gulf, in the continental shelf level and in the deeper part (Lecca et al 1998).

7.3.2 Geomorphology

The DTM model, obtained from the "MaGIC" project MBES dataset, shows the main features of investigated areas. In the western area where SPi is located, the continental shelf is characterized by an irregular morphology with large outcrops of the Oligo-Miocene volcanic-sedimentary succession, bounded on the Southwest by the shelf edge situated at 180m.

Ten miles off Capo Sandalo (westernmost cape of San Pietro Island) the limit between inner and distal shelf is represented by an array of paleo-cliffs engraved in the volcanic substrate with the foot located at an average depth of 140 meters and the top at about 90 meters. The paleo-cliff lines shows several gravitative morphologies, caused by backwards erosional processes; the lithology of these cliffs implies a long-term evolution and polycyclic elaboration, which had likely started in late Middle Miocene and continued during the Plio-Pleistocene transgressive phases. However, the erosional and gravitative morphologies are mainly due to the last low standing stage of sea level fluctuations (MIS 2). During this stage, physical alteration processes played an important role on the rock surface, due to periglacial subaerial conditions. The tectonic features that controlled the orientation of the cliff walls (NNW-SSE and NE-SW oriented) caused rotational slides and it is possible to distinguish separation niches and tilted slide deposits (Orru' et al 2012).

The proximal area is characterized by large mesas, cuestas and other typically volcanic morphotypes such as calderas, necks and mega-dykes, separated by deep incisions often filled by coarse sediments of both bioclastic and terrigenous origin. The distal area, slightly sloping, (0.6° - 0.8° degrees) is filled at the basis by the Miocene sedimentary series and by the Plio-Pleistocene succession, constituted by many system tracts of clinoform patterns (Lecca 2000). Several depressions filled by fine-grained sediments that characterize this shelf area have been identified, and the passage between the distal and proximal sectors occurs at -130 m depth (Ulzega et al 1980).

In the east area where CCc is located, the continental shelf reaches a maximum width of about 2 km and is characterized by sub planar morphology with a slightly steep ground (about 3-4 %). The shelf edge lies at a depth of 120 m; except for the canyon heads areas where the edge is at the depth of 90 m. In the study area, the Carbonara canyon collects ten tributary channels, all interested by retrogressive evolution. In the CCc area, the morphology is strongly controlled by tectonic lineaments, which follows the main regional structures, both on the continental shelf and in upper slope areas. In particular the western shelf edge and the upper slope are oriented according to an important tectonic lineament N130° oriented, resulting in a steep (>40°) fault wall exposure.

7.3.3 Extensive ROV survey:

The survey was focussed on locating and quantifying occurrence, size structure, and density of red coral in southern coasts of Sardinia (central western Mediterranean Sea). The main objective was to compile an extensive set of data through non-destructive methods (without causing any destruction to the colonies), in contrast with fishery dependant data gathered in the majority of past studies on *Corallium rubrum*.

For each site the following parameters have been retrieved and measured:

1. Abundance: i) number of patches in 100 linear meters; the total distance covered by the survey was obtained from ROV track positioning and ii) number of colonies per USU (Gori et al 2011a).

2. Density, estimated per each site as the mean number of colonies within patches per square meter (i.e. mean of densities within USU). The maximum and the minimum number of colonies per USU have also been recorded.

3. Basal diameter and colony height (from the basis of the colony to the farthest tip), in order to determine the proportion of colonies with a diameter of more than 8 mm (Sardinian legal size limit for harvesting)(Follesa et al 2013a), and the portion of colonies that

reached the maximum reproductive output (i.e. 6 cm in height (Tsounis et al 2006a). Red coral colonies were measured only if properly positioned with respect to the picture perspective, while dead colonies were counted (and consequently included in density values) but had not been measured.

4. From size/frequency distribution, the population structure of sites was investigated through descriptive statistics including skewness, kurtosis and associated p-values. Significance for skewness and kurtosis was calculated using the software XLSTAT (function ‘descriptive statistic’).

5. Site complexity was evaluated through slope (expressed as “low”, < 25°, “medium”, from 25° to 45°, or “high”, >45°), and profile curvature (high or low alternation of concavity and convexities) as parameters, at different scales, from small scale DTM and from ROV videos.

6. Number and percentage of non–ramified, ramified, dead and alive colonies, and orientation of colonies (0°, 45°, 90°, 135°, 180°). As already done in other works (Rossi et al 2008): 0° orientation is perpendicular to the horizontal sea floor, facing straight up, while 180° in overhanging position, facing straight down.

7. Colony age was determined following growing rates, estimated for Sardinian populations (Cau, unpublished data). Determination of

growth rate was made by “thin section-organic matrix staining datation method” (Marschal et al 2004).

7.4 RESULTS

Two ROV dives (1 per site) of about 2 hours each (4 hours of filming in total) at an average speed of 0.34 knots were conducted, resulting in a total distance of 2.74 nautical miles (5.07 km). The useful sampling distance (i.e. rocky bottoms, discarding soft bottoms and non-clearly visible images) covered by the ROV was 1.15 out of 1.22 nautical miles in SPi and 1.20 nautical miles out of 1.52 in CCc.

A total of 178 high definition frames were used for the image analysis. In detail, 66 SPi during a 2.13 km long transect and 112 in CCc (within a 2.22 km useful transect). By summing all the USU, a surface of 16.5 m^2 was investigated in SPi and 28 m^2 in CCc. The minimum number of USU for describing species diversity and patterns within coralligenous assemblages was considered a proper reference for the description of red coral patches in each site (i.e. minimum of 4000 cm^2 per site, using 20 x 20 cm squares)(Kipson et al 2011).

Red coral colonies were found in both sites, in different scenarios: along rocky ridges of carbonatic outcrops and boulders in SPi and over steep walls in CCc.

Among all frames investigated, a total of 1801 colonies were counted (330 and 1471 in SPi and CCc, respectively). Moreover, a total of 448 colonies were measured (having the proper position with respect of the laser beams): 81 colonies in SPi and 367 in CCc.

San Pietro Island: The survey was carried out at depths ranging between 80 and 85 m, with an average depth of 82 m. As shown in Figure 7.2, the site is the top surface of a lava flow attributed to the Miocenic volcanic sequence. At a small scale it did not show a particular roughness, while a larger scale analysis underlined an irregular surface, although overall complexity remains low. The site is characterized by small steps (i.e., 1-meter tall steps) with sparse small boulders, slope values ranged between 1° and 5° (low) on the surface of the lava flow, with high values (45° to 90°) along steps ridges. A total of 13 patches of red coral were found along the transect, 0.61 patches per 100 m, the mean density in examined patches was 12.88 ± 16.18 colonies/m^2, while within USU (50x50 cm squares) a maximum of 49 and a minimum of 2 colonies were found. Red coral occurred along steps ridges (Figure 7.3), with colonies oriented 45°, 90° and 135° (respectively 32.6%, 34.8%, and 17.4% of the total measured colonies. While no overhanging colony was found, 15.2% of colonies were oriented in vertical position on the top surface of boulders (i.e. 0°, facing straight up). In detail within used frames, 5 colonies were dead (2.51% out of the total counted colonies), while 78 were non-ramified (39.20%) and 116 were ramified colonies (58.29%).

Analysing the frequency distribution, the modal class in basal diameter was that of 6 mm (Figure 7.5), which was dominant in terms of number of colonies compared to other classes (kurtosis 9.42, p-value = 0), while in height the modal class was the 6–8 cm. The mean value for basal diameter and height of colonies were 7.9 ± 4.9 and 6.96 ± 4.05 respectively. The positive value of skewness in the size/class distribution indicates a predominance of small sized colonies within the population; this data is confirmed by the fact that 57.8% of the measured colonies had a basal diameter lower than 8 mm, which is the actual limit in force in Sardinian waters. Regarding the age structure of this population, the mean age among measured colonies was 25.4, and the modal class was 20 years old (Figure 7.7). The youngest colony was estimated to be 10 years old, and the oldest 94 years old.

Capo Carbonara canyon: The survey was performed at depths ranging from 88 to 115 m. The dive site is located in the shelf edge area on a NW-SE oriented regional fault wall, in the top of a deep and steep V-shaped channel indented into the continental shelf for 900 m. This canyon is controlled by a secondary tectonic feature oriented NE-SW (Figure 7.2). The CCc site shows high roughness both at small and large scale; the DTM analysis underlines an irregular surface and the overall complexity is high. The small-scale analysis describes an area characterized by a 50 meter high rocky wall overlooking the channel head; slope values ranges between 13° and 29° (medium) on the flatter parts, with high values (45° to 68°) along the rocky wall (Figure 7.2). Through a larger scale view, the site shows a high turnover of concavity and convexity throughout vertical walls with high values of slope, where red coral occurred. A total of 16 patches of red coral were found (0.72 patches per 100m on average) along the track, over steep walls concavities and on crust ridges (Figure 7.3). In all patches, the majority of colonies were oriented 90° (20.6%) and 135° (60.2%), with a minority oriented 45° (5.1%, Figure 7.4). In detail, out of a total of 1471 colonies, 454 colonies were non-ramified, 573 were ramified and 444 were dead (respectively 30.8%, 39.1% and 30.1%). The mean density for this site was 54.14 ± 32.36 colonies/m^2, while a maximum of 97 and a minimum of 2 colonies were found within USU. The modal size class in basal diameter was that of 6 mm (Figure 7.6). The size/class frequency distribution emphasizes the predominance of small size colonies (skewness 1.26, p-value = 0) and few size/classes compared to the total (i.e. positive kurtosis: 2.77, p-value = 0). Furthermore, the 26.1% of measured colonies has a basal diameter larger than 8mm while the modal size/age class was 20 years old. The mean age for this population was 19.8 and the oldest colony was estimated to be 60 years old, while the youngest 7.3 (Figure 7.7).

7.5 DISCUSSION

This work examines through a non-destructive and interdisciplinary approach, distribution, abundance, size and age structure of two red coral populations dwelling between 80 and 115 meters in two sites from south Sardinian continental margin (Central western Mediterranean Sea).

Through DTM analysis sites with different geomorphological setting were described, showing how these sites host red coral patches with different population structures and densities. SPi, which is the shallower site, showed low level of geomorphological complexity together with a lower mean density compared to CCc (12.88 vs 54.14 col/m^2; Figure 7.7). Conversely, CCc showed higher complexity with steep rocky walls where red coral occurs with perpendicular and overhanging position. Although direct measures of current and sedimentation rates were not taken in the investigated sites, differences in the current intensity were easily deducted from the accumulation of silt. In particular, the accumulation was almost absent in SPi, while was on the gently sloping surfaces of the canyon in CCc. This observation is confirmed by a higher frequency of vertically growing colonies in SPi, that are actually absent in CCc, possibly because of the accumulation of sediment does not make the substrate suitable for settling of *C. rubrum*. The canyon shape described in CCc probably provides more vertical rocky walls, where the accumulation of silt cannot occur and the currents provide good organic matter availability (Tsounis et al 2006c). In addition, smaller colonies are present in higher density in CCc, compared to SPi where the suitable surface for red coral is larger.

Concerning size structure of investigated populations, results indicate that a large portion of colonies do not reach the minimum commercial size of 8 mm (43.2% of SPi and 26.1% of CCc colonies are above this limit). Moreover, mean value for colony height is very low in CCc (3.87 ± 2.30 cm), which is over 2 cm lower than 6 cm, the measure at which deep colonies reach the 100% of fertility and can ensure the maximum reproductive output (Tsounis et al 2006c). On the other hand the mean height for SPi is almost 1 cm higher than the previously cited limit. The two populations were significantly different (P=<0.01,Table 7.2), showing a significant positive skewness values in the size frequency distribution (2.74 in SPi and 1.26 in CCc) which underlines that both populations are composed mainly by small and consequently young colonies (Zhao et al 2014). In detail, the higher skewness revealed in SPi is justified by the presence of large sized colonies that are absent in CCc, which influence the overall size class distribution. A higher occurrence of young colonies was found in CCc (Figure 7.6), along with elevated densities, and a considerable number of dead colonies (more than 30%).

7.6 CONCLUSIONS

The two populations of *Corallium rubrum* found at different depths showed different pattern of density and population structure with respect to what is known from literature (Rossi et al 2008; Follesa et al 2013a; Priori et al 2013). The shallower site, SPi, showed lower densities and larger colonies than those found in the deeper site, CCc. These preliminary results demonstrates that it is not possible to define a general pattern using depth as a single factor influencing red coral distribution. In the two investigated sites, geomorphological complexity seems to be the most important factor influencing hydrodynamic and, in turn, the presence and density of red coral colonies. In conclusion, we strongly stress that a strict conjunction of geomorphological studies coupled with biological survey could give a better overview of distribution patterns of this species, being a priceless tool for conservation and management plans.

7.7 FIGURES

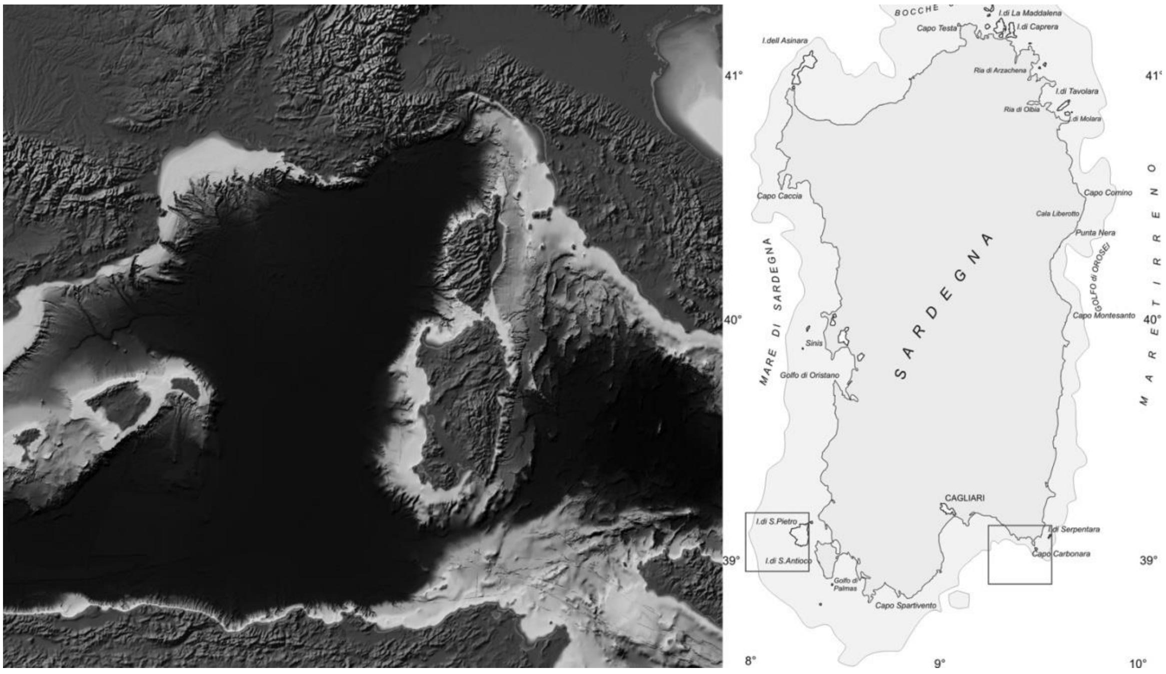

Figure 7.1 Geographic framework of the Island of Sardinia (left) and of the investigated sites (right), located on the southern continental shelf of the island of Sardinian - western Mediterranean Sea.

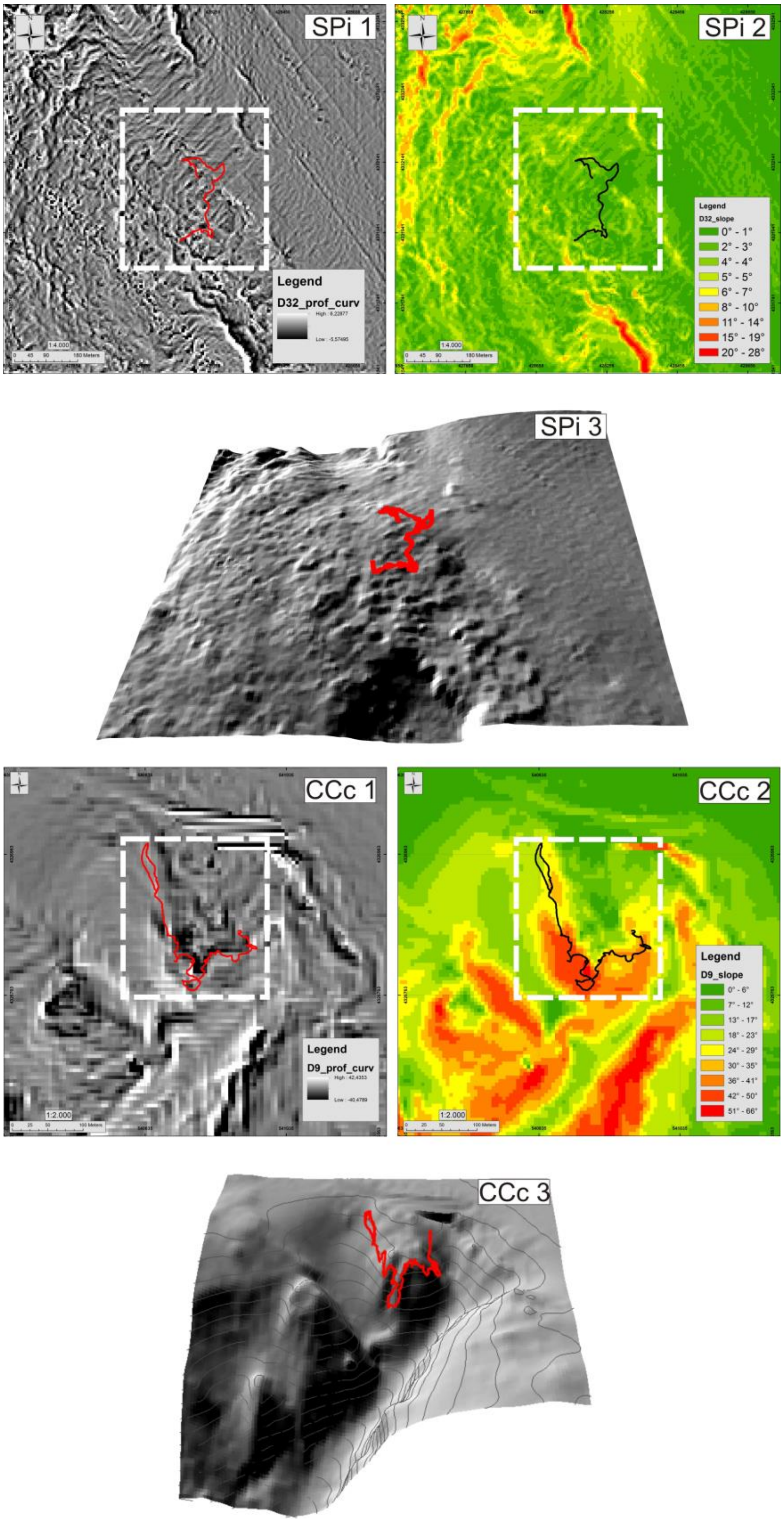

Figure 7.2 SPi 1) Profile curvature, SPi 2) slope and SPi 3) 3D view maps of SPi (vertical exageration 1.5x). The survey was conducted on the top surface of a lava flow; despite localized excerptions, overall complexity of the site is low. CCc 1) Profile curvature, CCc 2) slope map and CCc 3) 3D view of CCc (vertical exageration 1.5x), conducted on the head of a V-Shaped, strongly deepened, erosive channel. Overall complexity of the site is high.

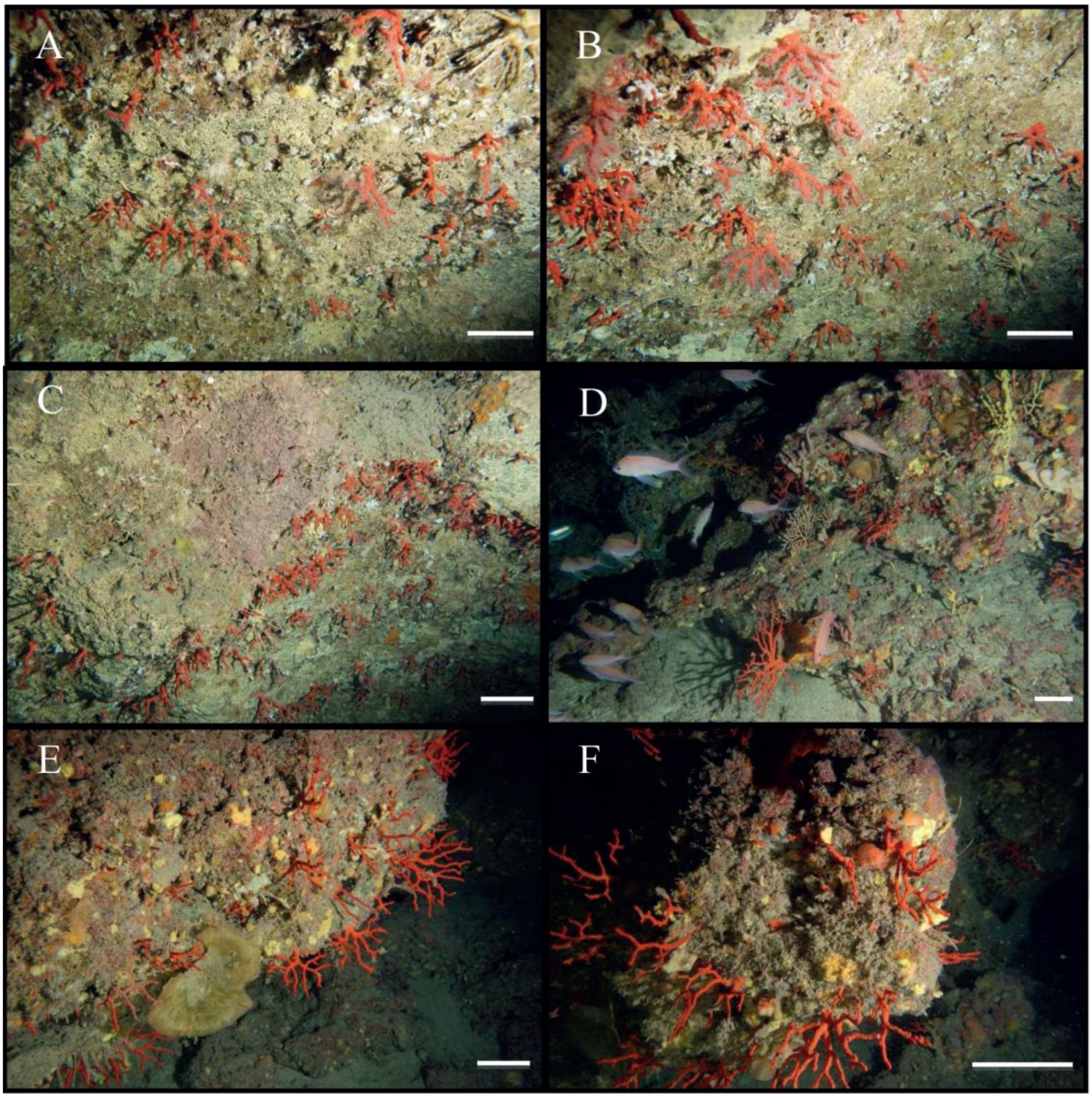

Figure 7.3 Red coral colonies of the studies sites. A, B, C) High density patches of C. rubrum with colonies oriented 145° along highly sloping walls in CCc; D,E,F) Vertical, 90° and 145° oriented colonies in red coral patches in SPi, where colonies occurred mostly along ridges of small steps that characterized this site. Scale Bar: 10 cm.

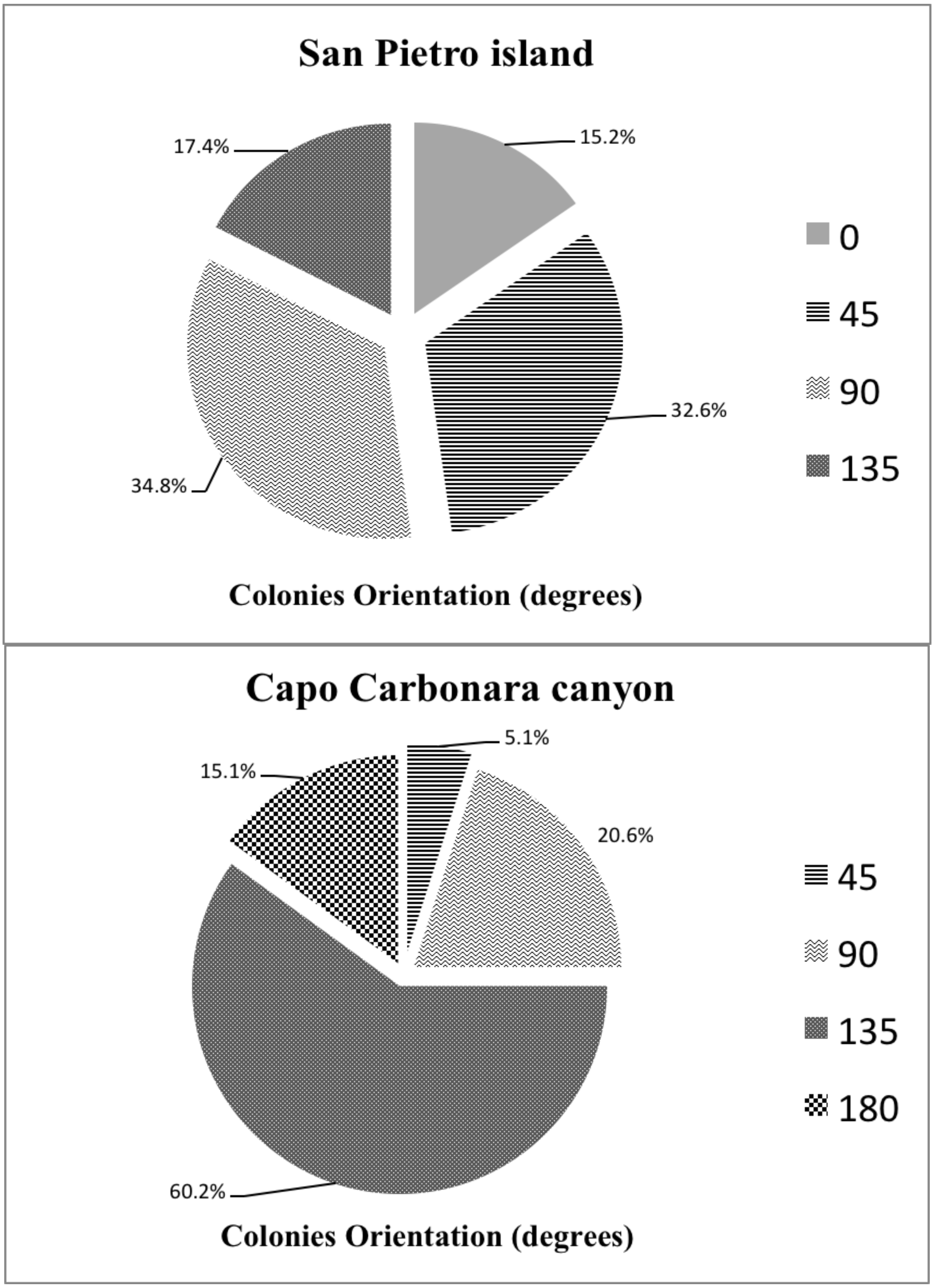

Figure 7.4 Percentage of colonies with different orientation with respect to the substratum (0°, 45°, 90°, 135°, 180°) in SPi (upper graph) and CCc (lower graph); 180° oriented were absent in SPi while 0° oriented colonies were absent in CCc.

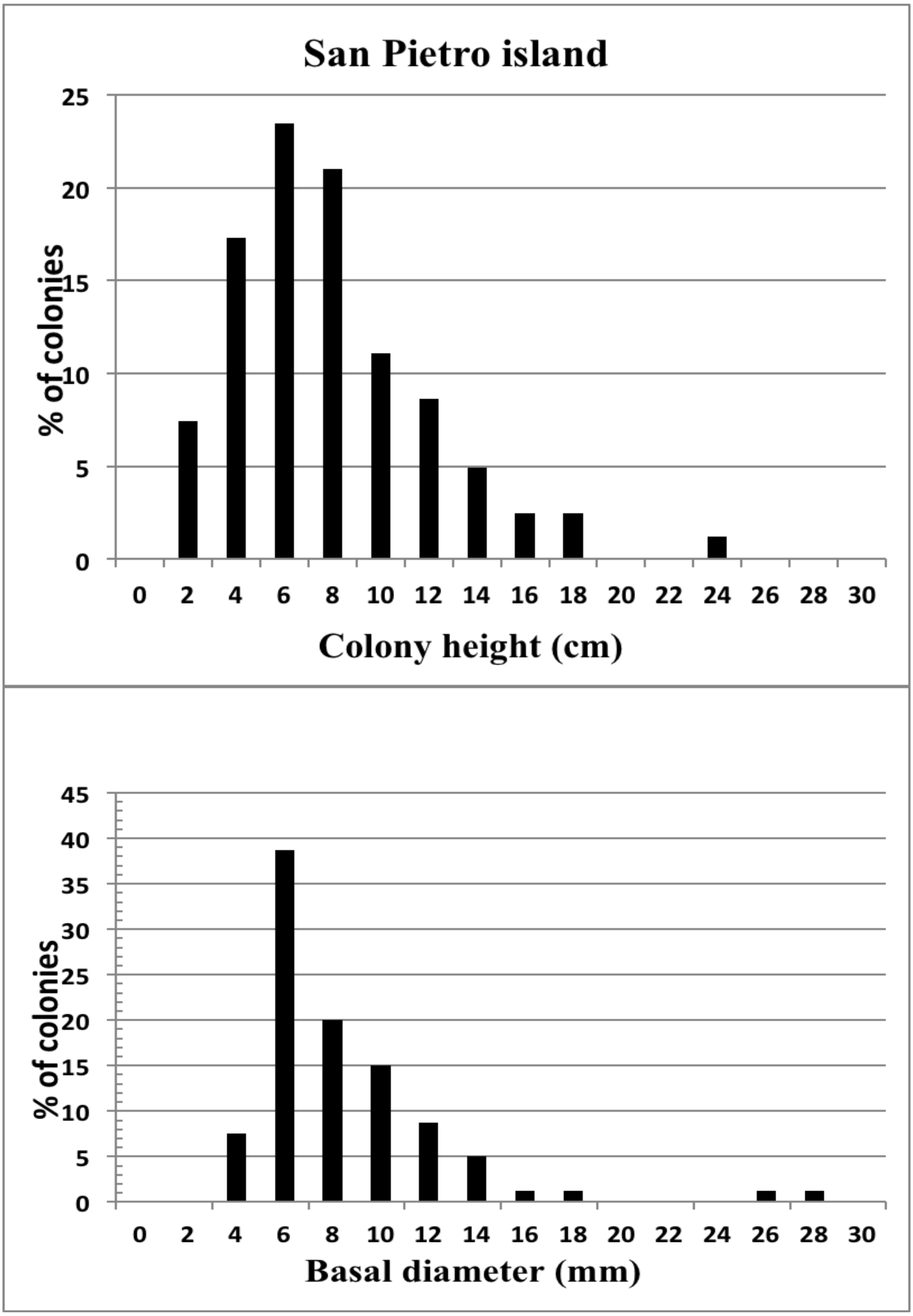

Figure 7.5 Size class % frequency for height (upper graph) and basal diameter (lower graph) of measured colonies in SPi.

