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# ECOLOGICAL PROCESSES AND STATUS ASSESSMENT IN CORALLIGENOUS HABITATS

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# Abstract

*The coralligenous biogenic reefs are among the richest, the most diverse and threatened habitats in the Mediterranean Sea. These reefs are the result of the interplay between the building activities, mainly sustained by Encrusting Calcareous Rhodophytes (ECRs), and physical and biological eroding processes. In the Mediterranean Sea, dense populations of the gorgonian* Paramuricea clavata *shape animal forests characterising the seascapes of coralligenous habitats. Despite the concerns for its health, the understorey of its forests and the ecological processes they promote are still little known. After the general introduction on the main topics address in this PhD thesis (Chapter 1), in Chapter 2 the abundance and composition of epibenthic assemblages inside and outside* P. clavata *forests were investigated across the central and western Mediterranean Sea, by applying a multifactorial sampling design. According to this study, the gorgonian understoreys share some common features, such as higher abundances of calcareous builder organisms and a reduced invasion by the non-indigenous alga* Caulerpa cylindracea*, compared to the adjacent unforested rocky bottoms. Furthermore,* P. clavata *showed non-linear density-dependent relationships with algal turfs and non-encrusting algae belonging to the genus* Peyssonnelia. *Moreover, by entrapping benthic mucilaginous aggregates with their branches, these gorgonians risk topic necrotic lesions but may reduce the suffocation risks for understorey organisms. Overall,* P. clavata *forests may enhance bioconstruction processes and increase resistance and resilience of the benthic assemblages in the Mediterranean coralligenous habitats.*

*However, Mediterranean gorgonian are threatened by anthropogenic disturbances and mass mortality events, like the one happened at Montecristo Island (Italy) on September 2017. These mortality events have often been linked to anomalies in the temperature profiles of the Mediterranean region. In Chapter 3, by utilising a combination of high-resolution oceanographic analysis, forecast models and citizen science initiatives, an early warning system for the concomitance of heat waves and mortality events can be put in place was proposed. A temperature-based coral disease surveillance tool could then be established for the entire Mediterranean Sea. Such a tool would allow for the timely study of mass mortality phenomena and the implementation of prompt mitigation and/or restoration initiatives.*

*The North Adriatic continental shelf host a large number of mesophotic biogenic reefs, spread on the sedimentary bottom. Little is known on the bioconstructions and bioerosion processes that occur in these biogenic reefs. The study of the microscale processes, driving the dynamic balance leading to the formation of coralligenous banks, requires sophisticated investigation techniques. In Chapter 4, an* in situ *experiment was performed to understand these processes. High-resolution Computed Tomography (CT) has been therefore used to analyse short and long-term (i.e., 3 and 12 years) bioconstruction and bioerosion processes occurring in experimental travertine tiles, used as a proxy of natural substrates, deployed on different typologies of mesophotic biogenic reefs in the northern Adriatic Sea. The most effective borers were sponges from the genus* Cliona *and the bivalve* Rocellaria dubia*, while the most important builders were ECRs, serpulid polychaetes, bryozoans, barnacles, the bivalves* Anomia ephippium*, oysters and vermetids. While builder organisms leave long-lasting calcium carbonate structures, borer organisms leave recognisable traces inside the substrates, allowing computing the eroded and building volume. The mean total eroded volumes varied among sites and between the two*

*exposure times. The mean erosion rate increased with time from the first three-year period to the following nine-year period, while the mean construction rate appeared to decrease. The bioconstruction-bioerosion net balance was positive in the 3-year exposure time, while negative in the 12-year exposure time. Experimental data combined with new technologies allow evaluating the role of the bioerosion and bioconstruction processes, which is a fundamental step towards the conservation of biogenic reefs in the Mediterranean Sea.*

*Citizen Science (CS) projects play a relevant role in environmental education and in increasing people awareness that lie at the basis of nature conservation. As shown above in Chapter 3, the CS could also be a useful warning and monitoring system of the mass mortality events. Furthermore, the growing need to assess the environmental quality status of Mediterranean coralligenous habitats threatened by multiple stressors and to fulfil the goals of the Marine Strategy Framework Directive (MSFD, 2008/56/EC), suggest the opportunity to develop innovative and reliable indices based on CS project that may support decision-makers in planning and applying conservation strategies, especially in Marine Protected Areas. Since 2006, trained scuba diver volunteers are collecting data on the distribution and abundance of 43 easily identifiable selected key marine species along the coasts of the Mediterranean Sea, by applying the Reef Check Mediterranean Underwater Coastal Environment Monitoring (U-CEM) protocol. In Chapter 5, based on U-CEM data and on the sensitivities of the selected species toward the sources of disturbance indicated by the MSFD, the Reef Check Mediterranean Species Sensitivity (MedSens) index is proposed. MedSens index, easily calculated on RCMed open access data in each area and period of interest, provides the mean sensitivity of the surveyed assemblages; helping to assess the environmental quality status and to identify the most likely disturbs acting in the study area. Thanks to MedSens, the marine CS project RCMed can provide a useful tool to support decision makers in adopting marine biodiversity conservation measures.*

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## Contents

# Chapter 1

## General introduction

## 1.1 Mediterranean Sea coralligenous habitats: threats and conservation

The Mediterranean Sea is an oligotrophic temperate sea that harbours almost 10% of world's marine species, and it is considered a marine biodiversity hotspot (Bianchi & Morri 2000, Boudouresque 2004, Ballesteros 2006, Coll et al. 2010). Coralligenous reefs, mesophotic biogenic structures (*sensu* Ballesteros 2006) are among the most important marine benthic subtidal habitats in the Mediterranean Sea because of their high species diversity, structural and functional complexity (see Ballesteros 2006, Ingrosso et al. 2018 and references therein). They are widespread along the Mediterranean Sea rocky coasts and also include outcrops developed on continental shelves, like occur in the silty-sand seabed in the North Adriatic Sea. In this biogenic reef, bioconstruction is mainly sustained by Encrusting Calcareous Rhodophytes (ECRs), growing at low irradiance levels and in relatively calm waters, counterbalanced by the physical and biological eroding processes (Ballesteros 2006, Ingrosso et al. 2018).

Benthic assemblages of the coralligenous reefs have two levels of complexity: a layer coating the substratum made by ECRs, bryozoans, scleractinian corals, serpulid polychaetes and vermetid gastropods, and an upper layer formed by species protruding from the substrate, like gorgonians and sponges (Ballesteros 2006). Coralligenous assemblages are dominated by long-lived, slow-growing and low-recruitment species with overall low population dynamics (Ballesteros 2006, Calvo et al. 2011, Teixido et al. 2011).

Coralligenous habitats provide ecosystem services (e.g. food provision and nutrient cycling; Micheli et al. 2013). Among them, coralligenous habitats play an essential role in the carbon cycle, thanks to the carbon sequestration by calcareous organisms.

However, coralligenous assemblages are highly threatened by anthropogenic disturbances (Claudet & Fraschetti 2010, Coll et al. 2010, Coll et al. 2012, Bevilacqua et al. 2018) such as nutrient enrichment (e.g. Gennaro & Piazzi 2011, Piazzi et al. 2018), increases in water turbidity and sedimentation rates (e.g. Balata et al. 2005, Mateos-Molina et al. 2015), invasion from non-indigenous species (e.g. Piazzi & Balata 2008, de Caralt & Cebrian 2013), mechanical damages by anchoring, fishing nets and divers frequentation (e.g. Bavestrello et al. 1997, Hinz 2017), and by global climate change-related disturbances like exceptional storms (Teixido et al. 2013), acidification and thermal anomalies (Martin & Gattuso 2009, Zunino et al. 2017). Thermal anomalies, which may induce physiological stress and increase the susceptibility to pathogens, coupled with the reduced food availability linked to the stratification of the water column in summers, seem to lie at the basis of sessile benthic communities mass mortality events (mainly sponges, cnidarians, bivalves, ascidians and bryozoans) recorded in recent decades in the north-western Mediterranean Sea (e.g. Cerrano et al. 2000, Garrabou et al. 2009, Calvo et al. 2011, Crisci et al. 2011, Turicchia et al. 2018).

Besides, the mass mortality events, acting from local to regional scale, lead to substantial change in the structure and functioning of coastal marine ecosystems (e.g. Lejeusne et al. 2010, Calvo et al. 2011).

The rising concern about the increase of anthropogenic pressures and their consequences on the European coasts (Airoldi & Beck 2007) has induced the European Union to propose and develop new conservation and recovering strategies. Since 2000, three are the directives embraced by the European Union concerning the coastal marine environments: the Water Framework Directive (WFD), the Marine Strategy Framework Directive (MSFD), and the Maritime Spatial Planning Directive (MSPD), which are oriented to assess the water quality, the good environmental status and the sustainable use plans, respectively. In 2008 the Barcelona Convention's Action plan was adopted for the conservation of coralligenous outcrops and other calcareous bio-concretions in the Mediterranean Sea. Although not legally binding, it asserts that 'coralligenous/maërl assemblages should be granted legal protection at the same level as *Posidonia oceanica* meadows' (UNEP-MAP-RAC/SPA 2008, updated in 2016).

## 1.2 Gorgonian forests

In the Mediterranean Sea, dense populations of gorgonians shape marine animal forests (*sensu* Rossi et al. 2017), characterising the seascapes of coralligenous habitats and acting as ecosystem engineers (Cerrano et al. 2010). By increasing the complexity of the environment, gorgonian forests modify the currents flow and the sedimentation rate and provide shelters for benthic and nekton organism (Valisano et al. 2016). However, their integrity is highly threatened by fishing lines and nets, anchorages and recreational divers causing mechanical damage (Bavestrello et al. 1997, Linares & Doak 2010, Tsounis et al. 2012), suffocation by mucilaginous benthic aggregates (Mistri & Ceccherelli 1996a, Giuliani et al. 2005), invasion from non-indigenous species (Cebrian et al. 2012), and increase in water turbidity and sedimentation rates due to run-off as a result of bad land management (Mateos-Molina et al. 2015). Moreover, gorgonian forests are threatened by global climate change-related disturbances such as increased frequency of exceptional storms (Teixido et al. 2013) and thermal anomalies (Cerrano & Bavestrello 2008, Linares et al. 2008a) that may induce physiological stress and increase their susceptibility to pathogens (Calvo et al. 2011, Vezzulli et al. 2013, Rivetti et al. 2014). The latter, coupled with reduced food availability due to the stratification of the water column in summer, seem to lie at the basis of the gorgonian mass mortality events recorded in recent decades in the north-western Mediterranean Sea (Cerrano et al. 2000, Martin et al. 2002, Linares et al. 2005, Garrabou et al. 2009, Calvo et al. 2011, Crisci et al. 2011, Huete-Stauffer et al. 2011, Rivetti et al. 2014, Marbà et al. 2015). The crises have not spared even the most remote gorgonian forests, far from direct anthropic disturbances (Turicchia et al. 2018).

The red gorgonian, *Paramuricea clavata*, the yellow gorgonian *Eunicella cavolini*, and the white gorgonian *Eunicella singularis* are characterised by low population dynamics (i.e. slow growth, late maturity and low fecundity) that in the face of increased disturbances could lead to low recovery capacity  (Coma et al. 1998, Linares et al. 2005, Linares et al. 2007). The gorgonians disappearance or rarefaction raise concern regarding the reducing of spatial complexity and biodiversity of coralligenous habitats and their benthic assemblages, with important ecological and economic consequences (Linares et al. 2005, Cerrano & Bavestrello 2008, Linares & Doak 2010). Currently, many gorgonian forests, mainly *P. clavata* forests, are fragmented in patches and are considered to be in a state of strong regression. Their loss can lead to a decrease of both benthic and vagile fauna associated and a shift toward a system dominated by filamentous algae (Cerrano & Bavestrello 2008, Ponti et al. 2014, Ponti et al. 2016, Ponti et al. 2018). Concerns for gorgonian forests loss and for the related consequences (e.g. impairment of fertilization and larval connectivity, shifts in the structure of benthic assemblages and of the related ecosystem functioning; Santangelo et al. 2015) are rising.

## 1.3 Biocostruction and bioerosion processes in coralligenous habitats

The growth of coralligenous bioconstructions depends on a positive balance between building and bioeroding processes (e.g. Garrabou & Ballesteros 2000). ECRs of the genera *Mesophyllum*, *Lithophyllum* and *Neogoniolithon* are the main contributors to coralligenous accretion (Ballesteros 2006) together with several species of the genus *Peyssonnelia* (e.g. *Peyssonnelia rosa-marina*). Also no-photosyntethetic organisms participate in the building process in coralligenous habitats such as cnidarians, bryozoans, molluscs and serpulid polychaetes, although with a minor role. The main bioeroders, which erode or dissolve coralligenous biogenic reefs in the Mediterranean Sea, belong to five main groups: microendoliths (e.g. *Hyella caespitosa*, *Mastigocoleus testarum*, *Leptolyngbya terebrans*), boring sponges (e.g. *Cliona* spp.), molluscs (e.g. *Hiatella arctica*, *Rocellaria dubia* and *Lithophaga lithophaga*), sipunculid polychaetes (e.g. *Aspidosiphon muelleri*) and grazing sea urchins (e.g. *Sphaerechinus granularis*) (Ballesteros 2006).

Although bioconstruction and bioerosion research are mainly concentrating on tropical coral reefs, the Mediterranean studies are growing up (e.g. Asnaghi et al. 2015, Farber et al. 2015, Casoli et al. 2016, Farber et al. 2016, Titschack et al. 2016). On overall, the calcium carbonate ($CaCO_3$) sequestration in the Mediterranean Sea ranges from 170 g $m^2$ $yr^{-1}$ to 660 g $m^2$ $yr^{-1}$, while the $CaCO_3$ loss by erosion range from 220 g $m^2$ $yr^{-1}$ in relative shallow waters to 20 g $m^2$ $yr^{-1}$ at a 60 m depth (Sartoretto et al. 1996, Ballesteros 2006). However, the study of bioconstruction and bioerosion processes requires sophisticated investigation techniques. Imaging 3-dimensional (Computed Tomography, CT) analyses have been recently applied to investigate the accretion and bioerosion from tropical to temperate biogenic reefs (e.g. Farber et al. 2016, Silbiger et al. 2016), but accretion and bioerosion rates remain challenging to estimate. Understanding these processes is a major step towards the conservation of biogenic habitats in the Mediterranean Sea.

## 1.4 Marine citizen science for the biodiversity conservation of coralligenous habitats

Marine Citizen Science (MCS) is the involvement of amateur (or not professional) scientists in scientific research and participatory monitoring related to marine environments. MCS has grown up in the last decades, and it has become more critical in conservation science (Bonney et al. 2009, Silvertown 2009). The growth factors are primarily the increasing understanding that volunteers are a free source of skills, labour-force and computational power and secondly the existence of informatics tools that can spread the information about projects easily and gathering data from the participants (Silvertown 2009). However, there is scepticism about the reliability of the data collected by the volunteers since they are often lack of experience and knowledge. Notably, the data generated by volunteers' surveys could contain high levels of bias (Ratnieks et al. 2016). The differences in skills among the volunteers would lead to decreased accuracy in measurements and misidentification of species. Actually, they are potentially a great scientific resource and not a means to acquire high-quality data cheaply. The science has neither the human nor the financial resources and the time to cope with the demands that biodiversity conservation requires. The volunteers then become a large workforce and could contribute to applied research through their participation in monitoring programs in which they are lead by experience scientists. So the citizens could help scientists to collect broad-scale data thereby bridging the funds and time lack (Mumby et al. 1995, Foster-Smith & Evans 2003, Dickinson et al. 2010, Zoellick et al. 2012, Bird et al. 2013, Holt et al. 2013, Tulloch et al. 2013).

MCS should be an essential contribution to scientific research not only for the data collections but also above all for the translation of science into policy and action laying the basis for participatory government. Citizen science projects can enhance the ability of decision-makers, stakeholders and no-government organisations to monitor, manage and protect natural resources, while citizen volunteers are increasingly involved in local issues and more awareness in environmental threats and careful about their everyday actions toward the environment (Conrad & Hilchey 2011, Alaback 2012, Tulloch et al. 2013).

Citizen science provides a large amount of data about species occurrence and distribution around the world and over long spans of time. Several projects have used these data for descriptive statistics, developing indices and, on overall, for advancing scientific knowledge.

Over the past three decades, the growing of scuba diving activities has encouraged the broad involvement of recreational divers for marine monitoring. Two broadly successful citizen science programs are those developed by Reef Check Foundation (www.reefcheck.org; Hodgson 1999, Hodgson 2001, Hodgson et al. 2006), based in California and with several national agencies around the World, and by Coral Watch no-profit organisation (www.coralwatch.org), based in Australia. The aim of both is to integrate global reef monitoring with participants education. Coral Watch has recruited volunteers from more than 60 countries, and its methodology has been applied in several published scientific papers (Hoegh-Guldberg 1999, Fabricius 2006, Siebeck et al. 2006, Leiper et al. 2009, Fabricius et al. 2011, Marshall et al. 2012). So far, Reef Check monitoring activities have provided 11123 surveys in more than 4500 reefs and 95 countries (http://data.reefcheck.us/; last accessed 11/10/2018) and contributed with the data collected by volunteers to increase knowledge on coral reef status (Hodgson 1999, Risk 1999, Wilkinson & Hodgson 1999, Shuman et al. 2005, Uychiaoco et al. 2005, Bruno & Selig 2007, Carpenter et al. 2008, Bruno et al. 2009, Eakin et al. 2010, Selig & Bruno 2010, Micheli et al. 2012, Marks et al. 2015, Johnson et al. 2016, Roelfsema et al. 2016, Done et al. 2017). Today both, Reef Check and Coral Watch are listed among the major monitoring programs for the tropical coral reef status assessment (Hill & Wilkinson 2004).

European examples of the involvement of volunteers in marine monitoring projects include the project NELOS (www.nelos.be) in Belgium and The Netherlands, Seasearch (www.seasearch.co.uk) in the United Kingdom, and the protocols proposed by Reef Check Mediterranean Sea (RCMed, www.reefcheckmed.org). Since 2006, the Citizen Science project RCMed involves volunteer divers to collect data underwater in the Mediterranean Sea, through the Underwater Coastal Environment Monitoring protocol (U-CEM protocol; Cerrano et al. 2017b). The RCMed mission is to collect data useful for scientific literature and decision-makers in marine conservation policies, while promoting environmental education (Cerrano et al. 2017b). The RCMed volunteers have provided a considerable amount of data on presence and abundance of selected key species along the Italian coasts (almost 43'000 observations; last accessed 11/10/2018) and the project is rapidly spreading through the Mediterranean Sea. The data collected by RCMed volunteers are freely available and have already been used in a number of studies mainly to investigate species patterns of abundance and distribution, and the role of MCS as a valuable environmental monitoring tool (Ponti et al. 2011b, Ponti et al. 2011c, Markantonatou et al. 2013, Turicchia et al. 2013, Pairaud et al. 2014, Giusti et al. 2015, Montefalcone et al. 2015, Özalp & Ateş 2015, Turicchia et al. 2015, Ozalp & Alparslan 2016, Turicchia et al. 2016, Cerrano et al. 2017a, Cerrano et al. 2017b, de Francesco et al. 2017, Di Camillo et al. 2018, Ponti et al. 2018, Turicchia et al. 2018, Ponti et al. 2019). Knowledge of species' distribution, abundance and absence at regional scale (i.e. Mediterranean Sea) is one of the major priority in the conservation of biodiversity, as it sets the baseline for the design and implementation of monitoring and intervention plans and conservation strategies.

## 1.5 Objectives and thesis structure

Today's marine environment is experiencing with multiple anthropogenic disturbances and stressors, such as wastewater discharge, pollution, trawling, the introduction of microbial pathogens and non-indigenous species. At the same time, the climate is changing due to anthropogenic emissions of greenhouse gasses that have consequences for the marine environment. Among them, the water temperature could become warmer on average, episodic extreme events such as heat waves and storms could become more frequent and severe. Marine organisms, which are adapted to different conditions, may not be able to resist these environmental changes (Gibelin & Déqué 2003, Beniston et al. 2007, Occhipinti-Ambrogi 2007, Somot et al. 2008, Coma et al. 2009).

Healthy coralligenous habitats may support ecological processes and high species diversity. However, the lack of knowledge on the ecological processes occurring in coralligenous habitats and the extents of the ecological roles of the main key species (e.g. gorgonians forests) that inhabit them are hindering the assessment of the impact associated. Shedding light on these processes is of paramount importance for the conservation of marine habitats.

This thesis is aimed to acquire baseline knowledge to develop effective conservation measures on coralligenous habitats and their habitat-forming species using different approaches. Indeed, the four studies composing this thesis focus on ecological processes and the assessment of the state of coralligenous reefs in the Mediterranean Sea from a conservation perspective. The scales investigated vary in space and time, from the regional scale, i.e. the Mediterranean Sea, to sub-basin, i.e. the North Adriatic Sea, and from decadal to annual processes. The approaches varying from field experiments, to Citizen Science approaches and to new technologies like the Computed Tomography.

In Chapter 2, we have investigated the spatial patterns of epibenthic assemblages inhabiting the understorey of Mediterranean *P. clavata* forest across the Mediterranean Sea. This was crucial to shed light on the conservation needs of this habitat-forming species and to preserve the biodiversity of coralligenous habitats. This chapter was published in Aquatic Conservation journal (Ponti et al. 2018).

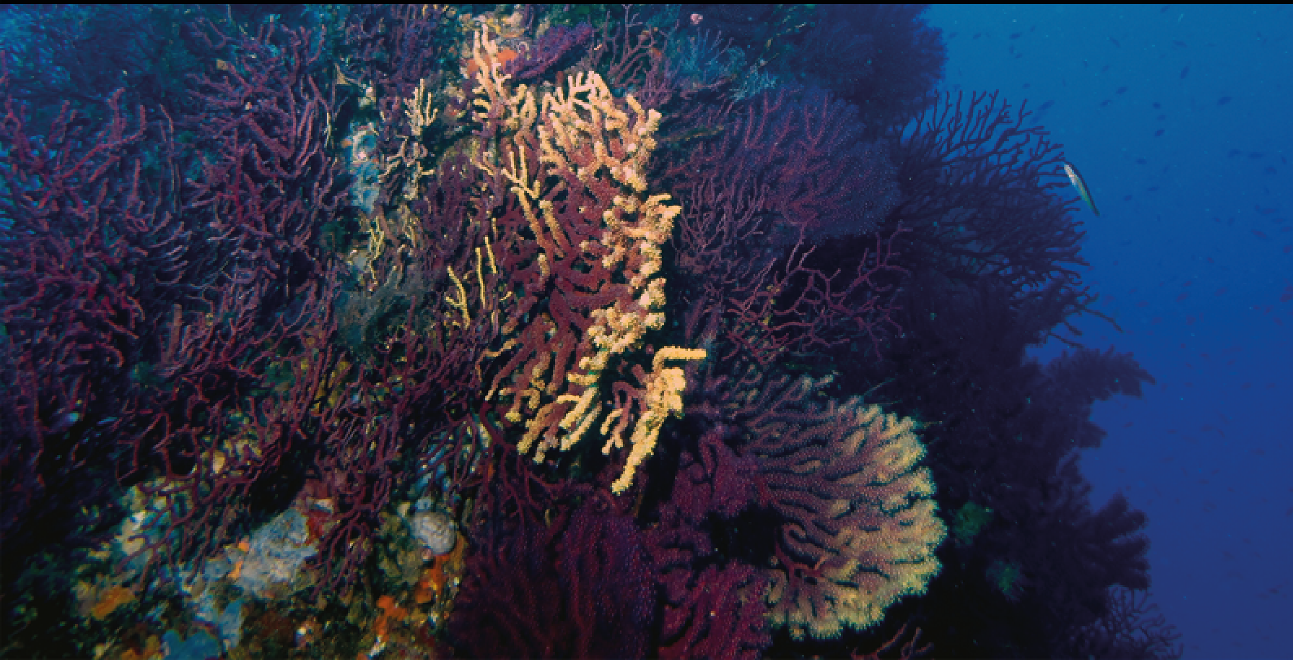
In Chapter 3, analysing a gorgonian mortality event, we propose to develop an early warning system for the concomitance of heat waves and mass mortality events of gorgonian forests by utilising a combination of high-resolution oceanographic analysis, forecast models and citizen science initiatives. This tool could be crucial for predicting change in coralligenous habitats and implementing conservation measures. This chapter was published in Disease of Aquatic Organisms journal (Turicchia et al. 2018).