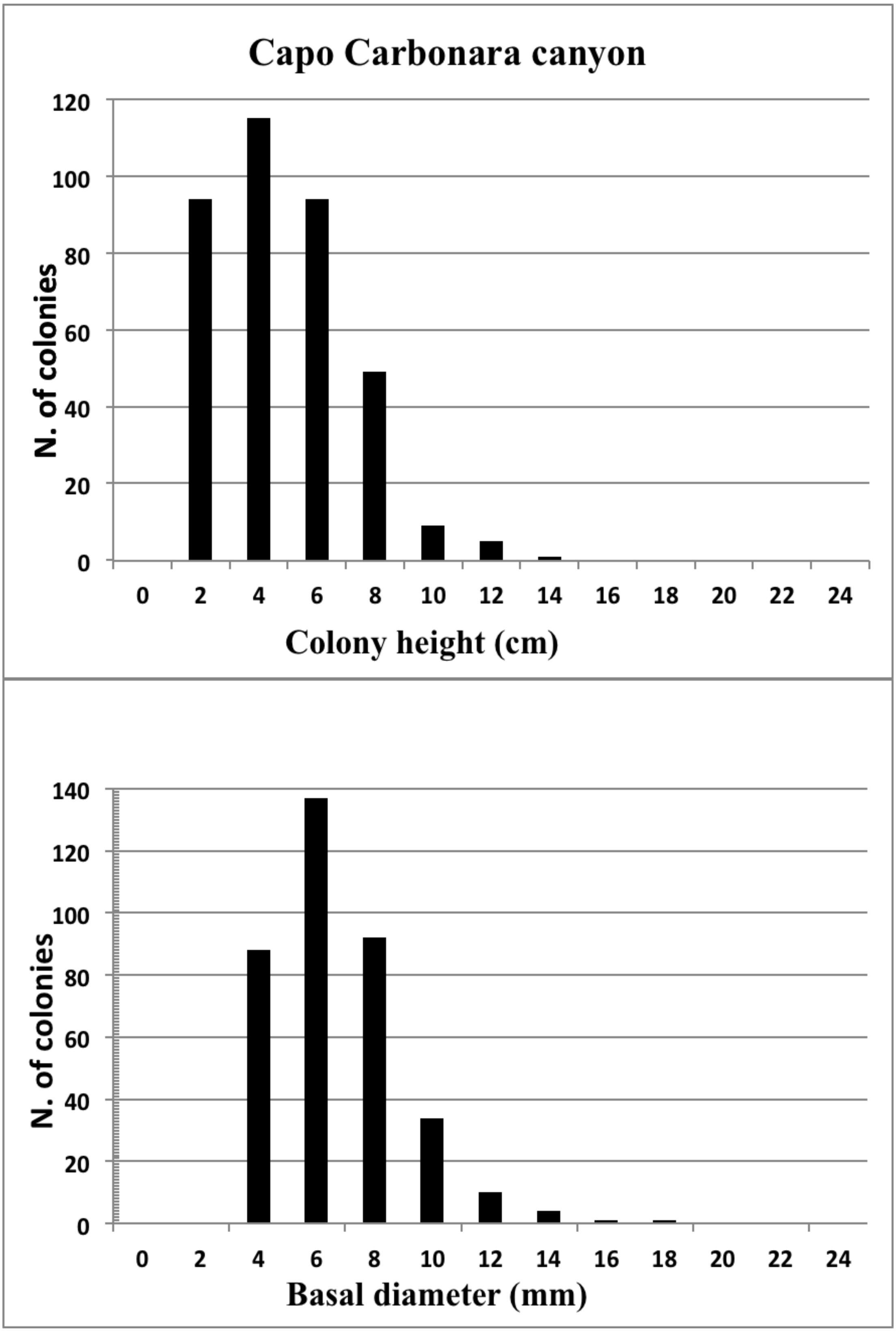

Figure 7.6 Size class % frequency for height (upper graph) and basal diameter (lower graph) of measured colonies in CCc.

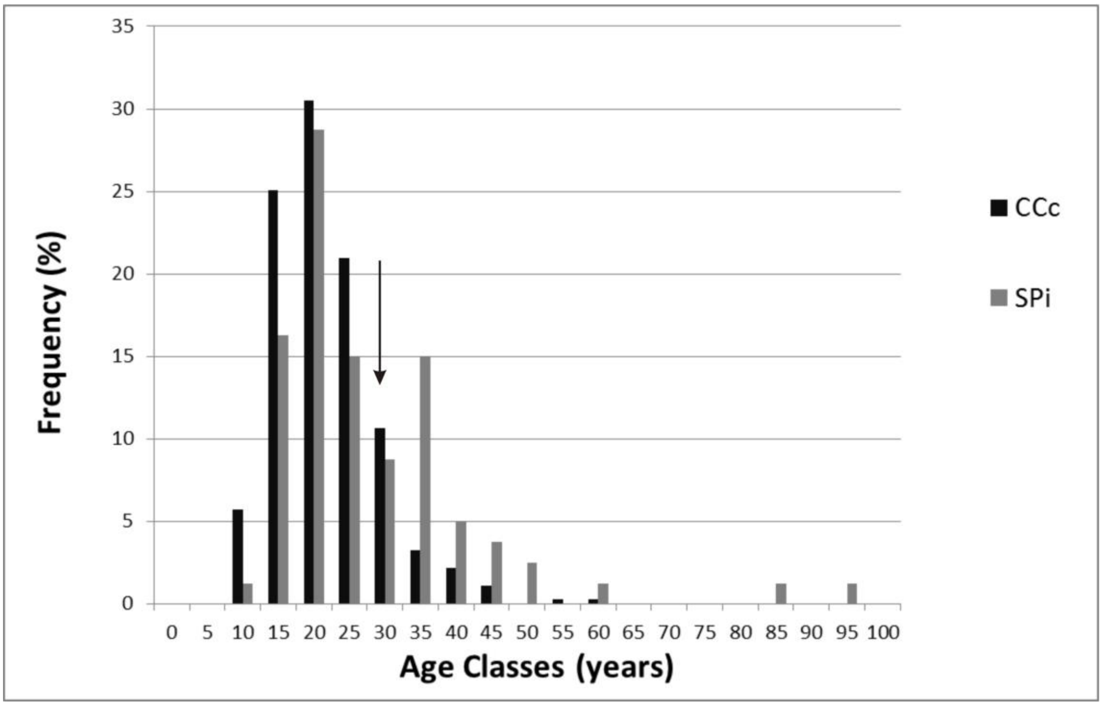

Figure 7.7 Frequency (%) of age classes (years) according to growth rate estimated for deep populations of red coral in Sardinian waters; the black arrow represents the age when colonies reach the minimum legal size for harvesting (i.e. 8mm in basal diameter), corresponding to 30 years of age.

7.8 TABLES

Table 7.1 Depth range, coordinates of the starting point of the performed ROV tracks. SPi and CCc are the code for the investigated areas: San Pietro island and Capo Carbonara canyon, respectively.

Area	Depth range (m)	Substrate	Coordinates	
SPi	80 - 85	Small boulders	N 39° 08' 113''	E 8° 10' 180''
CCc	90 - 115	Steep walls (canyon)	N 39° 05’ 27”	E 9° 28’ 21”

Table 7.2 Values of kurtosis and skewness for the size/class distribution (basal diameter) of red coral populations in SPi and CCc.

	San Pietro island	Capo Carbonara canyon
Kurtosis	9.42	2.77
Skewness	2.74	1.27
P-value	< 0.01	

8 Deep dwelling populations of Mediterranean red coral along different geological settings: back to the future

Alessandro Cau, L. Bramanti, M. Angiolillo, M. Bo, S. Canese, D. Cuccu, R. Cannas, M. C. Follesa, K. Guizien. *Scientific Reports* (in preparation)

8.1 ABSTRACT

The Mediterranean red coral, *Corallium rubrum*, is a long-lived habitat-forming species and one of the most economically relevant precious corals worldwide. Deep dwelling (>50 m depth), populations represent the current target of intensive professional fishery, though little is known about their ecology. It is generally assumed that coral populations follow a depth-related distribution, with small and dense colonies shifting to bigger and sparser colonies as depth increases. ROV footage coupled with multi-beam surveys in contrasting geological settings enabled us to analyse the relationships between *C. rubrum* populations' features and some abiotic and biotic drivers. Our results primarily show that water depth is not a major factor structuring red coral populations. Instead, other factors, possibly including competition for suitable rocky substrates, apparently influence density/size relationships along different geomorphologic structures, thus affecting the whole populations' shape. Our results show also that the distribution of the red coral can vary from patches characterised by high densities of small individual colonies to

patches exhibiting very low densities of large colonies. These results highlight the need for recasting conservation strategies, based on more refined environmental descriptors.

8.2 INTRODUCTION

Sessile organisms like hard branching corals, due to their three dimensional erect structure, enhance the benthic ecosystem's complexity, and play a pre-eminent role in the pelagic–benthic transfer of energy (Gili and Coma 1998; Bo et al 2009; Cerrano et al 2010; Rossi et al 2012). Very few corals, however, are regarded as precious items of professional fisheries (Tsounis et al 2010; Cannas et al 2014). Among these, the Mediterranean red coral *Corallium rubrum* (Linnaeus, 1758) has gained considerable attention from the scientific community because of its economic relevance for the jewellery market at global scale, which had led to an overharvesting mainly of shallow populations (<50 m depth) (Tsounis et al 2010). Due to the reduced density and size of colonies and to the protection measures applied above those depths, professional fishery of *C. rubrum* has progressively shifted to deeper populations (>50 m depth), which have been increasingly exposed to threat, and, as a consequence, are now under an adaptive management plan recently developed by the FAO/GFCM (Cau et al 2013a).

Ecology and distribution of deep populations of *C. rubrum* are still poorly known. However, recent development of submarine technologies like Remotely Operated Vehicles (ROVs) allowed scientists to extend their investigations to previously out-of-reach depths with non-invasive direct observations, particularly adapt for conservation issues. Although only few ROV-based investigations have been performed on the red coral populations in the Mediterranean sea (Rossi et al 2008; Follesa et al 2013a; Priori et al 2013; Bavestrello et al 2014c; Cau et al 2015), it appears that deep dwelling populations consist mostly of large, branched colonies, that can be distributed sparsely or form small patches on rocky surfaces (Santangelo et al 2007; Cannas et al 2011; Costantini et al 2013).

The distribution and size structure of *C. rubrum* populations is generally thought to be mostly driven by water depth, generally with shallow populations characterized by dense and small sized colonies gradually shifting to large, highly branched colonies, sparsely distributed with increasing water depth. On the basis of theoretical demographic models, it has been hypothesized that the structure of red coral shallow populations characterised by the dominance of dense but small colonies could be the consequence of centuries of harvesting: these led to the preferential loss of big colonies and the shift from the 'animal forest-like' shape of coral banks to grass plain-like structures (Bramanti et al 2009; Bavestrello et al 2014b).

The assessment of density-dependence of population dynamics through the analysis of the relationship between size or biomass and density, commonly used in forest ecology (Li et al 2000; Li 2002), has been recently used also in the marine environment (Linares et al 2008).

Intraspecific competition leads to changes in population structure according to the availability of space and resources (Ponti et al 2014). This is clearly observed in benthic suspension feeders in which an inverse relationship between the availability of food or space and colony size has been observed (e.g., *Paramuricea clavata* (Risso, 1826) and *Eunicella singularis* (Esper, 1791)(Linares et al 2008). However, the role of intra-specific competition in regulating shape and distribution of red coral populations in substrates approaching the carrying capacity (Li 2002), is still an unanswered question to be addressed in *Corallium rubrum.*

In this regard it is worth mentioning that heterogeneity and complexity of substrates have considerable influence on the distribution of benthic organisms (Edinger et al 2011), as they regulate the available space for settlement and also influence the food supply for the benthic fauna (Wilson et al 2007; Pusceddu et al 2010). Moreover, geomorphological attributes of the sea floor (e.g., slope, roughness) are important factors affecting bottom currents and rates of sedimentation; they can possibly affect larval settlement of benthic sessile species (Gili et al 2014; Ponti et al 2014), for instance by creating micro-habitats that act as shelters from predation (Cerrano et al 2010; Ponti et al 2014). As *C. rubrum* has a planktonic larval stage, we hypothesized that its distribution is influenced not only by water depth, but also by the type of the substrate, the availability of which could also lead to intra-specific competition.

To shed light on this issue, the objective of this work is to test three hypotheses: (1) *C. rubrum* population density is inversely related to depth, (2) density and mean individual size of *C. rubrum* population are inversely correlated and (3) *C. rubrum* population structure in the deep environment is related to different geological settings. To shed light on these hypothesis we take advantage of the new technological instruments, which give access to data on colony density and mean sizes of deep dwelling populations of *C. rubrum* and complement existing data on shallow populations.

8.3 RESULTS

A total of 3676 colonies of *C. rubrum* were counted and measured within 600 randomly positioned sampling units (50x50cm squares) to derive density and mean size per unit. Sampling units were distributed over 12 investigated sites (50 frames per site; Figure 8.1, Table 8.1) spanning from 85 to 170 m depth. The original dataset from the deep environment was complemented with the extensive dataset existing for shallow environment to test if a relationship between depth and population density exists (Table 8.2).

8.3.1 Is density of *C. rubrum* related to depth?

C. rubrum population density ranged from very low (1-2 colonies m^{-2}) to very high values (75 colonies m^{-2}) over the examined bathymetric range (Table 8.2, Figure 8.2). Depth, although significant, explained less than 11% of variance of population density ($R^2 = 0.10$, $P<0.001$), and when data were analysed for a restricted bathymetric range (85- 170 m), depth explanation power further decreased. Indeed, results from the General

Linear Model analysis show that despite the significant effect on density, the fraction of variance explained by the factor depth is very low and does not influence consistently either density or presence of red coral (Table 8.3).

Strikingly, in shallow sites (<50m), colony densities were always larger than 10 col. per sampling unit while in deep sites (>50m), both high (>10 colonies) and low densities (<10 colonies) can be found (Fig. 8.2).

8.3.2 Size and density relationship in *C. rubrum* populations

The relationships between density and i) branching (Figure 8.3) (here estimated as the ratio between the number of branches and the number of colonies per sampling unit) and ii) maximum height of colonies (Figure 8.3) are delimited by a rapidly decreasing curve. The data indicates that high densities are only observed when colonies are small, while large sized and highly branched colonies are only observed in patches with density lower than 10 col. per sampling unit. In between these two extremes, colonies of intermediate height and branching pattern can be found at intermediate population density.

8.3.3 Assessment of habitat constrains on *C. rubrum* presence and population stability

On the basis of the results indicating that both kind of populations of *C. rubrum* (dense small sized and sparse big sized ones) can be found in deep environment, we investigated how habitat constraints population density (presence) and structure, considering mesoscale and local scale habitat descriptors. At the mesoscale, two categories were identified according to bed slope: canyons or generally vertical walls environment (hereafter called 'Vwe') characterized by slope larger than 45°, and rocky pinnacles (hereafter called 'Pie') characterized by slope lower than 45°.

C. rubrum density varied significantly between the two mesoscale settings (Kruskall Wallys, H=36.19; P<0.001), with density in VWe (8.59± 0.71 per sampling unit, with a mean height of 4.37 ± 0.29 cm) much higher than those in PIe (2.93 ± 0.16 per sampling unit, with a mean height of 8.04 ± 0.57 cm) (Figure 8.4). Population structure differed between the two categories, with less dense and larger colonies in Pie than in Vwe. Such difference of population structure translate into significantly less skewed distribution of the colony size in Vwe than in Pie (Skewness= 0.787 in VWe and 1.98 in PIe; P<0.0001; Figure 8.4)

At the local scale, seabed slope, displayed by colony orientation, significantly affected *C. rubrum* population density and height (Kruskal-Wallis, H=65.58, P<0.0001). The density of colonies oriented in overhanging position (180°) was higher than in any other colony orientation and decreased significantly (Mann-Whitney, P<0.0001) with the colony growing vertically (Figure 8.5, Figure 8.6). On the contrary, the mean colony height decreased significantly (Mann-Whitney, P<0.0001) from vertically oriented colonies to those in overhanging position (Figure 8.7).

Finally, a GLM fitting was used to disentangle the influence of different local factors displaying potential co-variations (local slope, sediment accumulation, depth) on *C. rubrum* presence and population density. Results showed that accumulation of sediment negatively affect the presence of *C. rubrum* colonies (P<0.001), without significantly affecting the density (Table 8.3). In other words, sediment accumulation appears as an exclusive factor, strictly restricting *C. rubrum* habitat. Local slope of the substrate does not significantly affect *C. rubrum* presence (Table 8.3), while positively affects its density (only in Pie where all orientations have been observed).

8.4 DISCUSSION

We identified two different population structures of red coral population both occurring in the depth interval under investigation: i) high density patches of very small red coral colonies and ii) low density very large colonies. Despite the high heterogeneity in density documented in this study, the deep-dwelling populations are not numerically comparable to their shallow counterparts, which typically occur in from hundreds to thousands of colonies per square meter on vertical walls (Bramanti et al 2003; Tsounis et al 2006b; Bramanti et al 2014) (Figure 8.2).

However, in contrast to what has been usually reported in the literature over the last decade (Bramanti et al 2003; Santangelo et al 2004; Rossi et al 2008; Cannas et al 2011; Follesa et al 2013a; Costantini et al 2013), we show here that water depth (here considered as a surrogate descriptor of an array of many other environmental variables, like temperature, oxygen, salinity)(Huff et al 2013) does not explain much of the variation in red coral density.

Our results, providing evidence of a clear size/density relationship, allowed us to identify two different population structures of red coral population: i) the so called 'grass-plain like' structure) and ii) the 'forest like' structure. It is widely recognised that baseline information on the pre-fishing status of deep-dwelling red coral populations is absent, so that it is practically impossible to have a reliable picture of the possible structure of the pristine populations (Tsounis et al 2010; Bavestrello et al 2014b). Nevertheless, previous studies suggested that the 'forest like' structure populations of *C. rubrum* could resemble that of ancient 'pristine' populations, well before these were subjected to a strong and intensive harvesting pressure (Bramanti et al 2009; Linares et al 2012). Biotic factors, including negative intraspecific interactions, along with abiotic factors, including, among the others, substrate orientation and hydrodynamics are reported to have a major role in controlling the distribution and population structure of the red coral in the study area.

We report here significant variations in the coral colonies density with the height and branching of colonies as well as with their orientation. The reduction in coral density with increasing height and branching of the colonies could be interpreted as the result of intra-specific competition mechanisms already reported for long living benthic suspension feeders (Linares et al 2008; Bavestrello et al 2014b). In more details, it can be hypothesized that the increasing competition for resources in very dense patches could be thus one of the major factors causing the observed shift from ‘grass-plane’ (younger, denser, smaller) populations to the ‘forest’ (older, less dense, bigger) populations. The results of the GLM analyses, showing the presence of a large heterogeneity in the distribution of both grass-plane and forest-like populations which is independent from water depth, corroborate such hypothesis.

We report here that large and sparse colonies (>25 cm in height, those actually relevant for economic purposes) occur more often (in upright position) in environments characterized by a horizontal topography like rocky outcrops, and subjected to strong near-bottom currents. Instead, small size and dense patches of corals are more often found in the canyon, especially on steep walls where colonies are mostly oriented facing down (Figure 8.8). The different distribution of the two population structures could also the result of different effects of the different types of geological settings (i.e. *roche du large* ecosystems vs. canyons), having intrinsic variations in terms of ecological features and hydrodynamics (Canals et al 2006; Danovaro et al 2010; Pusceddu et al 2010; Bo et al 2012b). On the other hand, we report also that in presence of a consistent accumulation of deposits, like that observed in some of the horizontal plains on top of the rocky outcrop, corals were practically absent. Furthermore, as sloping walls becomes available a few meters below the top of the outcrop, red coral colonies can profit on an enhanced habitat complexity, hosting suitable microhabitats (i.e., available substrate) for settlement and a proper supply of organic matter. The presence of larger populations on the steep walls of the submarine canyon corroborate the hypothesis of the paramount importance of hydrodynamics in regulating the spatial distribution of red coral forests. In fact, it is known that submarine canyons, acting as a conduit of large amounts of nutrients exported from the continental shelf, can canalize high-speed bottom currents able to scour the sea bottom thus limiting the deposition of sediments especially in the upper part of the canyon (Canals et al 2006; Pusceddu et al 2010).

Connectivity in sessile species like *C. rubrum* may be limited by three successive bottlenecks: larval dispersal, settlement success and recruitment success (Pineda et al 2007; Kool et al 2013). Our results showed that the presence of *C. rubrum* (a proxy for successful larval settlement) is to a certain extent affected by sediment accumulation; patches of successful larval settlement could be higher in some geomorphological settings and lower in others because of the difficulty for the larvae to penetrate the accumulated sediment layer and reach the hard substrate (Pineda et al 2007). In addition to physical barriers such as sediment accumulation, settlement limitations deriving from intra-specific competition could also intervene (Bramanti et al 2003; Santangelo et al 2012; Bavestrello et al 2014b).

Finally, our results, based on non-invasive surveys, along different geological settings allowed us to challenge the paradigm of ‘large and sparse colonies along a depth gradient’ and to suggest a new assumption of a substrate-dependant distribution of red coral populations. Our results make light on the need of increasing the efforts to ascertain the actual distribution of deep-dwelling red coral populations in order to develop more reliable tools and strategies for their conservation, on the one hand, and ecologically sustainable harvesting, on the other one.

8.5 METHODS

8.5.1 Data acquisition

Biological data on *C. rubrum* were collected during a ROV campaign carried out on board the I.S.P.R.A r/v “Astrea” in southern waters of Sardinia during autumn 2011. 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) and three laser beams providing a 10 cm scale for the measurement of frames, sampling units and the colonies size. As positioning systems, ROV had a depth sensor, a compass and an underwater acoustic tracking positioning system (Tracklink 1500 MA, LinkQuest Inc.). Transects could not be linear as the survey was focused on the target species *Corallium rubrum*, which is distributed in patches in caves, steep walls and boulders.

A total of 12 sites were investigated (Table 8.1); the bathymetric range varied from 80 m in the proximal area to 170 m in the distal area of the continental shelf. More than 18 hours of ROV footage were performed, conducted at an average speed of 0.35 knots. The precise length of each track (from bottom-reaching to bottom-leaving time) was obtained throw ROV Track-link system.

8.5.2 Study area

Investigated area is located in the central-western region of the Mediterranean Sea, in southern waters of the island of Sardinia, between 80 and 170 meters depth (Figure 8.1). The mean and turbulent circulation of the shelf-slope region of the western coast of Sardinia was investigated by means of numerical simulation (Sorgente et al 2011; Olita et al 2013), describing a mean southward current along the west coast of Sardinia. Along south Sardinian coast, the westward Algerian current is reported to be dominant; this current merges with the southward currents forming the quasi permanent South Eastern Sardinian gyre (Sorgente et al 2011).

Southeaster and southwester coast of Sardinia are characterized by different seafloor morphology. On the western side, a vast shelf area characterized by volcanic outcrops extends from the Gulf of Cagliari to the San Pietro Island area, while a smaller shelf area (2 km of extension), with several canyon heads occurs along the south eastern shelf margin, extending from Capo Carbonara all the way north, along the investigated eastern coast (Sulli 2000; Mascle et al 2001).

8.5.3 Image analysis

The frame analysis was performed with CPCe Software (Coral Point Count with Excel extensions) (Kohler and Gill 2006). Random frames were extracted from the ROV footage and classified according to the quality, discarding overlaying and low-visible ones and keeping the analysable ones (useful). For each site, a total of 50 randomly positioned 50x50 cm squares were used as sampling unit within frames. The use of 50x50 cm sampling units was a worthwhile working solution, as frames were not always larger than a square meter, because of the contemporary necessity of "close-viewing" frames to facilitate and improve measurement precision.

Overall, 600 sampling units were analysed; for each one, the following parameters were retrieved: i) density, estimated as number of colonies in 2500cm^2 (50x50cm square); ii) basal diameter and colony height (from the basis to the farthest tip); iii) Local slope of the substrate (expressed as vertical or horizontal wall); iv) orientation of colonies (0°, 45°, 90°, 135°, 180°). Although direct measures of the current flow and sedimentation were not taken, differences in the current intensity were evident and easily deductible from the accumulation of sediment. In addition, as the life span of *C. rubrum* can easily reach >100 years old, single or multiple measures of current regime in a short range of time could be actually misleading the actual regime that brought coral to reach the measured features. In the same way, slope of the substrata was detected as local per each sampling unit, not using the MB dataset because of the patchiness distribution of *C. rubrum*, which can vary within very small distances. Statistical analyses were performed to test the null hypothesis that *C. rubrum* presence/absence and density did not varied among different substrates. Through the multibeam dataset, geomorphology of each site was characterized. Depending on the average slope of the substrata, each site was defined canyon or "vertical walls" environment ("VWe"; when average slope was lower than 30°) or general rocky outcrops like shoals or pinnacles ("PIe"; when mean slope was lower than 30°).

8.5.4 Statistical analyses

Test for normal distribution of residuals were performed on the whole dataset and for the two chosen substrates categories. The software XLSTAT was used (function 'normality tests'), performing both Shapiro-Wilks and Anderson-Darling. Both tests were significant (P<0.0001). Once the non-normal distribution was verified, univariate non-parametric Mann-Whitney test using the software PAST 2.17 (Hammer et al 2001), was performed to test for differences in *C. rubrum* density (square rooted data), between the two investigated types of substrates: VWe vs. PIe.

The proportion of zero observation was higher than 50%, thus a General Linear Model (GLM) with Zero Altered Poisson distribution (ZAP; R software Version 2.15.1, 'hurdle' function) was used to describe how coral density varies in relation to different environmental factors and covariates. The Poisson distribution was verified through XLSTAT software, function 'distribution fitting' (P<0.0001). The 'hurdle' function was preferred to the 'zeroinfl', as it does not make distinctions between different types of zeros ('true zeros' vs. 'false zeros'), and it gave us the opportunity to evaluate processes influencing both 'non-zeros' (i.e. density) and, possibly most importantly, 'zeros' through a binomial model (Jackman 2009; Zuur et al 2009).

Our sampling design included exclusively high-resolution footage performed over rocky bottoms, which are all suitable substrates for *C. rubrum* settlement, reducing considerably the possibility of “false zeros” due to sampling errors. Two separate models were constructed for the VWe and PIe. The response variable was the squared rooted value of density (n° of colonies in each sampling unit), in relation to the following predictive variables and factors: accumulation of silt (presence/absence), the local slope (vertical/horizontal), and depth as covariate.

The model with the lowest Akaike’s information criterion (AIC) included depth as covariate, sediment deposition and local slope of each sampling units as factors, and it was run for both environments. The Vuong test (command ‘vuong’) showed that our hurdle model fitted our dataset significantly better than a standard GLM with Poisson distribution ($P<0.0001$).

Once the model was chosen, the variability in density within sampling units was evaluated through the correlation of density and the average branching (tot. branches/ n. of colonies) with the orientation of colonies. Differences in density, branching and maximum height of colonies in relation to different colonies orientations were also tested with a Mann-Whitney procedure, performed between 2 categories of orientations (from 45° to vertically oriented vs. 90° to facing down colonies).

8.5.5 Size distribution and review from literature.

The size distribution of the two investigated habitats was analysed with the software XLSTAT (‘data description’ function), using skewness and kurtosis as parameters. Skewness is a measure of the symmetry of a distribution, using its mean; if significant, the distribution is asymmetric. Positive skewness indicates a population mainly composed by small sized individuals, while negative skewness points to the dominance of large size classes (Ferguson et al 2008; Edmunds et al 2014).

A linear regression between the mean density and the sampling depth from 44 sites from available literature on population dynamics of *C. rubrum* was performed with the software ‘StatPlus’ (‘linear regression’ function).

8.6 FIGURES

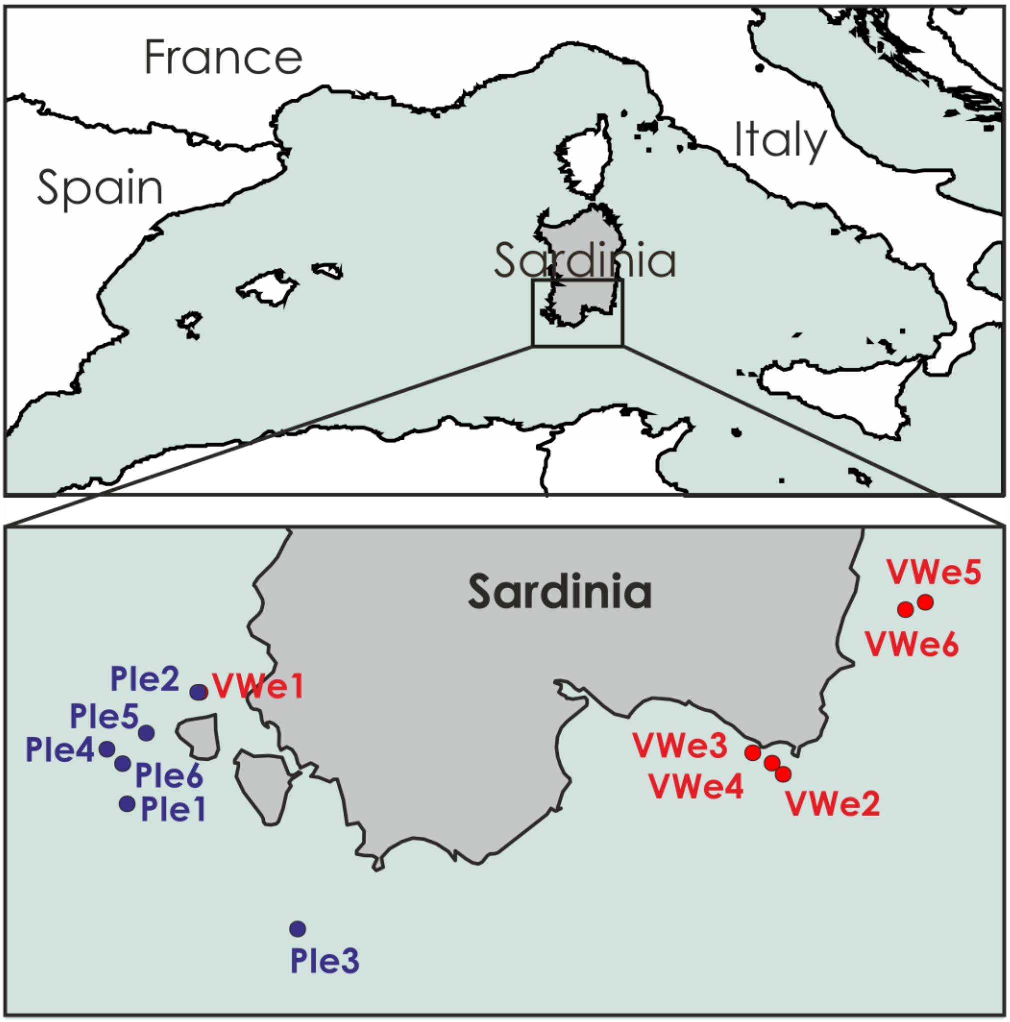

Figure 8.1 Map of the investigated sites. In red and blue the sites defined as 'vertical wall environments' VWe, and 'pinnacle environments' Pie, respectively

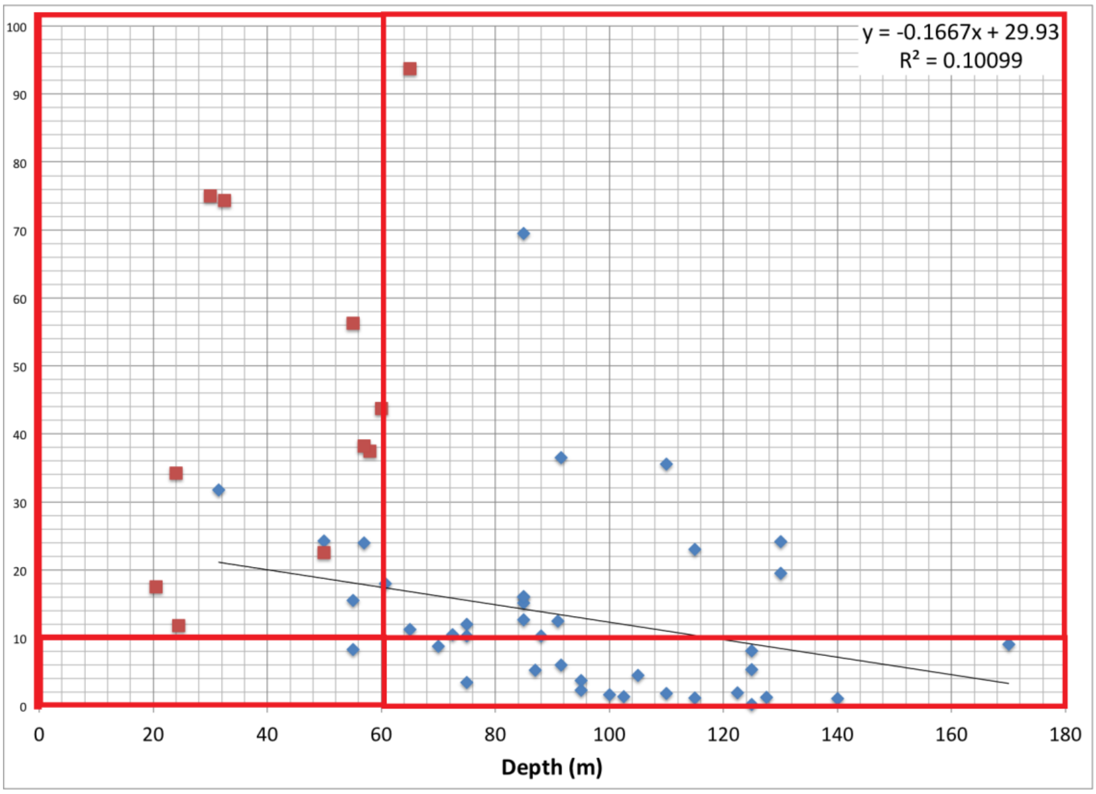

Figure 8.2 . Scatter plot of density (n. of colonies / 2500 cm^2) in relation to depth (m) from previously published datasets of shallow and deep dwelling populations of C. rubrum. The data summarize 51 sampling sites from Mediterranean basin (including coasts from Spain, France and Italy). The low variation (R^2 = 0.101) explained by the relationship with sampling depth and a slope of approximately 0.17 illustrates the absence of the bathymetric pattern.

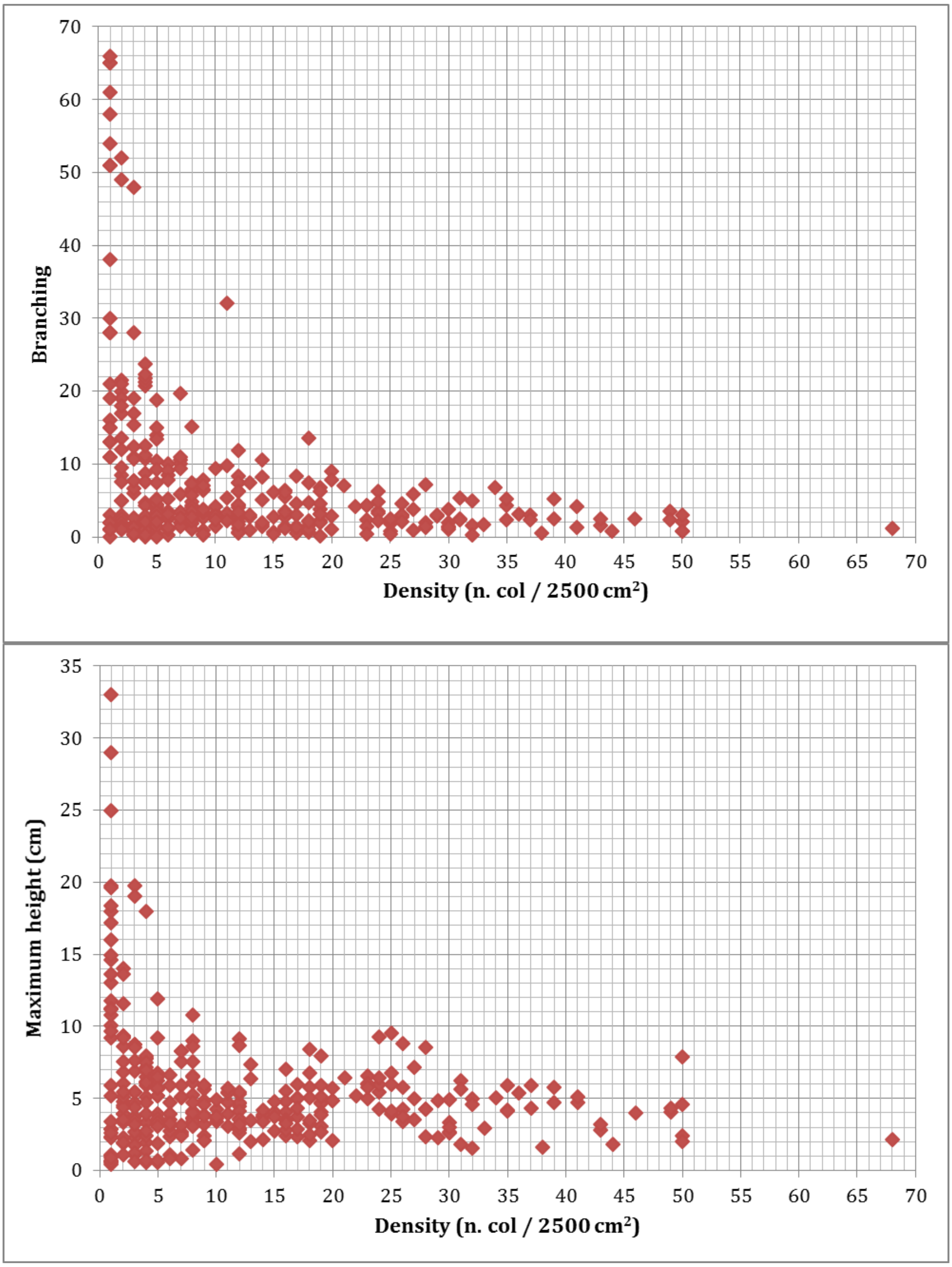

Figure 8.3 Scatter plot of branching and maximum height vs. density. Upper graph showing the relation between branching (tot. branches / n. of colonies, per sampling unit) and density; lower graph showing the relation between maximum height (cm) vs. density.

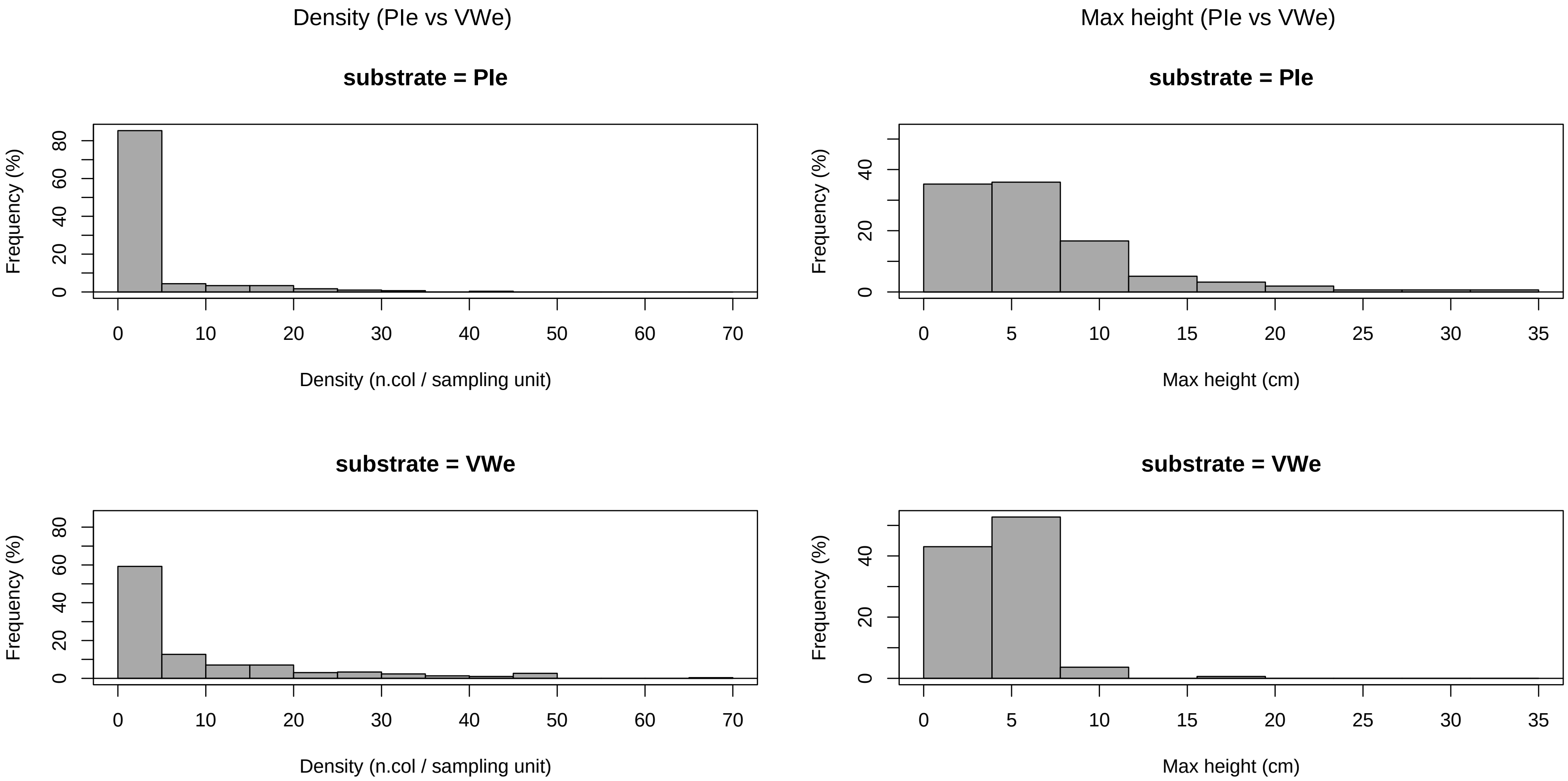

Figure 8.4 Density and maximum height structures of C. rubrum populations from the two investigated substrates (PIe on the upper graphs and VWe on the lower graphs).

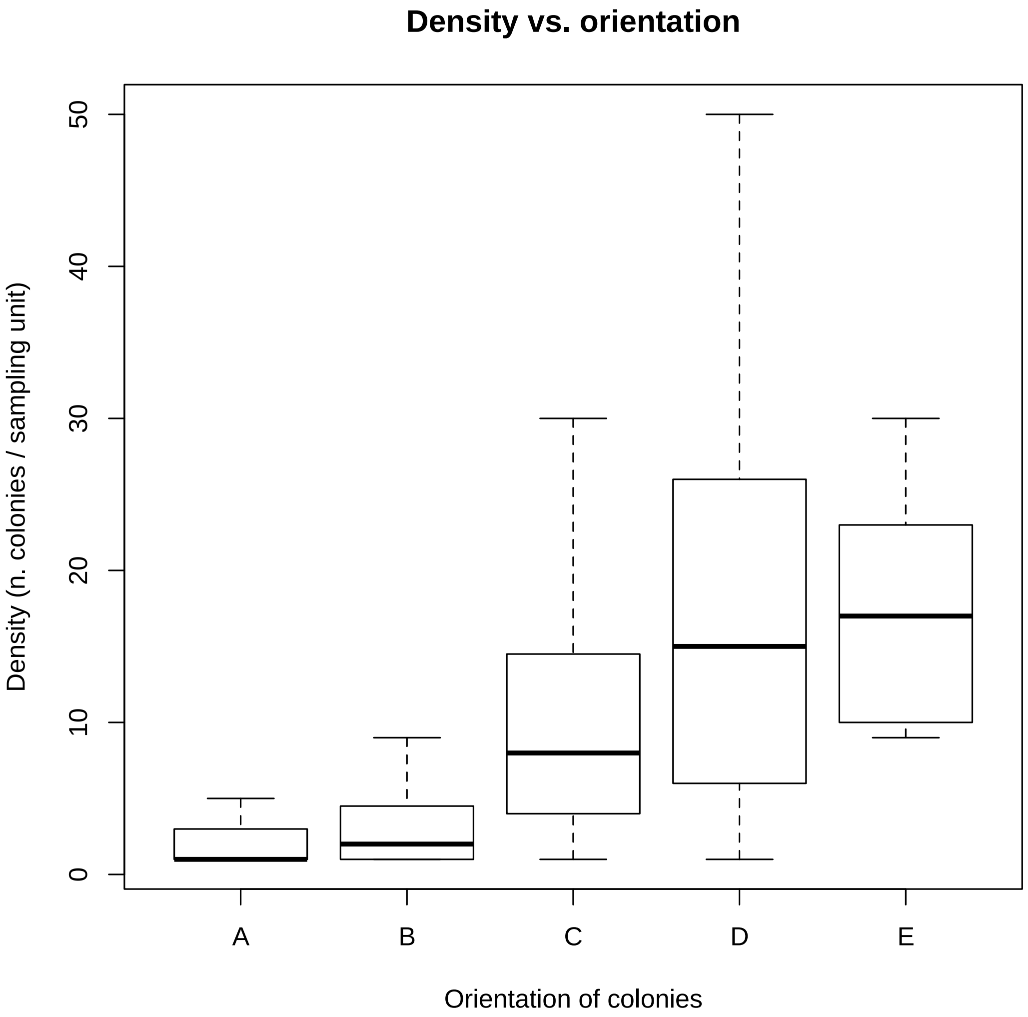

Figure 8.5 Variation in density in relation to colonies orientation (A=0°, B=45°, C=90°, D=135° and D=180°)

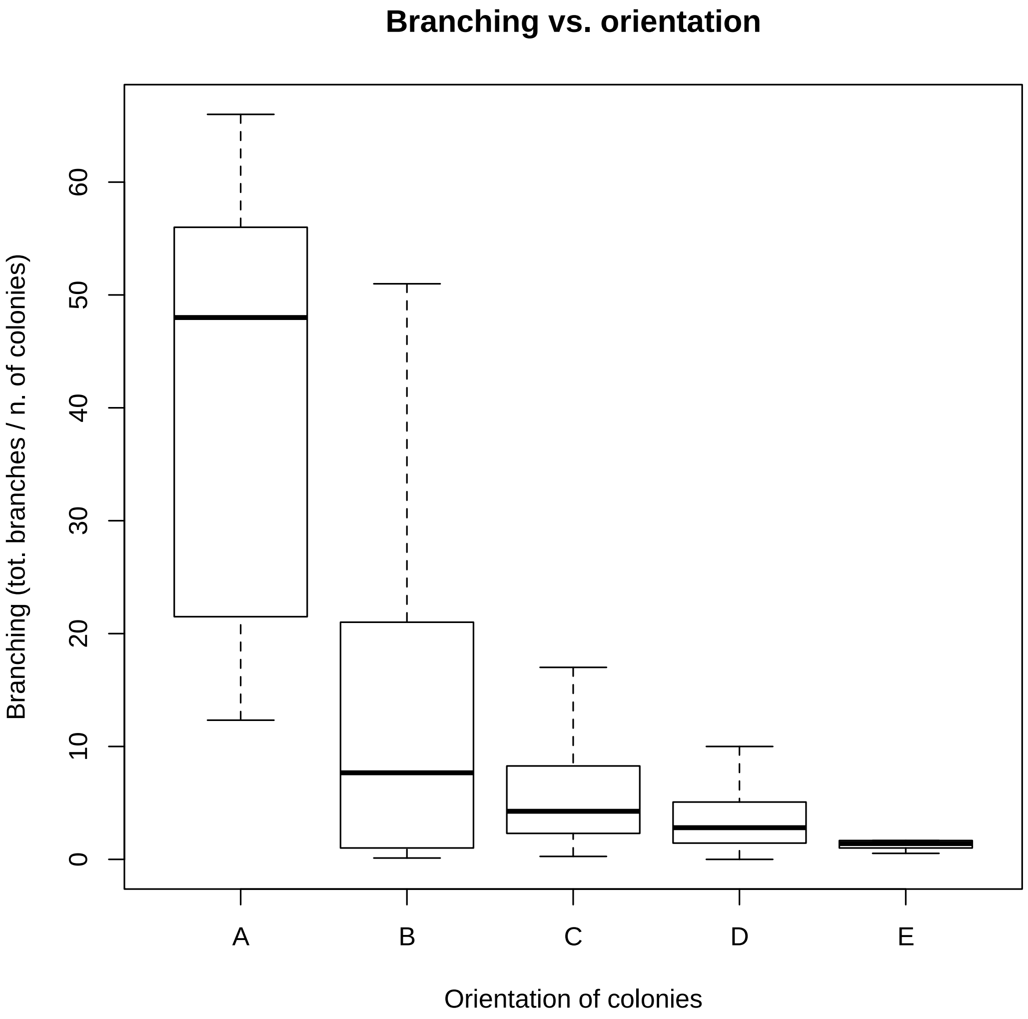

Figure 8.6 Variation of the mean number of colonies branches (tot. number of branches /n. of colonies, per sampling unit) in relation to colonies orientation (A=0°, B=45°, C=90°, D=135° and D=180°).

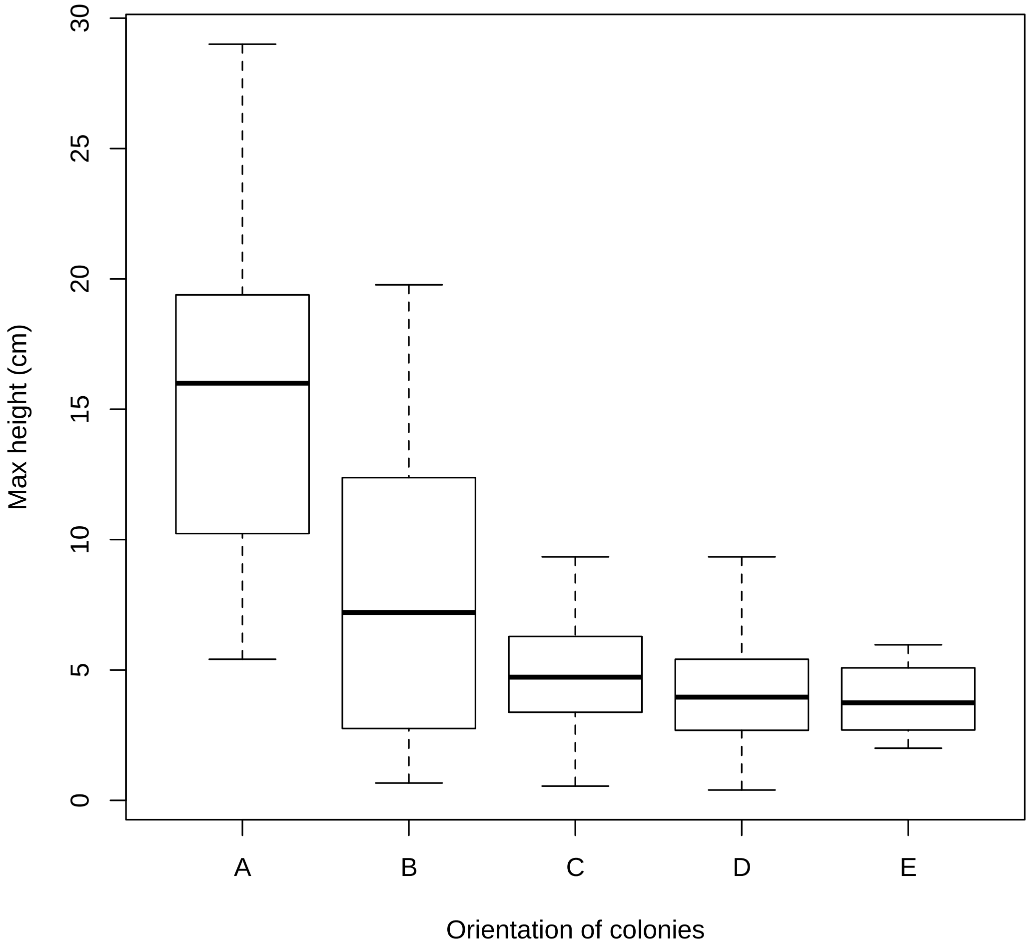

Figure 8.7 Variation of the maximum height (cm) of C. rubrum colonies in relation to their orientation (A=0°, B=45°, C=90°, D=135° and D=180°).

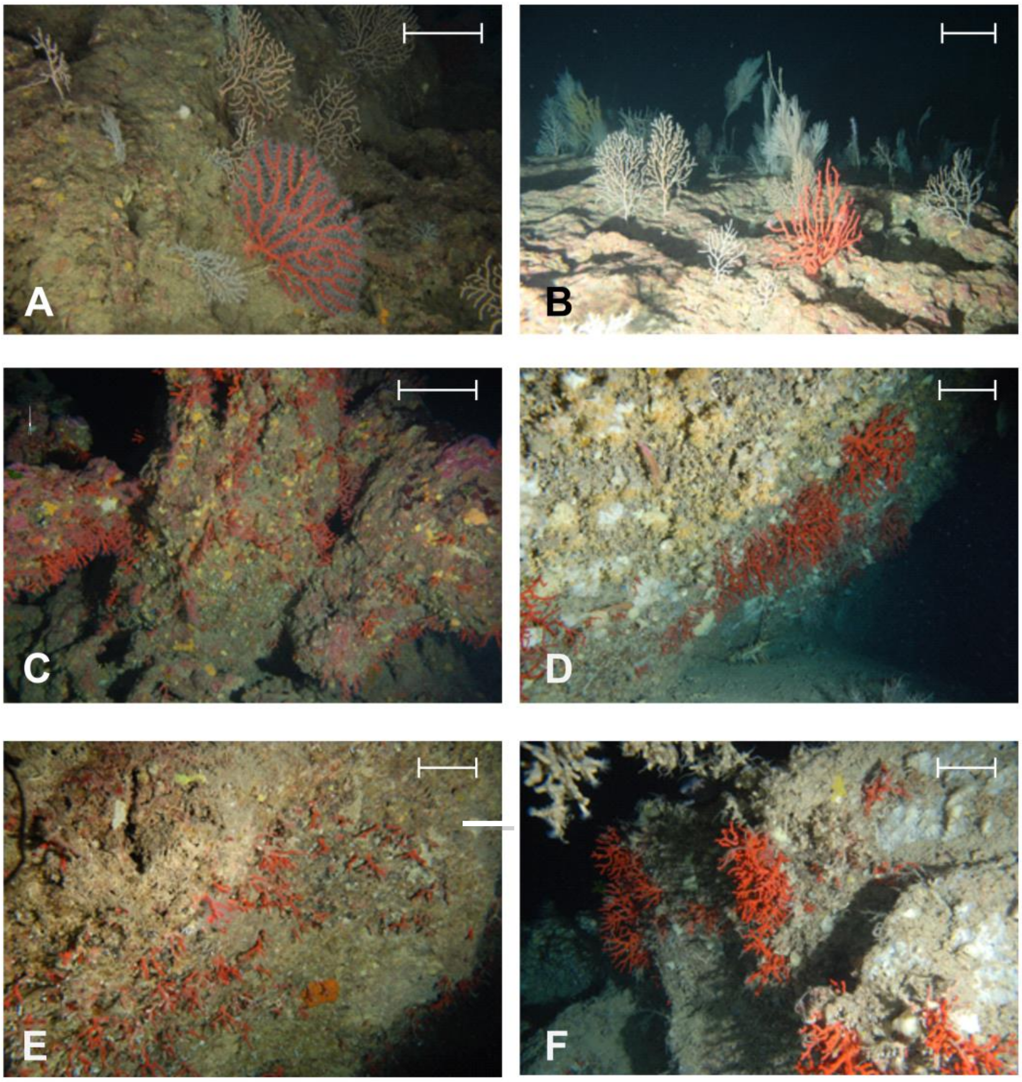

Figure 8.8 Shape of different populations of red coral dwelling along different geological setting.

8.7 TABLES

Table 8.1 Geographical coordinates and depth range of the 12 investigated sites.

Site	Latitude (N)	Longitude (E)	Depth (m)
VWe_1	39°14'369	8°16'684	80-90
VWe_2	39°03'998	9°29'775	120-130
VWe_3	39°06'719	9°25'970	115-130
VWe_4	39°05'414	9°28'327	120-140
VWe_5	39°25'765	9°47'474	120-130
VWe_6	39°24'832	9°44'994	115-130
PIe_1	38°59'816	8°07'720	155-170
PIe_2	39°14'381	8°16'379	90-100
PIe_3	38°44'425	8°29'025	110-120
PIe_4	39°07'173	8°08'258	125-130
PIe_5	39°09'213	8°10'180	80-85
PIe_6	39°05'349	8°07'227	120-130

Table 8.2 Mean density values (n° colonies/2500 cm^2) for red coral populations from different sites, countries and depths. Data from the literature and present study.