In Chapter 4, we have investigated the bioconstruction and bioerosion processes occurring in northern Adriatic coralligenous reefs, by means of a field experiment started in 2005 employing travertine tiles and using high-resolution computed tomography to analyse volumes built and eroded by different species. Understanding these processes is a major step towards the conservation of biogenic habitats in the Mediterranean Sea. Indeed, the persistence of coralligenous habitats depends on the interplay between bioconstruction and bioeroding processes.

The growing need to assess the environmental quality status of Mediterranean coralligenous habitats threatened by multiple stressors, as requested by the Marine Strategy Framework Directive (MSFD, 2008/56/EC), suggest the opportunity to develop innovative and reliable indices based on Marine Citizen Science project that may support decision-makers in planning and applying conservation strategies. In Chapter 5, we propose the RCMed Sens indices, which represent the first attempt to derive biotic indices from data collected by volunteers (i.e. Reef Check Mediterranean Sea volunteers) in the Mediterranean Sea.

# Chapter 2

## The understorey of gorgonian forests in mesophotic temperate reefs



## 2.1 | Introduction

In many marine habitats erect organisms may form dense populations resembling terrestrial forests. They include canopy-forming algae (e.g., kelps, fucoids), but also branched sessile invertebrates (e.g., sponges, cnidarians, bryozoans) able to form dense mono- or pluri-specific "animal forests" (Rossi et al. 2017). Forests forming species directly or indirectly affect availability of abiotic and biotic resources by structural changes and therefore may be considered ecosystem engineers (Jones et al. 1994; reviewed in Romero et al. 2015). Terrestrial forest control the understorey by providing favourable microclimates, with less fluctuations of environmental variables (e.g. humidity, temperature, organic matter, nutrient, shading) compare to habitats outside tree assemblages, and by driving interactions among animals and plants (Wright 2002). In the marine environment, kelp forests and the multitude of species interactions that they support may control ecosystem structure and functioning, mitigating environmental stress and enhancing resistance towards habitat loss or fragmentation (e.g., Steneck et al. 2002, Bennett et al. 2015). In kelp forests, sedimentation and accumulation of detritus (Duggins & Eckman 1994, Airoldi 2003) are considered key features in structuring benthic assemblages. Moreover, shading effects (Clark et al. 2004, Arkema et al. 2009) and depth gradient (Graham 2004, Rodgers & Shears 2016) control their food webs, driven by the primary production provided by kelps and their epiphytes.

Ecological processes in terrestrial and kelp forests were largely investigated, while little is known about marine animal forests. Marine animal forests are made by dense assemblages of benthic suspension-feeders, filtering large quantities of seston and substantially contributing to the benthic-pelagic coupling (Gili & Coma 1998, Gili et al. 2001). They include sponge gardens (Marliave et al. 2009), mono-specific forests of hydroids (Rossi et al. 2012, Di Camillo et al. 2013), black corals (e.g., Bo et al. 2014, Ingrassia et al. 2016), mesophotic zoantharians (e.g., gold coral) and gorgonians (Cerrano et al. 2010, Cartes et al. 2013). One of the most peculiar elements characterising Mediterranean coralligenous habitats (mesophotic biogenic reefs, sensu Ballesteros 2006) are the forests of the gorgonian *Paramuricea clavata* (Risso 1826) (Musard et al. 2014). *P. clavata* is a long-lived, slow-growing species (Linares et al. 2007). Colonies, irregularly branched and purple/yellow coloured, may be more than 1.5 m height and 100 years old (Linares et al. 2007). Pristine forests may reach densities higher than 50 colonies m$^{-2}$ and dry masses greater than 1,700 g m$^{-2}$ (Coma et al. 1998, Linares et al. 2008a). Biology and ecology of this species have been largely studied: geographic and bathymetric distributions (Gori et al. 2011, Kipson et al. 2015, Boavida et al. 2016, Roelfsema et al. 2016, Di Camillo et al. 2018; see also Supplementary material S1, Fig. S1.1 and Fig. S1.2), reproduction, growth, secondary production and survivorship (Mistri & Ceccherelli 1994, Coma et al. 1998, Linares et al. 2007, Cupido et al. 2008, Linares et al. 2008a, Fava et al. 2010, Cupido et al. 2012, Santangelo et al. 2015), feeding ecology (Coma et al. 1994, Ribes et al. 1999, Cocito et al. 2013), respiration rate (Coma et al. 2002, Previati et al. 2010), and population genetic structure (Mokhtar-Jamai et al. 2011, Perez-Portela et al. 2016, Pilczynska et al. 2016).

Integrity of *P. clavata* forests is threatened by mechanical damages caused by fishing lines and nets, anchorages and recreational divers (Bavestrello et al. 1997, Linares & Doak 2010, Tsounis et al. 2012, Markantonatou et al. 2016), suffocation by mucilaginous benthic aggregates (Mistri & Ceccherelli 1996a, Giuliani et al. 2005), invasion from non-indigenous species (Cebrian et al. 2012), and increase in water turbidity and sedimentation rate due to bad land management (Mateos-Molina et al. 2015). Moreover, gorgonian forests are threatened by global climate change-related disturbances such as increased frequency of exceptional storms (Teixido et al. 2013) and thermal anomalies (Cerrano & Bavestrello 2008, Linares et al. 2008a) that may induce physiological stress and increase the susceptibility to pathogens (Calvo et al. 2011, Vezzulli et al. 2013, Rivetti et al. 2014). The latter, coupled with reduced food availability due to the stratification of the water column in summer, seem to lie at the basis of the gorgonian mass mortality events recorded in recent decades in the north-western Mediterranean Sea (Cerrano et al. 2000, Martin et al. 2002, Linares et al. 2005, Garrabou et al. 2009, Calvo et al. 2011, Crisci et al. 2011, Huete-Stauffer et al. 2011).

Currently, *P. clavata* forests are often fragmented in patches and considered in strong regression due to their low resilience after disturbances (Cerrano et al. 2005, Linares et al. 2005, Cupido et al. 2009). Concerns for gorgonian forests, and for the consequences of their loss on the associated assemblages and on the ecological processes occurring in the understorey is rising, even if little is known on the structure, diversity and abundances of the benthic assemblages associated to *P. clavata* forest. Manipulative experiments highlighted that *P. clavata* forests significantly affect the early-stage recruitment of sessile epibenthic assemblages, although effects may vary across sites exposed to different environmental conditions (Ponti et al. 2014).

The aims of the present study were to explore spatial patterns of epibenthic assemblages inhabiting the understorey of Mediterranean *P. clavata* forests, compared to adjacent not forested rocky areas, and to shed light on the conservation needs of this habitat-forming species to preserve biodiversity of coralligenous habitats.

## 2.2 | Materials and methods

### 2.2.1 Sampling design and survey method

Epibenthic sessile assemblages inside and outside *Paramuricea clavata* forests were investigated by applying a multifactorial sampling design. Nine sites, in the Ligurian Sea, Tyrrhenian Sea and Adriatic Sea were randomly selected among those reported in the Reef Check database (Cerrano et al. 2017b; see Supplementary material S1, Fig. S1.1) (Fig. 1).

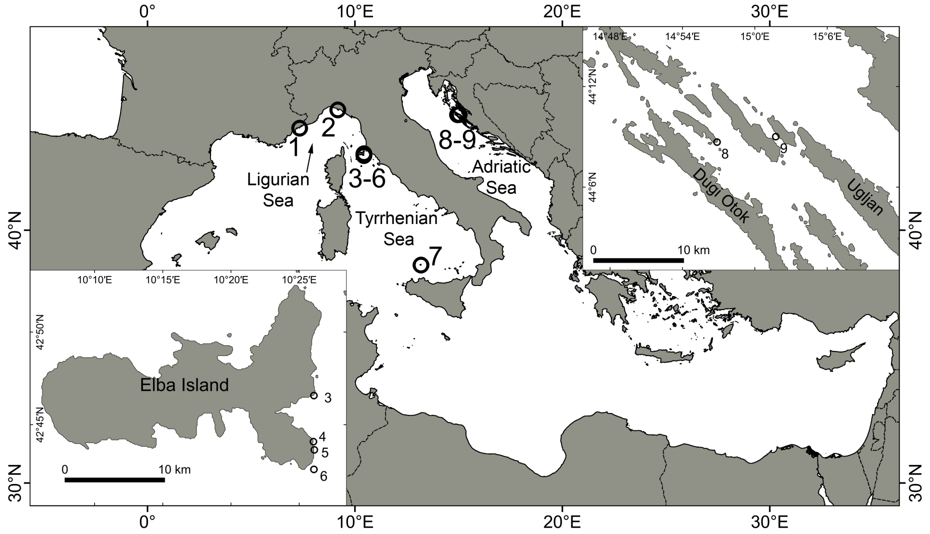


Figure 1. Study area and study sites (circles): 1, P.te Causinière; 2, Colombara; 3, Punta delle Cannelle; 4, Capo Calvo; 5, Picchi di Pablo; 6, Scoglio del Remaiolo; 7, Punta San Paolo; 8, Zverinac Juzni Rt; 9, Rivanjski Kanal (Mercator Projection, Datum WGS 84).

At each site 4 areas inside and 4 outside *P. clavata* forests, with similar orientation, inclination and depth (± 3 m), were selected. Four photographic samples (21 × 28 cm) were collected within each area (Fig. 2a) using either a Canon PowerShot G12 or a Canon PowerShot G15 digital cameras (10 to 12 Mpixel, respectively) equipped with aluminium underwater case, S-TTL strobe (Inon D-2000) and steel frame. Inside gorgonian forests height and percent cover of *P. clavata* colonies were measured in the field and by photographic samples (50 × 50 cm), respectively (Fig. 2b).

Percent cover of sessile organisms was quantified by superimposing a grid of 400 equal-sized squares, using the software PhotoQuad (Trygonis & Sini 2012). Percent cover was related to the total readable area of each image, obtained subtracting dark and blurred zones or portions covered by motile organisms as in Ponti et al. 2011a). Organisms were identified to the lowest possible taxonomic level, and grouped into morpho-functional groups (modified from Garrabou et al. 2002, Teixido et al. 2011, Casas-Guell et al. 2015). All calcifying taxa were also included in the builder group.

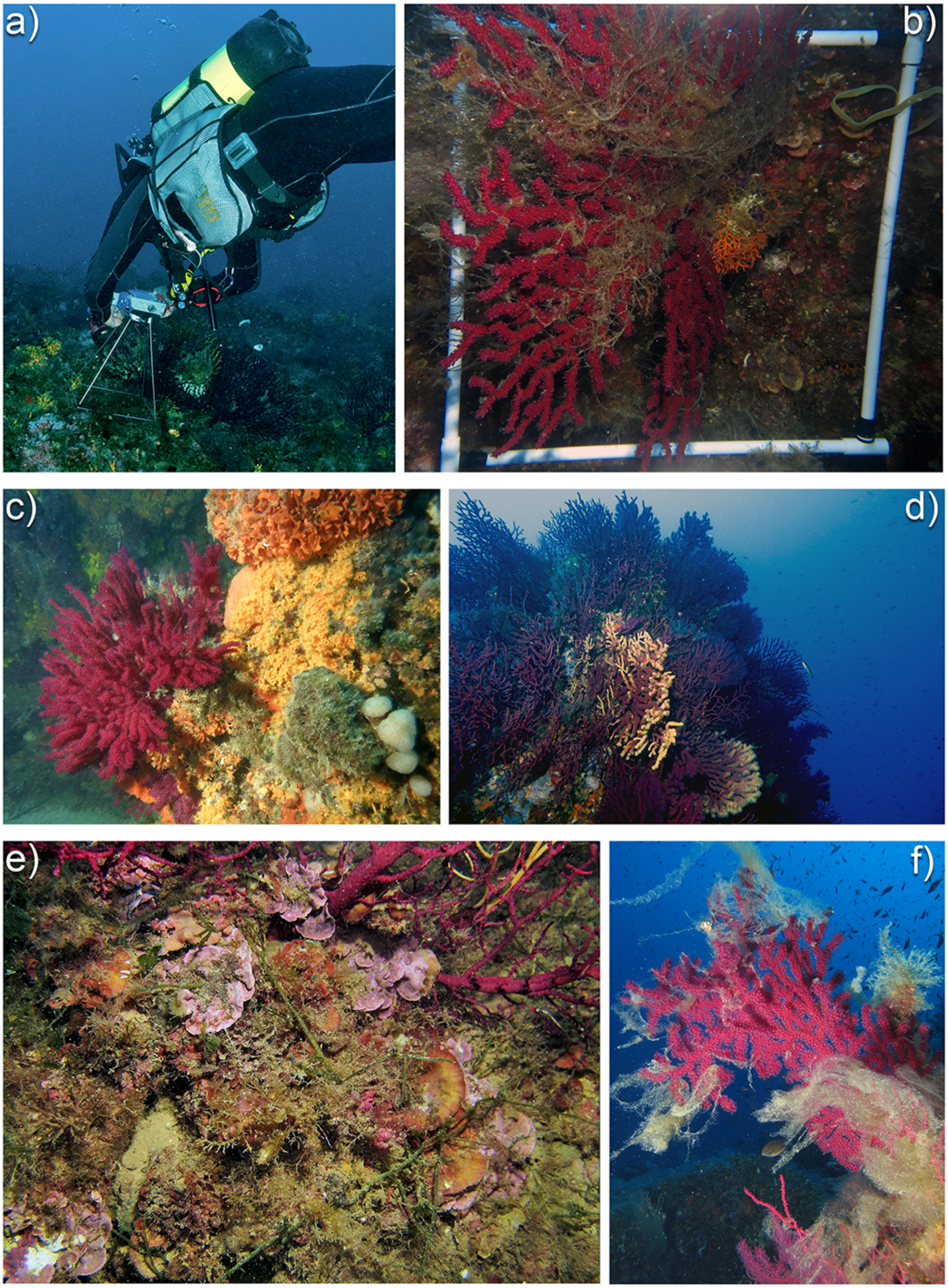


Figure 2. Photographic sampling method (a); a sampled area inside a gorgonian forest at Punta San Paolo, Ustica Island (b); benthic assemblages at Rivanjski Kanal, northern Adriatic (c); a dense gorgonian forest in the Tyrrhenian Sea (d); some thalli of *Caulerpa cylindracea* at the basis of *Paramuricea clavata* at Punta delle Cannelle, Elba Island (e); mucilaginous aggregates entrapped by gorgonian branches (f).

### 2.2.2 Data analysis

Differences in height and percent cover of *Paramuricea clavata* forests among sites were assessed by one-way Analysis of Variance (ANOVA, $\alpha = 0.05$; Underwood 1997).
Benthic assemblage similarities were calculated for each pair of samples using the Bray-Curtis coefficient applied to square root-transformed percent cover data and excluding *P. clavata* from the analysis (Clarke 1993). Similarity patterns of benthic assemblage inside and outside gorgonian forest and among sites were represented by both unconstrained ordination plot, using the Principal Coordinate Analysis (PCoA; Gower 1966), and constrained ordination plot, using the Canonical Analysis of Principal coordinates (CAP; Anderson & Willis 2003), calculated on the centroids of similarities among replicates from the same sampling area. CAP detects the axis that best discriminate between the two *a priori* groups, i.e. benthic assemblages inside and outside gorgonian forest. Differences in assemblage structures inside and outside gorgonian forest (Go: 2 levels, fixed), among sites (Si: 9 levels, random), in the interaction (Go × Si), and among areas within this interaction (Area(Go × Si)) were assessed by permutational multivariate analysis of variance (PERMANOVA, $\alpha = 0.05$; Anderson & ter Braak 2003). When less than 999 unique values in the permutation distributions were available, asymptotical Monte Carlo *p*-values ($p_{MC}$) were used instead of permutational *p*-values.
Species richness (number of species, *S*), Shannon's species diversity (*H'*, $\log_2$) and the corresponding Pielou's evenness component (*J'*) were calculated for each sample (Magurran 2004).
Differences in single species percent cover, morpho-functional groups percent cover and species diversity indices were assessed by mixed multifactorial ANOVA ($\alpha = 0.05$) following the same design adopted for PERMANOVA. Cochran's C test was used to check the assumption of homogeneity of variances and, when necessary, data transformations were applied. If variances were slightly heterogeneous even after transformations (Cochran's C test $p < 0.05$), the analyses were run at $\alpha = 0.01$ for significance test, while when variances were high heterogeneous (Cochran's C test $p < 0.01$) only not significant results were retained (Underwood 1997). In the ANOVA, when the term Go × Si was not significant ($p > 0.25$) it was pooled with the term Area(Go × Si) (Underwood 1997). When the term Go × Si was significant, the Student–Newman–Keuls (SNK) method was used for the *post-hoc* comparisons.
Possible relations between the percent cover of *P. clavata* and those of other taxa were explored by local polynomial regression fitting (LOESS), which is a smoothing method that summarizes the association between variables by fitting a multitude of regression models to adjacent subsets of the data (Cleveland & Devlin 1988). For this purpose, percent cover data inside gorgonian forests were averaged by area (i.e., 4 areas per site).

Multivariate analyses were performed using PRIMER 6 with PERMANOVA+ add-on package (Anderson et al. 2008). LOESS and univariate analyses were made in R (R Core Team 2017) with GAD, a specific package for General ANOVA Designs (Sandrini-Neto & Camargo 2014). Mean values were always reported along with their standard errors (s.e.).

## 2.3 | Results

### 2.3.1 The investigated gorgonian forests

The study sites were located in a wide geographical area, which spans 5.6° in latitude and 8.2° in longitude, from the north-western Mediterranean Sea (P.te Causinière at Cap Ferrat, Villefranche-sur-Mer and Colombara at Portofino, in the Ligurian Sea, Punta delle Cannelle, Capo Calvo, Picchi di Pablo and Scoglio del Remaiolo at Elba Island in the central Tyrrhenian Sea) to the central Mediterranean (Punta San Paolo at Ustica Island) and to the northern Adriatic Sea (Zverinac Južni Rt and Rivanjski Kanal in Croatia). At study sites, gorgonian forests occur at different depths, with upper limits from 22 to 40 m, either on vertical or horizontal substrates, with different orientations. Geological and environmental features at each site were reported in the Supplementary materials (S2, Fig. S2.1, Table S2.1 and S2.2).

Gorgonian forests significantly differed among study sites both in terms of colony height ($F_{8,27} = 6.38$, $p = 0.0001$) and percent cover ($F_{8,27} = 2.48$, $p = 0.037$). The mean gorgonian height ranges from 25.4 cm (± 4.3) to 122.1 cm (± 22.6); while their mean percent cover ranges from 22.3 % (± 5.1) to 56.8 % (± 7.4; Fig. 2c,d; Fig. 3).

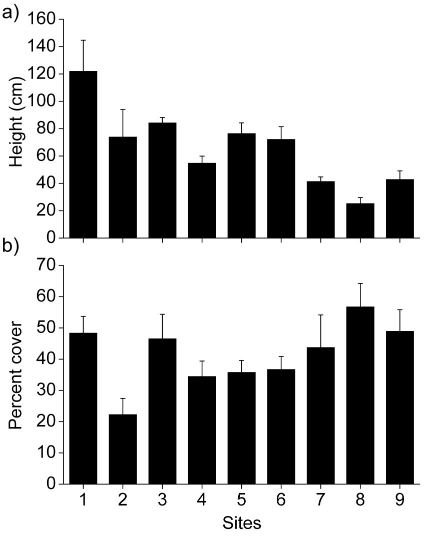


Figure 3. Mean (± s.e.) height (a) and percent cover (b) of *Paramuricea clavata* inside the gorgonian forest at each study site.

### 2.3.2 Epibenthic assemblages inside and outside gorgonian forests

Besides *Paramuricea clavata* 97 taxa were recorded and quantified, of which 45 were identified to species level. The allocation of taxa to morpho-functional groups has been reported in the Supplementary materials (S3, Table S3.1).

The most abundant and widely distributed taxa were encrusting calcareous rhodophytes belonging to Corallinaceae and Peyssonneliaceae families, and erect algae as *Flabellia petiolata* (Turra) Nizamuddin 1987, *Pseudochlorodesmis furcellata* (Zanardini) Børgesen 1925 and *Halimeda tuna* (J.Ellis & Solander) J.V.Lamouroux 1816. Some species, though locally abundant, were found only in nearby sites. It was the case of the colonial ascidians *Polycitor adriaticus* (Drasche 1883) and *Aplidium conicum* (Olivi 1792) that were recorded only in the northern Adriatic Sea (Fig. 2c), and of the invasive alga *Caulerpa cylindracea* Sonder 1845 that was found at the Elba Island (Fig. 2e).

Benthic assemblages showed a large variability at local scale (i.e., among sampling areas) but also significant differences in relation to the occurrence of gorgonian forest across sites (Table 1).

Table 1. PERMANOVA test on differences of epibenthic assemblage structures between inside and outside gorgonian forest (Go: 2 levels, fixed), among sites (Si: 9 levels, random), their interaction (Go × Si) and among areas within this interaction (Area(Go × Si)) (square root-transformed percent cover data, Bray-Curtis coefficient).

| **Source** | **df** | **SS** | **MS** | **Pseudo-*F*** | ***p*** | **Unique perms** |
|---|---|---|---|---|---|---|
| Gorgonian forest (Go) | 1 | 22,252 | 22,252.00 | 2.61 | 0.0280 | 9944 |
| Site (Si) | 8 | 235,020 | 29,378.00 | 12.89 | 0.0001 | 9856 |
| Go × Si | 8 | 68,109 | 8,513.60 | 3.74 | 0.0001 | 9823 |
| Area (Si × Go) | 54 | 123,080 | 2,279.20 | 3.76 | 0.0001 | 9539 |
| Res | 216 | 130,930 | 606.16 | | | |
| Total | 287 | 579,390 | | | | |

Patterns of similarity among assemblages was strongly affected by differences among sites rather than inside and outside gorgonian forests, as it is well represented in the PCoA ordination plot (Fig. 4a). In the unconstrained plot, the data cloud is driven by the large geographical differences in assemblage structures (i.e., the first two axes) and it masks the effects of the presence of gorgonians, detected by PERMANOVA. Conversely, the constrained ordination plot, obtained by CAP, revealed a clear differentiation between assemblages inside and outside gorgonian forest, with a large canonical correlation of 0.87 (Fig. 4b).

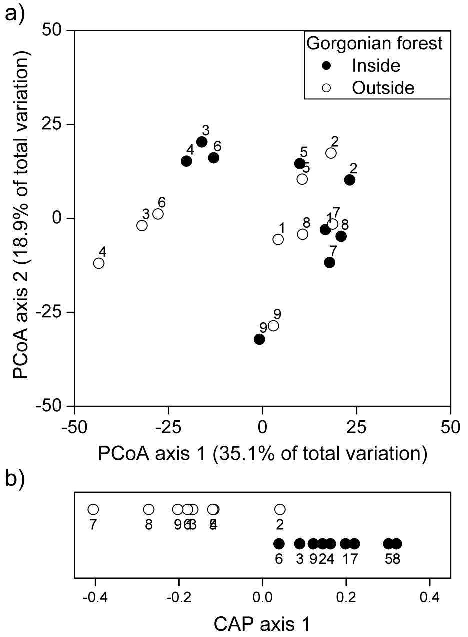


Figure 4. Unconstrained (a; PCoA) and constrained (b; CAP) ordination plots of benthic assemblage data from inside and outside forests of the gorgonian *Paramuricea clavata* at 9 sites in the Mediterranean Sea. Each symbol represents the centroid of 4 areas with 4 replicated samples each.