Sites	Country	Depth	Mean Density	References
Banyuls	France	24	34.25	(Linares and Doak 2010)
Carry	France	24.5	11.75	(Linares and Doak 2010)
Scandola	France	20.5	17.5	(Linares and Doak 2010)
Masina	Spain	72.5	10.5	(Rossi et al 2008)
Massa d'oros	Spain	65	11.25	(Rossi et al 2008)
Cap de Creus	Spain	32.5	74.37	(Bramanti et al 2014)
Costa Brava	Spain	31.5	31.75	(Tsounis et al 2006b)
Tuscany archipelago	Italy	91	12.5	(Priori et al 2013)
Ischia	Italy	85	16.12	(Priori 2012)
Elba-Pianosa	Italy	85	12.63	(Priori 2012)
Amalfi	Italy	110	35.56	(Priori 2012)
Alghero	Italy	102.5	1.33	(Priori 2012)
VWe_1	Italy	85	15.1	Present study
VWe_2	Italy	125	8.08	Present study
VWe_3	Italy	122.5	1.9	Present study
VWe_4	Italy	130	19.5	Present study
VWe_5	Italy	125	5.34	Present study
VWe_6	Italy	110	1.8	Present study
PIe_1	Italy	170	9.08	Present study
PIe_2	Italy	95	2.27	Present study
PIe_3	Italy	115	1.12	Present study
PIe_4	Italy	127.5	1.25	Present study
PIe_5	Italy	95	3.7	Present study
PIe_6	Italy	125	0.22	Present study
Calabria_1	Italy	100	1.60	(Bo et al 2009)
Calabria_2	Italy	105	4.51	(Bo et al 2009)
Calabria_3	Italy	130	24.15	(Bo et al 2009)
Capo Carbonara	Italy	115	23	(Cau et al 2015)
San Pietro Island	Italy	85	16	(Cau et al 2015)
Portofino promontory_1	Italy	30	75	(Bavestrello et al 2009)
Portofino promontory_2	Italy	50	22.5	(Bavestrello et al 2009)
Portofino promontory_3	Italy	70	0.5	(Bavestrello et al 2009)
Capo di conca	Italy	55	15.5	(Bavestrello et al 2014c)
Secca del Pampano	Italy	140	1.025	(Bavestrello et al 2014c)
Scoglio D'Ischia	Italy	75	3.45	(Bavestrello et al 2014c)
Punta S.Angelo	Italy	91.5	6	(Bavestrello et al 2014c)
Punta Solchiaro	Italy	57	24	(Bavestrello et al 2014c)
Punta Pizzato	Italy	55	8.25	(Bavestrello et al 2014c)
Punta S.Angelo_2	Italy	91.5	36.5	(Bavestrello et al 2014c)
Punta del faro	Italy	75	10.25	(Bavestrello et al 2014a)
Isuela shoal	Italy	50	24.25	(Bavestrello et al 2014a)

Maledetti shoal	Italy	85	69.5	(Bavestrello et al 2014a)
Bordighera	Italy	70	8.75	(Bavestrello et al 2014a)
North Pianosa Is	Italy	75	12	(Bavestrello et al 2014a)
Sante shoal	Italy	87	5.25	(Bavestrello et al 2014a)
Montecristo sh.	Italy	60.7	18	(Bavestrello et al 2014a)
Tuna paradise	Italy	88	10.25	(Bavestrello et al 2014a)
Portofino_Isuela Sud	Italy	65	56.75	(Bavestrello et al 2014b)
Portofino_Cala oro est	Italy	65	93.75	(Bavestrello et al 2014b)
Portofino_Torretta	Italy	65	37.5	(Bavestrello et al 2014b)
Portofino_Dragone	Italy	60	43.75	(Bavestrello et al 2014b)
Portofino_Casa del Sindaco	Italy	60	38.25	(Bavestrello et al 2014b)

*Table 8.3 Output of the GLM ran for both environments (i.e., PIe and VWe); (NS Not Significative; * P-value<0.05; ** P-value<0.01; *** P-value<0.001)*

Call for "PIe" environment			
hurdle(formula = density ~ depth + local slope + accumulated sediment | depth + local slope + accumulated sediment, data)			
Count model coefficients (truncated Poisson with log link):			
	Estimate	**Std. Error**	**P Value**
Depth	0.006732	0.002504	**
Local slope (vertical)	0.319	0.162	*
Accumulated sediment (yes)	-0.1465	0.212	N.S
Zero hurdle model coefficients (binomial with logit link):			
	Estimate	Std. Error	P Value
Depth	0.0381	0.006816	***
Local slope (vertical)	0.1272	0.299199	N.S
Accumulated sediment (yes)	-2.9157	0.395397	***

Call for "VWe" environment			
hurdle(formula = density ~ depth + local slope + accumulated sediment | depth + local slope + accumulated sediment, data)			
Count model coefficients (truncated Poisson with log link):	**Estimate**	**Std. Error**	**P Value**
Depth	0.001	0.412	N.S
Local slope (vertical)	0.19	0.004	N.S
Accumulated sediment (yes)	-9.76	74.61	N.S
Zero hurdle model coefficients (binomial with Logit link):			
	Estimate	**Std. Error**	**P Value**
Depth	-0.0026	0.012867	N.S
Local slope (vertical)	0.2672	0.51186	N.S
Accumulated sediment (yes)	-4.379	0.812419	***

9 Deep-water corals biodiversity in different *roche du large* ecosystems along the south Sardinia continental margin (CW Mediterranean Sea).

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9.1 ABSTRACT:

Deep-sea corals are engineer species that, along with other benthic organisms, create important three-dimensional structures that enhance ecosystem's overall complexity, hosting a rich fauna that includes also commercially relevant species. In the deep circa-littoral zone, rocky pinnacles arising from soft bottoms have proven to act as oases of coral biodiversity. Nevertheless, information about the levels of deep-water coral species turnover (i.e. beta diversity) among different rocky pinnacles are, if any, very scarce. To provide insights on this still mostly unaddressed issue, we tested the null hypothesis of no differences in the coral biodiversity encountered on five rocky pinnacles characterized by different geological settings, dwelling between 120 and 170 m depth along south Sardinia continental margin. Moreover, a visual census of anthropogenic debris was also carried out. Eleven target hard-bottom coral species (four black corals and seven gorgonians), among the most common encountered in the investigated area, were used as surrogate descriptors of deep-water coral biodiversity of the five pinnacles. Overall, total coral abundance varied from 1.35 to 7.23 colonies m^{-2}. Gorgonians *Callogorgia verticillata* followed by *Eunicella cavolinii* and *Corallium rubrum* were the most abundant species. Among black corals, *Antipathella subpinnata* was the most abundant species, followed by *Parantipathes larix*. Uni- and multi-variate analyses revealed the presence of significant differences in deep-water corals biodiversity in investigated sites. The census of debris revealed that lost fishing gears represented the most frequently observed typology of anthropogenic debris. These results allowed us to reject the null hypothesis, and to suggest that isolated rocky pinnacles while providing an important contribution to the levels of gamma diversity of the twilight zone of Mediterranean Sea, are possibly exposed to high levels of anthropogenic disturbance. We stress here that further and more focused strategies of conservation oriented to protect these hot spots of coral biodiversity should be soon identified.

9.2 INTRODUCTION

Mediterranean Sea coral communities have been widely investigated in the last several decades and information about the biology and distribution of the most common coral species of the photic zone of the Mediterranean basin has been progressively accumulated, underlining the paramount ecological role of these communities in benthic food webs (Harmelin and Marinopoulos 1994; Gili and Coma 1998; Coma et al 2000; Garrabou and Harmelin 2002; Tsounis et al 2006b; Linares et al 2008; Rossi et al 2008; Gori et al 2011a; Gori et al 2011b). The knowledge on the ecology of corals dwelling in the so-called twilight zone (50-150 m depth; (Bo et al 2009) of the Mediterranean Sea is still limited due to the practical difficulties of investigations in deep waters. Nevertheless, the awareness of their ecological relevance is progressively increasing (Bo et al 2012b; Bianchelli et al 2013).

Because of the disappearance of 'vegetal' forests' with increasing depth as a result of light attenuation, in the twilight zone benthic sessile organisms become progressively more important contributors to the three-dimensional complexity of mesophotic ecosystems, finally building up the so-called 'animal forests' (Rossi et al 2012; Rossi 2013). Indeed, just like terrestrial forests, these habitats create hot spots of biodiversity within their extension, provide refuges for numerous species (Bo et al 2011a; Cau et al 2013b) and chapter 11 of this thesis) and represent a nursery area for several commercial fish (Gili and Ros 1985; Gili et al 1989; Gili and Coma 1998; Ballesteros 2006; Etnoyer and Warrenchuk 2007; Baillon et al 2012; Baillon et al 2014). Deep-water corals are long-lived species that play an important role in the pelagic–benthic transfer of energy (Cerrano et al 2010); besides this, they also amplify the ecosystem's overall complexity along continental margins by providing new colonizable surface for the benthos (Gili and Coma 1998; Buhl-Mortensen et al 2010; Cerrano et al 2010). In the deepest part of the circa-littoral environment, which is included in the twilight zone, hard bottom coral assemblages dwell also on isolated rocky pinnacles commonly included among the so-called 'roche du large' ecosystems. In these ecosystems large anthozoans, such as gorgonians and black corals, represent the most frequently observed coral taxa. Rocky pinnacles in the deepest part of the continental shelf represent one of the least known ecosystems within Mediterranean mesophotic environments (Bo et al 2012b). Distribution of coral communities along these geological features may be determined by the combination of both biological (Glynn 1976; Neudecker 1979; Sheppard 1979) and environmental factors (Glynn 1976; Adjeroud 1997) that can synergistically affect the birth, larval settling, growth, and death rates of individuals (Hutchinson 1953). In particular, it has been recently postulated that the geomorphological characteristics of settlement substrates could be important factors structuring the biodiversity of the hosted animal forests, by affecting sediment accumulation rates, bottom currents, and, ultimately, the rates of food supply (Wilson et al 2007). Furthermore, as these habitats host some commercially relevant species, they are becoming increasingly subjected to fishing activities that directly damage these fragile communities (Bo et al 2014a).

Till a few decades ago, quantitative and qualitative information on sessile mesophotic fauna were hard to obtain because of the poor reliability and spatial resolution of traditional destructive benthic sampling techniques (e.g. trawls and dredges), in addition to their unknown capture rate (Ninio et al 2003; Matarrese et al 2004; Rossi et al 2008). The recent and fast development of underwater remotely operated vehicles (ROV) enabled to perform controlled sampling and detailed observation of specific mesophotic habitats with non-invasive protocols, which are particularly relevant for conservation interest habitats (Gori et al 2011b; Bo et al 2011b; Bo et al 2012b).

Along the Sardinian continental shelf (central western Mediterranean) investigations on coral assemblages are limited to the target species *Corallium rubrum* (Cannas et al 2011; Follesa et al 2013a; Cannas et al 2014; Cau et al 2015), whereas the status of communities of large anthozoans such as gorgonians and black corals are mostly unknown. Moreover, information about the levels of deep-water coral species turnover (i.e. beta diversity) among different rocky pinnacles are, if any, very scarce. Thus, to provide insights on this still mostly unaddressed issue, based on a combination of ROV footage and multi-beam inspections, we tested the null hypothesis of no differences in the coral biodiversity encountered on five rocky pinnacles, dwelling between 120 and 170 m depth and characterized by different geological settings, along the south Sardinia continental margin. Finally, to identify the possible anthropogenic factors of disturbance of the communities under scrutiny, we carried out the visual census of allochthonous debris deposited on the five rocky pinnacles.

9.3 MATERIALS AND METHODS

9.3.1 Study area

The study area is located along the southwestern continental margin of Sardinia (central-western Mediterranean Sea; Figure 9.1). Within the area, 5 rocky pinnacles (henceforth called S1, S2, S3, S4, and S5) were identified at depths ranging from 120 to 170 meters. The continental shelf in the southwestern waters of Sardinia is characterized by an irregular morphology, with large outcrops of the Oligo-Miocene volcanic sedimentary sequence, and it is bounded on the southwest by the shelf edge situated at 180 meters depth. From a geomorphological point of view the continental shelf can be divided into two parts: the proximal continental shelf (where S4 and S5 are located) is characterized by large mesas, cuestas and other typically volcanic morphotypes such as calderas, necks and mega dykes, whereas the distal continental shelf (where S1 and S2 are located) is slightly sloping (i.e. 0.6° - 0.8° degrees), and its surface is covered by the Miocenic sedimentary series (Ulzega and Fais 1980; Lecca 2000). In the southern part of the investigated area, the continental shelf is characterized by a sub-planar morphology and low gradient (about 2%), with extensive areas of outcropping and sub-outcropping bedrock. A large rocky outcrop (where the pinnacle S3 is located), interpreted as an emission center located close to the shelf edge, emerges oriented N-S for 5.5 kilometers; its summit reaches 105 m while the basis, rather articulated, is at a depth of 150 m.

The literature specifically devoted to the variability of the local circulation of this area of the Mediterranean Sea is poor. Olita et al. (Olita et al 2013), by means of numerical simulation, identified a southward current occurring along the western coast of Sardinia, with a maximum intensity in correspondence of the waters surrounding the San Pietro’s Island, nearby the area under scrutiny in this work.

9.3.2 Geophysical analyses and sea bottom mapping

The geophysical dataset was collected during two cruises carried out within the framework of the “Magic project” (MArineGeohazard along Italian Coasts) in 2009 and 2010 by CoNISMA’s R/V Universitatis in the southern waters of Sardinia, using the following acoustic systems: (1) the RESON SEABAT 8160 MultiBeam Echo-Sounder (MBES); (2) the GEOACOUSTIC CHIRP II Sub-Bottom Profiler (SBP).

The data obtained through MBES were used to locate the ROV transects in respect to the slope of the sea bottom. The raster resolution was a 5 x 5 meters cell; all maps in Figure 9.2 represent a grid of 292 x 332 cells, equal to 1452 x 1658 meters, and a surface of ca. 2.41 km^2.

9.3.3 Sampling

The video material was obtained during two ROV missions conducted along the south Sardinia continental margin in October 2011 and June 2013 (Table 9.1). Two different ROVs were used: i) the ROV “Pollux III” equipped with a digital camera (Nikon D80, 10 megapixel), a strobe light (Nikon SB 400), a high definition video camera (Sony HDR-HC7) and ii) the ROV “Seaeye Falcon” equipped with 3 cameras: i) a default color camera, ii) a color camera equipped with laser beams, over a 180° tilt platform and iii) an independent high definition video camera (GoPro 3+). Both ROVs were equipped with track-link system, depth sensor, compass, and two parallel laser beams providing a constant 10-cm reference scale in the video frame, for the measurement of the frame area.

Each of the five rocky pinnacles was investigated through a different number of ROV dives (from a min. of 1 to a max. of 3), within the same day, to collect enough video material for the further image analysis (Table 9.1).

The software DVDVIDEOSOFT was used to extract video frames every 30 seconds; overall, a total of 11 hours of ROV footage were analyzed. Frames with non-clear visibility, compromised resolution/focus or not-suitable substrate (i.e. soft bottoms), 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 m^{-2} ± standard error, henceforth col m^{-2} ± s.e.) and the species composition of the coral community for each frame.

9.3.4 Biodiversity analyses and human pressure

A total of 667 video-frames were obtained over the rocky bottom of different investigated sites (Table 9.1). To characterize the alpha and beta biodiversity of the hard bottom coral fauna on the rocky pinnacles, eleven target species were chosen: 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)] and 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. Coralliidae), *Eunicella cavolinii* (Koch, 1887) (fam. Gorgoniidae), *Bebryc emollis* (Philippi, 1842) (fam. Plexauridae) and *Villogorgia bebrycoides* Koch, 1887 (fam.Plexauridae)]. As per Bo et al. 2012, the two species of gorgonians *Villogorgia bebrycoides* and *Bebryce mollis* were pooled together and hereafter referred to the *V. bebrycoides-pool*, because they are scarcely distinguishable one from each other on the basis of ROV images only.

We chose these large anthozoans, as: i) anthozoans represent one of the most conspicuous components of deep-dwelling animal forests (Rossi et al 2012; Rossi 2013); ii) their taxonomic identification through ROV is relatively easy and iii) their ecological role has been already clarified in other similar habitats (Bo et al 2012b).

Although we acknowledge that the selected set of anthozoan species represents a fraction of the complex hard-bottom community hosted on the investigated rocky pinnacles, 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 2012b). More in details all of the selected species are long-living organisms representative of mature animal forests, which are composed of taller and highly branched corals that can alter major current flows and particle retention, thus hosting more zooplankton, eggs, larvae, juveniles and adults of vagile species than in their surroundings (Baillon et al 2012; Rossi 2013).

From the same ca. 700 frames used for the biodiversity analysis, the level of anthropic pressure was also estimated by enumeration and classification of allochtonous debris. Observed items were divided in two categories: i) fishing gears and ii) recreational litter (e.g. plastic bottles, cans, etc.); in addition, every damaged or entangled colony was annotated in order to estimate the total number of impacted colonies within the investigated coral assemblages.

9.3.5 Statistical analyses

Non-parametric, uni-variate analysis of variance (Kruskal-Wallis procedure) and non–parametric permutational multivariate analysis of variance (NPMANOVA), based on target species abundance data matrix and using pinnacles as the unique source of variation, were used to test for differences in: i) target species richness, ii) total coral abundance and iii) coral community composition turnover among the investigated pinnacles. The permutational multivariate analysis was based on Bray-Curtis similarity of previously square root transformed data. Non-parametric methods based on permutation tests such as the one performed by the NPMANOVA, are preferable since they allow to partition the variability in the data according to a complex design or model and to base the analysis on a multivariate distance measure that is reasonable for ecological data sets (Anderson 2001; McArdle and Anderson 2001). Later, to identify the taxa explaining the differences between various pinnacles, a SIMPER analysis was carried out based both on presence/absence and on sqrt(x)-transformed abundance data matrix with 90% cut-off.

Bray-Curtis matrices from multivariate analysis were used as inputs to perform unconstrained (Non-metric Multidimensional scaling) and constrained (Canonical Correspondence Analysis - CCA) multivariate ordination analyses of coral assemblages. Various environmental variables (latitude, longitude, distance from the coast, mean slope, and water depth of the sites) were also tested as possible explanatory factors of the observed differences in abundance and composition of the coral assemblages among the rocky pinnacles (Bo et al 2012b).

Statistical analyses were performed using the software PAST 2.17 (Hammer et al 2001) and PRIMER 6+ (Plymouth Marine Laboratory).

9.4 RESULTS:

9.4.1 Pinnacles morphology

S1 is located in the outer (distal) continental shelf, ca. 10 miles from the nearest coastline (the western side of the island of San Pietro, south-west Sardinia). This site is a small rocky outcrop that rises out from an extended surrounding muddy bottom area from -170 meters (sea bottom) to -155 meters (top of the pinnacle). Slope values are very low (maximum 5°) and the profile curvature underlines just the presence of the outcrop (Figure 9.2). A closer view of the morphology of the site through ROV footage shows the presence of more complex sea bottom morphology. The pinnacle, in fact, shows vertical walls suddenly arising from the bottom to the top of the outcrop, where gently sloping areas are present. Vertical walls are not detectable from the multibeam cells, because of the exiguity in size of this outcrop compared to the mesh size.

Also S2 is located in the outer continental shelf, ca. 12 nautical miles from the nearest coastline (the southwestern coast of San Pietro Island), and 1 nautical mile southwest from S1. Here, the ROV survey was performed within a depth range from 157 m (top of the pinnacle) to 165 m (bottom of the pinnacle); the profile curvature underlines the presence of a medium to low overall sea bottom complexity and a maximum slope of 5°. S3 is the farthest pinnacle from the nearest coastline: 13 nautical miles from Capo Teulada, southwestern Sardinia, at an average depth of 135 m. The survey here was performed both at the bottom of the pinnacle, which is characterized by sparse boulders arising from muddy substrates and at the top of it, where a more irregular morphology is observed. Slope value ranges from 2° at the base of the pinnacle to 13° on the top. Survey in S4 was conducted on its summit and the eastern side of a dyke (Figure 9.2). S4 is located 3 nautical miles off the western coast of San Pietro Island, which is the nearest coastline. Slope values range here from 10° on the top of the dyke to 25° along the investigated side. The profile curvature confirms the presence of a higher sea bottom complexity along the side of the dyke than in top of the pinnacle. S5 is located ca. 5 nautical miles from the closest coastline (San Pietro Island), and consists of a top paleo-rotational landslide body that affected the paleo-cliffs (Orrù et al. 2012); the slope ranges from 5° to 30° degrees along the entire ROV transect, and the profile curvature shows an irregular area characterized by numerous outcrops (Figure 9.2).

9.4.2 Biodiversity analyses

The area of each frame, estimated *a posteriori,* was on an average 3.94 ± 0.19 m^{-2}, covering a total of 2570 m^{-2} of analyzed surface. A total of 3357 colonies of target corals were counted, with an average abundance of 2.99 ± 0.24 colonies m^{-2}. The Kruskal-Wallis test showed that the total coral abundance (Figure 9.3) varied significantly among the five pinnacles (Tab. 2, $P < 0.001$). In particular, the highest coral abundance was observed in S1 with 7.23 ± 1.41 corals m^{-2}, followed by S4 (4.98 ± 0.45 col m^{-2}) and S5 (2.38 ± 0.33 colm^{-2}), S2 (1.61 ± 0.39 col m^{-2}) and S3 (1.35 ± 0.12 col m^{-2}). The abundance of *P. larix*, *A. dichotoma* and *L. glaberrima* did not vary among the investigated pinnacles whereas the abundance of all the remaining taxa varied significantly among the pinnacles (Table 9.2 Results of the non-parametric univariate analysis of variance (Kruskal -Wallis procedure) for differences among total abundance of corals and target-species abundance among five investigated pinnacles (***= $P<0.001$; ** = $P<0.01$; N.S = not significant). The test was not performed for *L. glaberrima* singularly, as it was present exclusively in S5.).

The NPMANOVA results reveal the presence of significant differences in the coral assemblage composition among the five pinnacles (P<0.0001, F=22.19; Table 9.3).

In S1, 6 out of the 10 target species were observed, with the precious red coral *Corallium rubrum* being the most abundant one (5.75 ± 1.46 col m^{-2}) followed by *Eunicella cavolinii* and *Callogorgia verticillata* (1.04 ± 0.22 col m^{-2} and 0.29 ± 0.06 col m^{-2}, respectively) (Figure 9.4). Among the black corals, which were less abundant than to gorgonians, *A. subpinnata* was the most abundant species (0.11 ± 0.06 col m^{-2}), and only few and sparse colonies of *A. dichotoma* and *P. larix* were present (both < 0.1 col m^{-2}).

Four out of the 10 target species were found in S2. *C. verticillata*, was the largely dominant species (1.58 ± 0.39 col m^{-2}) with more than 600 counted colonies encountered (Figure 9.4, Figure 9.5), whereas the remaining species were few and sparse (all <0.1 col m^{-2}). *P. larix* was the only black coral encountered in S2.

In S3, where 8 out of the 10 target species were present, *C. verticillata* was the most abundant target species (1.04 ± 0.08 col m^{-2}), followed by the *V. bebrycoides-pool* (0.18 ± 0.06 col m^{-2}) (Fig. 9.5). Only few and sparse specimens of *C. rubrum*, *E. cavolinii*, *A. hirsuta* and black corals *A. dichotoma*, *A. subpinnata*, and *P. larix* were encountered in S3 (all with <0.1 col m^{-2}).

In S4, 9 out of the 10 target species were observed with gorgonians representing the most abundant taxon. Among these >750 colonies of *C.*

verticillata were encountered, with an average abundance of 2.67 ± 0.30 col m^{-2}, followed by *E. cavolinii* (1.07 ± 0.27 col m^{-2}), *C. rubrum* (0.68 ± 0.35 col m^{-2}), *V. bebrycoides-pool* (0.21 ± 0.07 col m^{-2}) and *A. subpinnata* (0.13 ± 0.06 col m^{-2}). The gorgonians *A. hirsuta, V. flagellum* and the two black corals *A. dichotoma* and *P. larix* showed all abundance 0.1 col m^{-2}).

In S5, 7 target species were found, again with gorgonians as the most abundant taxon, and *C. verticillata* as the most abundant species (1.00 ± 0.16 col m^{-2}), followed by *E. cavolinii* (0.88 ± 0.24 col m^{-2}), *A. hirsuta* (0.20 ± 0.05 col m^{-2}), *V. flagellum* (0.53 ± 0.2 col m^{-2}) and *C. rubrum* (0.19 ± 0.04 col m^{-2}). The black coral *P. larix* and *L. glaberrima* were present with very low abundances (<0.1 col m^{-2}).

It is worth noting that the black coral *L. glaberrima* was observed only in S5*,* whereas the whip-like gorgonian *Viminella flagellum* was present in sparse patches in S4 and S5 only (up to 4.8 col m^{-2} as maximum).

The SIMPER analysis revealed, based either on sqrt(x) transformed abundance or presence/absence data, that differences among five investigated pinnacles were mostly explained by variations in the abundance of the gorgonian *C. verticillata* (Figure 9.5;Table 9.4).

The nMDS plot shows a clear separation of pinnacles S1, S2, S3 and a cluster including S4 and S5 (Figure 9.6A). The bi-plot (Figure 9.6B) obtained after the Canonical Correspondence Analysis (CCA) confirms an evident segregation of S1, characterized by a higher abundance of *C. rubrum* and *A. subpinnata*, from all other pinnacles, altogether characterized by the dominance of *C. verticillata*. S4 and S5 are distributed along the slope axis due and show high abundances of *E.cavolinii,* and *A. hirsuta*, whereas S2 and S3 for *C. verticillata*, *V. bebrycoides-pool, and A. dichotoma.* The set of environmental factors included in the CCA explain altogether about 86% of the total inertia in the composition of target coral assemblages of the five pinnacles. All of the tested explanatory variables contributed significantly also to the discrimination of the five pinnacles in terms of assemblage composition (permutation test $P<0.001$).

Although current velocity and sedimentation rates were not measured during this study, differences in the current speed were deduced from the different levels of silt accumulation estimated from the video inspections. High silting and low-speed currents characterized all investigated pinnacles; more in details, pinnacle S2 was characterized by the thickest coverage of silk, whereas S1, the deepest pinnacle under scrutiny with vertical walls and gently sloping surfaces on the top, was the one characterized by the lowest amount of silt.

Three out of the five pinnacles showed clear signs of anthropogenic pressure (i.e. S1, S2, S3), whereas S4 and S5 appeared clear from any litter of anthropogenic litter or fishing gear. The totality of observed items were lost fishing gears, with no recreational item found (e.g. glass/plastic bottles, aluminum cans). A total of 14 lost gears were recorded: 2 lines (possibly lost from damaged trawling nets), 1 long-line and 11 pieces of nets, which were not recognized as parts of gill nets, trawls or traps.

Although the considerable distance from the coastline (i.e. 13 nautical miles) S3 was the most impacted site with the presence of 10 fishing gears, representing ca. 71% of the total number of observed items. S1 (10 nm from the coast) and S2 (12 nm) hosted 3 (21% of the total number of items) and 1 (7%) lost gears, respectively. In spite of these signs of anthropogenic pressure, no coral colonies observed in the five pinnacles showed to be broken, damaged or entangled.

9.5 DISCUSSION

Although several investigations have been conducted on Mediterranean corals dwelling > 50 m depth, very few studies have been conducted so far on coral assemblages dwelling down to the deeper part of the continental shelf (Bo et al 2012b). Our study enhances knowledge on hard-bottom coral assemblages of a poorly investigated area and an almost neglected depth range (i.e. 120-170 meters depth). So far, few studies have been conducted in Sardinian waters, mostly dealing with the red coral *Corallium rubrum* (Cannas et al 2011; Follesa et al 2013a; Cannas et al 2014; Cau et al 2015), whereas investigations on mesophotic coral assemblages is still lacking.

Although the depth range investigated in this study is different from that considered elsewhere, we report here that the values of total coral abundance along the south Sardinian continental margin (W Mediterranean) appear to be lower than those reported in the gulf of St. Eufemia (S Tyrrhenian Sea; ca. 120 m depth; (Bo et al 2012b) and similar or slightly higher than those reported from the NW coasts of Sicily, though at much deeper depths (200-250 m depth; (Bo et al 2014c). Although with some variations, all investigated pinnacles were covered by silt, which suggests the presence of only slightly turbulent bottom currents. In such conditions, some coral species may reach a large size, as observed for *C. verticillata*, *A. dichotoma* and *P. larix* in other Mediterranean Sea areas (Bo et al 2012b; Bo et al 2014b; Bo et al 2014c). In our study we found generally dense populations of *Callogorgia verticillata*, whose abundance is higher than that observed in other Mediterranean areas (Bo et al 2012b; Fabri et al 2014; Bo et al 2014b; Bo et al 2014c). We report here also that pinnacles characterized by the lowest values of coral abundance (S2 and S3), show also the dominance of this fan-like gorgonian (Figure 9.3, Figure 9.5). This finding could be possibly explained by the capability of these voluminous populations, which by far provide a general 'forest-like' aspect to the coral communities, to intercept more efficiently suspended food particles, thus outcompeting other suspension feeders for both settlement and food requirement. In such a morphologically heterogeneous *scenario*, also complicated by the three-dimensional structure of animal forests themselves, we observed that variations in coral biodiversity among pinnacles (see also SIMPER analysis; Table 9.2) match to a certain extent the differences in the pinnacles' geological setting. For instance, the pinnacle S1, which particular structure does not allow a massive accumulation of detritus, hosts a consistent population of *Corallium* *rubrum* (here the most abundant species with a maximum of 64 col m^{-2} in a single frame) settled along highly sloping walls, which indeed represent a favorable substrate for this species (Tsounis et al 2006a; Cannas et al 2011; Follesa et al 2013a; Bramanti et al 2014; Cau et al 2015). On the other hand, the gently sloping top of this pinnacle is characterized by a clear accumulation of silt and here the dominant species are the black corals *P. larix, A. subpinnata,* and *A. dichotoma*. The noticeable abundance of red coral specimens in this pinnacle is among the highest reported so far at similar depths (Rossi et al 2008). Both pinnacles S2 and S3, with a generally horizontal topography, were the most silted sites; they were characterized by very similar composition of the target species assemblages, and both hosted dense populations (>85% of the total coral abundance; Figure 9.5) of the fan-like gorgonian *C. verticillata*, even along the steep slope. A slightly different condition is observed in pinnacles S4 and S5, which both showed a certain level of silt accumulation but also a more complex topography than in all other pinnacles. These pinnacles were indeed characterized by steeper slopes (Figure 9.3), possibly limiting silt deposition and, in this case, favoring the settlement of the black coral *A. hirsuta* and the gorgonians *E. cavolinii*. Along heavily silted horizontal rocky surfaces (e.g. top of boulders) we observed also the presence of dense patches of *V. flagellum*, which is the first record of this species in Sardinian waters, and the third in the whole Mediterranean Sea after the records by Giusti et al.

(Giusti et al 2012) and Bo et al. (Bo et al 2014c), both signaled in Sicilian waters.

Thus, the overall picture emerging from our investigation allows discriminating in the study area: i) pinnacles with high levels of silt deposition and a non-complex topography which are dominated by few specialized species (e.g., *C. verticillata*, *P. larix* and *A. dichotoma;* (Aguilar et al 2013); ii) pinnacles with a more complex morphology and variable levels of silt deposition, which host more heterogeneous coral communities characterized by the coexistence of species adapted to from low to moderate bottom currents (e.g. *C. verticillata* with *E. cavolinii* and/or *A. subpinnata*, respectively)

9.5.1 Human impact

The study area is characterized by the presence of a relatively exiguous number of anthropogenic litter, entirely constituted by lost fishing gear. The unique impacted sites (S3, S1 and S2) were also the pinnacles located more distant from the coasts (almost in international waters, i.e. >12 nm from the nearest coastline). The other two investigated sites (S5 and S4) were indeed completely free of any anthropogenic impact. This difference might be explained by the presence in more offshore waters of intense fishing activities, which are practiced especially with gill-nets for lobster harvesting (Follesa et al 2011; Follesa et al 2015). This hypothesis is, on the one hand, corroborated by the presence in the study area of numerous specimens of the spiny lobster *Palinurus elephas* (Fabricius, 1787), observed during the ROV surveys, and is in accordance with the recent observation by which the high diversity of commercial species sheltered by the structure-forming corals within the animal forest is highly attractive for local fisheries, even if considerable distances (tens of nautical miles) separate the fishing area from the nearest coastline (Fabri et al 2014). Nevertheless, the absence of entangled corals in the investigated area suggests that the investigated pinnacles, though exposed to a certain pressure from fisheries (as witnessed by the presence of lost fishing gear), are still far from being severely compromised. In this regard, we stress here also that no a single specimen of *C. verticillata*, which, because of its large and fragile fan shape (up to 1m wide) oriented in the direction of the predominant current (Sanchez et al 2009; Bo et al 2012b), is more likely subjected to mechanical damage, was injured or even partially entangled with lost fishing gear. Moreover, the presence of several healthy colonies of red coral in some of the investigated pinnacles, despite the generally intensive poaching and illegal harvesting of this species with non-selective gear along the Mediterranean continental shelves (Tsounis et al 2013; Rossi 2013), confirms the general good environmental status of the investigated pinnacles.