Few species, individually analysed, showed a clear pattern in relation to the occurrence of gorgonian forest. The green algae *Codium bursa* (Olivi) C.Agardh 1817 was found at 5 sites out of 9, and always only outside *P. clavata* forest (Fig. 5a). The yellow gorgonian *Eunicella cavolini* (Koch 1887) was found at 6 sites out of 9, and at 5 of these was present only inside *P. clavata* forest (Table 2, Fig. 5b). Overall, the percent covers of encrusting sponges and encrusting bryozoans were very variable at local and regional scale, i.e. among areas and sites, and in both cases they were significantly more abundant inside the gorgonian forests (Table 2, Fig. 5c,d). Erect bryozoans showed a less clear pattern with a large variability at local scale and their abundance inside and outside gorgonian forests was not consistent across sites (Table 2). The SNK test revealed that their percent covers were significantly higher inside the gorgonian forests at 3 sites, while at the 6 remaining sites no differences were detected (Fig. 5e). Mixed turf, which includes many filamentous algae and hydroids, showed large variability at local scale and their abundance inside and outside gorgonian forests was not consistent across sites (Table 2). In this case, the SNK test revealed that their percent covers were significantly higher outside the gorgonian forests at 7 sites, while at the 2 remaining sites no differences were detected (Fig. 5f). Percent cover of the encrusting calcareous rhodophytes (ECR) varied among sites and showed higher values inside gorgonian forest (Table 2, Fig. 5g). Similar results were obtained by summing to encrusting calcareous rhodophytes all the other builder organisms that may contribute to the coralligenous framework (Table 2, Fig. 5h).

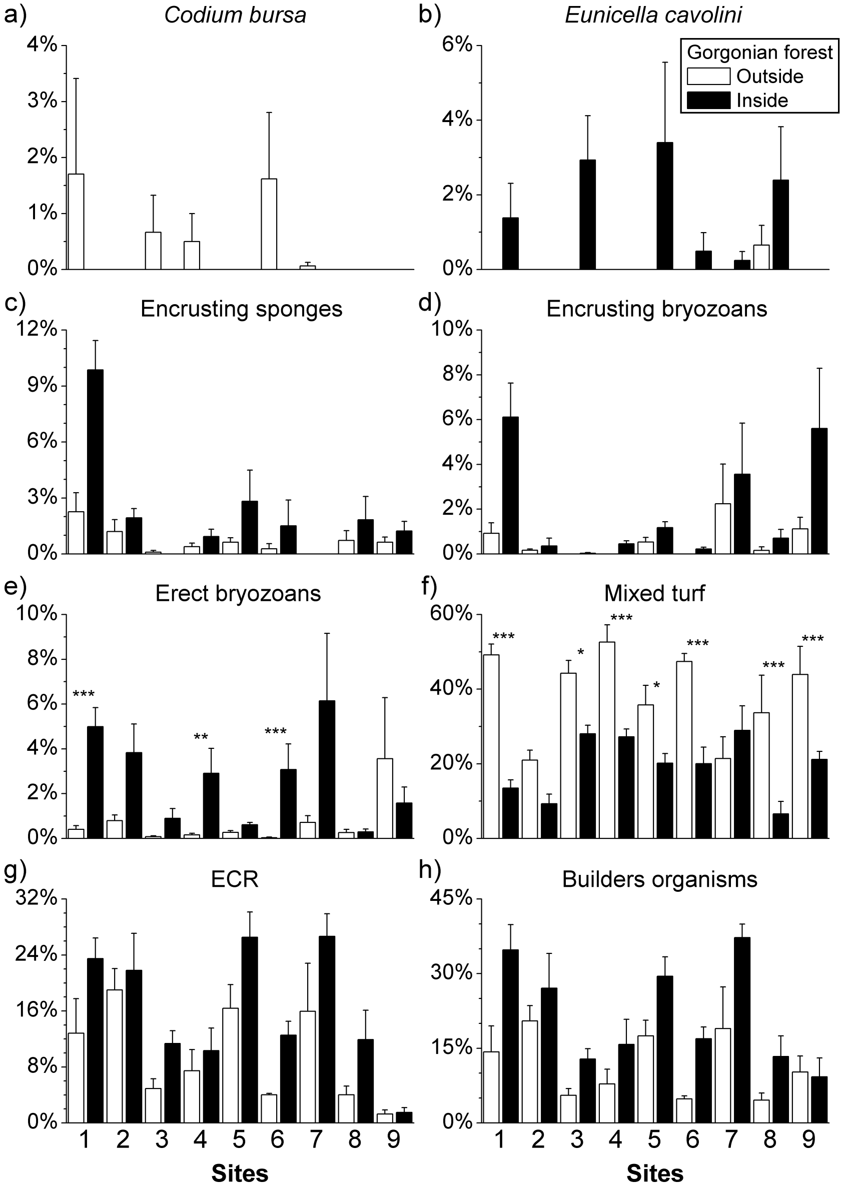


Figure 5. Mean (± s.e.) percent covers of *Codium bursa* (a), *Eunicella cavolini* (b), encrusting sponges (c), encrusting bryozoans (d), erect bryozoans (e), mixed turf (f), encrusting calcareous rhodophytes (ECR, g) and all builder organisms together (h), inside and outside forests of the gorgonian *Paramuricea clavata* at 9 sites in the Mediterranean Sea. Significant levels in SNK tests inside *vs.* outside gorgonian forests within sites were indicated by the following symbols: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Percent covers of green algal turfs and non-encrusting algae belonging to the genus *Peyssonnelia*, although not significantly different between inside and out outside the gorgonian forests, showed a peculiar pattern in relation to the cover of *P. clavata* inside the forests. At low gorgonian cover (up to 30%), green algal turfs seemed to be facilitated by *P. clavata*, while *Peyssonnelia* spp. was not affected (Fig. 6a,b).

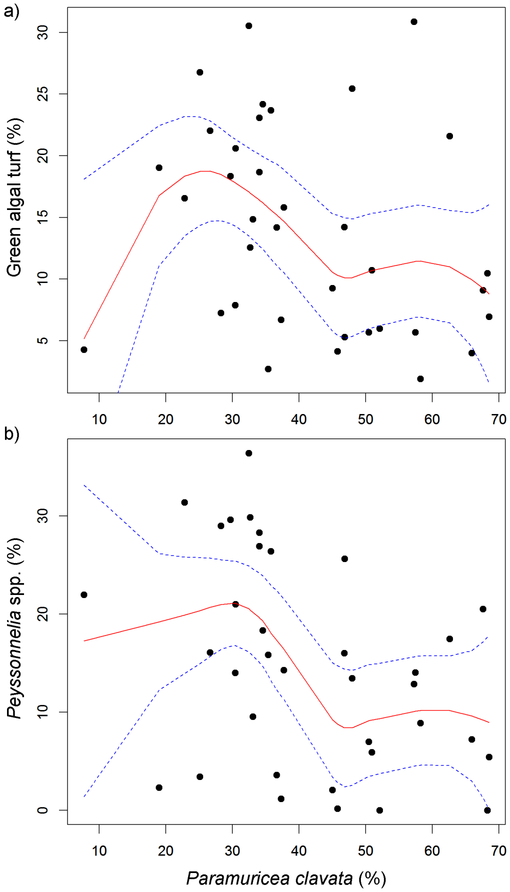


Figure 6. Polynomial regression fitting (LOESS) between percent cover of *Paramuricea clavata* and percent cover of green algal turf (a) and non-encrusting algae of the genus *Peyssonnelia* (b). The dashed lines delimit the confidence intervals at 95%.

At higher gorgonian cover, the percent covers of both algal groups decreased dramatically, down to about 10%. The non-indigenous alga *Caulerpa cylindracea* was found only at three sites located at the Elba Island. In these three sites, the percent cover of *C. cylindracea* showed large variability at local scale (i.e., significant differences among areas) and significantly lower values inside the gorgonian forests, regardless the sites (Table 2, Fig. 7a). Mucilaginous aggregates were found at only three sites: Portofino, Ustica Islands and one at Elba Islands (Fig. 2f). At these three sites, the percent cover of these aggregates showed large variability at local scale and a significant variation across sites and inside *vs* outside gorgonian forest (Table 2). The SNK test revealed that percent covers were significantly higher outside the gorgonian forests at 2 sites out of 3 (Fig. 7b).

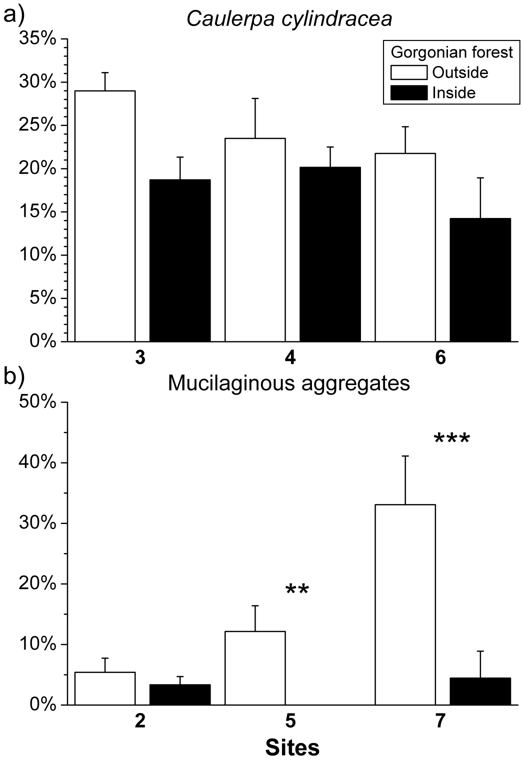


Figure 7. Mean (± s.e.) percent covers of *Caulerpa cylindracea* (a) and mucilaginous aggregates (b), inside and outside forests of the gorgonian *Paramuricea clavata* at 3 sites in the Mediterranean Sea. Significant levels in SNK tests inside *vs*. outside gorgonian forests within sites were indicated by the following symbols: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

All the species diversity indices (*S*, *H'* and *J'*) showed significant variability at local and regional scale, i.e. among areas and sites, and were significantly higher inside the gorgonian forests (Table 2, Fig. 8).

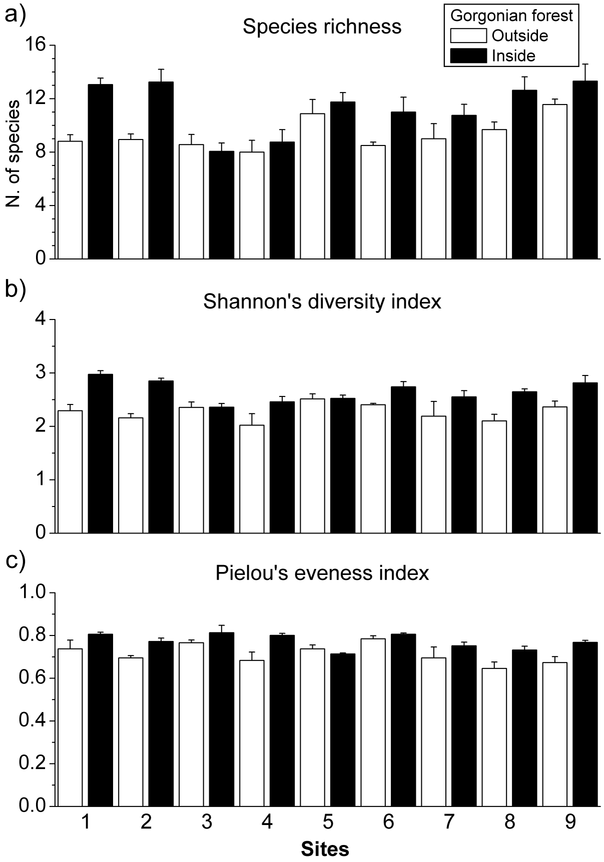
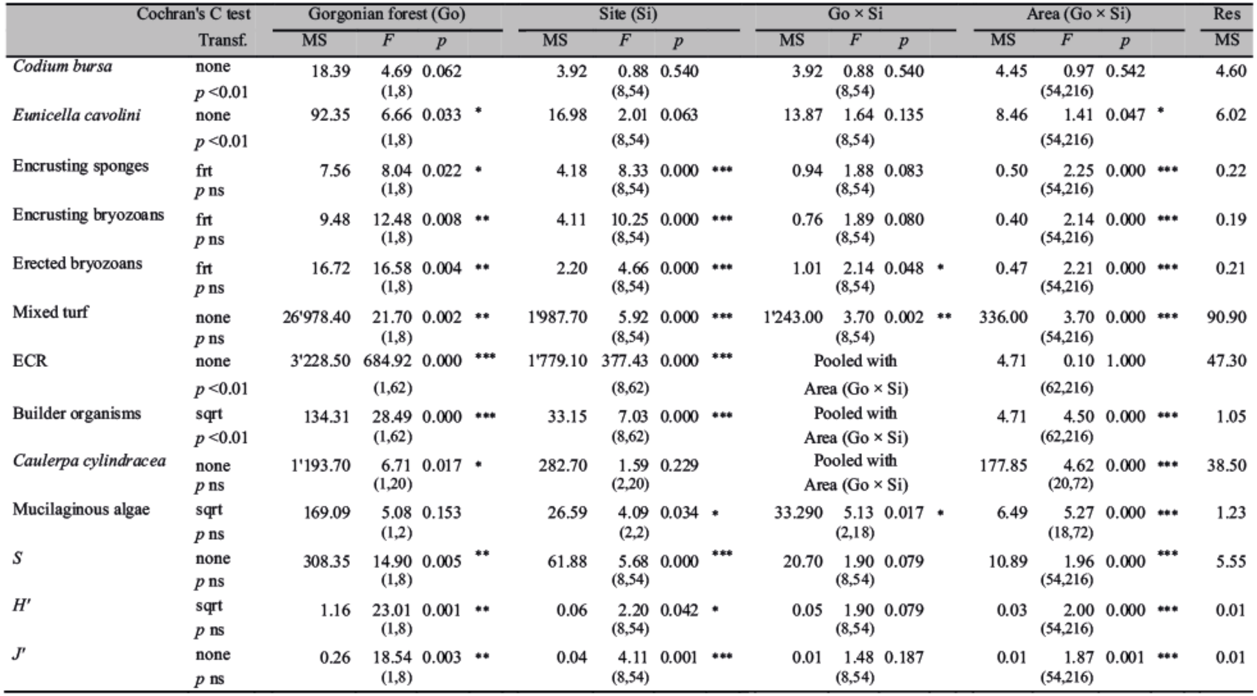


Figure 8. Mean (± s.e.) species richness (*S*, a), Shannon's diversity (*H'*, b) and Pielou's evenness (*J'*, c), inside and outside forests of the gorgonian *Paramuricea clavata* at 9 sites in the Mediterranean Sea.

Table 2. Summary of ANOVA tests. Data transformation: sqrt = square root; frt = four root. Degrees of freedom of numerator and denominator are given in parentheses. Significant levels were indicated by the following symbols: ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.



| | Cochran's C test | Gorgonian forest (Go) | | | Site (Si) | | | Go × Si | | | Area (Go × Si) | | | Res |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Transf. | MS | *F* | *p* | MS | *F* | *p* | MS | *F* | *p* | MS | *F* | *p* | MS |
| *Codium bursa* | none<br>*p* <0.01 | 18.39 | 4.69<br>(1,8) | 0.062 | 3.92 | 0.88<br>(8,54) | 0.540 | 3.92 | 0.88<br>(8,54) | 0.540 | 4.45 | 0.97<br>(54,216) | 0.542 | 4.60 |
| *Eunicella cavolini* | none<br>*p* <0.01 | 92.35 | 6.66<br>(1,8) | 0.033 * | 16.98 | 2.01<br>(8,54) | 0.063 | 13.87 | 1.64<br>(8,54) | 0.135 | 8.46 | 1.41<br>(54,216) | 0.047 * | 6.02 |
| Encrusting sponges | frt<br>*p* ns | 7.56 | 8.04<br>(1,8) | 0.022 * | 4.18 | 8.33<br>(8,54) | 0.000 *** | 0.94 | 1.88<br>(8,54) | 0.083 | 0.50 | 2.25<br>(54,216) | 0.000 *** | 0.22 |
| Encrusting bryozoans | frt<br>*p* ns | 9.48 | 12.48<br>(1,8) | 0.008 ** | 4.11 | 10.25<br>(8,54) | 0.000 *** | 0.76 | 1.89<br>(8,54) | 0.080 | 0.40 | 2.14<br>(54,216) | 0.000 *** | 0.19 |
| Erected bryozoans | frt<br>*p* ns | 16.72 | 16.58<br>(1,8) | 0.004 ** | 2.20 | 4.66<br>(8,54) | 0.000 *** | 1.01 | 2.14<br>(8,54) | 0.048 * | 0.47 | 2.21<br>(54,216) | 0.000 *** | 0.21 |
| Mixed turf | none<br>*p* ns | 26'978.40 | 21.70<br>(1,8) | 0.002 ** | 1'987.70 | 5.92<br>(8,54) | 0.000 *** | 1'243.00 | 3.70<br>(8,54) | 0.002 ** | 336.00 | 3.70<br>(54,216) | 0.000 *** | 90.90 |
| ECR | none<br>*p* <0.01 | 3'228.50 | 684.92<br>(1,62) | 0.000 *** | 1'779.10 | 377.43<br>(8,62) | 0.000 *** | Pooled with Area (Go × Si) | | | 4.71 | 0.10<br>(62,216) | 1.000 | 47.30 |
| Builder organisms | sqrt<br>*p* <0.01 | 134.31 | 28.49<br>(1,62) | 0.000 *** | 33.15 | 7.03<br>(8,62) | 0.000 *** | Pooled with Area (Go × Si) | | | 4.71 | 4.50<br>(62,216) | 0.000 *** | 1.05 |
| *Caulerpa cylindracea* | none<br>*p* ns | 1'193.70 | 6.71<br>(1,20) | 0.017 * | 282.70 | 1.59<br>(2,20) | 0.229 | Pooled with Area (Go × Si) | | | 177.85 | 4.62<br>(20,72) | 0.000 *** | 38.50 |
| Mucilaginous algae | sqrt<br>*p* ns | 169.09 | 5.08<br>(1,2) | 0.153 | 26.59 | 4.09<br>(2,2) | 0.034 * | 33.290 | 5.13<br>(2,18) | 0.017 * | 6.49 | 5.27<br>(18,72) | 0.000 *** | 1.23 |
| *S* | none<br>*p* ns | 308.35 | 14.90<br>(1,8) | 0.005 ** | 61.88 | 5.68<br>(8,54) | 0.000 *** | 20.70 | 1.90<br>(8,54) | 0.079 | 10.89 | 1.96<br>(54,216) | 0.000 *** | 5.55 |
| *H'* | sqrt<br>*p* ns | 1.16 | 23.01<br>(1,8) | 0.001 ** | 0.06 | 2.20<br>(8,54) | 0.042 * | 0.05 | 1.90<br>(8,54) | 0.079 | 0.03 | 2.00<br>(54,216) | 0.000 *** | 0.01 |
| *J'* | none<br>*p* ns | 0.26 | 18.54<br>(1,8) | 0.003 ** | 0.04 | 4.11<br>(8,54) | 0.001 *** | 0.01 | 1.48<br>(8,54) | 0.187 | 0.01 | 1.87<br>(54,216) | 0.001 *** | 0.01 |

## 2.4 | Discussion

This study compared the epibenthic faunal assemblages inside and outside gorgonian forests across a broad range of central and western Mediterranean coralligenous habitats, differing in environmental conditions and belonging to different biogeographic sectors (sensu Bianchi & Morri 2000). Investigated gorgonian forests vary in colony density, and dwell on rocky substrates differing in inclination, orientation and depth. Overall, understory assemblage of the animal forests showed a large variability at different spatial scales. None of the species found in this study is exclusive of either forested or not forested habitats, nor closely associated to the presence of *P. clavata*. Nevertheless, at the local scale, the structures of the epibenthic assemblages significantly differed between inside and outside the gorgonian forests.

The Mediterranean yellow gorgonian *Eunicella cavolini* is often associated to the *P. clavata* forests. Interspersion between colonies of *E. cavolini* and *P. clavata*, as observed in the present study, is a common finding through all the Mediterranean Sea (Di Camillo et al. 2018). This suggests a large overlap in their ecological niche and limited interspecific competition, at least at low colony densities. *E. cavolini* colonies are smaller in size, have a lower growth rate, an higher P/B ratio and a lower turnover rates compared to *P. clavata* (Coma et al. 1998 and references therein). These two gorgonians are affected by the same local and global threats (Cerrano et al. 2000, Garrabou et al. 2009, Sini et al. 2015), although *E. cavolini*, in a field experiment, showed greater resistance to thermal stress, with a lower mortality and a higher recovery capacity compare to *P. clavata* (Fava et al. 2010). Forests of *P. clavata* being larger in size may protect *E. cavolini* and other fragile species, such as erect bryozoans, from mechanical damage (e.g., fishing lines, nets and divers), as already documented for *Pentapora fascialis* (Pallas 1766) by Garrabou et al. 1998).

Compared to the unforested rocky bottoms, the understorey of *P. clavata* forests host higher percent covers of encrusting sponges, encrusting bryozoans and encrusting calcareous rhodophytes. All of them are relevant components of the Mediterranean coralligenous habitats and contribute to their frameworks (Ballesteros 2006). Recruitment of encrusting sponges and encrusting bryozoans may be facilitated by the presence of *P. clavata*, as consistently observed in early-stage recruitment experiments carried out in the Ligurian and the Tyrrhenian Sea (Ponti et al. 2014).

Overall, by summing all organisms able to deposit calcium carbonate it turns out that these forests support higher abundances of builder organisms. These findings suggest that gorgonian forests may largely contribute to maintain and develop coralligenous biogenic habitats. On the contrary, mixed turfs of filamentous algae and hydroids, which normally tend to compete for space with encrusting sponges, encrusting bryozoans and encrusting calcareous rhodophytes, were often more abundant outside the gorgonian forests.

*Codium bursa*, was found only outside the forests. The growth of this long-lived and slow-growing alga, is generally limited by nutrient availability (especially phosphorus), rather than by light (Vidondo & Duarte 1995). This suggests that the limiting effect of *P. clavata* towards this seaweed go well beyond the simple shading and likely acts at the early recruitment stage.

At high gorgonian density, edaphic conditions (e.g., physical factors such as light, currents, sedimentation rates) may be strongly modified, increasing habitat complexity that supporting many necto-benthic organisms (Cerrano et al. 2010, Ponti et al. 2016, Valisano et al. 2016). This study highlighted the importance that may have the gorgonians density, as in the cases of green algal turfs and non-encrusting peyssonneliacean algae. Indeed, the effects of *P. clavata* on erect and filamentous algae seemed not univocal, as observed in the early-stage recruitment experiments (Ponti et al. 2014). In these forests, density-dependent interactions are far from being known and deserve further investigations.

Higher values of species diversity indices were found inside gorgonian forests, compared to surrounding rocky bottoms. This trend is consistent across the investigated geographical area, corroborating the hypothesis of a positive relationship between three-dimensional habitat complexity and species diversity (Kovalenko et al. 2012).