9.6 CONCLUSIONS

Our results provide new insights on the potential role of deep coral assemblages hosted on isolated rocky pinnacles disseminated along the Sardinia continental shelf. Our results also corroborated previous findings showing the relevant role of different environmental factors in the distribution of deep coral communities on these peculiar habitats. We have also provided some evidence of the potential importance of the variation in the geological setting of these pinnacles on the biodiversity of mesophotic coral forests. Nevertheless, as the number of records of rocky outcrops disseminating the Mediterranean Sea continental shelf is progressively increasing (Martin et al 2014), more and more insights are still likely to emerge. Although we are still far from a real comprehension of animal forests' ecosystem functioning because of a poor holistic vision of these communities (Rossi 2013) and a scares knowledge of their resilience to human disturbance, we have to date collated a sufficient information to consider these habitats as important as other more complex and large coralligenous habitats in maintaining the high levels of biodiversity along the Mediterranean Sea continental margin. We anticipate, therefore, that future research efforts should be paid to understand better the driving factors deep coral biodiversity in the Mediterranean Sea and to comprehend how, at different spatial scales, the disappearance of animal forests could represents a point of no-return whose consequences are still not clear to the scientific community (Rossi 2013; Bo et al 2014a).

Our results stress also the need of investigating more accurately the distribution of deep coral communities throughout a comprehensive census of biodiversity in different geological structures of the continental shelf, with a special attention of waters out of the national jurisdiction, as also claimed by the IUCN/WCPA/WWF High Seas Marine Protected Areas Project (Bo et al 2014a).

9.7 FIGURES

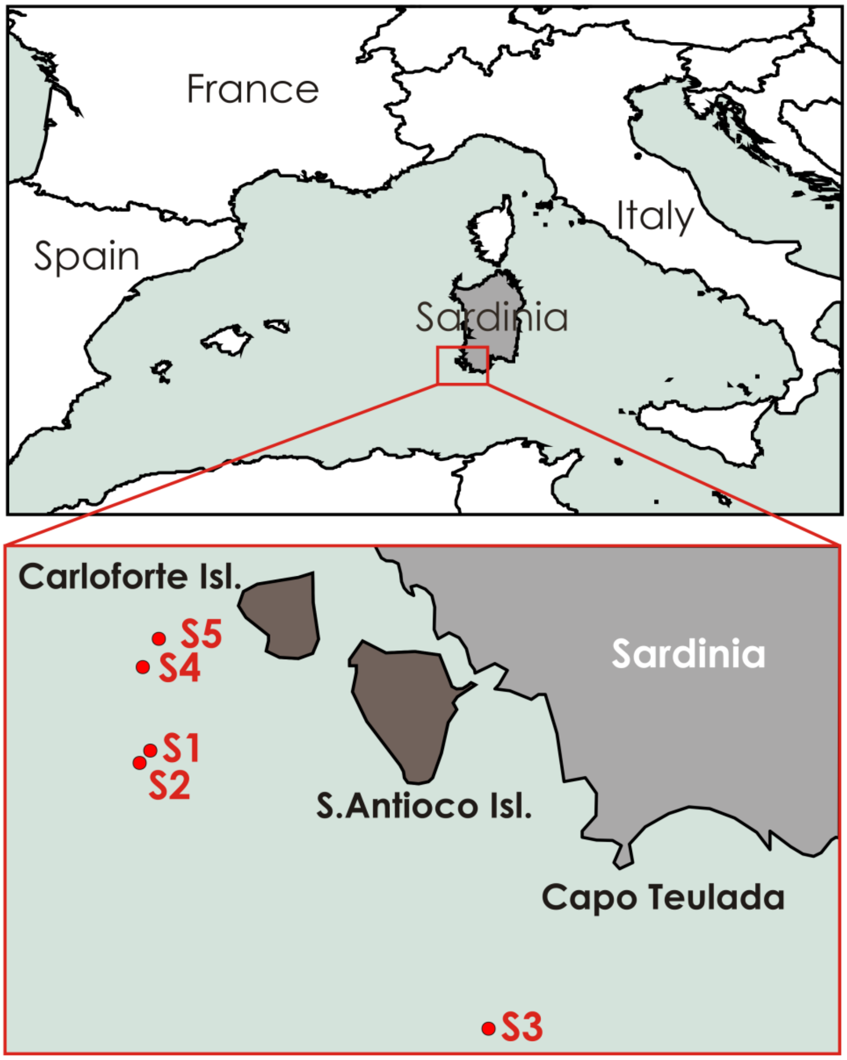

Figure 9.1 Map of the investigated area, showing investigated locations (red dots).

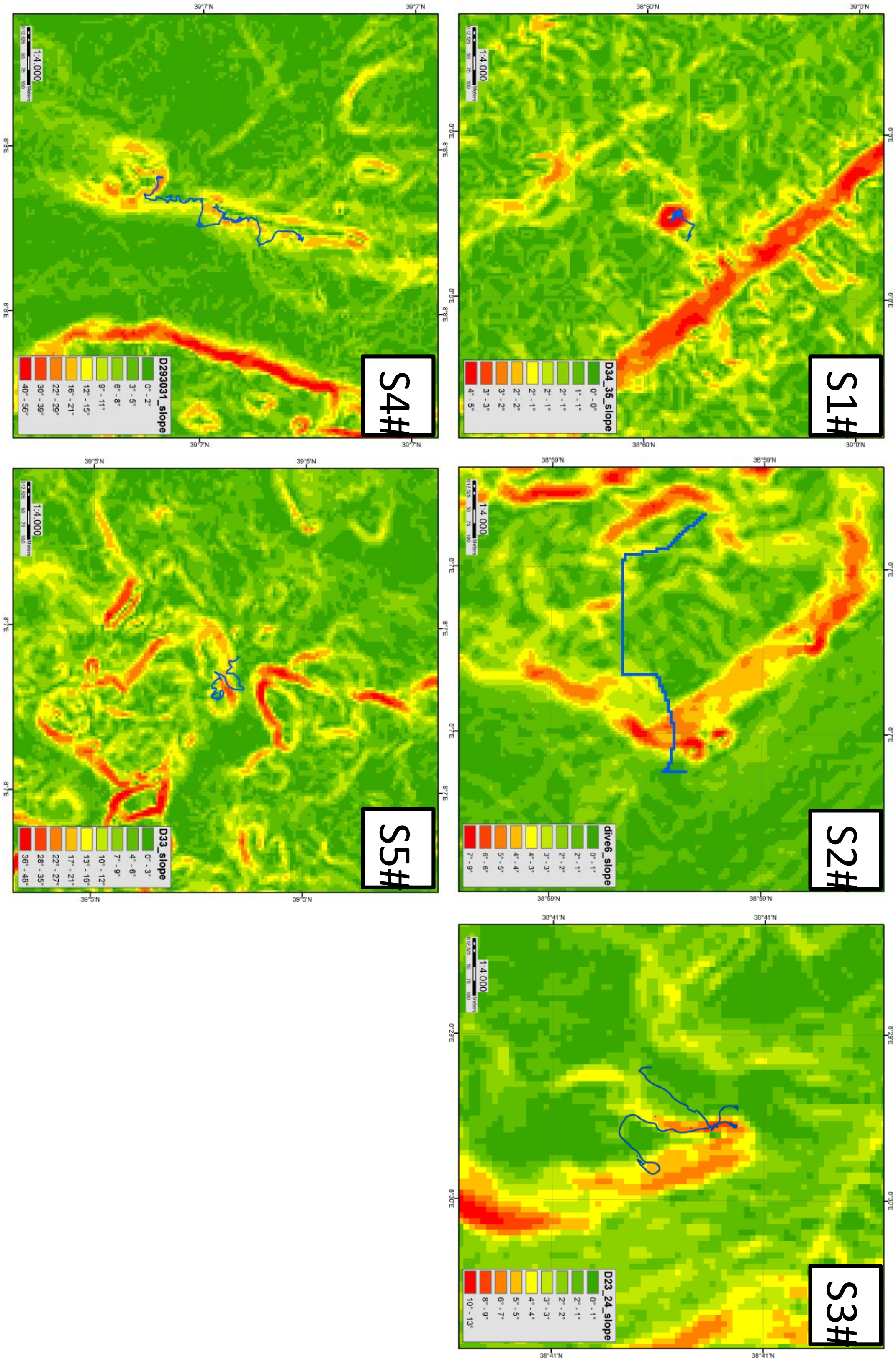

Figure 9.2 ROV surveys tracks (blue lines) reported over slope maps of investigated sites.

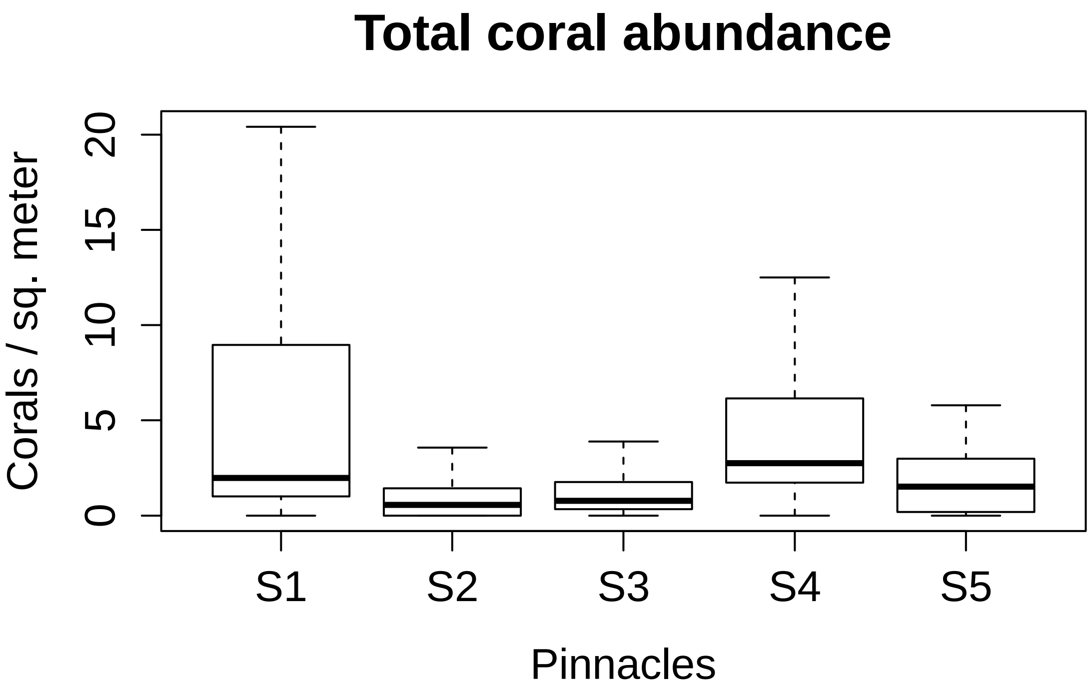

Figure 9.3 Box plot of total coral abundance (number of colonies per m-2) in the five investigated pinnacles.

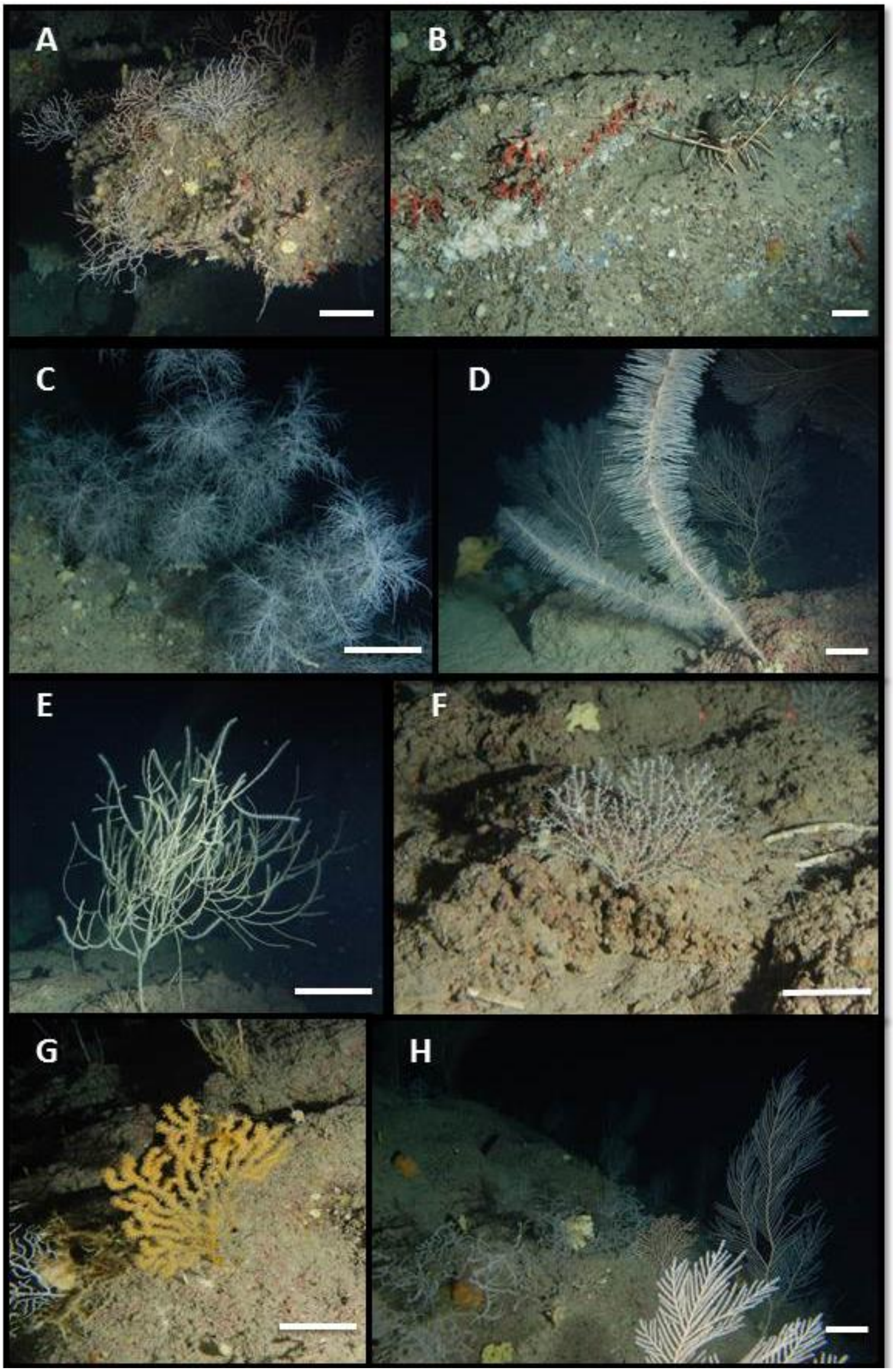

Figure 9.4 Coral assemblages of investigated pinnacles. A) Colonies of E. cavolinii with small non ramified colonies of C. rubrum; B) A specimen of spiny lobster Palinurus elephas with small colonies of red coral; C) Arborescent colonies of the black coral A. subpinnata; D) A specimen of P. larix surrounded by colonies of the fan-like gorgonian C. verticillata; E) A. dichotoma; F) A high detail image of V. bebrycoides G) A. hirsuta; H) Two small colonies of the black coral L. glaberrima, surrounded by colonies of E. cavolinii and C. vertillata. In all pictures, a 10 cm scale (i.e., white bar) is provided.

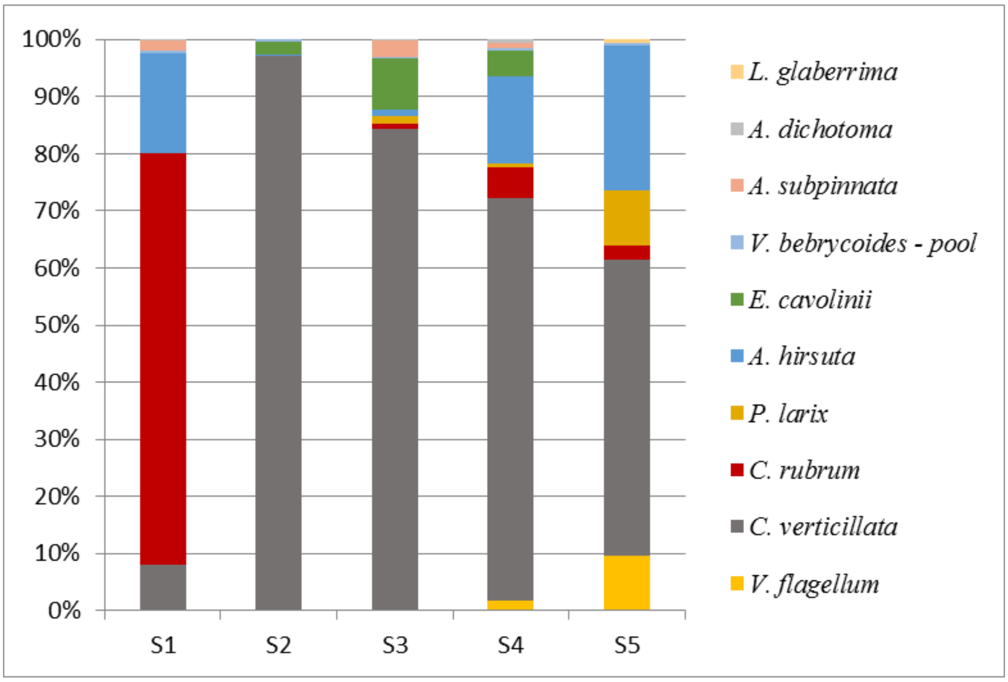

Figure 9.5 Species composition (percentage) of coral assemblages over the 5 investigated rocky pinnacles.

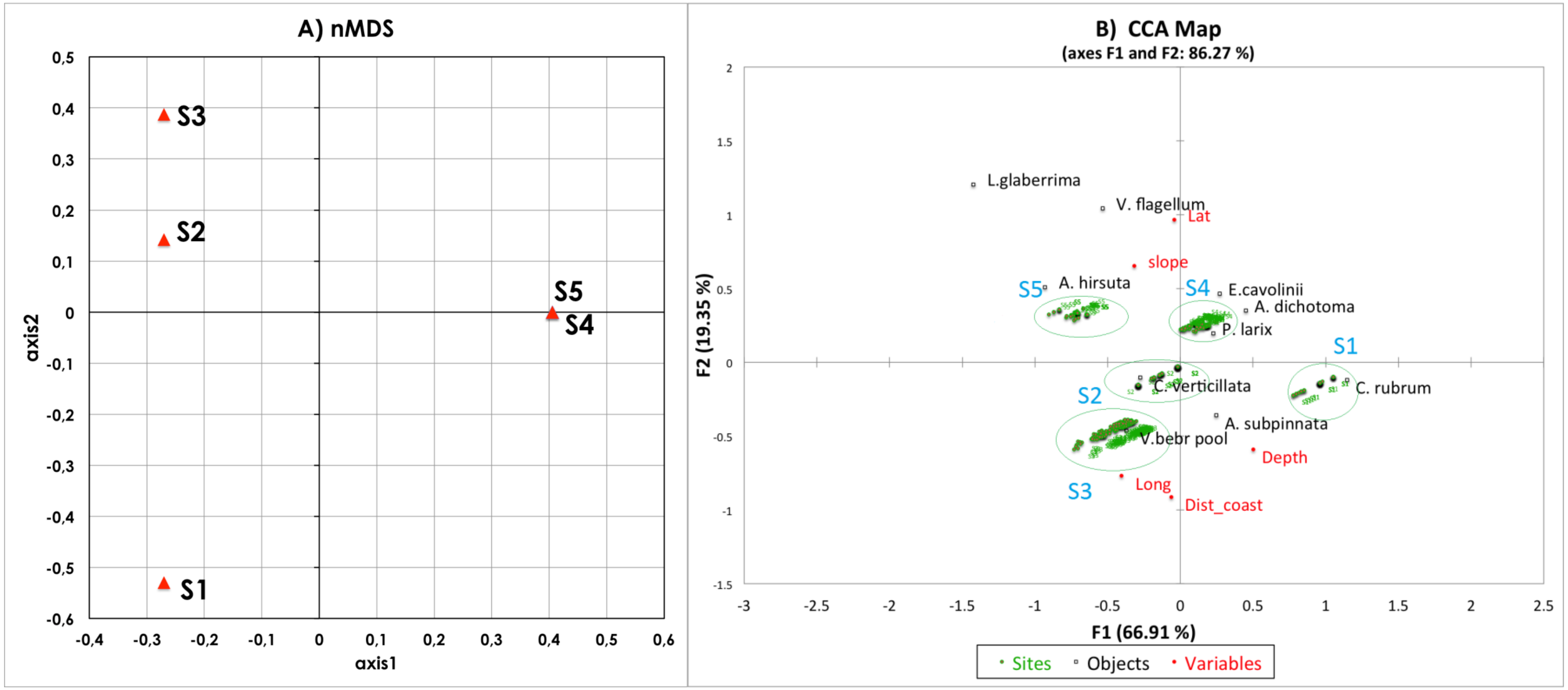

Figure 9.6 Results of nonmetric MultiDimensional Scaling (nMDS) (A) and Canonical Correspondance Analysis (CCA) (B): blue dots indicate the species, red triangles the sites, and grey lines the tested variables. See details on the main text.

9.8 TABLES

Table 9.1 Pinnacles location, n. of ROV dives performed and depth-range per each rocky pinnacle, time filming, number of useful frames and relative photographed area (m-2), number of counted corals and average abundance (n. of colonies / m-2± std. error) per each pinnacle, number of species counted among target corals.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Total**
Coordinates	38°59'816 N 8°07'720 E	38°59'00 N 8°07'02 E	38°41'394 N 8°29'469 E	39°07'221 N 8°08'280 E	39°05'349 N 8°07'227 E	
N. of dives	1	1	2	3	1	8
Survey	2011	2013	2011	2013	2011	
Depth range	150-170	150-170	135-145	120-130	120-130	120-170
Time filming	1h 36'	2h 59'	2h 58'	3h 20'	1h 10'	11h 36'
N. of frames	72	189	178	137	77	653
Photographed area (m^{-2})	122.13	926.00	824.10	436.15	262.47	2571.14
Counted corals	516	628	678	1109	426	3357
Coral abundance (col m^{-2} ± std. error)	7.23 ± 0.41	1.61 ± 0.39	1.35 ±0.12	4.98 ± 0.52	2.38 ±0.33	2.96 ± 0.24
Species richness	6	4	8	9	7	

*Table 9.2 Results of the non-parametric univariate analysis of variance (*Kruskal -Wallis* procedure) for differences among total abundance of corals and target-species abundance among five investigated pinnacles (***= P<0.001; ** = P<0.01; N.S = not significant). The test was not performed for L. glaberrima singularly, as it was present exclusively in S5.*

Species	***H***	***P-value***	**Pairwise comparison**
Total	148.9	***	
V. flagellum	2.54	***	S5 > S4; absent in S1, S2, S3
C. verticillata	83.69	***	S4 > S2 > S3 > S5 > S1
C. rubrum	25.58	***	S1 > S4 > S5 > S3; absent in S2
A. hirsuta	11.01	***	S5 > S4 > S3; absent in S1, S2
E. cavolinii	55.81	***	S4 > S5 > S1 > S3 > S2
V.bebr pool	5.47	***	S3 > S4 > S2; absent in S1, S5
P. larix	-	N.S	S4 > S2 > S3 > S1 > S5
A. subpinnata	2.54	***	S3 > S4 >S1; absent in S2, S5
A. dichotoma	-	N.S	S4 > S3 > S1; absent S5, S2
L. glaberrima	-	-	S5, absent in S1, S2, S3, S4

Table 9.3 Results of the pairwise comparison of the NPMANOVA analysis: P-values (below the diagonal) and F values (above the diagonal).

P/F	**S5**	**S4**	**S3**	**S2**	**S1**
S5		9.846	14.07	14.17	11.73
S4	0.0001		16.12	26.39	28.92
S3	0.0001	0.0001		4.159	46.24
S2	0.0001	0.0001	0.0174		51.08
S1	0.0001	0.0001	0.0001	0.0001	

Table 9.4 Results of the SIMPER analyses showing dissimilarities between couples of investigated areas and the hard bottom target-coral species mostly responsible for the observed differences.

Contrast	Dissimilarity (%)	Species	Contrib.(%)	Cum.(%)
S5 vs S4	**72.09**	*C. verticillata*	45.59	45.59
		E. cavolinii	23.51	69.1
		C. rubrum	9.68	78.78
		A. hirsuta	6.98	85.75
		V. bebr pool	4.81	90.56
S5 vs S3	**73.97**	*C. verticillata*	51.07	51.07
		E. cavolinii	17.9	68.97
		A. hirsuta	8.94	77.91
		C. rubrum	8.08	85.99
		V.bebr pool	5.5	91.49
S4 vs S3	**64.54**	*C. verticillata*	51.73	51.73
		E. cavolinii	18.54	70.27
		V. bebr pool	9.99	80.26
		C. rubrum	6.84	87.1
S5 vs S2	**74.64**	*C. verticillata*	57.65	57.65
		E. cavolinii	18.85	76.5
		A. hirsuta	8.48	84.98
		C. rubrum	8.13	93.11
S4 vs S2	**66.29**	*C. verticillata*	58.32	58.32
		E. cavolinii	18.94	77.26
		V. bebr pool	6.76	84.01
		C. rubrum	6.25	90.27
S3 vs S2	**59.86**	*C. verticillata*	79.29	79.29
		V. bebr pool	9.19	88.48
		A. subpinnata	4.45	92.92
S5 vs S1	**83.66**	*C. rubrum*	31.36	31.36
		E. cavolinii	28.43	59.8
		C. verticillata	25.61	85.41
		A. hirsuta	5.31	90.72
S4 vs S1	**81.76**	*C. verticillata*	34.92	34.92
		C. rubrum	27.59	62.51
		E. cavolinii	23.85	86.36
		A. subpinnata	4.57	90.93
S3 vs S1	**86.51**	*C. verticillata*	30.68	30.68
		C. rubrum	30.44	61.12
		E. cavolinii	25.8	86.92
		A. subpinnata	5.92	92.84
S2 vs S1	**87.72**	*C. verticillata*	32.52	32.52
		C. rubrum	31.64	64.16
		E. cavolinii	28	92.16

10 Beta-diversity of deep-water animal forests along the south Sardinia margin (Mediterranean Sea): role of contrasting environmental drivers

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10.1 ABSTRACT

Within mesophotic environment, one of the least studied environments worldwide, benthic sessile communities constitutes the most important contributors to biomass and three-dimensional development of the habitat, greatly enhancing all levels of biodiversity. These community, known as ‘animal forests’, although renowed as biodiversity hotspot, still need supporting knowledge on their distribution patterns and ecology in order to develop proper conservation measures.

In this study we investigated 8 sites from two locations from south the Sardinia continental margin characterized by highly contrasting geological settings. We hypothesized that coral forests dwelling along canyon heads could differ from forests dwelling on *roche du large* ecosystems of the continental shelf. Survey was conducted by means of Multibeam echosounder and ROV footage, using 12 species of gorgonians and black corals as target: a non-invasive approach that is particularly relevant for such delicate ecosystems. Through multivariate analyses (CAP and CCA),

we generally observed two patterns, where species like *C. rubrum* and *A. subpinnata* occur more likely along ventilated canyons vertical walls and *C. verticillata*, *A. hirsuta* along silted surfaces of rocky pinnacles. However, through the 2-level PERMANOVA with nested design, it was emphasized that the factor site, nested within locations, mostly explains the observed variability. In addition, presence of anthropogenic debris was also documented merely from a quantitative point of view, showing that canyons are the most impacted sites from lost fishing gear. Results highlighted that is not possible to discriminate "pinnacle communities" from "canyon communities" as the high heterogeneity observed among sites overcomes the variability observed among locations, emphasizing the need for further investigations on animal forest interactions with the environment. In conclusion, based on present results, site-specific conservation measures are required to deal with such biodiversity variability observed.

10.2 INTRODUCTION

Knowledge on deep-sea coral communities has improved greatly over the last two decades, but it still remains limited due to the practical difficulties of investigations in deep waters (Danovaro et al 2010; Ramirez-Llodra et al 2010; Pusceddu et al 2014). Anthozoans often represent the dominant taxon of the Mediterranean deep circa-littoral hard-bottom communities and play a key ecological role: they indeed act as ecosystems engineers, enhancing the structural heterogeneity of the substrate and thus promoting a diversification of the associated fauna (Henry and Roberts 2007; Buhl-Mortensen et al 2010; Rossi 2013). At the same time, the three-dimensional structural complexity of deep-water coral communities influence the water flow at the local scale and thus increase the residence time of suspended particles (Gili and 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 over the continental shelf, as well as in submarine canyons and seamounts (Baillon et al 2012; Rossi et al 2012; Rossi 2013).

The spatial distribution and habitat selection by different anthozoan taxa in the deep circa-littoral zone of the Mediterranean Sea have been most often explained in terms of variations in the hydrodynamic regime and the type and availability of substrate for settlement (Bo et al 2009; Bo et al 2011b; Bo et al 2012b). 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 2011a). Nevertheless, whether different geomorphological settings of substrates for corals propagules’ settlement might have a role on deep-water corals’ distribution is still largely an unexplored issue.