### 2.4.1 A focus on the non-indigenous species *Caulerpa cylindracea*

The non-indigenous species (NIS) have been recognised as a threat to the integrity of Mediterranean native communities. Establishment of NIS can drastically change the structure of marine communities, affecting species growth rates, survival and reproduction (Occhipinti-Ambrogi 2007, Butchart et al. 2010). The green alga *Caulerpa cylindracea* is listed among the most threatening Mediterranean invaders (Streftaris & Zenetos 2006), and represents one of the major concerns for the coralligenous habitats (Piazzi et al. 2012, Piazzi et al. 2016). Manipulative experiments showed that the presence of *C. cylindracea* negatively affects the *Paramuricea clavata* fitness by increasing percentage of necrosis and lowering biomass and survivorship (Cebrian et al. 2012). However the role of gorgonian forests in preventing the settlement and invasion of *C. cylindracea* has never been investigated. At all sites where it occurred *C. cylindracea* was significantly less abundant inside gorgonian forests. The result seems fairly robust and suggests a resistance of the gorgonians to the invasion of this seaweed. Piazzi et al. 2016), in a recent review on biotic and abiotic interactions of *C. cylindracea* in the Mediterranean Sea, suggested that canopy-forming algae and seagrasses may limit the spread of *C. cylindracea* by reducing photosynthetic performance by the shading. Gorgonian forests may cause a similar shadow effects. Moreover, *P. clavata* may directly or indirectly reduce the recruitment of *C. cylindracea* by releasing allelochemicals

(Rodriguez 1995) or limiting the abundances of algal turfs, which may enhance the spread of the invader (Piazzi et al. 2016 and references therein).

### 2.4.2 Entrapment of mucilaginous aggregates

Developments of mucilaginous aggregates are recurrent events in the Mediterranean Sea (Rinaldi et al. 1995, Sartoni et al. 2008). Deposition of mucilaginous aggregates on the seabed may cause severe damage to many benthic organisms, by reducing light availability, suffocating sessile invertebrates and establishing anoxic conditions (Schiaparelli et al. 2007). Gorgonians are very sensitive to mucilage, which can cause necrosis of coenenchyme and leave portions of axial skeleton bare (Mistri & Ceccherelli 1996a, Giuliani et al. 2005). *Paramuricea clavata* colonies in a few years may recover from damages caused by mucilage through recruiting juvenile colonies (Mistri & Ceccherelli 1996b). The present study shows that gorgonians, by trapping the mucilaginous aggregates with their branches, may reduce the accumulation in the understorey. Although at their expenses, gorgonian forests may therefore limit the damage from suffocation to many associated benthic invertebrates.

## 2.5 | Conclusions

The ecological role played by habitat-forming species cannot be easily disentangled, either from a physical or biological perspective. Generally, increased habitat complexity leads higher species diversity in the associated assemblages, by increasing the available space and the number microhabitats (Kovalenko et al. 2012). Moreover, the habitat complexity built up by ecosystem engineers induces physical changes, but may also modify many biological processes, with unpredictable ecological effects (Kelaher 2003). Although the mechanisms underlying these processes are still not well known, forests of *Paramuricea clavata*, besides modifying microscale hydrodynamics, may provide additional food resources, increase refuges and available surface area (Ponti et al. 2016, Valisano et al. 2016), as well as affect the recruitment processes (Ponti et al. 2014). According to the present results animal forests are supporting high species diversity and promote bioconstruction processes in their understorey. Presence of these long-lived organisms reduces the temporal variability, as it was observed in different Mediterranean coralligenous habitats (Piazzi et al. 2004, Ponti et al. 2011a, Teixido et al. 2011, Casas-Guell et al. 2015). Indeed, pristine gorgonian forests may also oppose to the spread of non-indigenous species and mitigate the effects of adverse events, like mucilage formations. Gorgonian species play an ecological role that goes well beyond the aesthetic value, which attracts a large number of recreational divers (Musard et al. 2014). The obtained results support the concern raised by several scientists about the loss of habitat complexity caused by extensive mass mortalities of

Mediterranean gorgonians (Cerrano & Bavestrello 2008). The fragmentation and the local disappearance of gorgonian forests could cause severe and sudden modifications of the epibenthic communities. It may lower the species diversity, alter the ecosystem functioning and reduce the resistance and resilience of the assemblages with concomitant losses of ecological goods and services, over different spatial and temporal scales. *Paramuricea clavata* is considered a "vulnerable" species according to the Mediterranean Red List (ver. 3.1) provided by IUCN, mainly because of its low recruitment, and the *facies* with *P. clavata* is listed in the habitats that deserve special protection in the frame of the Barcelona Convention (RAC/SPA, Tunis). Evidences from this and many other studies strongly suggest to consider the health status of this species and its forests, threatened by several anthropogenic disturbance and by the global climate changes, as essential elements of the ecological dynamics of the Mediterranean Sea, and therefore worthy of specific and effective transboundary protection measures. The European Marine Strategy Framework Directive (2008/56/EC), the environmental pillar of the Blue Growth strategy, defines the need of each member state to reach the Good Environmental Status (GES) of their marine waters. This achievement should require an urgent regulation of fishing activity, anchorages and scuba diving where gorgonian forests are present and restoration activities when evident alterations are documented. The results here presented clearly indicate that *P. clavata* represent a key species to maintain the GES of the Mediterranean temperate reefs, the coralligenous habitats.

## Acknowledgments

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# Supplementary material Chapter 2

## S1 Distribution of *Paramuricea clavata*

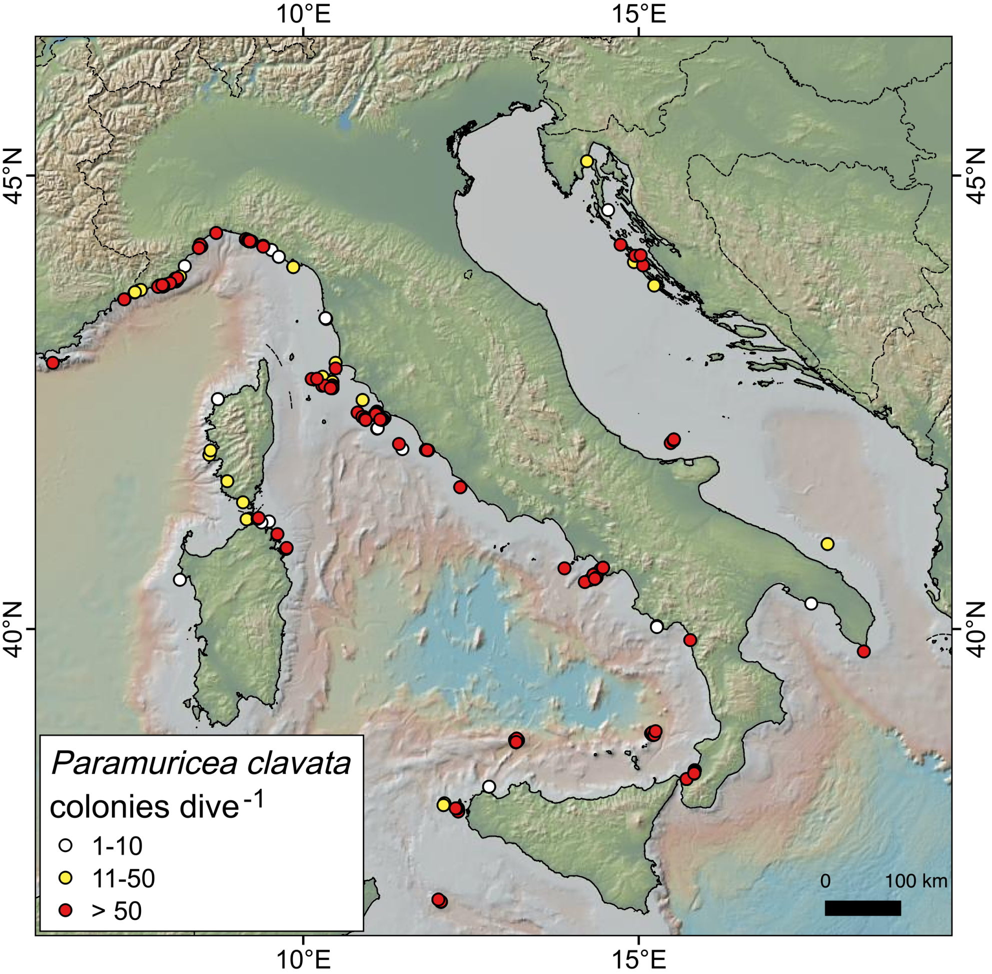


Figure S1.1. Reports of *Paramuricea clavata* colonies provided by volunteer divers according to the Reef Check protocol (www.reefcheckmed.org; Cerrano et al. 2017b) along Italian, French and Croatian coasts from 2006 to 2016 (n = 698). Abundances are reported in terms of number of colonies sighted per dive by independent observers (base map: Global Multi-Resolution Topography, GMRT (Ryan et al. 2009); Mercator Projection, Datum WGS 84).

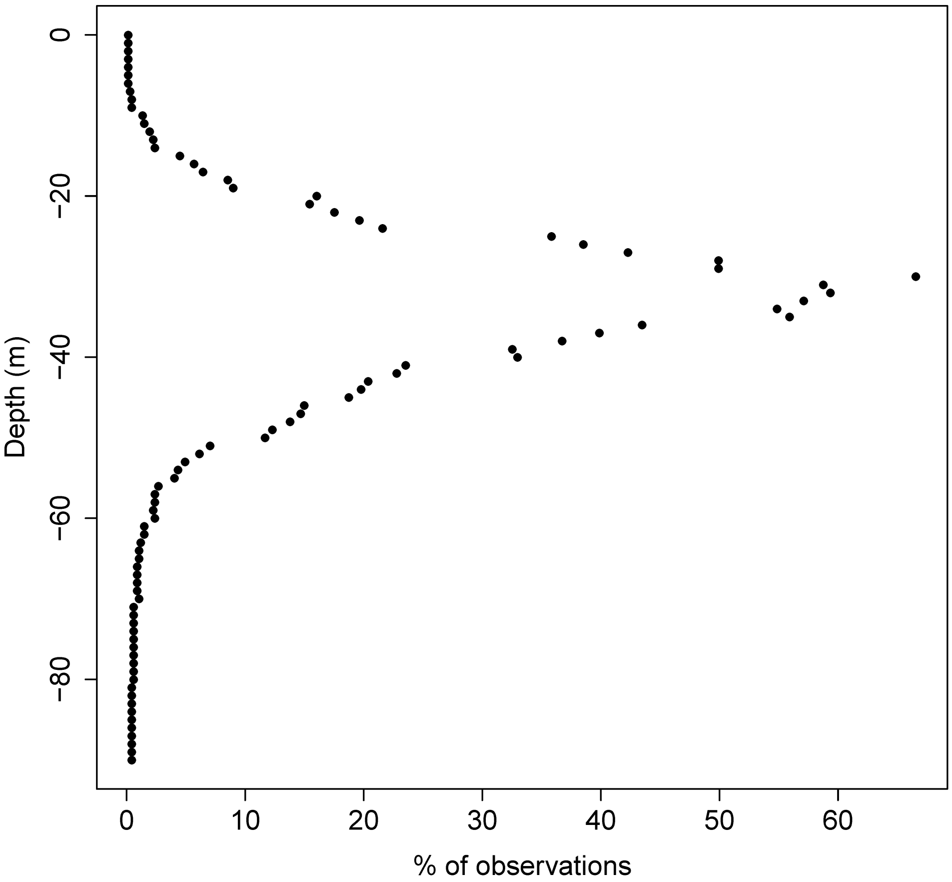


Figure S1.2. Bathymetric distribution of *Paramuricea clavata*. Percentage of observations provided by volunteer divers according to the Reef Check protocol (www.reefcheckmed.org; Cerrano et al. 2017b) along Italian, French and Croatian coasts from 2006 to 2016 (n = 667).

## S2 Geological and environmental features at each study site

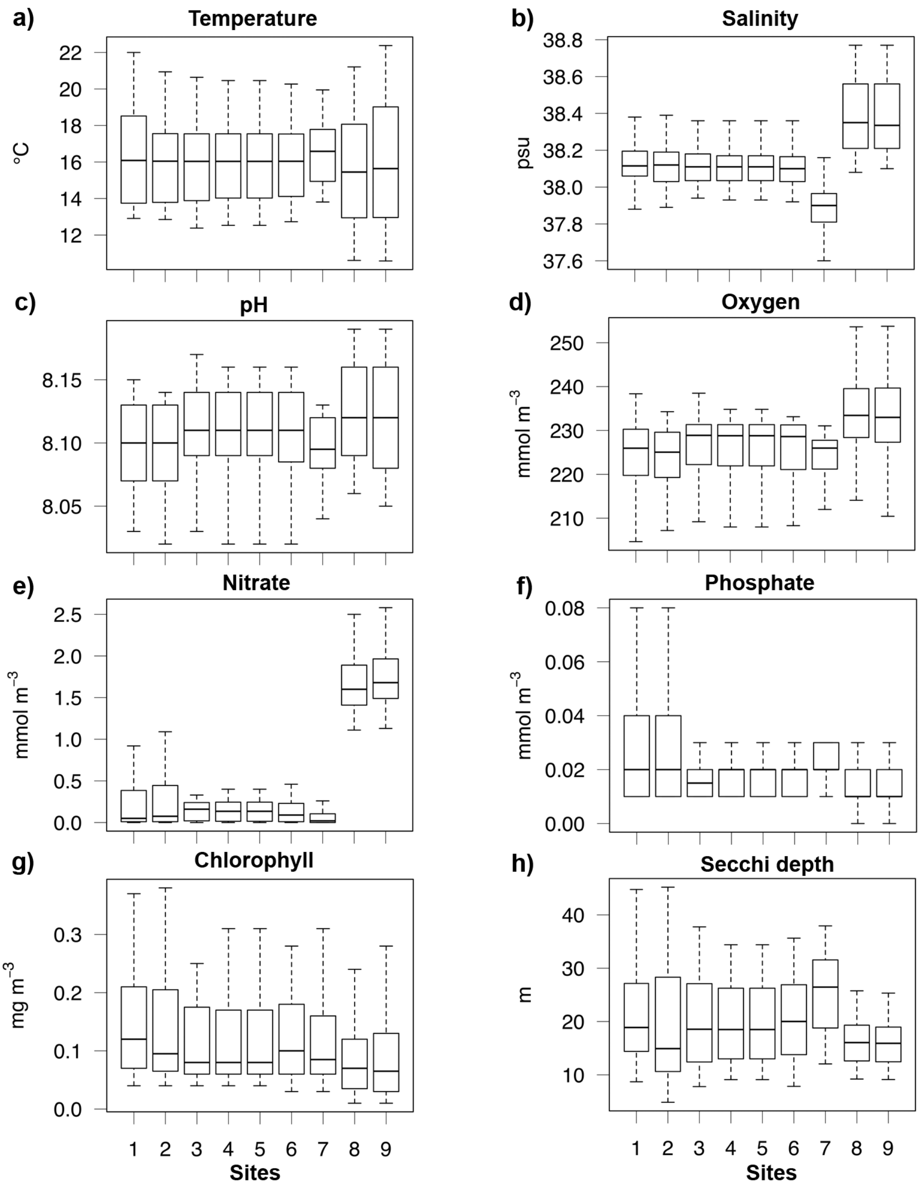


Figure S2.1 Boxplot of the monthly mean temperature (a), salinity (b), pH (c), concentration of dissolved Oxygen (d), Nitrate (e), Phosphate (f), and Chlorophyll (g), and water transparency as Secchi depth (h), estimated at each sampling site and depth in the period January 2009 - December 2014 (n = 72, except for Secchi depth at Site 1, n = 67, and at Site 2 and 8, n = 71).

All data were retrieved from the E.U. Copernicus Marine Service Information (http://marine.copernicus.eu/). Temperature and salinity were derived from the Mediterranean Sea Physics Reanalysis numerical model (Spatial resolution: 0.06°; Vertical resolution: 72 levels) provided by the MED-INGV-BOLOGNA-IT unit

(Fratianni et al. 2015); oxygen, nutrients and chlorophyll concentration were obtained from the Mediterranean Sea Biogeochemistry Reanalysis numerical model (Spatial resolution: 0.06°; Vertical resolution: 72 levels) provided by the MED-OGS-TRIESTE-IT unit; water transparency data come from the Global Ocean and Optics GlobColour-OSS2015 satellite observations (Spatial resolution: 4 km; Vertical resolution: surface) provided by the OC-ACRI-NICE-FR unit.

Table S2.1 Geological features at the sampling sites.

| Site | Geological formation | Period | Source |
|---|---|---|---|
| 1 | Marl-limestone | Jurassic | Geological Map of France http://infoterre.brgm.fr |
| 2 | Sandstone and conglomerates | Palaeogene | Geological Map of Italy http://www.pcn.minambiente.it |
| 3-6 | Metamorphic limestones and marls sometimes with shales and radiolarytes | Jurassic | Geological Map of Italy http://www.pcn.minambiente.it |
| 7 | Terrigenous-skeletal limestones like "Panchina" | Pleistocene | Geological Map of Italy http://www.pcn.minambiente.it |
| 8-9 | Carbonate (limestone, dolomite, and carbonate breccia) | Cretaceous | Pikelj & Juracic 2013 http://www.hgi-cgs.hr |

Table S2.2. Sampling site names and locations. Sampling depths, substrate inclinations and orientations are also reported.

| No. | Site name | Location | Sea | Country | Latitude | Longitude | Depth | Inclination | Orientation | Sampling date |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | North | East | m | | | |
| 1 | P.te Causinière | Cap Ferrat | Ligurian Sea | FR | 43.67603° | 7.33505° | 32-34 | Vertical | 135° | 16/10/2014 |
| 2 | Colombara | Portofino | Ligurian Sea | IT | 44.31159° | 9.17580° | 35-36 | Vertical | 225° | 13/06/2014 |
| 3 | Punta delle Cannelle | Elba Island | Tyrrhenian Sea | IT | 42.77617° | 10.43467° | 36-38 | Horizontal | 112° | 06/11/2015 |
| 4 | Capo Calvo | Elba Island | Tyrrhenian Sea | IT | 42.73465° | 10.43412° | 36-38 | Horizontal | 67° | 08/11/2015 |
| 5 | Picchi di Pablo | Elba Island | Tyrrhenian Sea | IT | 42.72733° | 10.43517° | 36-38 | Horizontal | 67° | 07/11/2015 |
| 6 | Scoglio del Remaiolo | Elba Island | Tyrrhenian Sea | IT | 42.70968° | 10.43467° | 36-38 | Horizontal | 157° | 07/11/2015 |
| 7 | Punta San Paolo | Ustica Island | Tyrrhenian Sea | IT | 38.69627° | 13.18653° | 40-42 | Vertical | 135° | 27/08/2014 |
| 8 | Zverinac Južni Rt | Zverinac Island | Adriatic Sea | HR | 44.14483° | 14.94783° | 33-35 | Vertical | 157° | 09/08/2014 |
| 9 | Rivanjski Kanal | Sestrunj-Rivanj Island | Adriatic Sea | HR | 44.15020° | 15.02890° | 22-23 | Vertical | 315° | 10/08/2015 |

## S3 Allocation of taxa to morpho-functional groups

Table S3.1 Allocation to the morpho-functional groups of the identified taxa and their role as builders or borers (n.a. = not assigned).

| **Taxa** | **morpho-functional groups** | **Builders/ Borers** |
|---|---|---|
| Microalgal mat | Microalgal mats | n.a. |
| Green algal turf | Mixed turfs | n.a. |
| Mucilaginous aggregates | Mucilaginous aggregates | n.a. |
| Dictyotales | Erect ochrophytes | n.a. |
| cfr. *Dictyopteris lucida* | | |
| Dictyotales | Erect ochrophytes | n.a. |
| *Padina pavonica* | Erect ochrophytes | n.a. |
| Ectocarpales | Erect ochrophytes | n.a. |
| cfr. *Spermatochnus paradoxus* | | |
| *Zanardinia typus* | Erect ochrophytes | n.a. |
| Red algal turf | Mixed turfs | n.a. |
| *Amphiroa rigida* | Erect rhodophytes | Builders |
| *Ellisolandia elongata* | Erect rhodophytes | Builders |
| Encrusting Corallinaceae | Encrusting calcareous rhodophytes | Builders |
| Ceramiales | Erect rhodophytes | n.a. |
| cfr. *Chondria capillaris* | | |
| *Osmundaria volubilis* | Erect rhodophytes | n.a. |
| *Peyssonnelia* spp. | Erect rhodophytes | n.a. |
| Encrusting Peyssonneliaceae | Encrusting calcareous rhodophytes | Builders |
| *Botryocladia* cfr. *botryoides* | Erect rhodophytes | n.a. |
| *Botryocladia* cfr. *chiajeana* | Erect rhodophytes | n.a. |
| Gigartinales | Erect rhodophytes | n.a. |
| cfr. *Dudresnaya verticillata* | | |
| *Phyllophora crispa* | Erect rhodophytes | n.a. |
| *Palmophyllum crassum* | n.a. | n.a. |
| *Acetabularia acetabulum* | Erect chlorophytes | n.a. |
| *Bryopsis plumosa* | Erect chlorophytes | n.a. |
| *Caulerpa cylindracea* | Erect chlorophytes | n.a. |
| *Codium bursa* | Erect chlorophytes | n.a. |

| **Taxa** | **morpho-functional groups** | **Builders/ Borers** |
|---|---|---|
| *Codium* cfr. *decorticatum* | Erect chlorophytes | n.a. |
| *Codium* cfr. *effusum* | Erect chlorophytes | n.a. |
| *Halimeda tuna* | Erect chlorophytes | Builders |
| *Flabellia petiolata* | Erect chlorophytes | n.a. |
| *Pseudochlorodesmis furcellata* | Erect chlorophytes | n.a. |
| *Valonia macrophysa* | Erect chlorophytes | n.a. |
| *Clathrina* spp. | Massive and erect sponges | n.a. |
| *Cliona* spp. | n.a. | Borers |
| *Polymastia* spp. | Massive and erect sponges | n.a. |
| *Tethya* spp. | Massive and erect sponges | n.a. |
| *Chondrosia reniformis* | Massive and erect sponges | n.a. |
| *Crella (Grayella) pulvinar* | Encrusting sponges | n.a. |
| *Hemimycale* spp. | Encrusting sponges | n.a. |
| *Phorbas tenacior* | Encrusting sponges | n.a. |
| *Tedania (Tedania) anhelans* | Massive and erect sponges | n.a. |
| *Axinella* spp. | Massive and erect sponges | n.a. |
| *Axinella cannabina* | Massive and erect sponges | n.a. |
| *Dictyonella incisa* | Encrusting sponges | n.a. |
| *Agelas oroides* | Massive and erect sponges | n.a. |
| *Haliclona* cfr. *(Halichoclona) fulva* | Massive and erect sponges | n.a. |
| *Petrosia (Petrosia) ficiformis* | Massive and erect sponges | n.a. |
| *Ircinia* spp. | Massive and erect sponges | n.a. |
| *Sarcotragus* spp. | Massive and erect sponges | n.a. |
| *Dysidea* spp. | Massive and erect sponges | n.a. |
| *Pleraplysilla spinifera* | Massive and erect sponges | n.a. |
| *Aplysina* spp. | Massive and erect sponges | n.a. |
| Massive sponge sp. 1 cfr. *Clathria (Clathria) compressa* | Massive and erect sponges | n.a. |
| Massive sponge sp. 2 | Massive and erect sponges | n.a. |
| Encrusting sponge sp. 1 cfr. *Spirastrella cunctatrix* | Encrusting sponges | n.a. |
| Encrusting sponge sp. 2 cfr. *Crambe crambe* | Encrusting sponges | n.a. |

| **Taxa** | **morpho-functional groups** | **Builders/ Borers** |
|---|---|---|
| Encrusting sponge sp. 3 | Encrusting sponges | n.a. |
| Encrusting sponge sp. 4 | Encrusting sponges | n.a. |
| *Oscarella lobularis* | Massive and erect sponges | n.a. |
| Hydroids turf | Mixed turfs | n.a. |
| *Pennaria disticha* | n.a. | n.a. |
| *Cornularia cornucopiae* | Encrusting corals | n.a. |
| *Alcyonium acaule* | n.a. | n.a. |
| *Maasella edwardsi* | Encrusting corals | n.a. |
| *Corallium rubrum* | Tree-like corals | Builders |
| *Eunicella cavolini* | Tree-like corals | n.a. |
| *Eunicella singularis* | Tree-like corals | n.a. |
| *Cerianthus* spp. | n.a. | n.a. |
| *Caryophyllia (Caryophyllia) smithii* | Solitary hard corals | Builders |
| *Balanophyllia (Balanophyllia) europaea* | Solitary hard corals | Builders |
| *Leptopsammia pruvoti* | Solitary hard corals | Builders |
| *Epizoanthus* spp. | Encrusting corals | n.a. |
| *Parazoanthus axinellae* | Encrusting corals | n.a. |
| *Filograna implexa* | Tube forming polychaetes | Builders |
| Sabellidae | Tube forming polychaetes | n.a. |
| Serpulidae | Tube forming polychaetes | Builders |
| Vermetidae | Vermetids | Builders |
| *Rocellaria dubia* | n.a. | Borers |
| *Beania* spp. | Encrusting bryozoans | Builders |
| *Adeonella calveti* | Erect bryozoans | Builders |
| *Smittina cervicornis* | Erect bryozoans | Builders |
| *Pentapora fascialis* | Erect bryozoans | Builders |
| *Myriapora truncata* | Erect bryozoans | Builders |
| *Reteporella* spp. | Erect bryozoans | Builders |
| Encrusting bryozoan sp. 1 cfr. *Schizomavella (Schizomavella) mamillata* | Encrusting bryozoans | Builders |

| Taxa | morpho-functional groups | Builders/ Borers |
|---|---|---|
| Encrusting bryozoan sp. 2 cfr. *Schizobrachiella sanguinea* | Encrusting bryozoans | Builders |
| Encrusting bryozoan sp. 3 | Encrusting bryozoans | Builders |
| Erect branched bryozoan cfr. *Schizoretepora solanderia* | Erect bryozoans | Builders |
| *Pycnoclavella* spp. | n.a. | n.a. |
| *Polycitor adriaticus* | Massive ascidians | n.a. |
| *Aplidium conicum* | Massive ascidians | n.a. |
| *Microcosmus* spp. | Massive ascidians | n.a. |
| *Halocynthia papillosa* | Massive ascidians | n.a. |
| Colonial ascidians sp. 1 cfr. Polyclinidae | Massive ascidians | n.a. |
| Colonial ascidians sp. 2 | Massive ascidians | n.a. |
| Colonial ascidians sp. 3 | Massive ascidians | n.a. |
| Colonial ascidian sp. 4<br>cfr. *Polysyncraton* spp. | Encrusting ascidians | n.a. |
| Colonial ascidians sp. 5 | Encrusting ascidians | n.a. |