Recent interest has focused on the variability of coral communities associated with different geomorphological features (e.g. canyons, seamounts, and rocky outcrops arising from muddy bottoms) distributed throughout the Mediterranean continental shelf. Rocky elevations like pinnacles, and sparse rocky shoals can host rich coral fauna and levels of biodiversity much higher than those in the surrounding soft-bottom substrates, so that they have been at times defined as deep coral oases (Bo et al 2012b; Bianchelli et al 2013). 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; Pusceddu et al 2010) Mediterranean canyons that extend across the continental shelf are known to be particularly steep and landward extended compared to worldwide canyons. Such particular feature allows benthic communities associated with submarine canyons to intercept organic-matter-rich-sediments being transported along the inner shelf zone (Harris and 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 2010).

We hypothesized that different deep-water coral communities characterize the intrinsic geomorphological characteristics of different hard substrates occurring on the top of canyons and on isolated rocky pinnacles emerging from the continental shelf. To test such hypothesis we compared the community composition of deep-water corals from two locations, off 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 (Bo et al 2014a; Ponti et al 2014), the presence of anthropogenic impacts was documented in both sites in order to prompt the need of protection measures of these vulnerable habitats.

10.3 MATERIALS AND METHODS

10.3.1 Study area

We compared coral communities from two different areas, located off the south Sardinia continental margin (central-western Mediterranean; Figure 10.1), characterized by different geomorphological settings: 1) isolated rocky pinnacles located along the southwestern Sardinian margin, and 2) submarine canyons along the southeastern Sardinian margin. The two areas are also characterized by conspicuously different seafloor morphologies with the western area being a vast shelf area characterized by volcanic outcrops, and the southeastern coast of Sardinia characterized by a narrow continental shelf (2 km on average) deeply incised by a number of submarine canyons. 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 140 m).

10.3.2 Sampling

This study is based on direct observations with ROV footage and image analysis, a non-invasive approach that is particularly preferred in high conservation interest habitats like the coral communities under investigation.

The video material was obtained during two surveys onboard the R/V "Astrea" conducted along the south Sardinia continental margin in autumn 2011 and summer 2013 (Table 10.1). 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) and 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. 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 in a reasonable time interval.

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 2267 m^2 (Table 10.1). The total coral abundance (number of colonies m^{-2} ± standard error, henceforth col m^{-2} ± s.e.) and the species composition of the coral community, after taxonomic identification as described below, were estimated for each frame using the CPCe software (Kohler and Gill 2006). The coral abundance measures were estimated for total number of target corals per m-2 ± standard error (henceforth col m-2 ± s.e.) and for each target species.

10.3.3 Coral community composition and levels of anthropogenic pressure

We limited our investigation to the analysis of large anthozoans as they represent the most conspicuous component of mesophotic animal forests of the Mediterranean Sea (Rossi et al 2012; Rossi 2013); the relatively easy identification of deep-water corals 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 of the Mediterranean Sea. 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 2012b).

Alpha and beta biodiversity levels were based on counts of twelve most representative species (Figure 10.2): 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. Coralliidae), *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. Because of the scarce visual distinguishability of the two gorgonians *Villogorgia bebrycoides* and *Bebryce mollis* on the sole basis of ROV images (Bo et al 2012b), 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 (Bo et al 2012b; Baillon 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). While the presence of REi was considered merely from a descriptive point of view, the sites were discriminated for the level of impact from FGe using the following values: no impact (0, no items), low impact (1; <10 items per site) and high impact (2; >10 items per sites).

10.3.4 Statistical analyses

The software XLSTAT (function 'normality tests') performing both Shapiro-Wilks and Anderson- Darling tests, was used to ascertain the normal distribution of the whole data. Both tests showed significant non-normality distributions of the data ($P<0.0001$). Thus, 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 test for differences in the total coral abundance (Bray-Curtis similarity matrix of sqrt(x) transformed data), using location (L, with two fixed levels) and site (with four random levels nested in L) as sources of variation. The same design was also used also to analyze variations in the coral community composition between locations and among sites. The analysis was carried out with the PERMANOVA routine based on Bray-Curtis resemblance matrixes of either presence/absence and sqrtx transformed abundance data. Non-parametric methods based on permutation tests such as the one performed by the PERMANOVA, are preferable since they allow to partition the variability in the data according to a complex design or model and to base the analysis on a multivariate distance measure that is reasonable for ecological data sets (Anderson 2001; McArdle and Anderson 2001). 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 and Willis 2003), using the same Bray-Curtis based distance/ similarity matrices used for the PERMANOVA tests. 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 (Canonical Correspondence Analysis - CCA) multivariate ordination analyses of coral assemblages using the software XLSTAT. The environmental variables included in the CCA were mean slope, water depth, distance from the coast, silt (expressed as presence or absence according to the number of frames where it was present), and level of human impact in terms of n. of lost fishing gear, which is supposed to indicate a more intense fishing activity within the area.

10.4 RESULTS

10.4.1 Anthozoans abundance and community composition

The area of each single frame used for image analysis, estimated *a posteriori,* was on an average 3.37 ± 0.16 m^{-2}, covering a total of ca. 2257 m^{-2} of analyzed surface. A total of 5391 colonies of target corals were cumulatively observed in the 8 sites. The highest total coral abundance was observed at E4 (12.8 ± 1.5 colonies m^{-2}), whereas the lowest was observed in W1 (1.36 ± 0.4 colonies m^{-2}) (Figure 10.3). Since the PERMANOVA showed that variations in the total coral abundance were significant among sites within each location ($P < 0.001$), but not among locations ($P = 0.347$; Table 10.2), we analysed separately the abundance of each single taxa separately for E and W sites.

The percentage histogram showing community composition of different sites is shown in Figure 10.4.

In E4, four over the twelve target species were observed: the precious red coral *Corallium rubrum* was the most abundant species (14.5 ± 2.1 colonies m^{-2}) followed by *E. cavolinii* and *C. verticillata* (1.5 ± 0.2 colonies m^{-2} and 0.05 ± 0.02 colonies 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 ± 0.12 colonies m^{-2}). *C. rubrum* was the most abundant species (4.08 ± 0.7 col m^{-2}) also in E1, which was also characterized by the highest abundance of black corals among all investigated sites, with 1.58 ± 0.32 colonies of *A. subpinnata* m^{-2}. 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 ± 1.2 colonies 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 colonies m^{-2}, was the unique black coral observed in E3. The coral community in E2 was dominated by *E. cavolinii* (1.38 ± 0.27 colonies m^{-2}) followed by *V. flagellum* (0.8 ± 0.33 colonies m^{-2}) and another four gorgonians including *C. rubrum* (0.51 ± 0.26 col m^{-2})*, A. hirsuta* (0.14 ± 0.07 col m^{-2}), *B. mollis-pool* (0.12 ± 0.05 col m^{-2}) *and C. verticillata* (0.11 ± 0.07 col m^{-2}).

W3 was characterized by the highest value of total coral abundance among the western sites, with *E. cavolinii* as the dominant species (11 ± 0.76 colonies m^{-2}*),* followed by *A. hirsuta* and *P. clavata* (1 ± 0.02 and 1 ± 0.03 colonies m^{-2}, respectively). W2 was characterized by the highest number of target species among all investigated sites the dominance of *E. cavolinii and C. verticillata* (3.13 ± 0.34 and 3.04 ± 0.399 colonies m^{-2}, respectively), and a very high density of *C. mediterranea* (1.80 ± 0.30 colonies m^{-2}) and *V. flagellum* (1.63 ± 0.52 col m^{-2}. *C. rubrum* (0.57 ± 0.10 col m^{-2}), *A. hirsuta* (0.2 ± 0.04 col m^{-2}) and *B. mollis-pool* (0.70 ± 0.16 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 colonies m^{-2}. Four out of the thirteen target species were found over W4. However, apart for *C. verticillata*, which was the dominant species (5.25 ± 0.36 col m^{-2}) with more than 457 counted colonies; the remaining species were few and sparse gorgonians with abundance values lower than 0.1 col m^{-2} (*C. rubrum, B. mollis-pool*, *E. cavolinii*). 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. In W1, seven target species were found with *A. hirsuta* as the most dominant one (1.15 ± 0.17 colonies m^{-2}), followed by *E. cavolinii* (0.68 ± 0.15 colonies m^{-2}). The remaining few and sparse gorgonians showed very low abundance values (cumulatively < 0.1 colonies m^{-2} of *B. mollis-pool* and *C. rubrum*). *P. larix* was the largely dominant black coral (0.53 ± 0.12 colonies m^{-2}), but also *A. subpinnata* and *A. dichotoma* were observed (cumulative abundance <0.1 colonies m^{-2}).

10.4.2 Deep-water corals beta diversity

Although 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 (Table 10.3), the bi-plot produced after the CAP analysis (Figure 10.5), shows a segregation of the eastern sites from the western ones. It is noticeable that the lack of significant differences among locations obtained after the PERMANOVA test is due to the community occurring in E2. In fact, as highlighted by the cross validation test obtained after the CAP, only 79% of the eastern sites observations follow the *a priori* assignation, whereas about 90% of the observations assigned *a priori* to the western sites were indeed clustered into the western group. The SIMPER analysis carried out on the presence/absence data shows that *C. verticillata*, *C. rubrum* and *E. cavolinii* contribute (altogheter) to 67% of the dissimilarity (85.21%; Table 10.4) between locations. The same analysis performed on the abundance data matrix shows the same result: *C. rubrum*, (explaining 23% of the dissimilarity), *E. cavolinii* (21.6 %) and *C. verticillata* (21.4%). Among the four eastern sites, differences in the benthic coral assemblages were mostly explained by variations in the abundance of the species *C. rubrum* (contribution to dissimilarity 55.7%; Table 10.5) 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%; Table 10.6), which was almost totally dominant (>95%) in W4. 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 (Figure 10.6) 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*.

10.4.3 Anthropogenic pressure

Regarding anthropogenic pressure, the eastern sites were considerably more impacted (cumulatively 69 items) compared to the western ones (10 items; Table 10.7). 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 nets (n=24). E2 is the second most impacted site (with 3 REi and 16 FGe). Overall, 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.

10.5 DISCUSSION

As the awareness of the ecological role of deep-coral communities dwelling along the continental shelf is exponentially increasing (Bo et al 2012b; Bianchelli et al 2013; Bo et al 2014c), our observations conducted along south Sardinian continental margin enhance greatly our knowledge on the distribution and composition of coral forests in these poorly known ecosystems, according to the renowned necessity to cope with a more holistic vision of these ecosystems supporting ‘animal forest’ communities (Rossi 2013). Our investigation provides the first comparative analysis among contrasting geological features of the continental shelf.

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. However, this statement is not always true, as in some cases other studies have shown no significant differences in biomass and abundance of benthic fauna between the slope and canyon habitat (Houston and Haedrich 1984) or lower biomass and abundance in the canyon (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 2010). On the other hand, if the awareness of seamounts and canyons as biodiversity hot-spot is consolidated (Bo et al 2011b; Misic et al 2012), recent studies have proved how even way smaller rocky outcrops like *roche du large* ecosystems could become oases for benthic biodiversity, enhancing ecosystem' overall complexity (Bo et al 2012b; Bo et al 2014c). 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.

In this work, we aimed to test if different locations characterized by different geological settings could host different deep coral forests. Here we show through the CAP analysis that 90% of western observation followed the *a priori* grouping while for the eastern sites it dropped to 79%. The further step made through the nested design of the 2-level

PERMANOVA allowed us to emphasize that most of the variability occurs among sites within each location rather than among locations. This result was observed for all investigated features of the animal forest: the total coral abundance, the species richness and the community composition (Table 10.2, Table 10.3). The elevated heterogeneity observed among *roche du large* sites is in accordance with the few investigations performed so far (Bo et al 2012b).

The hydrodynamic regime is considered to be one of the key factors determining the spatial distribution patterns of benthic suspension feeders, that are entirely dependent from the transport of particles from water current (Gili and Coma 1998). In this regard, within eastern location, a noticeable environment was observed in E2. This site showed the presence of large amounts of accumulated *Posidonia oceanica* (Figure 10.7C, D, E), accumulated along canyon walls outcrops, transported from more shallow waters, along with thousands of specimens of *Plesionika spp* shrimps (Figure 10.7A, 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). These observations could possibly emphasize a strong ‘flushing effect’ (Canals et al 2006; Pusceddu et al 2010) of the canyon E2 that possibly shaped the coral community of this particular site. The reason for this particularly consistent transport might be the closer position to the coastline (see CCA analysis, Figure 10.6), compared to other sites. The different species composition in this site may reflect the high variability that can be found among canyons (Zúñiga et al 2009). Within the western location, the geological setting is represented by very small to medium sized rocky outcrops arising from the surrounding muddy bottom characterized by a slightly turbulent environment. Overall, 3 out of 4 western sites were covered by silt and this might explain the low abundance of species like *C. rubrum* and *A. subpinnata* (Aguilar and Zegna 2006) that, on the other hand, dominate the eastern location. These silted sites showed a high abundance of the fan like gorgonian *C. verticillata* (W4) and *E. cavolinii* (W3). A remarkable finding was the presence of the bamboo coral *Isidella elongata* 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 (Bo et al 2015).

W2 is the only western site that is not covered by silt and shows a much higher heterogeneity in the species composition, with species that are typical of environment with an intense current regime like the red coral *C. rubrum*. 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), along with the geological setting of the site (a rocky pinnacle that arises for 25 meter from the surrounding muddy bottom) could be used as explanatory factor for this heterogeneity compared to the other sites. In W2, red coral was present in large sized colonies oriented vertically (Figure 10.7 E, H), differently from the eastern sites where the abundance was higher with smaller colonies, confirming a sort of adaptation to this species to different geological settings (Cau et al 2015).

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).

As showed by CAP, species that are more likely to dwell in an environment characterized by a medium/strong current regime (i.e. *C. rubrum* and *A. subpinnata*) (Bo et al 2014c) were consistently more abundant in the eastern location, characterized by canyon heads. On the other hand, facies dominated by *E. cavolinii* and *C. verticillata* were characteristics of rocky outcrops from the western sites. However, two sites (E2 and W2) showed differences from other sites from the same location (Figure 10.4, Figure 10.5, Figure 10.6), with a higher heterogeneity in the species composition and abundance; this result could be explained with the combination of hydrodynamic features and a higher substrate complexity of these sites, where both species like *V. flagellum*, *C. verticillata* and *P. larix,* dwelling in silted surfaces with low current regime (Bo et al 2014b; Bo et al 2014c) coexist with species typical of intense current regimes like *C. rubrum*, showing how geological setting could provide shelter for certain species, also in a theoretically unlike environment, creating suitable micro-habitat.

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 2015). 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 loss of the fishing gear, as they have to be positioned not over rocky outcrops like in the western location but along canyon margins.

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 variability among sites overcoming the macroscale highlights that a substrate-based policy where particular topographies are object of conservation rather than others, could not be enough for preserving 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.

10.6 FIGURES

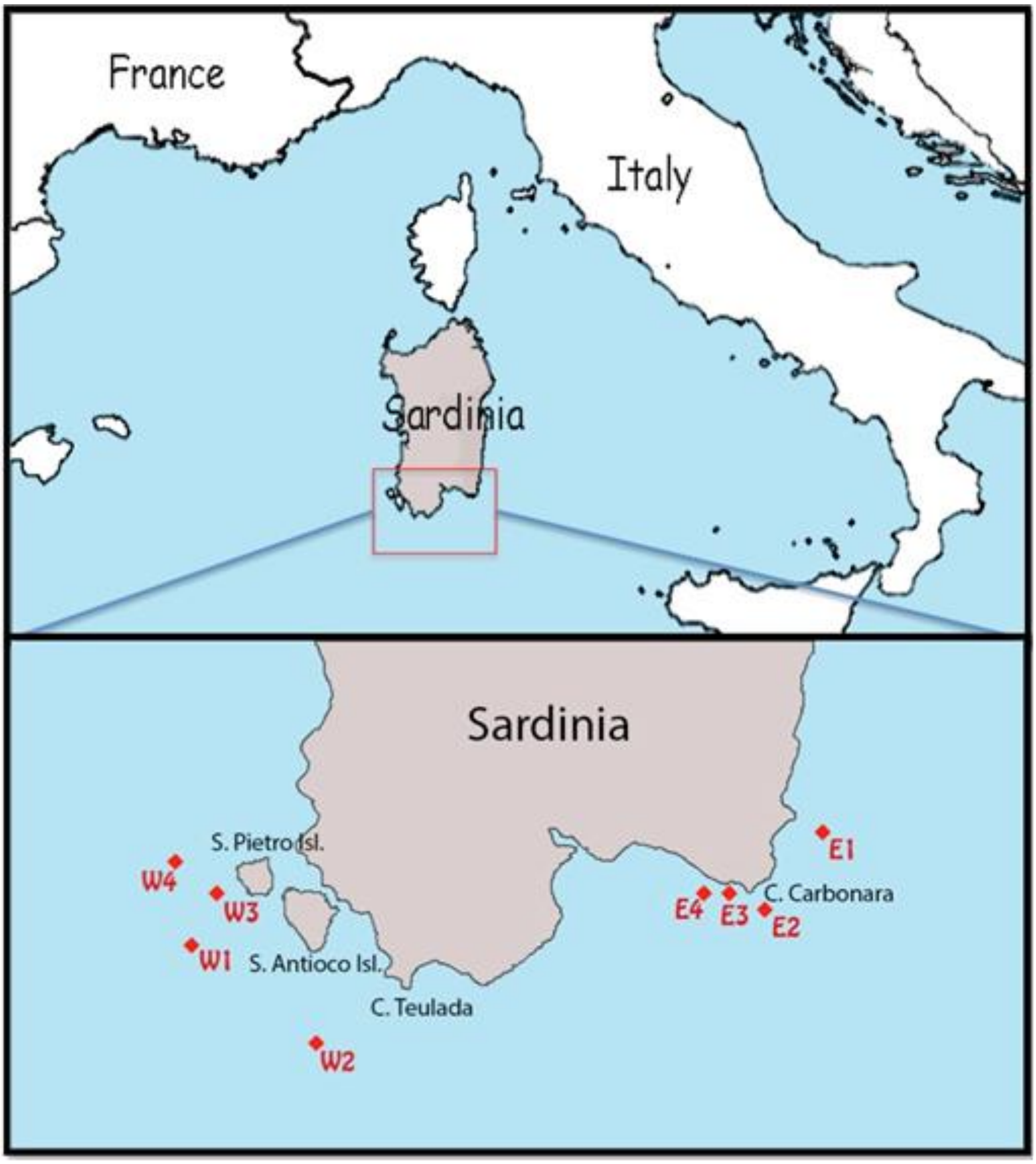

Figure 10.1 Investigated area. Map of the investigated area, showing sites within western and eastern locations.

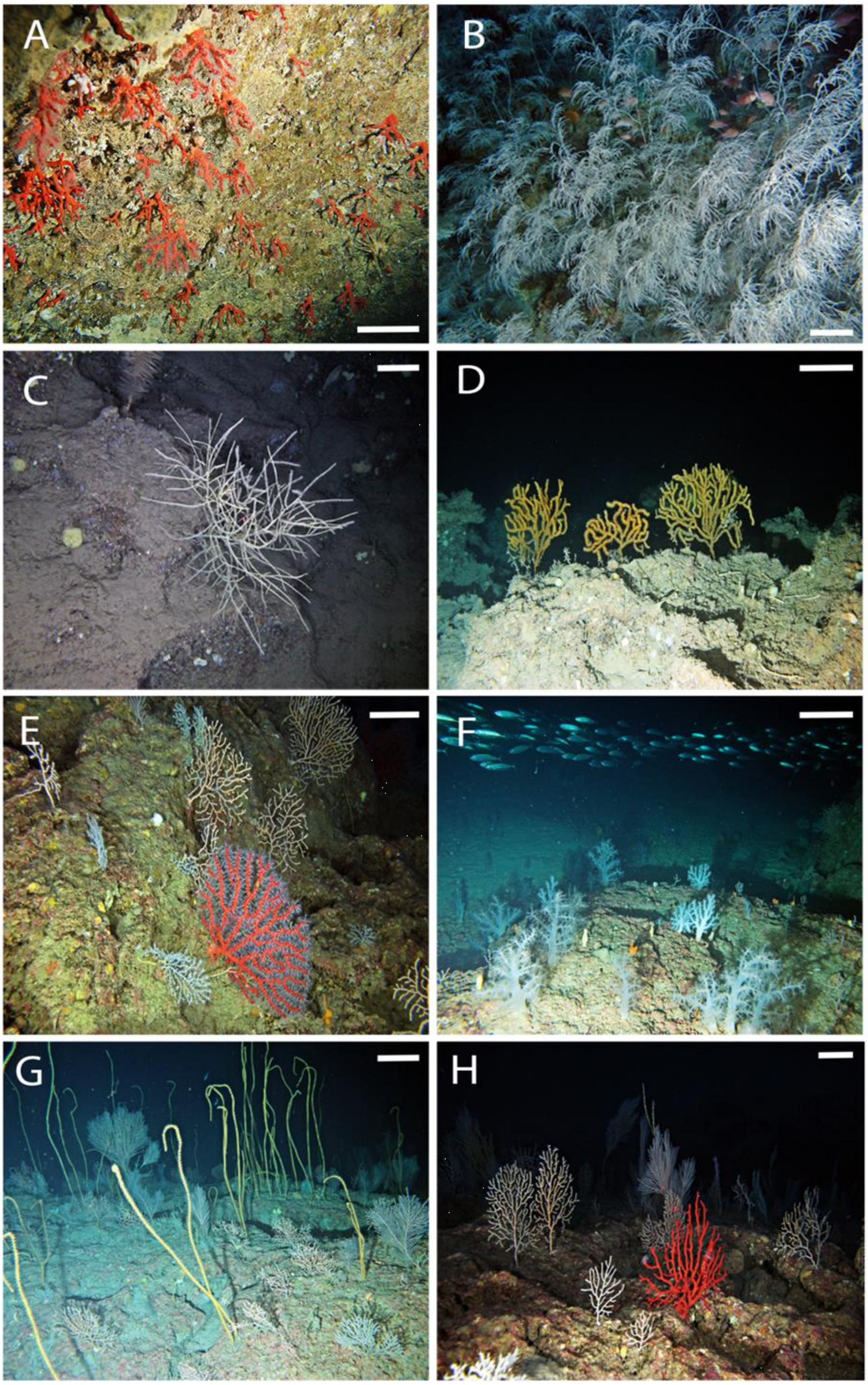

Figure 10.2 Coral assemblages of investigated sites. ***A)*** *Vertical wall colonized by small colonies of C. rubrum;* ***B)*** *Arborescent colonies of black coral A. subpinnata;* ***C)*** *Specimens of A. dichotoma and P. larix ; D) Three big colonies of A. hirsuta ;* ***E)*** *One big colony of red 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. vertillata around a big colony of C. rubrum . In all pictures, a 10 cm scale (white bar) is provided.*

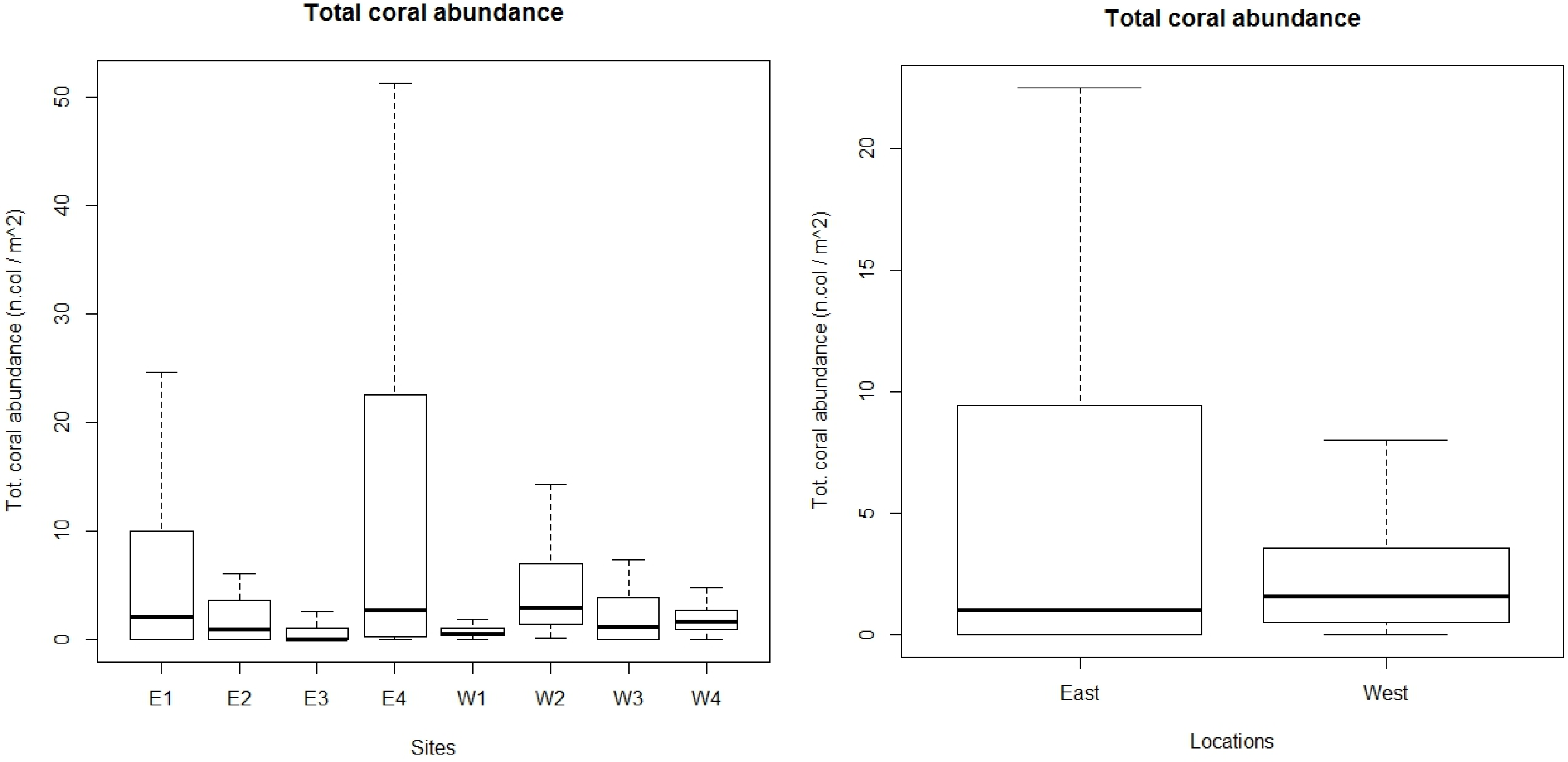

Figure 10.3 Total coral abundance. Boxplot showing differences of the total coral abundance (n° col m^{-2} ± sd) among different sites from eastern and western locations.

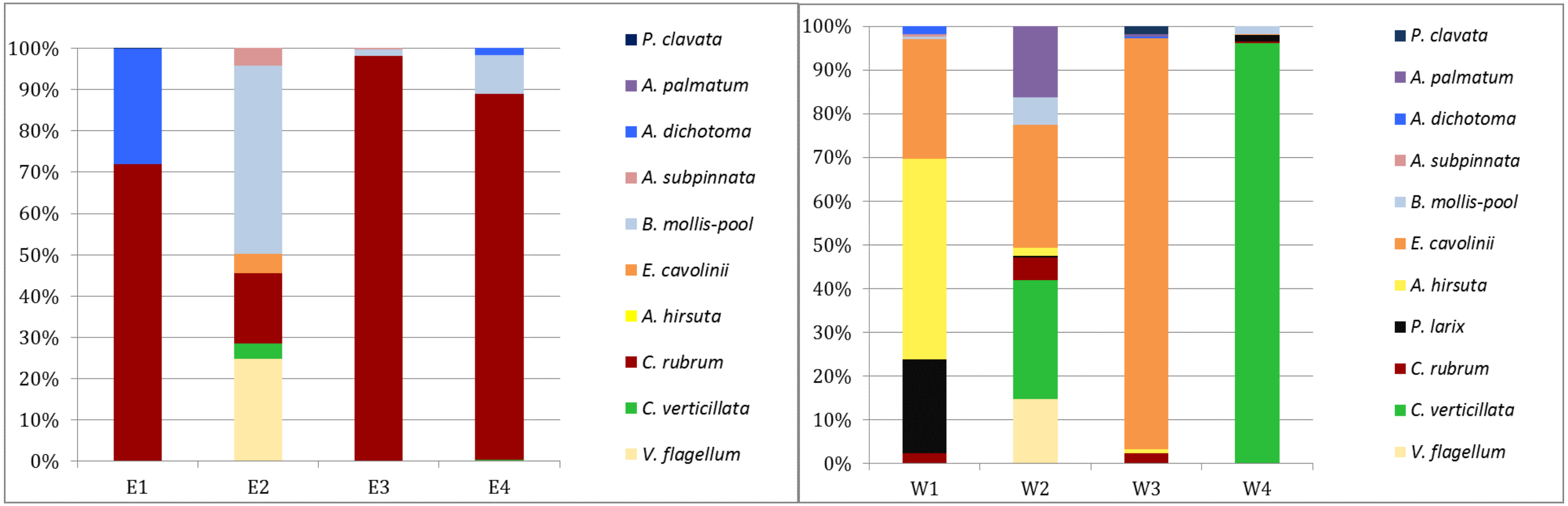

Figure 10.4 Community composition. Percentage histogram showing community composition of different sites.

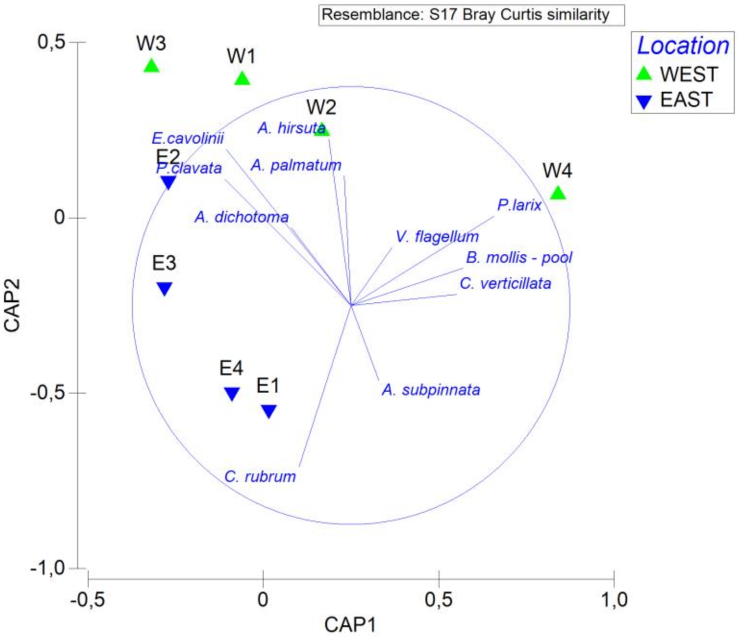

Figure 10.5 Canonical analysis of the principal coordinates. Bi-plot produced after the multivariate analysis showing segregation of sites from different locations according to variations in abundance of target coral species.