# Chapter 3

## Mass mortality hits gorgonian forests at Montecristo Island

## 3.1 | Introduction

Montecristo Island is one of the least accessible and most protected areas in the Mediterranean Sea. The island is the core of the Tuscan Archipelago National Park (Italy), has been a State Natural Reserve since 1971, and is designated as a Special Protection Area (IT5160014; European Directive 79/409/EEC). The distance from the mainland and other populated islands (i.e., 41 km south of Elba Island, the nearest populated area), coupled with its impervious nature has meant that historically only a few have inhabited this island. The island is also inaccessible to tourists and far away from major human disturbances which have been shown to threaten the vast majority of the rest of the Mediterranean coast (e.g., coastal development, destructive fishing practices, and land based pollution; Micheli et al. 2013). For these reasons, Montecristo Island has been considered among the best-preserved sites in this region, both on land and underwater. Its well-preserved benthic assemblages have therefore been used as a reference condition in the development of biotic indices for the ecological quality assessment throughout the western Mediterranean Sea (Cecchi et al. 2014 and references therein). To our knowledge, Montecristo has not been affected by severe benthic mass mortality events, which have been reported in many other locations within the Mediterranean over recent years (e.g., Cerrano et al. 2000, Calvo et al. 2011, Rivetti et al. 2014). That is with the exception of a localised and restricted mortality event, which affected the purple gorgonian *Paramuricea clavata* (Risso, 1826). This mortality event was reported in the late 80s by the film director and underwater photographer Gerry Guldenschuh (in Bavestrello et al. 1994). Here we report the early stage of a mass mortality event affecting two other gorgonian species, *Eunicella singularis* (Esper, 1791) and *Eunicella cavolini* (Koch, 1887) off Montecristo Island. We further assessed if the anomalous water temperature that occurred at the end of summer 2017 could be one of the causes of the mass-mortality.

## 3.2 | Material and Methods

Two scuba diving surveys were conducted by 6 scientific divers on the 5th of September 2017 at Montecristo Island (Tyrrhenian Sea; Fig. 1): one on the north-western side of the island, Punta del Diavolo (42°21.19' N 10°17.97' E), and the other on the south-eastern side, Punta Forata (42°19.11' N 10°19.60' E). Surveys were initially conducted in order to collate data on the abundance and depth distribution of selected ecological indicator species (including gorgonians). This was undertook by conducting visual observations along random paths down to a depth of 40 m – in accordance to the Reef Check monitoring protocol for the Mediterranean Sea (Cerrano et al. 2017b). Photos and videos were recorded by two Canon PowerShot cameras (models: G15 with a Patima case, and a G7 X with a Fantasea case), both equipped by Inon' strobes. *In situ* depth and temperature were simultaneously recorded by two dive computers at 4-seconds intervals (models: Scubapro/Uwatec Aladin 2G and Tec 3G), with the clocks synchronised to the cameras. At each site, mean temperature (± s.e.) at one-meter depth intervals was obtained by combining the data of the two dive computers.

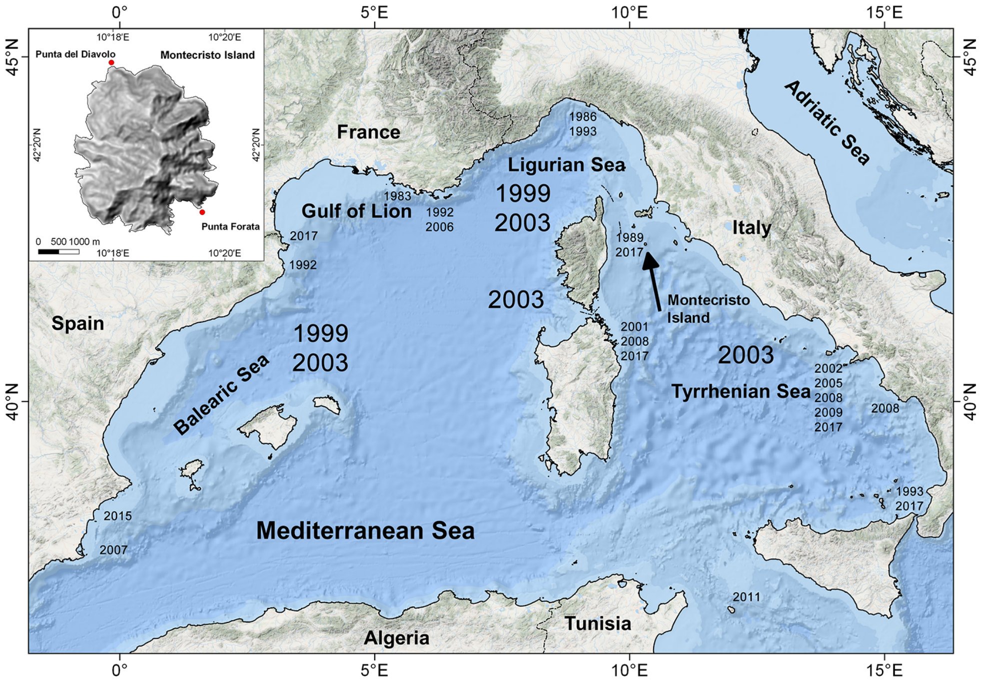


Figure 1. Location of study sites, Punta del Diavolo and Punta Forata, at Montecristo Island, in the Tyrrhenian Sea. Numbers indicate the location and year of mass mortality events that have affected gorgonians in the western Mediterranean Sea as reported in supplementary Table S1. The larger the font of the number the wider the regional event (open source base maps: MapSurfer ASTER GDEM-SRTM Hillshade and ESRI Ocean; Mercator projection, Datum WGS84).

In order to analyse the temporal trend of water temperature and any observed anomalies, daily mean water column temperature in the sampling area was downloaded from the European Union Copernicus Marine Environment Monitoring Service (CMEMS). The daily mean temperatures for 2017 were obtained from the Mediterranean Sea Physical Analysis and Forecasting Product, which is based on a hydrodynamic model with a horizontal grid resolution of 1/24° (ca. 4.6 km in latitude) and 141 unevenly spaced depth levels (Clementi et al. 2017). By comparison, the daily climatological temperatures between 1987-2015 were computed from the Mediterranean Sea Physical Reanalysis Product (MEDREA), based on a hydrodynamic model with a horizontal grid resolution of 1/16° (ca. 6.9 km in latitude) and 72 unevenly spaced depth levels (Simoncelli et al. 2014). The coastline of Montecristo Island falls into 4 calculation cells of the first hydrodynamic model and in 2 cells of the second. Since data from these cells were almost identical, in both cases the data from the first upper-left cell was utilised. It should be noted that the accuracy of these models varies both in space and depth. The Root-Mean-Square Error (RMSE) throughout the water column is on average 0.3 °C, with a peak of 0.8 °C at 30 m depth (Simoncelli et al. 2014, Clementi et al. 2017). Furthermore, a higher error ratio is likely to occur nearest to the coast. All data was analysed and plotted using the geographical information system QGIS and the computational software R, with the package OceanView (Soetaert 2016).

## 3.3 | Results and Discussion

Along the investigated submerged cliffs at Montecristo Island, the white gorgonian *Eunicella singularis* formed dense populations that shaped 'marine animal forests' (see Ponti et al. 2018). Colonies of *E. singularis* were found from 10 m to more than 40 m depth with higher densities (up to 10 ind. m$^{-2}$) between 15 and 25 m. In contrast, the yellow sea whip *E. cavolini* was much less dense than *E. singularis* but colonies were found across a similar depth range (from 15 m to more than 40 m). Relatively higher densities of this species were found between 25 and 35 m (up to 5 ind. m$^{-2}$). Colonies of *Paramuricea clavata* were also observed but only in areas deeper than 25 m.

On the 5th of September 2017, colonies of *E. singularis* and *E. cavolini* exhibited signs of 'rapid tissue loss' at Punta Forata and Punta del Diavolo. The coenenchyme of affected colonies exhibited either partial or total detachment from the skeleton (Fig. 2). No encrusting epibionts were observed on any of the denuded skeletons assessed suggesting the lesion progression was rapid in a similar manner to the tissue loss often seen in scleractinian corals on tropical reefs (e.g., Work et al. 2012). Interestingly in a few colonies the coenenchyme appeared to remain attached when initially observed. However, the tissue was easily dislodged by minor water movement (e.g. by a diver's hand moving in front of the coral; see Video S1). This may be indicative of an early stage of the disease in question. Colonies that were affected by this rapid tissue loss were more commonly found above the thermocline, which reached to depths of 30 m at the sites surveyed.

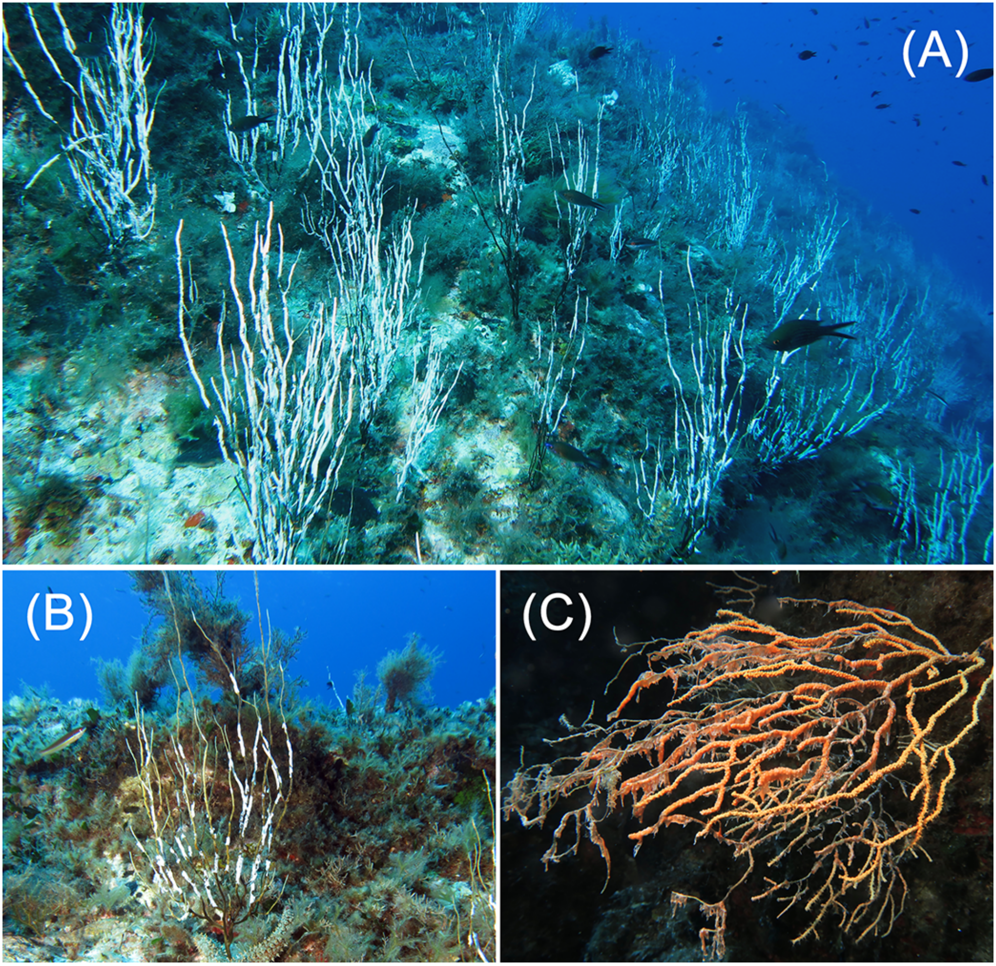


Figure 2. Examples of colonies of *Eunicella singularis* (A, B) and *E. cavolini* (C) at Montecristo Island suffering from 'rapid tissue loss'.

At Punta Forata, diseased colonies of both *E. singularis* and *E. cavolini* were observed down to 17 and 21 m depth respectively. These corresponded to water temperatures of 23 and 22 °C (Fig. 3a). At Punta del Diavolo affected colonies were observed down to 25 m for *E. cavolini*, and 30 m for *E. singularis*, and these correlated with temperatures of 21 and 19 °C, respectively (Fig. 3b).

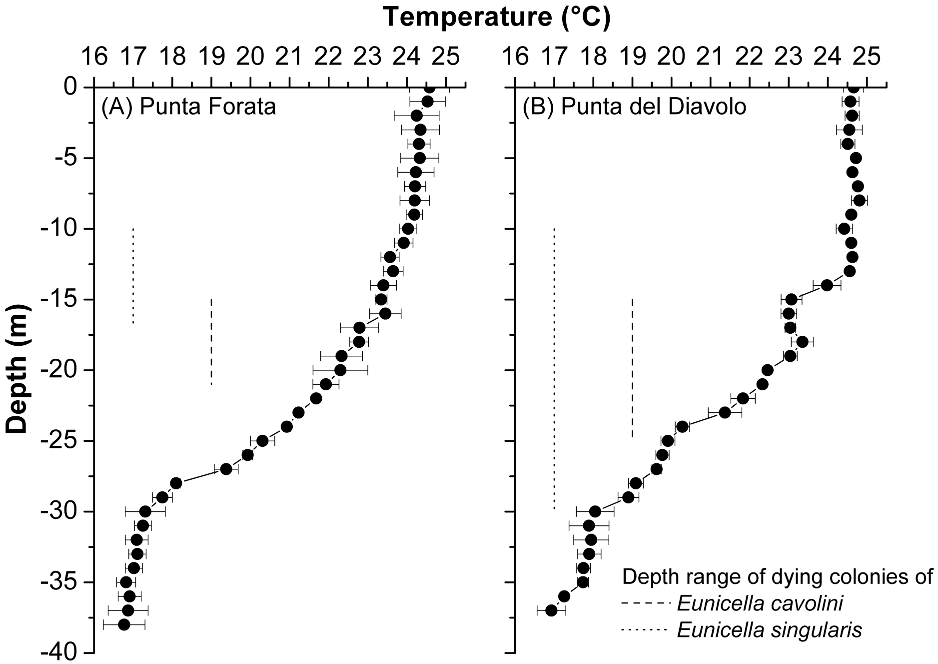


Figure 3. Vertical water temperature profiles and depth range associated with diseased colonies of *E. cavolini* and *E. singularis* at Punta Forata (A) and Punta del Diavolo (B) on the 5th of September 2017. In situ mean water temperatures (± standard errors) were obtained from two dive computers at 1 m depth intervals.

The vertical profiles of daily mean water temperature (collected from the 1st March to the 30th November 2017 at Montecristo Island), showed an anomalous thermocline formation (Fig. 4) when compared to the climatology for the same period between 1987 and 2015. In March 2017, water temperatures were almost 1 °C warmer than the climatological reference period from the surface down to 60 m depth. In April, this anomaly increased to 2.5 °C at 10 m depth and by the end of June the shallow waters rose to 3 °C above the average. From July the warming moved to greater depths until reaching the temperature anomaly of 4°C at 25–30 m depth at the beginning of September.

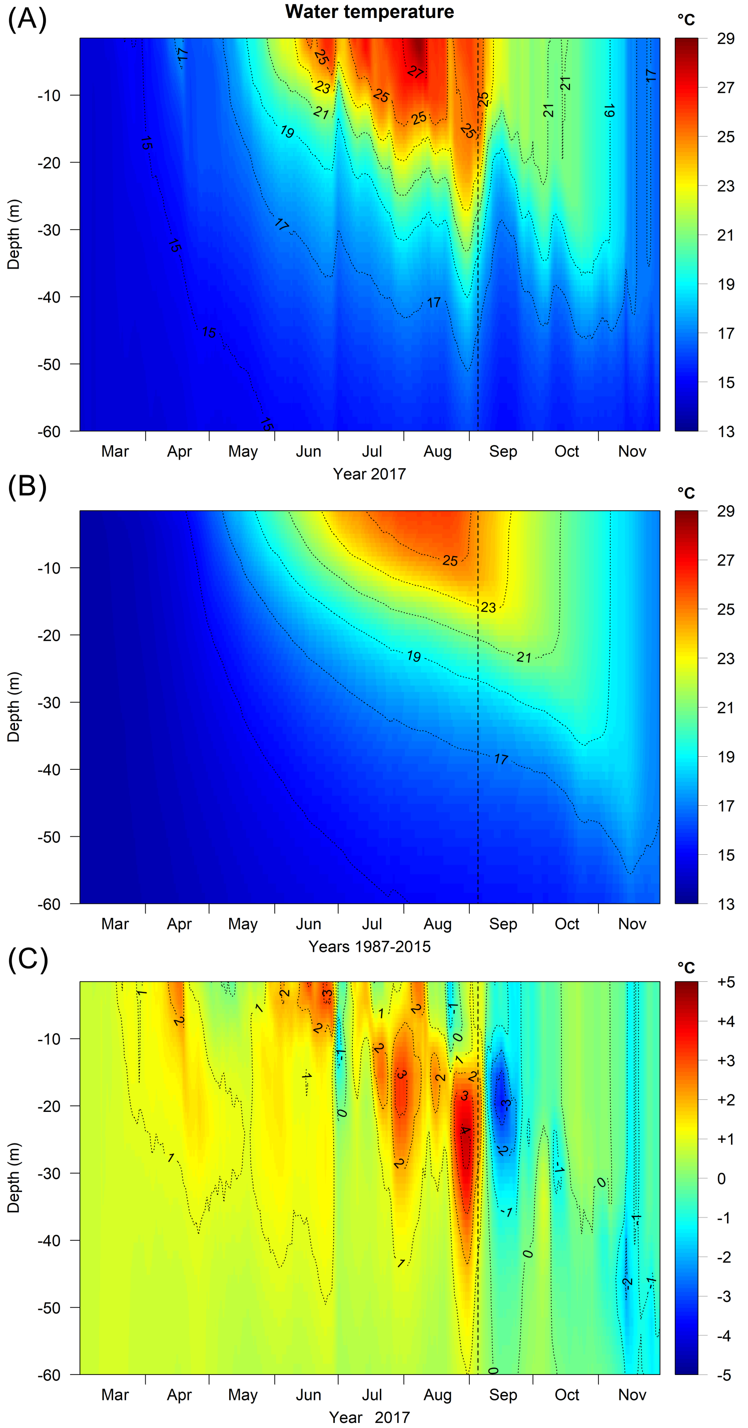


Figure 4. Vertical profiles of water temperature at Montecristo Island from 1st March to the 30th November: (A) daily averages from 2017 CMEMS analysis; (B) daily climatological values (1987-2015) from CMEMS reanalysis; (C) daily thermal anomalies in 2017. Dashed line indicates the surveys date.

Mass mortality events of gorgonians are not un-documented. Indeed such events have been recorded throughout the western Mediterranean over recent decades, as reported in Fig. 1 (see supplementary Table S1 and references therein for an updated review). The first documented case of mass mortality in gorgonians occurred in the north–western Mediterranean (the Gulf of Lion and Ligurian Sea) in the 1980s. And colonies of *P. clavata* and *E. singularis* were the most commonly affected species. However, *Eunicella* die offs were never reported at Montecristo Island, even during the most extensive mass mortality events, which occurred in the region in 1999 and 2003 (Cerrano et al. 2000, Garrabou et al. 2009).

It is generally agreed that thermal stress plays an important role in the onset of gorgonian mass-mortality events (e.g., Cerrano et al. 2000, Coma et al. 2009, Garrabou et al. 2009, Vezzulli et al. 2010, Calvo et al. 2011, Rivetti et al. 2014, Rubio-Portillo et al. 2016). Longer-lasting hotter summer periods result in positive seawater temperature anomalies, strong water column stratification and a decrease in the available oxygen for benthic organisms. These stressors have been shown to have a direct effect on the health of many benthic organisms including gorgonians (Cerrano et al. 2000, Cerrano & Bavestrello 2008, Garrabou et al. 2009). For example, during these periods gorgonians have been shown to exhibit reduced energetic reserves, a result likely due to investment in reproduction and the low food availability (Coma et al. 2009). Furthermore, due to physiological stress, the gorgonians may lose the capacity to regulate their microbiome through the production of antimicrobial and quorum-sensing interfering compounds (van de Water et al. 2018 and references therein). For example alteration of the microbiome to a pathobiome state has been well documented to result in the onset of disease in various marine organisms (Sweet & Bulling 2017). Indeed recent studies have shown that gorgonians harbour relative stable and core microbial communities (La Rivière et al. 2015, van de Water et al. 2017), which likely play important roles in the health and fitness of these organisms. However the true function of these core microbes remains to be fully understood (La Rivière et al. 2015 and references therein).

Although the purpose of this study was not to ascertain the causal agent of this die off – other studies have assessed possible causal agents of the previous events. For example, increases in microbes from the genus *Vibrio* (a commonly referenced marine pathogenic genus) have been found in diseased *P. clavata* and *E. singularis* when compared to healthy individuals from the same locations (Vezzulli et al. 2010, Rubio-Portillo et al. 2016). In another case a heterogeneous consortium of filamentous cyanobacteria (including representatives from the genera *Synechococcus*, *Arthrospira* and other unculturable species belonging to the order Oscillatoriales) were more prevalent in diseased colonies of *E. singularis* and *E. cavolini* (Carella et al. 2014) when compared to health individuals. Regardless of the causal agent or agents, the trend in epidemics and pandemics in both marine and terrestrial systems is worrying and any outbreak especially mass die offs warrants further study.

Gorgonian marine forests are species-rich habitats and therefore ecologically important (Ponti et al. 2014, 2016, Ponti et al. 2018). The increase in frequency, intensity and depth of thermal anomalies in these habitats is therefore alarming (Coma et al. 2009, Calvo et al. 2011, Rivetti et al. 2014). However, it is often argued that the resulting decline in reef health (cold water, temperate or tropical) is from a combined effect of climate change and other more local stressors. In order to attempt to untangle such a complex network of stress effects it is essential that reliable baselines are available for any given ecosystem (Harvell et al. 2002). Especially at sites where other anthropogenic stressors (non-climate related) are reduced or non-existent, i.e. location such as the likes of Montecristo Island.

Such baseline studies, coupled with the advent of CMEMS and the availability of real-time analysis, forecasting and reanalysis products for the whole Mediterranean Sea, may allow the development of an efficient temperature-based disease surveillance tool (Maynard et al. 2016). Access to high spatial resolution oceanographic data as well as a 10-day forecast capability, means an early warning system for the most sensitive of locations can be put in place, especially where these marine forests exist, such as the one studied here. Such a tool should likely include other variables, which may shift in the face of climate change. For example, oxygen levels, salinity, turbidity and availability of plankton biomass. The involvement of citizen scientists could also be utilized to develop such a management tool (Cerrano et al. 2017b, Di Camillo et al. 2018).

In conclusion, we highlight a new mass mortality die off of gorgonian forests in the Mediterranean Sea and indicate that thermal anomalies are likely key triggers in the onset of the disease in this instance. We also illustrate that with a better understanding of the environmental conditions and other stressors (which may lead to future disease outbreaks) an ecosystem-based management plan can be adopted which will hopefully prevent or mitigate the ecological damage caused by loss of these marine forests.

## Acknowledgements

We thank Daniela Pica, Ubaldo Pantaleo, Chiara Luciani and Pier Giacomo De Cecco who attended the underwater expedition supported by the University of Bologna and Reef Check Italia *onlus*, and authorised by Tuscan Archipelago National Park (Permission #345/2017). This study is part of ET PhD and it has been conducted using E.U. Copernicus Marine Service Information.

# Supplementary material Chapter 3

## Video supplementary

Video clip showing seascape of affected gorgonian colonies and their detaching coenenchyme (www.int-res.com/articles/suppl/d131p079_supp/).

## S1 Updated chronology of gorgonians mass-mortality events

Table S1. Updated chronology of mass mortality events hitting gorgonians in the western Mediterranean Sea (modified and updated from Calvo et al. 2011).

| **Year** | **Locations** | **Scale** | **Depth range (m)** | **Species** | **References** |
|---|---|---|---|---|---|
| 1983 | La Ciotat (Ligurian Sea) | Local | 0 to 20 | *Eunicella singularis*<br>*Corallium rubrum* | Harmelin 1984 |
| 1986 | Portofino Promontory (Ligurian Sea) | Local | 0 to 20 | *Eunicella cavolini* | Bavestrello & Boero 1988 |
| 1989 | Montecristo Island (Tyrrhenian Sea) | Local | - | *Paramuricea clavata* | Guldenschuh in Bavestrello et al. 1994 |
| 1992 | Medes Islands (north-western Mediterranean Sea), Port-Cros National Park | Local | 0 to 14<br>10 to 45 | *Paramuricea clavata* | Coma & Zabala 1992<br>Harmelin & Marinopoulos 1994 |
| 1993 | Strait of Messina (Tyrrhenian Sea), Portofino Promontory (Ligurian Sea) | Local | 20 to 39 | *Paramuricea clavata* | Mistri & Ceccherelli 1996a<br>Bavestrello et al. 1994 |
| 1999 | Coast of Provence and Ligurian Sea, Balearic Islands (north-western Mediterranean Sea), Gulf of La Spezia, Port-Cros National Park, coast of Calafuria (Tyrrhenian Sea) | Regional | 0 to 45 | *Paramuricea clavata*<br>*Eunicella singularis*<br>*Eunicella cavolini*<br>*Eunicella verrucosa*<br>*Corallium rubrum*<br>*Leptogorgia sarmentosa* | Cerrano et al. 2000<br>Perez et al. 2000<br>Garrabou et al. 2001<br>Linares et al. 2005<br>Bramanti et al. 2005<br>Coma et al. 2006a<br>Cupido et al. 2008<br>Crisci et al. 2011 |
| 2001 | Tavolara Island (Tyrrhenian Sea) | Local | 10 to 45 | *Paramuricea clavata*<br>*Eunicella cavolini* | Calvisi et al. 2003 |

| Year | Locations | Scale | Depth range (m) | Species | References |
|---|---|---|---|---|---|
| 2002 | Ischia and Procida Islands (Tyrrhenian Sea) | Local | 15 to 20 | *Paramuricea clavata*<br>*Eunicella singularis* | Gambi et al. 2006 |
| 2003 | Gulf of Genova, Provence coast, Corsica−Sardinia, Gulf of Naples, Balearic Islands, Catalan coast, Medes Islands, Gulf of La Spezia | Regional | 0 to 40 | *Paramuricea clavata*<br>*Eunicella singularis*<br>*Eunicella cavolini*<br>*Eunicella verrucosa*<br>*Corallium rubrum*<br>*Leptogorgia sarmentosa* | Bally & Garrabou 2007<br>Gambi et al. 2006<br>Cupido et al. 2008<br>Garrabou et al. 2009<br>Bensoussan et al. 2010<br>Crisci et al. 2011<br>Sini et al. 2015 |
| 2005 | Ischia and Procida Islands (Tyrrhenian Sea) | Local | 16 to 22 | *Eunicella cavolini*<br>*Eunicella singularis* | Cigliano & Gambi 2007 |
| 2006 | Coast of Provence (north-western Mediterranean Sea) | Local | 20 | *Paramuricea clavata* | Bensoussan et al. 2010 |
| 2007 | Cabo de Palos-Cabrera Island (north-western Mediterranean Sea) | Local | 0 to 45 | *Eunicella singularis* | Coma et al. 2007 |
| 2008 | Tavolara Island (Tyrrhenian Sea) | Local | 15 to 43 | *Paramuricea clavata* | Vezzulli et al. 2010<br>Huete-Stauffer et al. 2011 |
|  | Grotta Azzurra, Palinuro (Tyrrhenian Sea) | Local | 15 to 20 | *Eunicella cavolini* | Gambi et al. 2010 |
|  | Gulf of Naples (Tyrrhenian Sea) | Local | 18 | *Eunicella cavolini*<br>*Eunicella singularis* | Carella et al. 2014 |
| 2009 | Gulf of Naples (Tyrrhenian Sea) | Local | 28 | *Eunicella cavolini*<br>*Eunicella singularis*<br>*Paramuricea clavata* | Gambi et al. 2010<br>Carella et al. 2014 |
| 2011 | Pantelleria Island (Strait of Sicily) | Local | >60 | *Paramuricea clavata* | Vezzulli et al. 2013 |
| 2015 | Tabarca, Spain (south-western Mediterranean Sea) | Local | 20 to 25 | *Eunicella singularis*<br>*Leptogorgia sarmentosa* | Rubio-Portillo et al. 2016 |

| Year | Locations | Scale | Depth range (m) | Species | References |
|---|---|---|---|---|---|
| 2017 | Montecristo Island (Tyrrhenian Sea) | Local | 10 to 30 | *Eunicella cavolini*<br>*Eunicella singularis* | present study |
| | Ischia Island (Tyrrhenian Sea) | Local | 15 to 22 | *Eunicella cavolini*<br>*Eunicella singularis* | Gambi et al. 2018 |
| | Banyuls sur mer, Cap de Creus (north-western Mediterranean Sea) | Local | | *Eunicella singularis* | L. Bramanti pers. com. |
| | Tavolara Island (Tyrrhenian Sea) | Local | 20-30 | *Eunicella cavolini*<br>*Eunicella singularis*<br>*Paramuricea clavata* | M. Romor pers. com. |
| | Strait of Messina (Tyrrhenian Sea) | Local | | *Paramuricea clavata* | F. Turano et al. (Internet social networks) |

# Chapter 4

## X-ray Computed Tomography to assess long term bio-construction and erosion processes in northern Adriatic coralligenous habitats

## 4.1 | Introduction

The coralligenous biogenic reefs are among the richest, diverse and threatened habitats in the Mediterranean Sea. These reefs are the result of the interplay between the building activities, mainly sustained by Encrusting Calcareous Rhodophytes (ECRs), and physical and biological eroding processes (Ballesteros 2006, Ingrosso et al. 2018). Coralligenous habitats are considered 'hot spot' of species diversity, shaped by geological events and climate changes (Boudouresque 2004, Coll et al. 2010). These habitats support ecological processes that lead to the formation of their assemblages and the maintenance of their diversity (Ballesteros 2006). Bioconstruction and bioerosion are key processes in mesophotic temperate reefs and act at different spatial and temporal scales. The net reef accretion depends on building process of reef calcification and the eroding process of reef dissolution and bioerosion (i.e. the erosion of hard substrate by living organisms; Neumann 1966). For reef to persist, the building process must exceed bioerosion rate (Garrabou & Ballesteros 2000). ECRs, at the end of their life cycle, contributed to sediments and limestone production by diagenesis and lithification, providing the greatest contribution to coralligenous bioconstruction (Ballesteros 2006, Ingrosso et al. 2018). Scleractinian corals, bryozoans, serpulids polychaetes and bivalves also play a role in the building process. Bioerosion generally comprises a suite of endolithic organisms, including both microborers (i.e. cyanobacteria, algae, and fungi; Glynn 1997, Pica et al. 2016) and macroborers (i.e. sponges, polychaetes, and bivalves; Cerrano et al. 2001, Calcinai et al. 2011, Casoli et al. 2016). Bioerosion process shows a temporal succession that begins with microborer communities, which usually take less than 1 year of exposure to establish (Tribollet & Golubic 2005, Farber et al. 2015, Grange et al. 2015), and continues with macroborer communities, which can develop a community after 2-3 years (Kiene & Hutchings 1994, Pari et al. 2002). Few long-term (~10 years) experiments have been conducted to study the bioerosion processes from tropical (Scott et al. 1988, Kiene & Hutchings 1994) to temperate sea (Farber et al. 2016). However, none of them has investigated simultaneously the variability of both bioconstruction and bioerosion rates among different assemblages using experimental approaches.

The northern Adriatic continental shelf host a large number of mesophotic biogenic reefs, spread on the sedimentary bottom (Ponti et al. 2011a). These biogenic reefs generally build above hard bottoms, which probably originates from the consolidation of sediments by cementation of calcium carbonate bound to methane and bacteria, nevertheless their primary origin is still debated (Gordini et al. 2012, Donda et al. 2015, Tosi et al. 2017). North Adriatic mesophotic coralligenous habitats grow between 15 and 40 m depth in dim light condition due to the high mean water turbidity characterising the area (Aubry et al. 2004, Falace et al. 2015). Their size range from few to thousands square meter, while the distance among outcrops from few meters to tens of kilometres. The diversity of assemblages of coralligenous outcrops in the northern Adriatic Sea has been documented in recent studies (e.g. Casellato et al. 2007, Ponti et al. 2011a, Falace et al. 2015). These habitats are characterised by high spatial heterogeneity and low temporal variability (Ponti et al. 2011a). A recruitment experiment shows that the species involved in the early colonisation stage largely depend on larvae availability and environmental condition in the area (Fava et al. 2016). Ten months after the deployment of tiles, pioneer species were gradually replaced or overgrown by long-lived slow-growing species, which contribute to increase the species richness, complexity and structural features of the assemblages. In this phase, the colonisation of travertine tiles mainly occurred via lateral encroachment, especially by sponges and colonial ascidians (Fava et al. 2016).

These assemblages are threatened by several anthropogenic disturbances, including several dystrophic crises mostly related to eutrophication caused by wastewater discharge (Zuschin & Stachowitsch 2009, Tomasovych et al. 2017), litter and intense trawling activities (Kollmann & Stachowitsch 2001, Melli et al. 2017) that can decrease the species diversity, inhibiting the ECRs growth and increasing the bioerosion rate (see Ballesteros 2006, Ingrosso et al. 2018 and references therein).

The study of the microscale bioconstruction and bioerosion processes together requires sophisticated investigation techniques (Silbiger et al. 2016). Investigation of boring organisms requires the analysis of the shape of holes and cavities hidden inside the substrates, and the signs left by organisms, of which most of the tissue vanishes after dead. High resolution Computed Tomography (CT), offers new opportunities to visualise the internal bioerosion and external morphology of the bioconstructions and to be applied in ecological researches (Gutierrez et al. 2018). This technology is widely used in medical, archaeological, geological and industrial applications, but the ecological quantitative application on bioconstruction and bioerosion processes are still scarce (e.g. Laforsch et al. 2008, Silbiger et al. 2014, Farber et al. 2016, Silbiger et al. 2016). CT is a non-destructive approach that combines the use of X-rays and computerised analysis of the images allowing the generation of a virtual 3-Dimensional (3D) volume reconstruction of the object and the investigation of the inner and outer structure of biogenic substrates at very fine scale.

The role of bioconstruction and bioerosion processes in the formation and maintenance of the heterogeneity of the northern Adriatic Sea coralligenous assemblages has been investigated in a long-term experiment (i.e. 3 and 12 years) by deploying travertine tiles on the three main typology of assemblages found (Ponti et al. 2011a, Fava et al. 2016) and applying a novel non-destructive approach (i.e. X-ray CT) to assess the bioconstruction-bioerosion net balance.

## 4.2 | Materials and Methods

### 4.2.1 Study area and experimental design

The heterogeneity of coralligenous assemblages off Chioggia and Venice (North Adriatic Sea) has been documented and according to the abundance of ECRs, algal turf, sponges and colonial ascidians, 3 main typologies of benthic assemblages have been recognised in Ponti et al. 2011a. In August 2005 experimental travertine tiles were deployed at 3 sites (i.e. MR08, P204 and P213, Fig. 1A and Fig. 1B), one site for each of the 3 typologies of assemblages founded (the description of the experiment is provided in Fava et al. 2016). The travertine is a commercially available limestone characterised by high porosity (mean porosity of the employed material 7%; Fava et al. 2016), which may provide a suitable substrate for many species living in the area. To quantify the bioconstruction and bioerosion processes a subset of the travertine tiles (4 tiles × 3 sites × 2 periods = 24 tiles) were retrieved in August 2008 and 2017, 3 and 12 years after deployment, respectively. Photographic samples (Fig. 1C) of each tile underwater were collected using Canon PowerShot G15 digital camera, equipped with an aluminium underwater case and an S-TTL strobe (INON D2000). Photographic sample of each tile were also collected in the laboratory before and after rinsing with fresh water and removing of soft epiliths. Spicular samples were collected to identify the sponge species present in the tiles. The tiles were then dried at 80 °C for 24 hours.

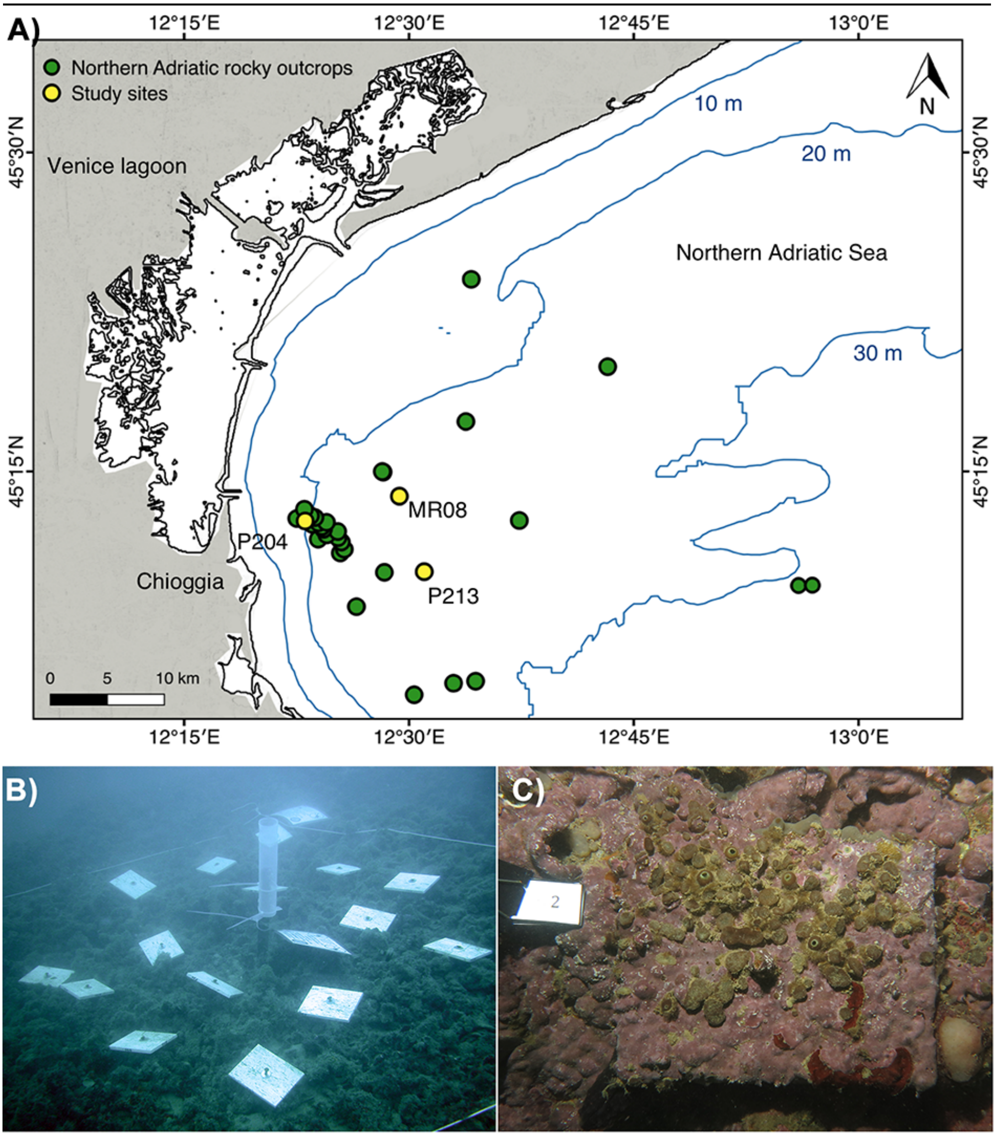


Figure1. Sites location and experimental setup. (A) Map of the study area showing the three experimental sites, yellow dots, and the northern Adriatic Sea outcrops out off Chioggia and Venice, green dots (Mercator Projection, Datum WGS 84); (B) example of a plot of travertine tiles; (C) example of a photographic sample of one tile underwater.

### 4.2.2 High-resolution Computed Tomography

The volume of construction and erosion by organisms in the 24 tiles were calculated using high-resolution Computed Tomography (CT).

Tiles were scanned using an experimental CT system composed by the X-ray radiation source (X-ray tube Kevex PXS10-65W), the X-ray flat-panel digital detector (VARIAN PS2520D) with a pixel size of 127μ, and the precision rotation stage (Physic Instrumente M-038). An X-ray source voltage of 130 kV, a current of 50 μA, and a pre-filter of 3 mm of aluminium were applied. Angular projections were acquired in a full 360° rotation in 0.4° increments. Images were reconstructed using PARREC software (i.e. developed by the X-ray Imaging group of the University of Bologna, Brancaccio et al. 2011). The resulting voxel (vx) size was 0.1 mm.

Calcareous deposits and holes were discriminated according to their shape and density. In order to measure the volume of each portion, the images were manually segmented, using the brush tool (Segmentation Editor) and the volumes were quantified with the Material Statistics module available in the AMIRA software (v. 5.0, Mercury Computer Systems, Inc., USA).

### 4.2.3 Data analyses

The percentages of bioeroded and built volumes ascribable to each taxon were calculated with respect to the initial volume of the tiles, taking into account the natural fractures of the travertine. Not having CT images before the experiment, the travertine natural fractures inside each tile were assessed at the end of the exposure time within less eroded portions, and assumed similar in the whole tile. However, being the natural fractures a possible preferential corridor for borer organisms, this method may lead to a slight underestimation of the pre-existing cavities.

Differences in percentage of bioeroded and built volumes of each taxon among sites (3 levels, fixed) and between exposure time (2 levels, fixed) were assessed by two-way analysis of variance (ANOVA, $\alpha = 0.05$; Underwood 1997). The homogeneity of variances was assessed by Cochran's C test, and data were transformed if required.

Multivariate spatial distribution and temporal variation of the build and eroded volumes by different taxa were analysed using the principal coordinate analysis (PCoA, i.e. metric multidimensional scaling) based on Bray-Curtis similarity of square root transformed data (Gower 1966, Clarke 1993). Distance-based two-way permutational multivariate analysis of variance (PERMANOVA; Anderson & Robinson 2001, Anderson & ter Braak 2003) was used to test for multivariate differences of build and eroded volumes by different taxa among sites (3 level, fixed) and between exposure times (2 level, fixed). Significant results were further analysed by *a posteriori* PAIR-WISE tests.

The mean built and erosion annual rate (% $yr^{-1}$) were calculated both for 3-year and 12-year exposure rime.

## 4.3 | Results

### 4.3.1 Bioerosion

In the travertine tiles deployed in the northern Adriatic Sea the volumes eroded by boring organisms varied among sites and with the exposure period, on average the mean total eroded volumes were 7.27 ± 2.21% (s.e.) and 46.32 ± 3.54% (s.e.) at MR08 site, after 3 and 12 years of deployment, respectively; 2.84 ± 0.85% (s.e.) and 28.73 ± 7.21% (s.e.) at P204 and 1.83 ± 0.67% and 14.78 ± 7.39% (s.e.) at P213 (Fig. 2A). The most effective borer was the sponge *Cliona viridis* at all study sites (e.g. Fig. 2B after 3 years of exposure and Fig. 2C after 12 years of exposure). ANOVA tests detect a significant effect of the exposure time, both in total eroded volume and in volume eroded by *Cliona viridis* (Tab. 1). In both cases, the highest bioerosion was in tiles with 12-year exposure time. The volume eroded by *Rocellaria dubia* (e.g. Fig. 2D in a tile surround by *C. viridis* chambers and Fig. 2E a particular of *R. dubia* cavities) and its abundance was significantly different among sites (Tab. 1). *Cliona rhodensis* was found just in just one tile at MR08 site with 12-year exposure time (Fig. 2A).