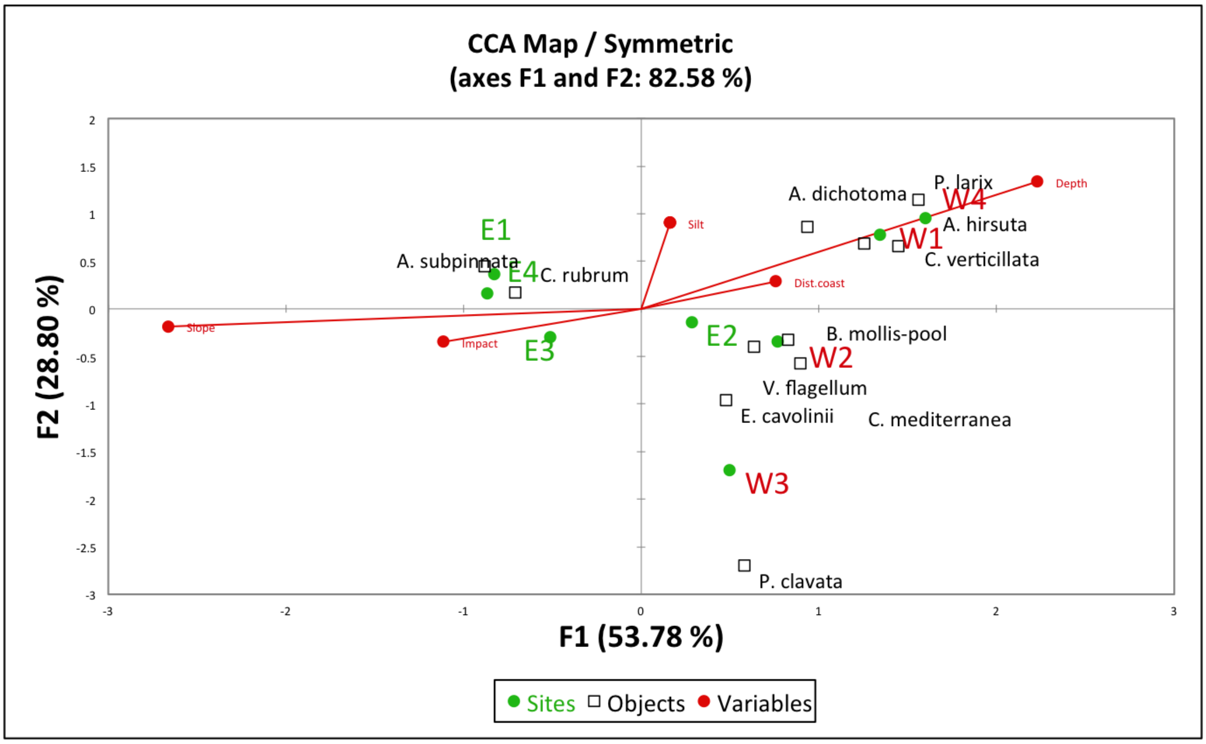

Figure 10.6 Canonical Correspondence analysis.

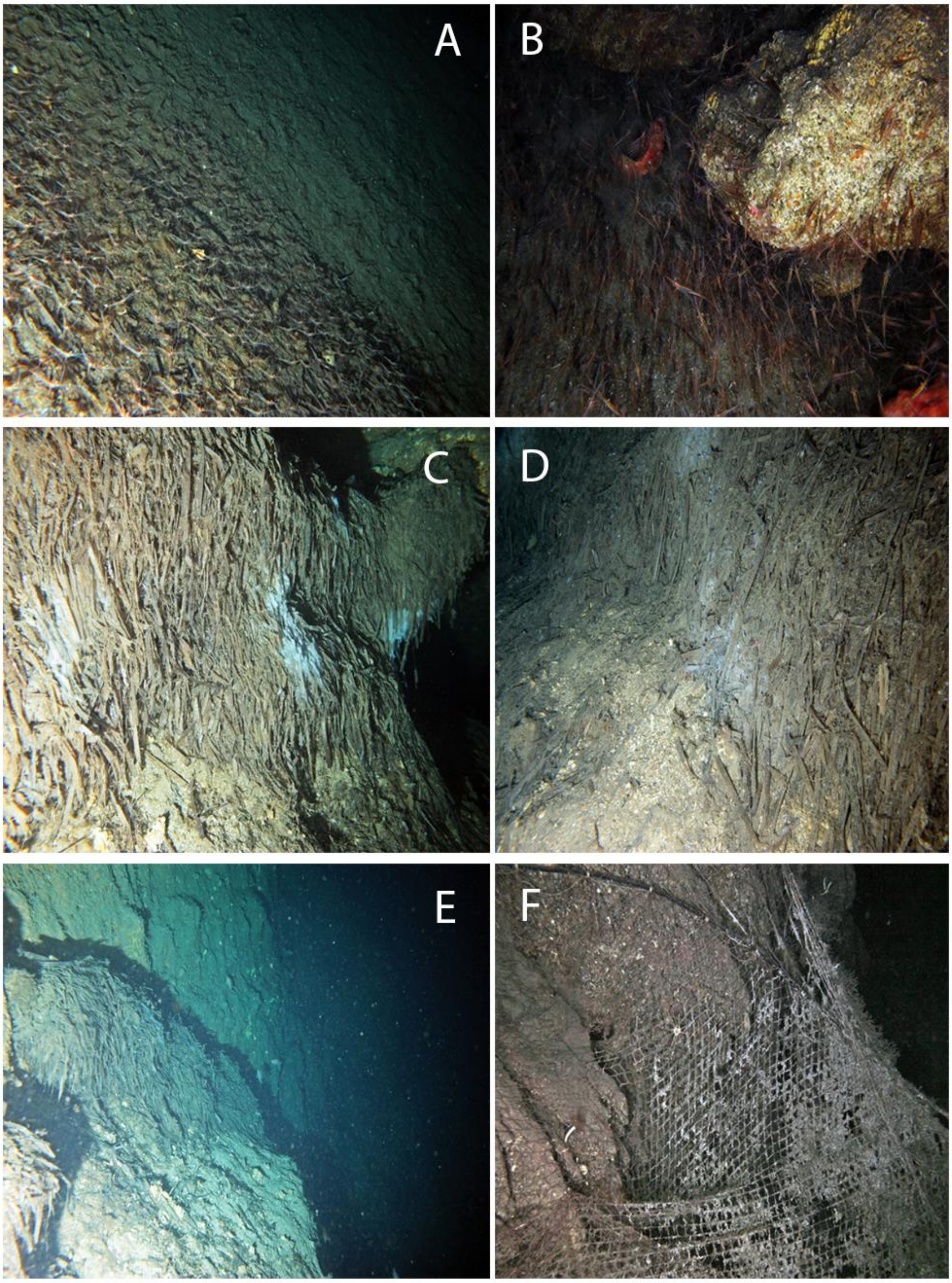

Figure 10.7 Site E2 snapshots.

10.7 TABLES

Table 10.1 Details of the investigated sites

	Site E1	**Site E2**	**Site E3**	**Site E4**	**Total**
Coordinates	39° 25' 340" N 9° 47' 594" E	39° 04' 780"N 9°33'760" E	39° 06' 719"N 9°25' 970" E	39° 05' 414"N 9° 28' 327" E	
N. of dives	2	2	4	1	9
Survey	2011	2013	2011	2011	
Depth range	90-110	110-145	90-140	100-140	
Time filming	2h 08'	3h 02'	5h 47'	2h 30'	8h 30'
N. of frames	97	54	72	133	356
Frames area (m^{-2})	145.4	135.69	250.47	311.58	843.14
N. of coral colonies	553	165	308	2225	3251
Coral abundance (colonies m^{-2} ± s.e.)	8.6 ± 1.5	3.0 ± 0.82	3.4 ± 1.14	12.8 ± 1.5	
Species richness	3	6	4	4	
	Site W1	**Site W2**	**Site W3**	**Site W4**	
Coordinates	39° 10' 122"N 8° 06' 133" E	38° 44' 425" N 8° 29' 025" E	39° 05' 345" N 8° 10' 538" E	38° 59' 064" N 8° 07' 270" E	
N. of dives	1	1	1	1	4
Survey	2013	2013	2013	2013	
Depth range	140-150	100-120	80-100	160-190	
Time filming	1h 20'	1h 50'	2h	2h 57'	7h 57'
N. of frames	67	110	49	87	313
Frames area (m^{-2})	284.8	648.74	150.31	330.15	1449.28
N. of coral colonies	168	1287	215	481	2151
Coral abundance (colonies m^{-2} ± s.e.)	1.36 ± 0.34	4.98 ± 0.55	5.53 ± 0.36	1.8 ± 0.15	
Species richness	7	9	3	6	

*Table 10.2 Results of the PERMANOVA testing for the effect of locations and sites on the total coral abundance (*** = ≤0.001; * = ≤0.05; ns= not significant).*

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

*Table 10.3 Results of the PERMANOVA testing for the effect of location and site on composition of the coral communities based on the abundance and the presence/absence matrixes (***= P<0.001; N.S.= not significant).*

Abundance matrix				
Source	**df**	**MS**	**Pseudo-F**	**P**
Location	1	$1.860E10^5$	2.306	ns
Site (Location)	6	94836	52.246	***
Residual	491	1815.2		
Total	498			

Presence/absence matrix				
Source	**df**	**MS**	**Pseudo-F**	**P**
Location	1	$1.590E10^5$	2.142	ns
Site (Location)	6	87182	37.535	***
Residual	491	2322		
Total	498			

Table 10.4 Results of the SIMPER analyses from abundance data matrix showing dissimilarities between the two investigated locations and the contribution of the different species to the observed differences.

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

Table 10.5 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.

Contrast	**Dissimilarity (%)**	**Species**	**Contribution (%)**	**Cumulative (%)**
E1 vs. E2	89.69	*C. rubrum*	39.3	7.6
		E. cavolinii	65.0	0.0
		A. subpinnata	85.1	0.9
		V. flagellum	92.4	0.0
		C. verticillata	95.5	0.0
		B. mollis	98.1	0.0
E1 vs. E3	83.03	*C. rubrum*	62.0	62.0
		E. cavolinii	18.1	80.1
		A. subpinnata	17.7	97.8
		C. verticillata	2.0	99.8
		B. mollis	0	100
		P. clavata	0	100
E1 vs. E4	69.2	*C. rubrum*	64.4	64.4
		A. subpinnata	29.5	93.9
		E. cavolinii	2.9	96.8
		A. dichotoma	1.8	98.6
		B_mollis	1.0	1.4
		P. clavata	0	100
E2 vs. E3	85.54	*C. rubrum*	49.4	49.4
		E. cavolinii	33.7	83.1
		V. flagellum	6.1	89.2
		C. verticillata	4.1	93.3
		A. subpinnata	3.2	96.5
		B. mollis	2.1	98.6
E2 vs. E4	80.18	*E. cavolinii*	39.7	39.7
		C. rubrum	36.2	75.8
		V. flagellum	10.6	86.4
		C. verticillata	5.1	9.5
		B. mollis	4.9	96.4
		A. hirsuta	2.3	98.7
E3 Vs. E4	79.88	*C. rubrum*	64.1	64.1
		E. cavolinii	26.9	91.0
		A. subpinnata	4.0	95.0
		C. verticillata	3.1	98.2
		A. dichotoma	1.1	99.3
		B. mollis	0.7	10.0

Table 10.6 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.

Contrast	**Dissimilarity (%)**	**Species**	**Contribution (%)**	**Cumulative (%)**
W1 vs. W2	93.7	*E. cavolinii*	23.5	23.5
		C. rubrum	19.0	42.4
		C. verticillata	17.6	60.0
		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	24.0	72.3
		A. hirsuta	19.0	91.3
		P. larix	3.7	94.9
		P.clavata	3.4	98.3
		A. dichotoma	1.1	99.4
W1 vs. W4	98.5	*C. verticillata*	50.3	50.3
		C. rubrum	22.1	72.4
		A. hirsuta	11.3	83.7
		E. cavolinii	12.5	92.6
		P. larix	1.6	98.0
		B. mollis	1.1	99.1
W2 vs. W3	86.4	*E. cavolinii*	40.1	40.1
		C. verticillata	20.1	60.2
		A. palamtum	12.5	72.7
		C. rubrum	8.0	80.7
		B. mollis	6.7	87.3
		V. flagellum	5.6	92.9
W2 vs. W4	82.8	*C. verticillata*	39.5	39.5
		E. cavolinii	20.8	60.3
		C. mediterranea	10.1	70.5
		B. mollis	7.0	77.4
		C. rubrum	7.0	84.4
		V. flagellum	5.6	89.9
W3 vs. W4	99.4	*C. verticillata*	52.5	52.5
		E. cavolinii	38.0	87.0
		C. rubrum	3.0	93.8
		P. clavata	2.9	96.7
		P. larix	2.0	98.6
		B._mollis	1.1	99.7

Table 10.7 Anthropogenic pressure analysis. Number of items (n) for the two categories of litter: recreational items (REi) and fishing gear (FIg).

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

11 Nursery area for the small spotted catshark *Scyliorhinus canicula* in a millenary forest of *Leiopathes glaberrima* in South-western Sardinia

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Biodiversity and conservation (in preparation)

11.1 ABSTRACT

During ROV exploratory surveys, over 740 egg capsules of the spotted catshark *Scyliorhinus canicula* have been found in the South West Coast of Sardinia (Carloforte Shoal), attached to colonies of the long lived black coral *Leiopathes glaberrima,* forming a dense forest. The egg-cases were counted and measured; a single capsule was collected and hatched in captivity.

The *S. canicula* nursery area is located on the southwestern side of a rocky elevation of the sea bottom, at depths between 188 and 210 meters. The existence of a dense black coral forest, with a nursery area for the small spotted catshark within the coral ramifications, together with the occurrence of a meadow of the soft bottom gorgonian *Isidella elongata*, indicates that this area have to be considered a pristine Mediterranean deep coral ecosystem, a coral sanctuary that would deserve special protection.

11.2 INTRODUCTION

Scyliorhinus canicula (Linnaeus, 1758) is chondrichthyan species that is frequently observed along the coasts of Europe in the Atlantic and Mediterranean Sea, including the Black Sea. It primarily lives over sandy, gravely or muddy bottoms at depths of a few metres down to 550 m, mainly from 50 to 250 m on the continental shelf (Serena 2005). It is particularly abundant (both in terms of biomass and density) in Sardinian waters (Relini et al 2010; Follesa et al 2013b). The species is a typical by catch of bottom trawl fishery, and represents an important part of shark landings in Europe (Serena 2005). In Mediterranean, the small spotted catshark is targeted for consumption. For instance in Sardinia it is locally marketed under the vernacular name of 'gattuccio' and has a relatively ‘high’ economic value.

S. canicula is an oviparous species that lays its eggs in protective egg-cases. These are deposited in pairs, and anchored to solid structures (Kousteni et al 2010). Females can spawn about a hundred eggs per year (range 90 to 115) (Serena 2005). In previous studies, *S. canicula* egg-cases were observed on macroalgae, and attached to benthic invertebrates such as poriferans, bryozoan and hydroids (Ellis and Shackley 1997). Several studies concordantly indicate that both in the Atlantic and in the Mediterranean the breeding season of *S. canicula* is protracted, with egg-laying almost continuous along the year (Leloup and Olivereau 1951; Rodriguez-Cabello et al 1998; Serena 2005; Capapé et al

2008; Bendiab et al 2012; Wearmouth et al 2013) and references therein).

S. canicula has been globally assessed as Least Concern' in the IUCN Red List, but in the Mediterranean Sea has been classified as 'Near Threatened'. Actually, in vast areas of the distributional range the populations of the species are stable and common (e.g., in Tyrrhenian Sea) but they are declining in other areas (e.g., the Adriatic Sea; (Ellis et al 2009). From a conservation point of view special attention should be given to the nursery areas, usually located on the upper slopes (~200 m). They have been identified around Corsica, Sardinia and Tuscany (Relini et al 2010 and references therein).

In the present study we describe a nursery ground for the small spotted catshark *S. canicula*. It is located on a rocky elevation at depths between 188 and 210 meters, characterized by the presence of dense patches (a 'forest') of the smooth black coral *Leiopathes glaberrima* (Esper, 1788).

In the Mediterranean Sea smooth black corals are considered quite common since they are frequent in the fishermen's bycatch (Deidun et al 2010; Sampaio et al 2012; Mytilineou et al 2014; Deidun et al 2014; Bo et al 2015). However, a comprehensive geographic mapping of the populations of *L. glaberrima* is lacking. Usually black coral forests occur within rich biocoenoses up to 500 m depth, only occasionally they form very abundant monospecific populations, especially on exposed rocky, bench terraces with low silting levels (D'Onghia et al 2010; Angeletti et al

2014; Bo et al 2014a; Mytilineou et al 2014; Bo et al 2015 and references therein).

From an ecological point of view, coral forests are considered as complex ecosystems representing important three-dimensional habitat, supporting high levels of biodiversity both within the area and in their neighboring areas (Gili and Coma 1998). These forests are also "nursery" places where many species (e.g. fishes, crustaceans, cephalopods, etc.) spend part or all the life cycle (Rossi et al 2012; Rossi 2013). The more mature forests consist of taller and more branched corals, with more structures that can alter major current flows and particle retention, thus concentrating more zooplankton, eggs, larvae, juveniles and adults in their surroundings (Baillon et al 2012).

To our best knowledge, this paper contains the first *in situ* documentation of a shark nursery in a dense forest of *L. glaberrima*. The reasons and urgent need to protect the site are discussed in detail.

11.3 MATERIALS AND METHODS

The shoal of Carloforte is a rocky elevation located 11 nautical miles from the nearest coastline, that is the western coast of the island of San Pietro in the south western waters of Sardinia (central western Mediterranean sea; Figure 11.1). The shoal of Carloforte (henceforth CS) is situated within the south west Sardinia continental margin, precisely in the outer shelf area, that is characterized by the presence of numerous rocky elevations emerging from a flat muddy bottoms at about 210 m depth.

The shoal has an oblong shape, NW/SE oriented, with a surface of about 0.5 km2. The higher elevation from the surrounding muddy bottom is encountered along the south-western side of the structure (Figure 11.1), that is characterized by a series of bench terraces only slightly covered by sediments, while, towards the eastward side, the shoal continues with a rocky plateau with only few rocky boulders emerging from patches of detritic sand (Bo et al 2015). The deepest area of the shoal is the north-western side, located at 220 m depth; this side of the shoal rises with gently sloping walls, all the way through the south-eastern side (Figure 11.1).

11.3.1 Data acquisition

Data were collected during three ROV surveys, that were carried out in October 2011, June 2013 and July 2013. Two different ROVs were used: i) the ROV "Pollux III" (October 2011 and July 2013) equipped with a digital camera (Nikon D80, 10 megapixel), a strobe (Nikon SB 400), a high definition video camera (Sony HDR-HC7) and ii) the ROV "Seaeye Falcon" (June 2013) equipped with 3 cameras: i) default colour camera, ii) color camera equipped with laser beams, over a 180° tilt platform and iii) independent high definition video camera (GoPro 3+). Both ROVs were equipped with track-link system, depth sensor, compass, and two parallel laser beams providing a constant 10 cm reference scale in the video frame, for the measurement of the frame area.

A total of 5 ROV dives were performed in the Carloforte shoal area, at depths between 186 and 221 meters. A total of 10h 49′ of filming were acquired and a total linear distance of 4725 meters was covered. The image analysis was performed with CPCe software (Kohler & Gill 2006), on 815 frames that were randomly extracted from the video footage with DVDVideoSoft. The videos were analyzed by quantifying the numbers of black colonies with and without egg cases and the number of egg cases per colony.

In the whole area, species composition and abundance of the coral communities were described, and measured as explained in Chapter 9 and 10.

11.4 RESULTS

During this study, all the sides of CS were investigated; the western slope was explored during two campaigns (October 2011, and July 2013) realized on board of the R/V 'Astrea' (ISPRA, Rome) equipped with the ROV "Pollux III", while the eastern slope was inspected in June 2013 by a chartered commercial boat and the ROV "Seaeye Falcon".

In the whole area, species compositions and abundances of the coral communities and associated megafauna were described and measured.

In particular, Bo and colleagues (Bo et al 2015) described in detail the south-western side of CS, while Cau and colleagues (Cau, this chapter) characterized the north-western side.

11.4.1 The CS south-western side

In CS south-western side three distinct habitats were investigated: the bench terraces, the rocky plateau, and the muddy bottom.

The bench terraces bordering the southwestern side of the CS shoal were dominated by the antipatharian *L. glaberrima* (79% of the total counted organisms, with an average density of 0.8±0.05 col m^{-2}). The community hosted also *C. verticillata* (9%), *B. mollis* (5%), *A. hirsuta* (3%), *P. larix* (3%), and *A. dichotoma* (1%) (Bo et al 2015). In the rocky plateau, the coral community was dominated by a mixed assemblage of *L. glaberrima and C. verticillata* (64% and 29%, respectively), with lower abundances than in the terraces (Bo et al 2015). The community included also *A. hirsuta* (4%), *E. cavolinii* (2%), and *B. mollis* (1%). A monospecific population of *I. elongata* was found in the muddy bottom surrounding the rocky elevations (Bo et al 2015).

11.4.2 The CS north-western side

The CS north-western side was characterized by a different morphology were the muddy (or heavily silted area) gently arises to the plateau area described by (Bo et al 2015). The only observable terrace, like the southwestern side, was located along the NW ridge side, where a ca 3m tall one was found. Along transects few small rocky outcrops were observed. The coral community was composed by *C. verticillata* (47.8% of the total coral), *A. hirsuta* (26.3%), *B. mollis* (5.6%), *E. cavolinii* (0.4%), *I. elongata* (5.6%) and the antipatharians, *P. larix* (3.4%), *A. subpinnata (10.3%)* and *A. dichotoma* (0.4%). *L. glaberrima*, which was dominant on the southwestern side, was not found on the northwestern slope.

11.4.3 Capsules

A total of 743 capsules was observed (Figure 11.2): 245 capsules in 2011 (Cau et al 2013b) and 498 in 2013.

The egg-cases were found attached to 363 colonies of black corals of the species *L. glaberrima*. Apart from a single capsule morphologically attribuable to a unidentified skate's species (family Rajidae), the size, shape (with long tendrils, and a bulging fusiform shape), and color of the capsules was similar to that reported for the deep-water catsharks *Scyliorhinus canicula* (Mellinger 1983).

Yolk was visible through the walls of many capsules, indicating that the embryos were still developing. Some capsules had a clean surface with a clearly visible yolk while others were degraded or covered with sediments. From the video frames, it was possible to measure only 86 capsules; the height and width ranged from 4.99 to 7 cm and from 1.56 to 3.11, respectively. However, the measurements of the eggs were quite problematic because the capsules were often strictly intertwined with the coral branches, therefore they should be interpreted with caution.

Over the total number, in 380 colonies capsules were absent while the maximum was 44 eggs over a 2 meters tall colony. A total of 8 specimens of *S. canicula* were observed in the area, moving on the muddy bottom surrounding the rocky pinnacle (Figure 11.3). Through the image analysis it was possible to measure the total length of 4 individuals of *S. canicula* (LT>38 cm). Other species than the spotted catshark were observed during the survey as a prove that other organisms take advantage of this habitat (Bo et al 2015).

11.4.4 Hatching in captivity

To further confirm the species identification of the capsules, an egg case specimen, containing the developping embryo, was collected with the ROV's jaw grabbers on September 4th 2013. It was immediately stored on board in sea-water tanks, then transferred in an aquarium at the Univerisity of Cagliari, and mainted until it hatched in controlled appropriate conditions (Capapè et al 2006) (Figure 11.4).

On date January 8th 2014 (after 126 days), the embryonic development was completed and hatching occurred (Figure 11.5). The length at bith was 8.2 cm. Unfortunately, the neonate of *Scyliorhinus canicula* survived only a few weeks.

11.5 DISCUSSION AND CONCLUSIONS

Elasmobranches consists of some of the most threatened marine species in the last century, with serious consequences on the entire ecosystem, because of their top position among the food web (Heithaus et al 2008). Because of this, the importance of identifying shark nursery areas is increasing in order to implement conservation or management measures. Heupel and colleagues (Heupel and Carlson 2007) developed a new nursery definition for sharks as follows: "Three criteria [must be] met for an area to be identified as a nursery: (1) sharks are more commonly encountered in the area than in other areas; (2) sharks have a tendency to remain or return for extended periods; (3) the area or habitat is repeatedly used across years''. These criteria are much easier to apply than the more general definition proposed in previous literature (Francis 2013 and references herein).

According to the results of the present study, the CS shoal meets all the criteria and can be designated as a 'nursery area' for *S. canicula*.

In the investigated area, over seven hundreds capsules at different stages of development were found. It is worth noting that the presence of eggs was first discovered in 2011 and then reconfirmed in 2013, therefore their finding is not fortuitous but several females continuously use the area to lay the eggs. During the ROV surveys, several specimens *S. canicula* (total length > 38 cm) have been observed near the CS rocky elevation; all were presumably sexually mature since males mature at 39 cm, females at 34 to 45 cm (Serena 2005). Once maturity is reached, spawning can take place almost immediately (Rodriguez-Cabello et al 1998).

11.5.1 Hatching in captivity

Despite a known variability among sites and geographical areas of the Atlantic and the Mediterranean Sea, the size, shape and colour of the eggs conformed to the features known for the species *S. canicula* (Collenot 1966 and references therein).

The identification of the capsules was further confirmed after the hatching of the neonate kept in aquarium. The size at hatching of the examined individual (8.2 cm) is in line with the data from the literature (from 8 to 10 cm TL) (Collenot 1966; Serena 2005).

The eggs took 4 months to hatch in aquarium, where they were kept in controlled conditions (pH and temperature of about 14±0.5° C). In the literature, the incubation has been reported to last from 3 to 11 months (Collenot 1966; Ellis and Shackley 1997; Serena 2005). The length of the development from the spawning to the hatching is variable, even among eggs spawned on the same day, because not all the embryos are at the same stage of development at the time of deposition. Unfortunately, it was impossible to rear the young specimen for long time, similarly to previous studies where rearing was quite unsuccessful, although a few specimens survived for c. 8 months (Collenot 1966).

11.5.2 Nursery ground

The Carloforte shoal is characterized by the presence of different benthic assemblage on the southwestern and northwestern slopes. Similar results were obtained for an off-shore rocky bank in Sicily, where black coral colonies were exclusively found on one side of the shoal (Bo et al 2014c). In particular, the western ridge was dominated by a dense meadow of the deep black coral *L. glaberrima* (2.3 ± 0.3 on average, range 0-4.4 col m-2, maximum height 1 m), together with scattered colonies of *A. hirsuta*, *V. flagellum* and dead corallites of *D. cornigera*. In general, the presence of distinct assemblages along the different slopes can been explained by the influence of different hydrodynamic factors on their distribution (Bo et al 2014c).

This study records the first documentation of eggs of *S. canicula* attached to smooth black coral colonies (*L. glaberrima*) in the Mediterranean Sea.

In several areas and depths, capsules of other species of scyliorhinids were found attached to elevated colonies of octocorals, gorgonians, and antipatharians (Etnoyer and Warrenchuk 2007; Concha et al 2010; Flammang et al 2011).

It is well known in literature that oviparous sharks preferentially deposit their eggs in well-ventilated and sheltered locations (Etnoyer and Warrenchuk 2007).

High vertical relief and increased water currents are important aspects of reproductive aggregation sites in *Scyliorhinus retifer* and some deep-sea teleosts and cephalopods. Water circulation may be especially important for providing adequate oxygenation for embryogenesis when egg cases are clumped together in large aggregates (Flammang et al 2007).

Moreover, in order to increase survivorship of the embryos, the eggs are laid in areas that may provide optimum current conditions for egg ventilation. In particular, the flexible organic skeleton of gorgonians and black corals offers a weak resistance to the current, allowing them to live in areas with a higher hydrodynamism (Bo et al 2011b; Bo et al 2014c; Bo et al 2015) and at the same time may potentially create local turbulence conditions among the ramifications (Scinto et al 2009; Buhl-Mortensen et al 2010; Cerrano et al 2010).

Furthermore, considering that embryonic development may last up to 2 years in an egg case without parental input or care, scyliorhinids are particularly vulnerable to interspecific predation (Flammang et al 2007). Therefore, nursery grounds are a strategy by which the vulnerability of individual egg cases can be reduced by being part of an aggregate (Flammang et al 2007).

11.5.3 The unicity of the CS forest

Recently, the presence of dense *L. glaberrima* populations has been described also in Sicily and Malta on rocky bench terraces in elevated positions (Bo et al 2014c; Deidun et al 2014). Though, in Carloforte there is the largest Mediterranean forest of *L. glaberrima*, hosting the oldest living organisms ever described in the Mediterranean Sea (Bo et al 2015). The black coral population has a heterogeneous distribution, with colonies aggregated in several small patches, particularly dense along the bench terraces (with peaks of 8 colonies m^2). The peculiarity of the Sardinian forest is the presence of very big old specimens. In short, the forest is composed of about 2600 colonies, which are up to 2 meter tall. A colony of 4 cm of diameter, analysed with the radiocarbon dating technique revealed an approximately age of 2000 years (Bo et al 2015). Bigger colonies were found up to a maximal basal diameter of nearly 7 cm. Therefore, major proportion of the colonies is hundreds of years old with few millennial specimens. Unfortunately, it was impossible to determine the exact age of the biggest individuals since the correlation among size (both height and diameter) and age is not linear (Bo et al 2015 and references therein). This finding is not new, given that black corals are well known for the longevity (the maximum age determined in the Atlantic for an *Antipathes* sp. colony was 4265 years) (Roark et al 2009).