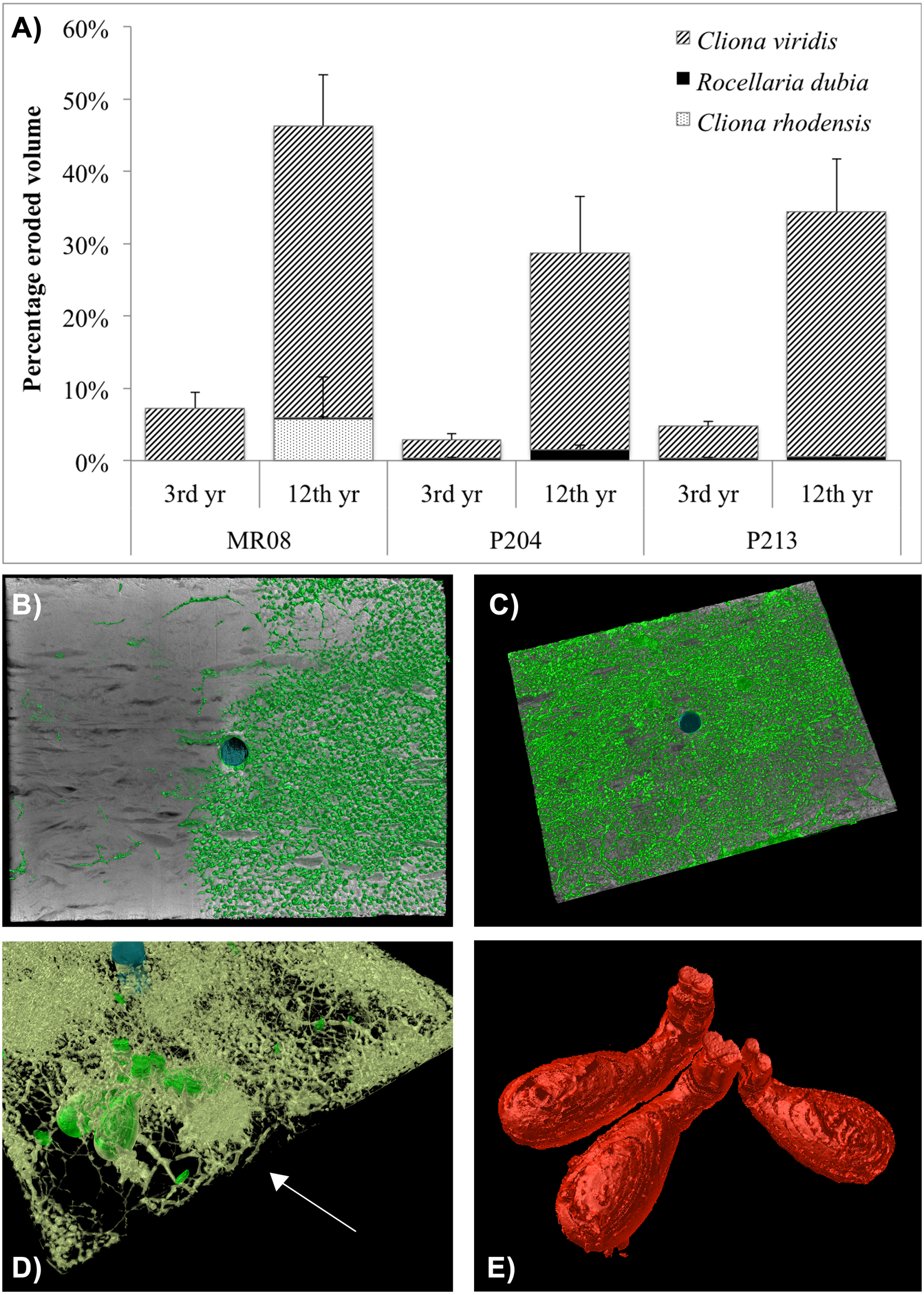


Figure 2. Eroded volume by taxa and CT visualisation. (A) Mean percentage eroded volumes by taxa (± s.e.); (B) 3-years tile with *Cliona viridis* eroded volume pattern; (C) 12-years tile with *C. viridis* eroded volume pattern; (D) 12-years tile with *C. viridis* volume pattern enclose the *Rocellaria dubia* eroded volume pattern showed by the row; (E) *R. dubia* volume particular.

Table1. Results from ANOVA on percent volumes bioeroded and built

| Source | *df* | Total eroded volume | | | | *Cliona viridis* chambers | | | | *Rocellaria dubia* chambers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | *p* | | MS | *F* | *p* | | MS | *F* | *p* | !! |
| Site (Si) | 2 | 0.027 | 3.22 | 0.0636 | ns | 0.017 | 1.47 | 0.2573 | ns | 0.007 | 6.21 | 0.0089 | "" ! |
| Exposure time (Et) | 1 | 0.634 | 75.95 | 0.0000 | *** | 0.543 | 47.76 | 0.0000 | *** | 0.005 | 4.32 | 0.0524 | #$! |
| Si × Et | 2 | 0.009 | 1.04 | 0.3748 | ns | 0.004 | 0.38 | 0.6860 | ns | 0.001 | 1.22 | 0.3192 | #$! |
| Res | 18 | 0.008 | | | | 0.011 | | | | 0.001 | | | |
| Transf. | | None | | | | None | | | | Sqrt | | | |
| SNK test | | 12th > 3rd | | | | 12th > 3rd | | | | P204 = P213 > MR08 | | | |

| Source | *df* | Overall built volume | | | | Encrusting organisms volume | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | *p* | | MS | *F* | *p* | |
| Site (Si) | 2 | 0.084 | 8.66 | 0.0023 | ** | 0.090 | 11.47 | 0.0006 | *** |
| Exposure time (Et) | 1 | 0.043 | 4.43 | 0.0497 | * | 0.043 | 5.53 | 0.0303 | * |
| Si × Et | 2 | 0.079 | 8.14 | 0.0030 | ** | 0.070 | 9.01 | 0.0019 | ** |
| Res | 18 | 0.010 | | | | 0.008 | | | |
| Transf. | | Sqrt | | | | Sqrt | | | |
| SNK test | | 3rd Si × Et: MR08 = P204 = P213<br>12th Si × Et: MR08 > P204 = P213<br>MR08 Si × Et: 12th > 3rd<br>P204 = P213 Si × Et: 12th = 3rd | | | | 3rd Si × Et: MR08 = P204 = P213<br>12th Si × Et: MR08 > P204 = P213<br>MR08 Si × Et: 12th > 3rd<br>P204 = P213 Si × Et: 12th = 3rd | | | |

Significant levels were indicated by the following symbols: ns = not significant,

* = $p < 0.05$;

** = $p < 0.01$;

*** = $p < 0.001$

### 4.3.2 Bioconstruction

The mean built volumes were 13.92% ± 2.87% (s.e.) and 45.57% ± 8.43% (s.e.) at MR08 site, after 3 and 12 years of exposure, respectively, 15.78% ± 5.05% (s.e.) and 8.71% ± 2.51% (s.e.) at P204 and 10.30% ± 2.14% and 14.18% ± 3.62% (s.e.) at P213 (Fig. 3A). The main contributors to calcium carbonate deposition were a pool of organisms not distinguishable in CT images due to the interpenetrated system they develop. They were collectively categorised as 'encrusting organisms', and include ECRs (Fig. 3B), serpulid polychaetes (Fig. 3C), bryozoans, barnacles and the bivalve *Anomia ephippium*. The other two identified categories were oysters (Fig. 3D), only found at P204 site, and vermetids (Fig. 3E), only found at MR08 site. The ANOVA tests detect an interaction across sites and exposure time for both overall built volume and volume built by encrusting organisms (Tab. 1). The post-hoc SNK tests revealed that the overall built volume and volume built by encrusting organisms were different between 3 and 12 years of exposure time only at MR08 site. Furthermore, at 3-year exposure time, these two categories were not different among sites, while at 12-year exposure time they were different at MR08 compared to the other two sites (Tab. 1).

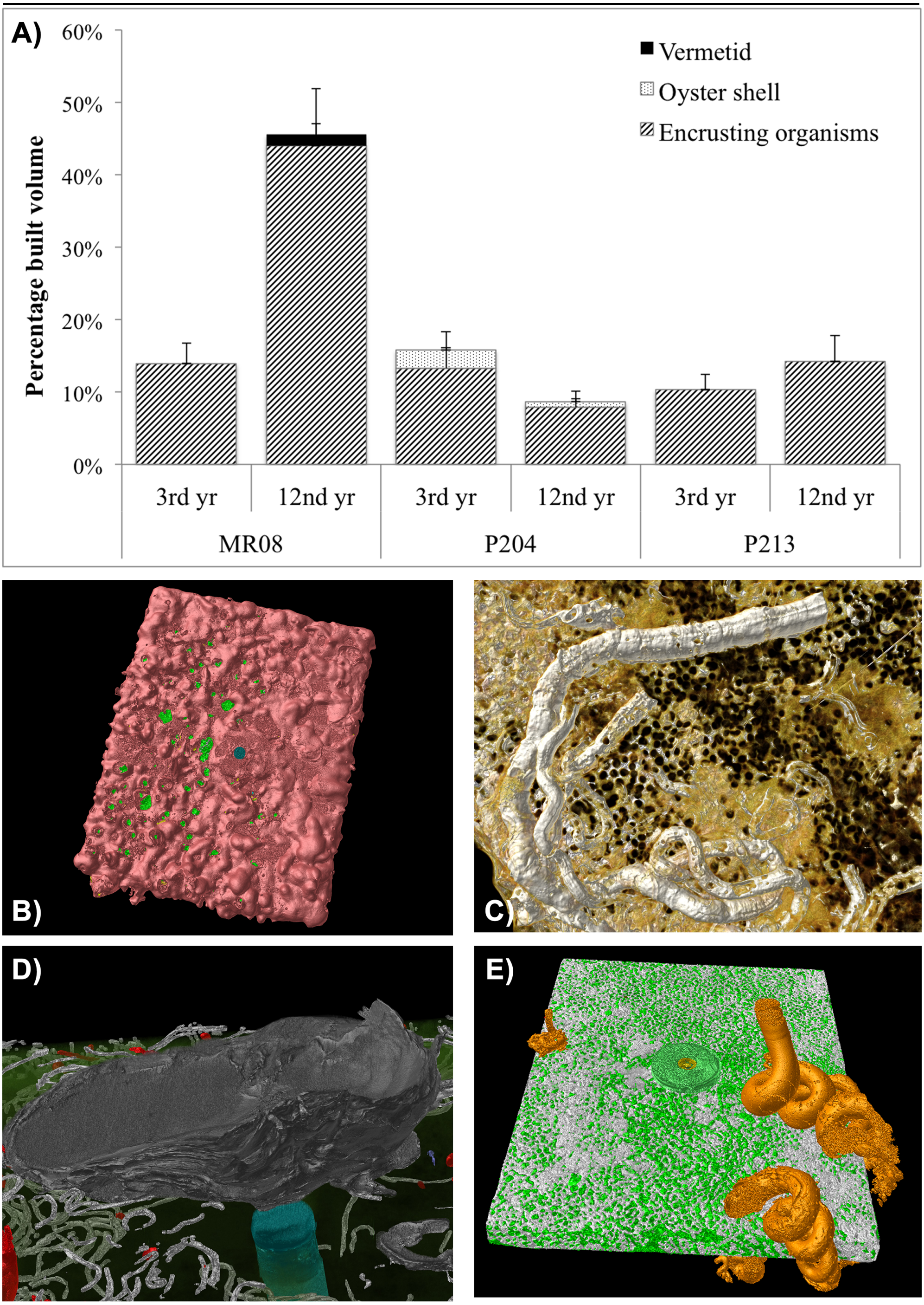


Figure 3. Constructed volume by taxa and CT visualisation. (A) Mean percentage bioconstructed volumes by taxa (± s.e.); (B) 12-years tile with ECRs in pink and *C. viridis* oscula holes in green; (C) 3-years tile with a particular of serpulid polychaetes calcium carbonate deposition; (D) 12-years tile with an oyster shell on top; (E) 12-years tile with vermetid shells pattern in orange and *C. viridis* volume in bright green, while at the tile centre, it is visible in green the metallic wheel that was embedded by bioconstruction.

## 3.3 Spatial-temporal variability of bioconstruction and bioerosion

Despite the high variability at local scale, pattern of similarity among the volumes eroded and built by different taxa were strongly affected by differences between exposure times (mainly driving differences along PCoA plot axis1 that explain 60.1% of the total variation) rather than among sites, and confirmed by the PERMANOVA test (Fig. 4, Tab. 2). The PAIR-WISE test revealed that bioerosion and bioconstruction processes were different between 3 and 12 years of exposure time at all sites. However, at 3-year exposure time, these processes were not different among sites, while at 12-year exposure time they were different at MR08 compared to the other two sites (Tab. 2).

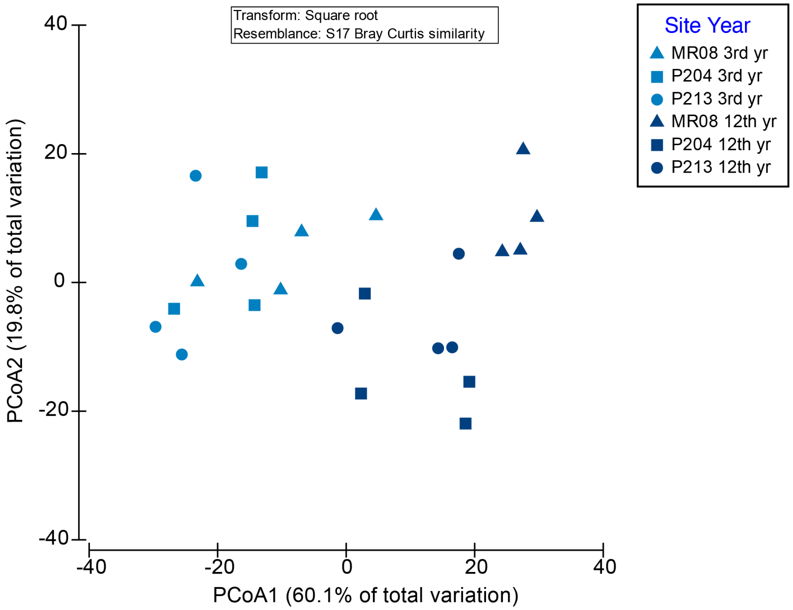


Figure 4. Similarities between volumes built and eroded on different sites and years of exposure. PCoA unconstrained ordination plot based on Bray-Curtis similarity of square root transformed volume percent data. Symbols represent sampling sites, while colours the years of exposure.

Table 2. Results from PERMANOVA based on Bray-Curtis dissimilarities of squared root transformed percent volumes bioeroded and bioconstructed data

| Source | *df* | SS | MS | Pseudo-F | *P* (perm) | | Unique perms |
|---|---|---|---|---|---|---|---|
| Site (Si) | 2 | 2'336.50 | 1'168.20 | 4.7602 | 0.0004 | ** | 9936 |
| Exposure time (Et) | 1 | 6'855.70 | 6'855.70 | 27.934 | 0.0001 | ** | 9938 |
| Si × Et | 2 | 1'100.20 | 550.08 | 2.2414 | 0.0424 | * | 9941 |
| Res | 18 | 4'417.60 | 245.42 | | | | |
| Total | 23 | 14'710.00 | | | | | |
| Transf. | | Sqrt | | | | | |
| PAIR-WISE test | | 3rd Yr × Si: MR08 = P204 = P213 | | | | | |
| | | 12th Yr × Si: MR08 > P204 = P213 | | | | | |
| | | (MR08, P204, P213) Si × Yr: 12th > 3rd | | | | | |

Significant levels were indicated by the following symbols: ns = not significant, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

### 4.3.4 Net balance

The mean erosion rate appeared to increase with the exposure time from 1.33% ± 0.34% $yr^{-1}$ in the 1st 3-year period to 3.04% ± 0.33% $yr^{-1}$ in the second 9-year period (i.e., between 3rd year and 12th year, Fig. 5A). While, the mean construction rate appeared to decrease with the exposure time from 4.44% ± 0.66% $yr^{-1}$ in the first 3-year period to 0.95% ± 0.19% $y^{-1}$ in the second 9-year period at the sites P204 and P213, while it appears to increase to 3.80% ± 0.70% $y^{-1}$ at the site MR08 (Fig. 5B).

The bioconstruction-bioerosion net balance was positive in the 3-year period, while negative in the 12-year period (Fig. 5C) of exposure. It significantly differs between years but not among sites (ANOVA test, Si: $p > 0.05$; Yr: $p < 0.01$; Si×Et: $p > 0.05$).

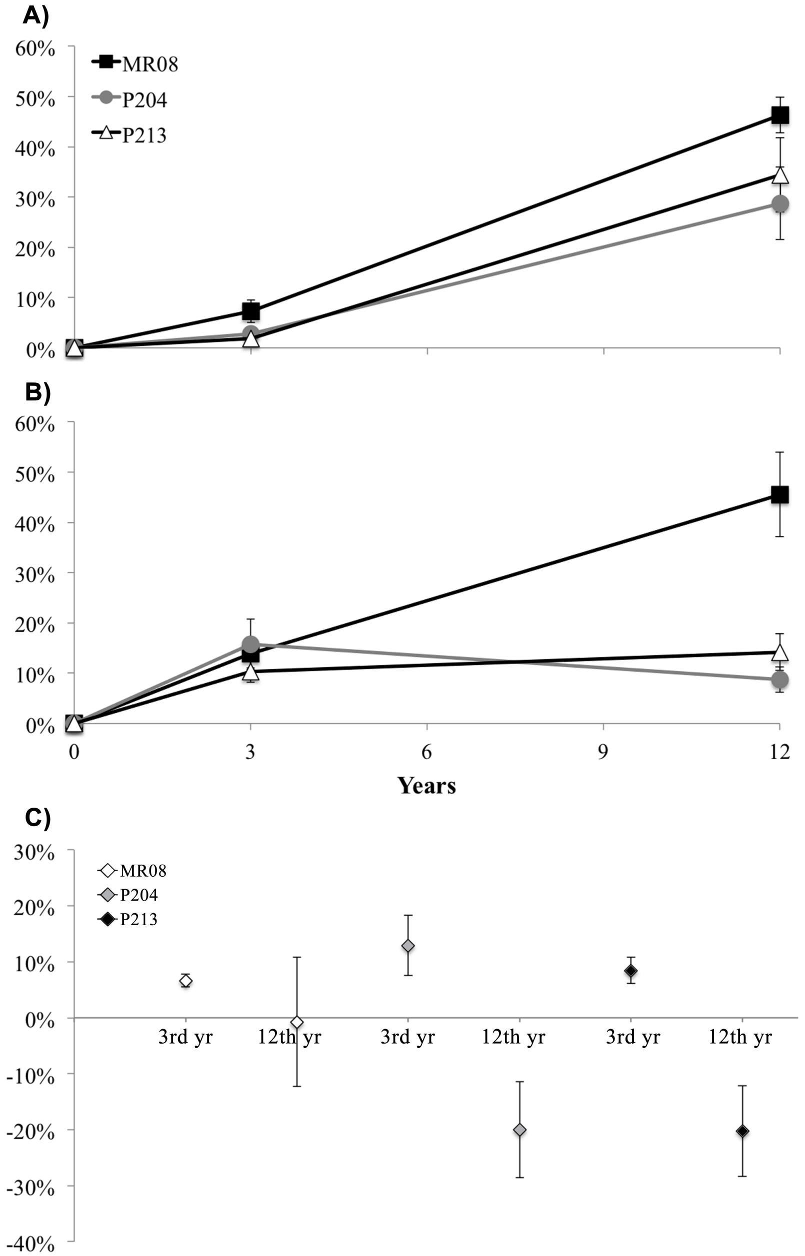


Figure 5. Temporal trend of bioeroded volumes (A) and bioconstructed volumes (B) among sites (± s.e.); (C) bioconstruction-bioerosion net balance (± s.e.) among sites after 3 and 12 years of exposure.

## 3.4 CT images: an in depth analysis

Besides estimating volumes, the CT technique offers novel perspectives in the study of bioconstruction and bioerosion process allowing for not only identify and measure every depositional structure and inner cavity but also analyses depositional sequences and possible interactions among species. As an example we may consider a specific tile collected at the MR08 site after 12 years of exposure (Fig. 6A). The main borers were the sponges *Cliona viridis* (22.01 %, Fig. 6B) and *Cliona rhodensis* (23.08 %, Fig. 6B), of which the eroding patterns are clearly recognisable in the 3D images rendering. Both sponges have distinguishable papillae protruding from the limestone, greenish-brownish in *C. viridis* and reddish in *C. rhodensis*, but most of them were covered by another sponge, *Tedania* (*Tedania*) *anhelans*, as visible in the picuture taken in the wild before tile retrieving (Fig. 6A). The CT picture allows seeing the two boring sponges fighting each other competing for substrate. In the meantime, the leading builders were the encrusting organisms (46.05%, in this case, mainly composed by a layer of ECRs, Fig. 6C, over serpulid polychaetes, ET personal observation) and vermetids (0.52%, Fig. 6D). Despite the vermetid shells were completely covered by late successional coralline algae and their volumes represent less than 1% of the whole bioconstruction, their morphology is distinguishable through the CT 3D image reconstruction (Fig. 6D). This is just an example of how CT may offer new perspectives and insight into understanding and representing ecological processes.

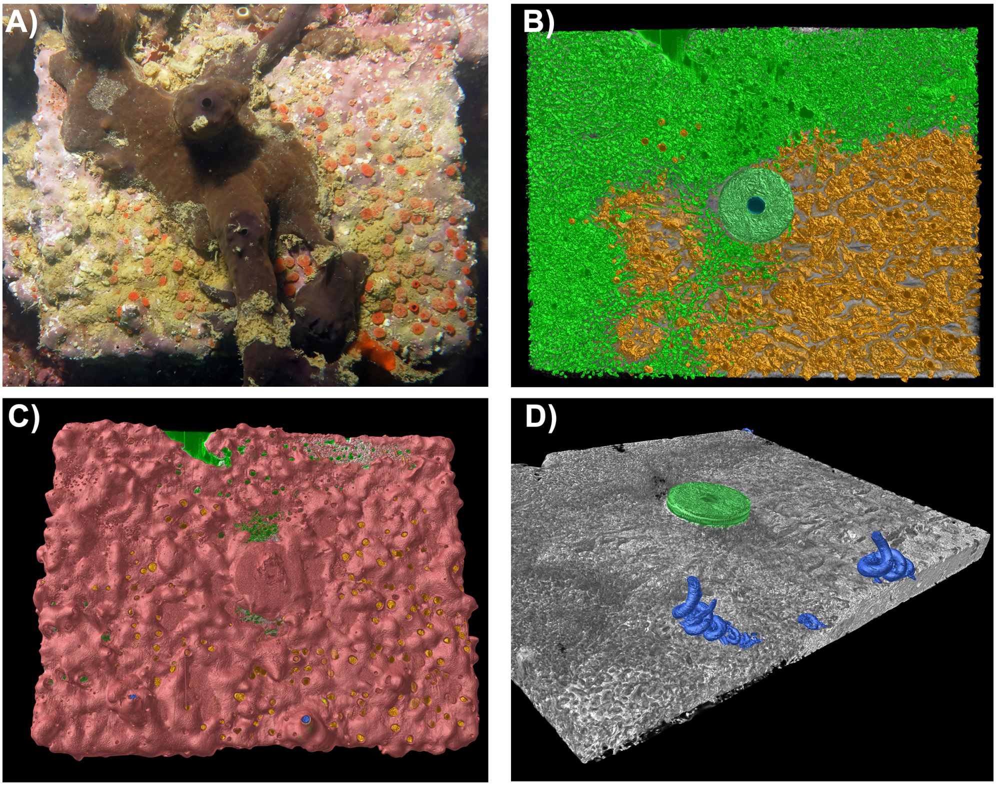


Figure 6. High resolution computed tomography visualisation of bioconcretions and bioerosion traces in an experimental tile deployed for 12 years at site MR08. (A) a photographic sample of the tile underwater before collecting. On the right side and partly in the bottom left, the *Cliona rhodensis* orange papulae are clearly visible, while on the left side and cover by the sponge *Tedania anhelans*, *Cliona viridis* greenish-brownish papulae are present; (B) 3D visualisation of internal tile bioerosion volumes and traces of *C. viridis* in bright green and *C. rhodensis* in orange; (C) encrusting organisms visualisation in pink; (D) vermetid shells volumes in blue on the top of the tile.

## 4.4 Discussions

In the northern Adriatic mesophotic biogenic reefs, three main typologies of epibenthic assemblages have been described (Ponti et al. 2011a, Curiel et al. 2012, Falace et al. 2015). Their species diversity and resilience appeared largely entrusted to recruitment of long-lived species (e.g., encrusting calcareous rhodophytes, sponges and colonial ascidians), characterised asexual reproduction (Fava et al. 2016). However, the long-time persistence of these mesophotic biogenic reefs emerging from sedimentary bottoms should rely on the dynamic equilibrium between bioconstruction and erosion processes. In these horizontal substrates, the net bioconstruction should also exceed the siltation rate. In the present experiment, a positive net construction results only in the first 3-years of exposure, while in the 12-years period the erosion processes assume more relevance. That suggests the existence of a potential calcification occurring at all sites probably dependent to the homogenous spatial distribution of reef builder organism at a regional scale (Ponti et al. 2011a), but a predominance of erosion processes in the long period. When the activities of bioeroder organisms exceed the actions of builder ones, the coralligenous reefs risk a shift from builder-dominant to a boring-dominant, leading to an unbalance of net accretion, as locally happen in the tropical sea (e.g. Rose & Risk 1985, Ward-Paige et al. 2005, Marulanda-Gómez et al. 2017).