A second peculiar feature of the CS forest is the population structure. At least four distinct cohorts are present, with very few juveniles and some very big individuals, a condition which is indicative of an overall stable population (Bo et al 2015).

On the contrary in Malta the black coral colonies were smaller (typically 60 to 100 cm high, although some colonies were up to 160 cm high), and less dense (maximum density of 4–5 adult colonies m−2). Moreover, within the *L. glaberrima* coral patches, numerous colonies belonging to the smallest size classes were identified (juveniles) as well as various newly settled colonies (less than 5 cm high) (Deidun et al 2014). The ongoing impact of the fishing activities combined with the known past commercial exploitation of the resource in the area could explain the described population structure, with a reduced presence of the largest size classes in Maltese waters (Deidun et al 2014).

Finally, in Sardinia relevant traces of fishing impact were not found in the *L. glaberrima* forest. Only 2.2 % of total video frames presenting lost gears (exclusively long lines) (Bo et al 2015).

On the contrary, both the Sicilian and Maltese populations were evidently impacted. For instance, the entire area of the Sicilian bank was strongly affected by fishing activities (focused on the red sea-bream *Pagellus bogaraveo*), with long lines trapped on about 29±6% of the colonies of *L. glaberrima* (Bo et al 2014c). Relevant traces of discarded fishing gears were described also in the Maltese site (Deidun et al 2014). The most commonly recorded gear was represented lines used by the fishery for the dolphin fish (*Coryphaena hippurus* Linnaeus, 1758). Moreover numerous dead or extirpated colonies were observed within this study area (Deidun et al 2014).

11.5.4 Urgent conservation measures

In summary, the Carloforte shoal is supposedly a very old, stable, but vulnerable ecosystem.

Firstly, the persistence of this dense old forest is guaranteed by the hardground hosting the black coral population that represents a physical barrier against the mechanical impact acted on the surrounding heavily exploited fishing grounds (Bo et al 2015).

Secondly, the existence of a nursery area for catsharks within the coral ramifications, the high biodiversity of structuring species and the rich associated fauna, along with the occurrence of a meadow of the now rare soft bottom gorgonian *Isidella elongata*, indicates that this ecosystem have to be considered a pristine Mediterranean deep coral ecosystem(Bo et al 2015).

At the same time, the extreme longevity of black corals, the slow growth rates and late maturity age, the limited larval dispersal and population connectivity of the populations, the low recovery ability due to natural fragmentation, the susceptibility of colonies to impact and habitat destruction renders this ecosystem highly vulnerable (Bo et al 2015).

At present, the species *Leiopathes glaberrima* is included in the CITES Convention (Appendix II – strictly controlling the trade) and in the Barcelona Convention (recently moved from Appendix III to Appendix II – list of endangered or threatened species).

However, it is time to move towards more tangible conservation and management actions. The Carloforte shoal meets the criteria for being recognised both as VME (Vulnerable Marine Ecosystem, (FAO 2009), and EBSA (Ecologically or Biologically Significant Area, (Dunn et al 2014). An effective way to protect the species and habitats should be to promptly include them in a deep marine protected area (deep coral sanctuary), where all the harmful gears and fishing practises are prohibited.

11.6 FIGURES

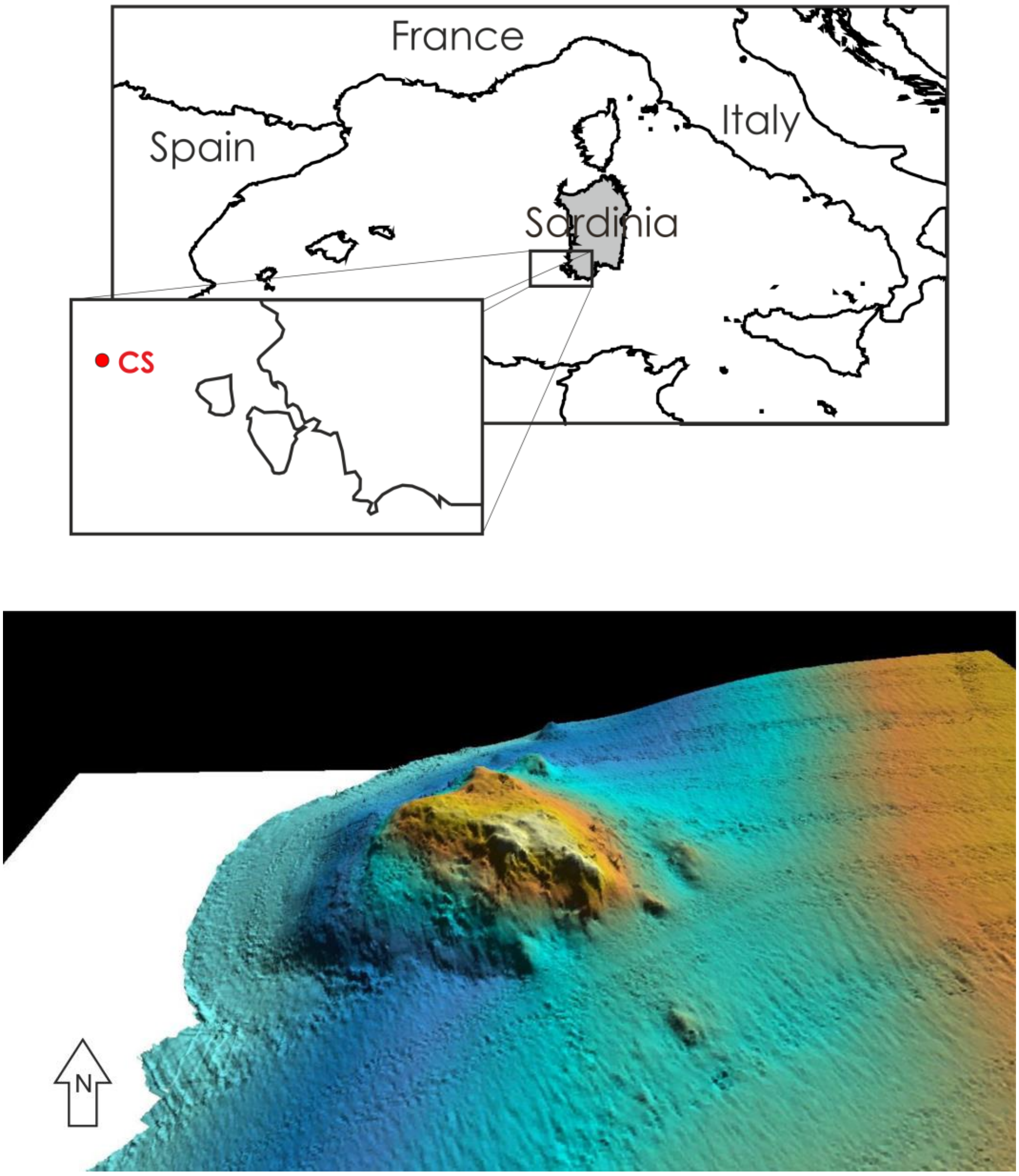

Figure 11.1 Map showing the position of the Carloforte shoal (CS) (upper figure). High-resolution bottom topography: multibeam maps of the studied shoal (lower figure).

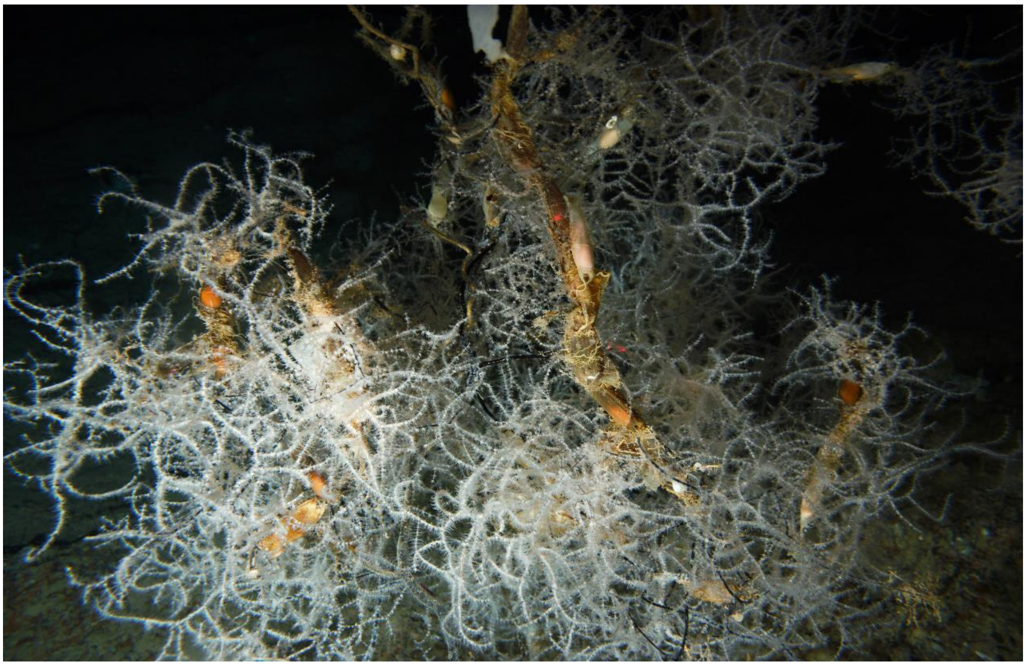

Figure 11.2 Capsules of S. canicula laying on a L. glaberrima colony.

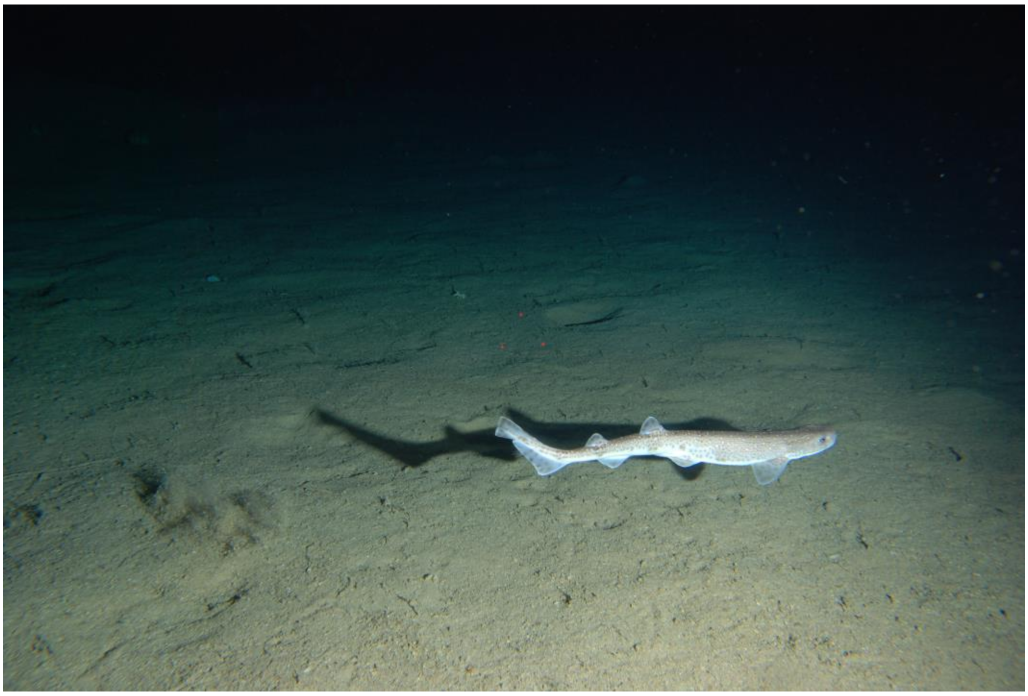

Figure 11.*3 Adult of S. canicula swimming over the muddy bottom around the rocky pinnacle*

Figure 11.4 Egg-case of S. canicula in aquarium

Figure 11.5 Photos of the *neonate* of *S. canicula in aquarium the day of birth: lateral view (upper photo) and close-up (lower photo).*

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Section three – general discussion and final conclusions

12 General discussion

Understanding how biodiversity varies at different spatial scales and the drivers behind these patterns is a crucial issue in ecology (Gambi et al 2014); variability patterns of biodiversity with depth across taxa and habitats suggest that multiple forces drive this distribution (Danovaro et al 2014). Knowledge of ecological processes in the coral forests dwelling in the mesophotic zone of the Mediterranean Sea is becoming increasingly important not only to preserve those habitats and safeguard the communities living there, but also to manage coastal fisheries in a more sustainable way because of the renewed link between such communities and other economically relevant species (Cerrano et al 2015).

Numerous key questions should deserve to be answered in order to build up proper conservations measures. In particular, among others, 1) if and where highly diverse and pristine habitats still exist; 2) what drives the biodiversity pattern in these habitats; 3) what is their importance on the biodiversity and ecosystem functioning of neighbouring areas.

We actually tried to response to some of these questions throughout this thesis.

In chapter 7, investigations conducted on the target species *Corallium rubrum* showed that populations of red coral dwelling in two different locations of the Sardinian continental margin were characterised by contrasting size population structures. Coupling the results of the DTM analysis, which showed the basic differences in the geomorphological setting of the two investigated area, with morphometric analyses of the observed colonies, we showed that the two areas host coral patches characterised by clear differences in the morphometric and populations characteristics.

Although differences at large spatial scales have already been documented in several studies (Bramanti et al 2014) and reference therein), we noticed that investigated populations did not follow the usual assumption that bigger colonies are supposed to be found at greater depths. In fact, bigger and sparse colonies showing the 'forest-like shape' were found in the shallower site while the 'grass-plain shape' was found in the deeper site.

This preliminary result suggests that we have still too localized information to depict general patterns in red coral distribution and population structure using merely water depth as a discriminant. In the two investigated areas, we found possible connections between the levels of geomorphological complexity and the structure of the red coral populations, possibly mediated by the presence of different current regimes.

In chapter 8, we wanted to tested with a multiple regression analysis the assumptions proposed so far from literature of a depth-dependant distribution, collecting also data from published literature on this species. The results showed not only that depth is not the structuring factor of deep dwelling populations of red coral. Our investigation proved that both population shapes (i.e., grass plain and forest like) are present in the deep environment; therefore, we wanted to test if environmental factor such as the geological setting might influence the distribution of these two population structures.

In this regard, our results from the General Linear Model showed how vertically oriented and very large sized colonies are likely to occur more on horizontal topographies such as rocky outcrops or boulders arising from soft substrates, and how dense colonies are more likely to occur on vertical walls such as canyons. In addition, the coverage of sediment along rocky surfaces is, independently from the geological setting, possibly the most negatively affecting factor for red coral settlement and consequently distribution. This particular result, although very basic (silt layer = no settlement), if combined with other evidences regarding the geological setting, could be the missing tile in a complex mosaic with genetic studies indicating very restricted realized larval dispersal (strong genetic structuring) in very small spatial scales (Cannas et al 2011; Costantini et al 2013; Cannas et al 2014) on one hand and, on the other, studies on red coral larval behaviour showing a great potential for dispersion (Martínez-Quintana et al 2014). It is assumed that connectivity drives the spatial structure of species distribution and abundance, in the absence of any other structuring factor (Guizien et al 2014). Present results highlighting the interaction of red coral presence and density with environmental factors could actually be those factors invoked as those overcoming the actual larval dispersal capability of a species. Results presented in chapter 8 give new relevant insight regarding distribution and ecology of this species that could be helpful for revising, or better, improving existing conservation strategies.

Apart from *Corallium rubrum*, we decided to widen the target of the investigation, extending the studies to the deep coral forests. In particular, the most conspicuous components of the animal forest were used as target and reference of our work: gorgonians and black corals.

In chapter 9, ROV surveys were conducted along similar sea bottom topographies (*roche du large*) within a relatively small spatial scale: the southwestern Sardinia continental margin. Results presented in this chapter are generally in accordance with the data available in literature (Bo et al 2012b; Bo et al 2014c; Deidun et al 2014). Indeed, if the number of investigations on deep coral communities is increasing, biodiversity comparisons among different communities within different spatial scales are still very sparse. However, according to other sites from St. Eufemia gulf in Tyrrhenian Sea (central Mediterranean), our results emphasized by means of uni- and multivariate analysis that both total coral abundance and community composition varied significantly among investigated sites within investigated spatial scale. What we actually noticed more is how intrinsic characteristics of these rocky outcrops such as the roughness and slope of the substrate, which indirectly have influence on the accumulation of sediment, generally match differences in species richness and beta diversity (see also CCA analysis, Fig. 9.6).

The investigation proposed in chapter 9 was further extended (chapter 10) along a wider spatial scale (southeast and southwest Sardinia continental margin). We actually present the first available comparison among coral communities from multiple sites within different locations. Through a nested experimental design, results showed how macroscale differences (east sites vs. western sites, >150km distant), and thus contrasting geological setting actually do not influence the animal forests, both quantitatively and qualitatively. Indeed, the 2-level PERMANOVA analysis performed on the total coral abundance, presence/absence and community composition clearly demonstrated that the macroscale variability (among location) is lower than in the mesoscale (among sites). These results partially confirm what previous chapters, both at species and community level, stated: although different communities can be recognizable (see CAP analysis), the variability is mostly due to differences at mesoscale rather than macroscale.

The last chapter 11 documented another example of such microscale diversity, showing the significant differences in composition and densities of coral communities from two sides of the same rocky elevation. Furthermore, the ecological role of a millennial forest of black corals, acting as nursery area for the spotted catshark *Schyliorhinus canicula* was described in detail. In isolated deep coral oases such as the *roche du large* investigated in chapter 11, physical protection against destructive anthropogenic practices like trawling allows hundreds of *L. glaberrima* colonies (some actually millennial) to dwell along the most current exposed surfaces of these rocky outcrops. The preference for such ventilated habitat by *L. glaberrima,* along with morphological features of this species (e.g., 1-2m tall, highly branched colonies) perfectly matches the requirements for elasmobranch egg cases. The presence of a nursery within this pristine, dense, millennial black coral forest, indicate that this particular area should deserve a very high priority status for conservation.

13 Final conclusions

According to chapter 7 and 8, our results emphasize that:

- The current paradigm, considering water depth as the unique discriminant for different populations of *Corallium rubrum,* should be revisited. Other factors possibly affecting red coral distribution and population structure, including geomorphological settings and hydrodynamic regimes should be taken in consideration.
- Further geomorphological studies coupled with biological non-destructive surveys could provide new insights on the biology and ecology of this species but also priceless information for new and more sustainable conservation and management plans for *Corallium rubrum* harvesting.

According to chapters 9 and 10:

- *Roche du large* ecosystems have confirmed to be oases of biodiversity along the continental margin, hosting deep coral forests that enhance the overall biodiversity.
- Geological setting and hydrodynamic seem to have a synergic paramount role controlling the horizontal distribution of mesophotic coral forests, and the necessity to further promote such interdisciplinary investigation is evident.
- The micro and mesoscale variability of beta diversity overcomes the macroscale. In this regard, conservation strategies should be based on direct observation, as the heterogeneity of such features of the

continental shelf does not allow proposing a “substrate-based” conservation plan.

According to chapter 11:

- Investigated *roche du large* ecosystem, because of the geographical position, surrounded by miles of trawled muddy bottoms becomes a *refugia* for deep corals and species that have specialized relations with this environment. In particular it appeared to be a nursery area for the spotted catshark *S. canicula* which, taking advantage of the specialized ecology of the black coral *Leiopathes glaberrima* used this ecosystem as spawning ground. It appears clear the ecological relevance of this particular environment should deserve special protection.

Section four: references and appendix

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APPENDIX: Bibliography of the candidate

13.1.1 Publications on topics pertinent to the present PhD thesis.

New insights into connectivity patterns of mesophotic *C. rubrum* populations

Rita Cannas, Flavio Sacco, **Alessandro Cau**, Elisabetta Coluccia, Maria Cristina Follesa, Angelo Cau

Hydrobiologia, **In Press** DOI: 10.1007/s10750-015-2198-0

Abstract

The present study investigates on mesophotic *Corallium rubrum* populations (>80 m of depth), subject to commercial exploitation in Sardinia (Western Mediterranean Sea). It compares samples from the western and eastern coasts of Sardinia, located in the Algero-Provençal and Tyrrhenian Basin, respectively. The genetic diversity and connectivity patterns among red coral populations are investigated using 12 microsatellite loci. The two basins and almost all locations have been found genetically differentiated (overall F_{ST} = 0.115, P-value = 0). Bayesian clustering analyses have pointed out the occurrence of four distinct genetic clusters. An isolation-by-distance (IBD) pattern has been identified, with significant spatial structuring at the scale of less than 10 km.

The connectivity patterns are discussed in light of the potential role of the complex hydrography that characterizes the zone, one of the most dynamic areas of the entire Mediterranean Sea.

The results of the present study can give important insights in describing genetic diversity and connectivity of mesophotic red coral populations; they represent a very useful baseline information for the current and future management of commercially exploited *C. rubrum* populations in Sardinian waters.

Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy)

Michela Angiolillo, Bianca di Lorenzo, Alessio Farcomeni, Marzia Bo, Giorgio Bavestrello, Giovanni Santangelo, Angelo Cau, Vincenza Mastascusa, **Alessandro Cau**, Flavio Sacco & Simonepietro Canese.

Marine Pollution Bulletin, **In press**

http://dx.doi.org/10.1016/j.marpolbul.2014.12.044

Abstract

Marine debris is a recognized global ecological concern. Little is known about the extent of the problem in the Mediterranean Sea regarding litter distribution and its influence on deep rocky habitats. A quantita- tive assessment of debris present in the deep seafloor (30–300 m depth) was carried out in 26 areas off the coast of three Italian regions in the Tyrrhenian Sea, using a Remotely Operated Vehicle (ROV). The dominant type of debris (89%) was represented by fishing gears, mainly lines, while plastic objects were recorded only occasionally. Abundant quantities of gears were found on rocky banks in Sicily and Camp- ania (0.09–0.12 debris m^{-2}), proving intense fishing activity. Fifty-four percent of the recorded debris directly impacted benthic organisms, primarily gorgonians, followed by black corals and sponges. This work provides a first insight on the impact of marine debris in Mediterranean deep ecosystems and a valuable baseline for future comparisons.

Persistence of pristine deep coral forests in the Mediterranean Sea (SW Sardinia)

M. Bo, G. Bavestrello, M. Angiolillo, L. Calcagnile, S. Canese; R. Cannas, **Alessandro Cau**, M. D'Elia, F. D'Oriano, M. C. Follesa, G. Quarta, A. Cau

Plos One, **In press**

Abstract

Leiopathes glaberrima is a tall arborescent black coral species structuring important facies of the deep hardgrounds of the Mediterranean Sea that are severely stifled by fishing activities. At present, however, no morphological in vivo description, ecological characterization, age dating and evaluation of the possible conservation actions have ever been made for any population of this species in the basin. A dense coral forest was reported during two ROV surveys conducted on a rocky bank off the SW coasts of Sardinia (Western Mediterranean Sea). *L. glaberrima* forms up to 2 m-tall colonies with a maximal observed basal diameter of nearly 7 cm. The radiocarbon dating carried out on a colony from this site with a 4 cm basal diameter revealed an approximately age of 2000 years. Considering the size-frequency distribution of the colonies in the area it is possible to hypothesize the existence of other millennial specimens occupying a supposedly very stable ecosystem. The persistence of this forest is likely guaranteed by the heterogeneous hardground hosting the black coral population that represents a physical barrier against the mechanical impacts acted on the surrounding muddy areas, heavily exploited as trawling fishing grounds. This favorable condition, together

with the existence of a nursery area for catsharks within the coral ramifications and the occurrence of a meadow of the now rare soft bottom gorgonian *Isidella elongata* in small surviving muddy enclaves, indicates that this ecosystem have to be considered a pristine Mediterranean deep coral sanctuary that would deserve special protection.

Genetic monitoring of deep-water exploited banks of the precious Sardinia coral *Corallium rubrum* (L., 1758): useful data for a sustainable management

Rita Cannas, Flavio Sacco, **Alessandro Cau**, Danila Cuccu, Maria Cristina Follesa and Angelo Cau .

Aquatic Conservation: Marine and Freshwater Ecosystems (2014), DOI: 10.1002/aqc.2522 on line first

Abstract

1. The precious coral *Corallium rubrum* is one of the most vulnerable resources in the Mediterranean Sea because of the high levels of human-related and natural mortality rates. The aim of the present study was to document the diversity and connectivity patterns among Sardinian *Corallium rubrum* populations focusing, in particular, on deep populations (>80 m depth) where the commercial exploitation is concentrated.

2. Microsatellite markers revealed high levels of inbreeding, heterozygote deficits, and significant genetic differentiation between and within areas at different depths, with an associated 'isolation by distance' pattern.

3. These results indicated that for precious coral populations in Sardinia the 'deep reef refugia hypothesis', that envisages the capacity for deep corals to act as seed banks for the shallower impaired (over-harvested) populations, is not supported.

4. The highest genetic diversity recorded in Sardinia for all areas and depths with respect to other Mediterranean areas indicates that the strict local management has been effective, since harvesting has not yet led to

a substantial erosion of the genetic pool.

5. Possible causes for the high levels of observed diversity in Sardinia are discussed in relation to hydrological conditions, its geographical position and its proximity to some putative glacial refugia.

6. Thus, given the occurrence in Sardinia of highly diverse and differentiated deep-water populations of *C. rubrum* whose genetic diversity are important for the long-term resilience of the species, the results of the present study strongly support the close monitoring of harvesting, along with the maintenance or even strengthening of the current management and conservation measures (no-take protected areas) already in place in the area.

Deep-water red coral from the island of Sardinia (north-western Mediterranean): a local example of sustainable management

Maria Cristina Follesa, Rita Cannas, **Alessandro Cau**, Claudia Pedoni, Paola Pesci and Angelo Cau.

Marine and Freshwater research, 2014, 64 (8): 706-715
http://dx.doi.org/10.1071/MF12235

Abstract

Since 1979, red coral harvesting in Sardinia has been regulated by regional laws. The regulations, including a minimum take size of 10-mm basal diameter, were imposed to limit and control harvesting. Our study indicated that 75% of the red coral colonies harvested in 2007–08 in the northwestern coast were above the legal minimum size for exploitation. Moreover, extensive surveys with remotely operated vehicles allowed us to document the real structure of wild populations. Large colonies (10mm) represented a big portion of the population (38% of the total), suggesting that harvesting effort did not affect yet the maximum size of colonies. All these results seem to be confirmed by the application of the model of Beverton and Holt. This model shows that the fishery of red coral in Sardinia reaches its maximum yield at an age at first capture of 20 years, an age significantly lower than the average age of 30 years calculated for the colonies actually harvested in the Sardinian coast. Our data confirmed that the measures enacted in the Sardinian regulations provide for an adequate management of this resource and, in addition, can represent a guideline for other countries that still lack a valid management plan.

Leiopathes glaberrima forest from south west Sardinia: a thousand years old nursery area for the small spotted catshark *Scyliorinus canicula*

Alessandro Cau, Maria Cristina Follesa, Marzia Bo, Simonepietro Canese, Andrea Bellodi, Rita Cannas, and Angelo Cau.

Rapp. Comm. int. Mer Médit. 40, 717 (2013)

ABSTRACT

Presence of over 200 spotted catshark Scyliorinus canicula egg capsules associated with long lived black coral *Leiopathes glaberrima* was observed in the South West Coast of Sardinia, describing a thousand years old nursery area for Elasmobranches. The forest, composed by 153 colonies, was discovered during a ROV survey carried out in October 2011 over a rocky elevation of the sea bottom, at depths between 188 and 210 meters. The described zone represents an important component of the ecosystem that deserves special protection.

13.1.2 Other publications on topics not pertinent to the present PhD thesis.

D. Cuccu, M. Mereu, C. Porcu, M.C.Follesa, **Al. CAU**, A. CAU Development of sexual organs and fecundity in *Octopus vulgaris* Cuvier, 1797 from the Sardinian waters (Mediterranean Sea). *Medit. Mar. Sci*., 14/2, 2013, 270-27.

Follesa M.C., Cannas R., **Cau Al**., Cuccu D., Mulas A., Porcu C., Saba S., Cau A. (2014) - Homing and orientation of *Palinurus elephas* (Fabr., 1787) in three no-take areas of the central-western Mediterranean: implications for marine reserve design*. Marine and Freshwater Research*, (in press), DOI:10.1071/MF13079

Angioni Alberto, **Alessandro Cau**, Marco Secci, and Piero Addis Contamination levels of PAHs in marine environment by sea urchin Paracentrotus lividus GCITMS analysis in Sardinia. *Mar. Poll. Bull.* 82 (2014) 201–207.

M. Renzi, **Alessandro Cau**, N. Bianchi and S. E. Focardi, "Levels of Mercury and Polychlorobiphenyls in Bluefin Tuna from the Western Mediterranean Sea: A Food Safety Issue?" *Journal of Environmental Protection*, Vol. 5 No. 2, 2014, pp. 106-113. doi: 10.4236/jep.2014.52014.

Alberto Angioni, **Alessandro Cau**, Piero Addis "Gas-Chromatographic mass spectrometry determination of geosmin and 2-methylisoborneol off-flavor in Mugil cephalus roe". Food Analytical Methods. DOI 10.1007/s12161-014-0025-2

Antonello Mulas, Andrea Bellodi, Rita Cannas, **Alessandro Cau**, Danila Cuccu, Maria Francesca Marongiu, Cristina Porcu & Maria Cristina Follesa. "Diet and feeding behaviour of longnosed skate Dipturus oxyrinchus in Sardinian waters (central-western Mediterranean)". Journal of Fish Biology 01/2015; 86(1): 121-38.

Cristina Porcu*, Maria C. Follesa, Rita Cannas, **Alessandro Cau**, Flavio Sacco, Paola Pesci, Angelo Cau. "Reproductive features of shallow and deep colonies in the Mediterranean gorgonian *Corallium rubrum* (Linnaeus, 1758)". Hydrobiologia (submitted).

Marco Mereu; Blondine Agus; Piero Addis; Serenella Cabiddu; **Alessandro Cau**; Maria C Follesa & Danila Cuccu. "Mark recapture experiment on movement patterns of Octopus vulgaris Cuvier, 1797". Journal of Experimental Marine Biology and Ecology. (Submitted)

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