The distribution, abundance and erosion rates of boring sponges are controlled by different environmental factors such as nutrient, temperature and turbidity (e.g. Schonberg 2008, Nava & Carballo 2013, Marlow et al. 2018 and reference therein). The continuous boring activities of clionids shape the coralligenous habitats through the erosion of the substrate, they increase the shelter for cryptic organisms (Cerrano et al. 2001, Calcinai et al. 2015), but in the same time reduce the mechanical stability of the reef (Scott et al. 1988).

In particular, *Cliona viridis* is known to be the most abundant and destructive sponge in the Mediterranean Sea (Rosell et al. 1999). On the experimental tiles, the organism mainly responsible for erosion was *Cliona viridis*, which eroded volume shows no difference among sites, but difference between the exposure times. *C. viridis* seems to be tolerant to high levels of turbidity (<4 m visibility) and sedimentation (Carballo et al. 1994, Carballo et al. 1996). On the tiles, *Cliona rhodensis* was so far represented by a single record found in the site further from the coast (MR08), where the sedimentation rate is lower than in the other sites (Ponti et al. 2011a). That in agreement with the fact that *C. rhodensis* seems to be sensitive against turbidity and sedimentation (Carballo et al. 1994, Carballo et al. 1996).

The endolithic bivalve *Rocellaria dubia* is a typical Mediterranean boring species, easily recognisable in the field through its 8-shapes calcareous holes. Nevertheless, its biology and ecology are poorly known. Its planktonic larvae can settle in crevices or holes in the substrate (Fava et al. 2016) and easily excavate biogenic and non-biogenic carbonate substrates (Morton et al. 2011, Ponti et al. 2015, Casoli et al. 2016). The volumes eroded by *R. dubia* in the experimental tiles were significantly different among study sites; in particular, they were higher at the site closest (P204) and the intermediate distance from the coast (P213) than at the farthest (MR08). That could be related to the tolerance of this species to high sedimentation rates, thanks to its long syphons protected by aragonitic siphonal tubes (Morton et al. 2011). Moreover, *R. dubia* seems to be a fast borer in the first 3-years (Casoli et al. 2016) when reach its maximum density.

On the overall, the mean bioerosion rate seems to increase over time. This could be due to the firm establishment of the boring sponges (Kiene & Hutchings 1994, Pari et al. 2002, Farber et al. 2016).

In the first 3-year period, the building rate was no different among sites. However, the site MR08 show higher volumes of bioconstruction after 12 years compared to the others two sites. The mean construction rate seems to increase in the second 9-year period just for the site MR08, and decrease for the others two sites. These differences could be due to the differences in species assemblages among the study sites (Ponti et al. 2011a). The sites closer to the coast (P204 and P213) are characterised by higher abundances of turf, fleshy algae, sponges and ascidians. This fast-growing species may have covered the others, limiting the growth of ECRs. That is in agreement with the conceptual model of recruitment processes proposed by Fava et al. 2016 that shows that in the early-recruitment-stage pioneer species (e.g., serpulid polychaetes and the bivalve *Anomia ephippium*) were the most abundant at all the study sites. Serpulid polychaetes are indeed important actors in developing and maintain the biogenic reefs (Laborel 1987, Cocito 2004). Then, if the abiotic and biotic conditions are favourable, long-lived slow-growing species gradually replaced or overgrown the pioneer species, increasing the complexity and structural features of the assemblages (Fava et al. 2016), as shown at the site MR08.

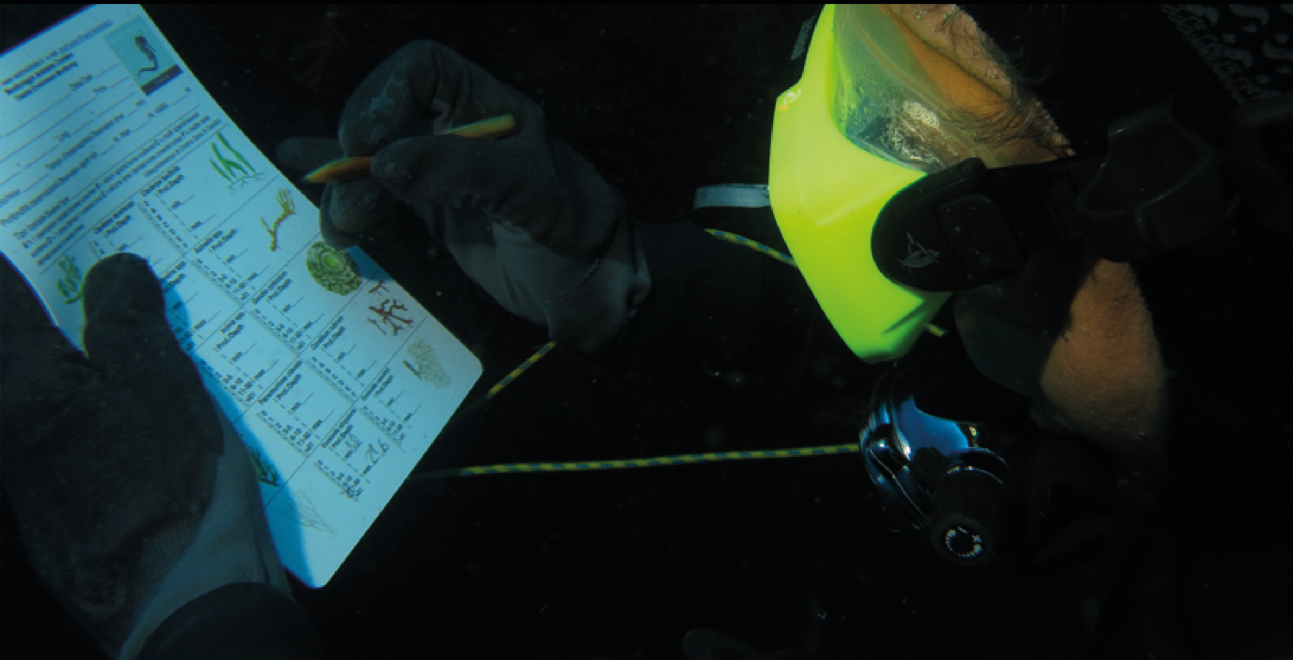
The erosion pattern morphology of clionid sponges could be affected by the microtexture of the calcareous substrates, and the pit sizes are reduced in the porous substrates (e.g. coralline algae) than in denser ones (e.g. marble; Calcinai 2004, Calcinai et al. 2008). Experimental data derived from the use of artificial substrates that resemble the natural ones (i.e. the travertine) combined with use of CT technology allow exploring the mechanism that underlies the formation and maintenance of the species diversity of these habitats and to increase our understanding and evaluation of the ecological processes underlying it. This is especially valuable for ecological processes less evident due to the spatiotemporal scales or the places where they occur.

The experimental data obtained by applying new technologies and artificial substrates that resemble the natural ones (i.e. the travertine) allow exploring the mechanisms that underlies the formation and maintenance of the species diversity of coralligenous reefs. This is especially valuable for ecological processes less evident due to the spatiotemporal scales or the places where they occur.

The experimental and innovative approach used could lead towards the implementation of appropriate conservation strategies of mesophotic coralligenous habitats in the Mediterranean Sea that are characterised by long-lived species, high spatial variability and limited temporal changes (Teixido et al. 2011) and are among the most vulnerable habitats (Micheli et al. 2005, Micheli et al. 2013) to the current increases of anthropogenic disturbances.

# Chapter 5

## The bridge between citizen science and management



## 5.1 | Introduction

Public participation in scientific research, also knows as Citizen Science (CS), is increasing worldwide in the last decades (Conrad & Hilchey 2011). This is particularly true for marine CS projects (MCS; Thiel et al. 2014). Engaging millions of people around the world and as the resources for monitoring fail to match the scale of the question at hand, CS has become more critical in conservation science by influencing and improving management (of, e.g. MPA or fisheries) and by substantially increasing the observation capacity available (Pattengill-Semmens & Semmens 2003, Cohn 2008, Conrad & Hilchey 2011, Miller-Rushing et al. 2012, Tulloch et al. 2013, Cigliano et al. 2015, Wright et al. 2016, Burgess et al. 2017, Lucrezi et al. 2018). The growth factors are primarily the rising awareness that volunteers are a free source of skills, labour-force and computational power and secondly the existence of informatics tools that can spread the information about project easily and gathering data from the participants (Silvertown 2009, Dickinson et al. 2012, Newman et al. 2012, Cerrano et al. 2017b). The dual aim of CS programs should be indeed the collection of reliable data and the increase in people's awareness and knowledge, which are essential requirements for responsible behaviours towards nature conservation.

Therefore, the involvement of citizens in environmental monitoring expands our ability to collect information and data in space and time (Pattengill-Semmens & Semmens 2003, Cerrano et al. 2017b, Di Camillo et al. 2018) and in contributing valuable information that can be used by researchers, decision-makers, stakeholders and the public (Bonney et al. 2014). However, there is scepticism about the reliability of the data collected by the volunteers since they often lack experience and knowledge (see Curtis 2006, Kamp et al. 2016 and references therein). Mainly, the data generated by volunteers' surveys could contain high levels of bias or variability (Crall et al. 2011, Tulloch et al. 2013, Forrester et al. 2015). The differences in skills among the volunteers would lead to decreased accuracy in measurements and misidentification of species, and the validation of the data collected is critical (Boudreau & Yan 2004). For these reasons, several studies have compared data collected by citizens and researchers (e.g. Bonter & Cooper 2012, Cox et al. 2012, Gillett et al. 2012, Forrester et al. 2015, Kosmala et al. 2016, Done et al. 2017, Tye et al. 2017, Johnston et al. 2018) as well as the kind of training that volunteers attend (Edgar & Stuart-Smith 2009, Holt et al. 2013, van der Velde et al. 2017). The results showed how well-trained citizens can be a great resource in providing valuable data on widespread environmental issues, such as marine litter (van der Velde et al. 2017), and that protocols suitable for volunteer projects can give results consistent with protocols used by the professional researchers (Holt et al. 2013). Thus, the keys to success of a citizen science project lie in simple and effective protocols (Bonney et al. 2009, Edgar & Stuart-Smith 2009, Holt et al. 2013), specially developed by scientists for the aims to be achieved, in proper trainings, including skills assessment, and in timely feedbacks on the progress and success of the actions, in order to keep high the involvement of the participants (Devictor et al. 2010, Cerrano et al. 2017b, van der Velde et al. 2017).

Although not as prevalent as its terrestrial counterpart (Cooper et al. 2014, Theobald et al. 2015, Sullivan et al. 2017, Bradter et al. 2018), the MCS projects have spread up from tropical coral reefs (e.g., Reef Check tropical EcoDiver program and Reef Environmental Education Foundation program; Hodgson 1999, Hodgson 2001, Pattengill-Semmens & Semmens 2003) to temperate habitats (e.g., British and Irish Seasearch protocols and Californian Reef Check; Pikesley et al. 2016). Since 2006, following the growing of scuba diving activities that has encouraged the broad involvement of recreational divers for marine monitoring, trained scuba diver volunteers are collecting data on the distribution and abundance of 43 easily identifiable selected key marine species along the coasts of the Mediterranean Sea, by applying the Reef Check Mediterranean Underwater Coastal Environment Monitoring (RCMed U-CEM) protocol (www.reefcheckmed.org; Cerrano et al. 2017b).

CS should be an essential contribution to scientific research not only for the data collections but also above all for the translation of science into policy and nature conservation action laying the basis for participatory government. Citizen science projects can enhance the ability of decision-makers, stakeholders and non-government organisations to monitor, manage and protect natural resources. While citizen volunteers are increasingly involved in local issues, and more awareness in environmental threats and careful about their everyday actions toward the environment (Alaback 2012, Conrad & Hilchey 2011, Whitelaw et al. 2003, Tulloch et al. 2013).

The request of the Marine Strategy Framework Directive (MSFD, 2008/56/EC) to assess the environmental status of coastal areas threatened by multiple stressors (Micheli et al. 2013), and the broad availability of data collected by RCMed volunteers along subtidal rocky habitats suggest the possibility to develop innovative and reliable indices based on RCMed project. That may support decision-makers in planning and applying conservation strategies, especially in Marine Protected Areas (MPAs).

After testing the reliability of the data collected by the volunteers, aim of this study is to develop and proposed the RCMed Species Sensitivity (MedSens) index based on U-CEM data and the sensitivities of the selected species toward the sources of disturbance indicated by the MSFD. This index is not intended to replace studies carried out by specialist but to provide a complementary tool for evaluating the ecological quality status of Mediterranean rocky habitats, freely available for the decision-makers, and involving the public thought a participative approach in nature conservation.

## 5.2 | Materials and Methods

### 5.2.1 The Reef Check Mediterranean U-CEM protocol

The RCMed scuba diver volunteers, the EcoDivers hereafter, collected the data according to the RCMed U-CEM protocol described in Cerrano et al. 2017b. The RCMed EcoDiver's observations are sent to the online RCMed database through an online form on the dedicated website (www.reefcheckmed.org) or a dedicated mobile app (free available on the Google Play store). The latter, if used close to the entry point into the water and with the built-in GPS, allows avoiding positioning errors. Each RCMed EcoDiver enters data autonomously and is nominally responsible for the provided data. This allows for contacting each data providers to ask for further information or solve data inconsistency, if needed.

### 5.2.2 Volunteers data survey validation

An experimental comparison was carried out to evaluate the reliability of data collected by RCMed EcoDivers. Ten participants were divided into three training levels: two participants belong to the training level "very experts" (i.e. marine biologists and RCMed' trainers), four to "expert" (i.e. marine biologists and RCMed EcoDiver), and four to "trained RCMed EcoDiver" (i.e. without any academic training in marine sciences).

At the Gallinara Island (Ligurian Sea), two dive sites were randomly selected. At each site, volunteers kept independent records of the presence, abundance and depth of 20 previously selected target taxa, among the ones in the RCMed U-CEM protocol, along a predefined belt-transect of 100 × 6 m. The dive profile varied from 3 to 30 m depth. Each participant recorded the data by applying the RCI U-CEM protocol, except for the constrained path, and registered them into the online database. Data were retrieved by the database following the procedure described below. Multivariate assemblages' data were analysed using principal coordinate analysis (PCoA) based on Bray-Curtis similarities without data transformations (Anderson & Willis 2003). Differences in assemblages found between the two sites (random factor) and the three volunteer levels (fixed factor) were assessed by a two way crossed permutational non-parametric multivariate analysis of variance (PERMANOVA, $\alpha=0.05$; Anderson & ter Braak 2003) under the hypothesis that difference among sites, if any, were similarly detected independently by the training levels of the observers. The analyses were performed using the software PRIMER v. 6 (Anderson et al. 2008).

### 5.2.3 Data mining

Data extracted from the RCMed database as comma-separated values (CSV) files are converted to ESRI shapefiles through a script that allow for automatic quality control and fixing some possible errors due to manual input (e.g., inversion between the minimum and maximum survey and founding depths). A shapefile for each taxon, one for all the surveys (i.e. single dives) and an overall one, with all the taxa together, are generated. After a further manual quality control, based on the correspondence between data point locations, site names and topography, shape files are made free available online through the Web-GIS and a file repository.

Data analysis and mapping were done using the free software environment for statistical computing and graphics R (R Core Team 2017), including the Shapefile package (Stabler 2013), and the free and open source geographic information system QGIS (QGIS Development Team 2017).

### 5.2.4 Territorial units and time periods

RCMed U-CEM data are unevenly distributed in spatial and time because they are affected by preferences and behaviour of the volunteers. Information collected by a single EcoDiver in a single place and date could be not representative of the mean abundance of the target species in the area. To overcome this issue, data collected by several independent EcoDivers within a defined territorial unit (TU) and time period (TP) have to be pooled and analysed together. TUs and TPs should be defined according to the scope of the intended analysis, as monitoring and management purposes, for instance. Therefore, TUs may coinciding with administrative territories like municipalities, provinces, Marine Protected Areas (MPAs), or designed management and monitoring zones within MPAs. The minimum TU size should be represented by the area usually explored by divers, taking into account the accuracy of the positioning they can employ. While nautical positioning may rely on the few-meter accuracy of the enhanced satellite system (WAAS/EGNOS-enabled GPS, Witte & Wilson 2005), underwater positioning is hampered by the difficulty of penetrating radio waves into water. Although there are underwater acoustic positioning systems (Meidinger et al. 2013) and new photographic methods of three-dimensional mapping of seabed are developing (Palma et al. 2018), their use is still limited to specialists. Therefore, recommended minimum TU size should be higher than approximately 0.25 km$^2$ (e.g. 500 × 500 m or 15" grid cells). TP could be range between few months, in case of intensive monitoring programs and several EcoDivers involved, to multi-years for broad-scale analyses. All the analyses presented in this work were carried out on the data collected from 2006 to 2018 and used as TUs the MPAs borders and management and monitoring zones within MPAs, when available.

MPAs boundaries were obtained from the World Database on Protected Areas (WDPA) made available by IUCN-UNEP-WCMC (www.protectedplanet.net), and the limits of the management zones by the official borders stated by Italian law for each MPAs.

### 5.2.5 Species sensitivity assessment

Following the Marine Evidence based Sensitivity Assessment (MarESA; Tyler-Walters et al. 2018) approach developed by the Marine Life Information Network for Britain and Ireland (MarLIN, www.marlin.ac.uk), a sensitive assessment has been done for 25 taxa inhabiting the Mediterranean subtidal rocky bottom, especially coralligenous habitats (Tab. 1). MarESA approach is based on the review of available literature on the life history, distribution, environmental preference and any possible effects of physical, chemical and biological pressures listed in the MSFD.

For each taxon and pressure, a resistance and a resilience value were attributed based on the literature review and to the MarESA standard benchmarks (Tyler-Walters et al. 2018). The species sensitivity toward each pressure was established by combining the resistance and resilience ranks according to MarESA method (Tyler-Walters et al. 2018). For each taxon, the Mean Sensitivity Value (MSV) toward physical ($MSV_{phy}$), chemical ($MSV_{chem}$) and biological ($MSV_{bio}$) pressures and the overall mean ($MSV_{tot}$) were calculated (see Supplementary material, S1).

Table 1. Taxa selected for the sensitive assessment and their typical habitats

| **Taxon** | **Typical habitats** |
|---|---|
| *Aplysina* spp. | rocky bottom, cave |
| *Arca noae* | rocky bottom |
| *Astroides calycularis* | rocky bottom |
| *Axinella* spp. | coralligenous |
| *Balanophyllia europaea* | rocky bottom |
| *Caulerpa cylindracea* | rocky bottom |
| *Caulerpa taxifolia* | rocky bottom |
| *Cladocora caespitosa* | coralligenous |
| *Corallium rubrum* | coralligenous, cave |
| *Diplodus* spp. | rocky bottom |
| *Eunicella cavolini* | coralligenous |
| *Eunicella singularis* | coralligenous |
| *Eunicella verrucosa* | soft bottom, coralligenous |
| *Geodia cydonium* | rocky bottom, detritic |
| *Hippocampus* spp. | rocky bottom, seagrasses |
| *Homarus gammarus* | coralligenous, cave |
| *Leptopsammia pruvoti* | coralligenous |
| *Palinurus elephas* | coralligenous, cave |
| *Paracentrotus lividus* | rocky bottom |
| *Paramuricea clavata* | coralligenous |
| *Parazoanthus axinellae* | rocky bottom |
| *Pinna nobilis* | soft bottom, seagrasses, rocky bottom |
| *Savalia savaglia* | coralligenous |
| *Sciaena umbra* | rocky bottom |
| *Scyllarides latus* | coralligenous, cave |

### 5.2.6 RCMed Species Sensitivity (MedSens) index

The MedSens index may be calculated toward physical ($MedSens_{phy}$), chemical ($MedSens_{chem}$), biological ($MedSens_{bio}$) and overall disturbs ($MedSens_{tot}$) is based on the corresponding MSV of taxa found in the computing TU and TP, weighted for their observed abundances classes. For each observation, the abundance class were converted in abundance score (*Sc*) from 0 to 6 (Tab.2). The index is calculated by the following equation:

$$\mathbf{MedSens_x = \sum (Sc_i \times MSV_{(x)i}) / \sum Sc_i}$$

where $x$ represents *phy*, *chem*, *bio* or *tot*, and $MSV_{(x)i}$ refers to the taxon in the *i*th observation having abundance score $Sc_i$ in the selected TU and TP.

To ensuring the robustness of the index, the following minimum requirements were imposed:

- minimum TU size: 0.20 km$^2$
- minimum involved Ecodivers: 3
- minimum number of observations (including absences): 20
- minimum searched taxa: 10 (up to 25)

-

The index ranges between 0 and 3, even if the extreme values are technically impossible to achieve. Index values increase with increasing of the mean sensitivity of the species sighted and, in less extent, with their abundance.

Table 2. Abundance numerical and descriptive class converted in abundance score (*Sc*)

| Abundance numerical class | Abundance descriptive class | Score (*Sc*) |
|---|---|---|
| 0 | absent | 0 |
| 1 | isolated specimen | 1 |
| 2 | some scattered specimens | 2 |
| 3-5 | several scattered specimens | 3 |
| 6-10 | a crowded area | 4 |
| 11-50 | some crowded areas | 5 |
| > 50 | several crowded areas | 6 |

### 5.2.7 RCMed MedSens index calibration and classification

In order to explore the real range of values assumed by the index, it was apply through the whole available dataset (43’196 observations, last access 14/10/2018) using as TUs a 15" grid (= 0.00417°, i.e., 1⁄4 of nautical mile in latitude) covering all the coast of the Mediterranean Sea and the entire period of data available. This computation resulted in 89 TUs assessed with MedSens index values ranging from 1.08 to 2.27. The thresholds of 5-class classification were defined by using quintiles on the entire values distribution considering all index versions. Therefore the colour was assigned to each class: red, orange, yellow, light green and dark green, in the order, to indicate increasing mean sensitivity of assemblages.

## 5.3 | Results

### 5.3.1 Reliability of data collected by RCMed volunteers

In order to assess the reliability of the RCMed dataset, ability of divers with different training level were compared in a field test carried out at the Gallinara Island, which included 2 surveys at 2 different sites and 10 independent observers. Patterns of similarities among observed assemblages are shown in the PCoA ordination plot (Fig. 1). The first two axes of the PCoA explained 34.8% and 28.9% of the total variation. The scatter plot shows some degrees of separation between the two sites, but not among the observer training levels.

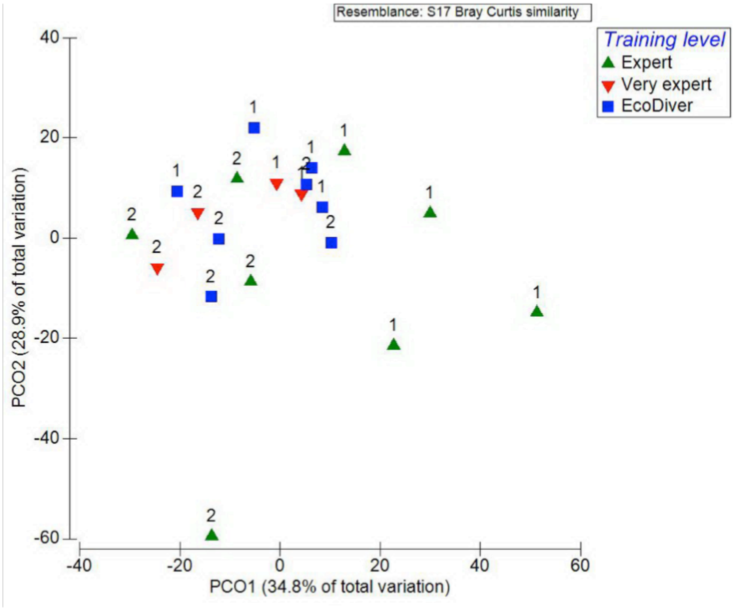


Figure 1. PCoA ordination plot based on Bray-Curtis similarities. The level of experience is indicated with symbols and colours, while the dive sites (Site1 and Site2) are indicated by numbers.

The PERMANOVA test confirmed the pattern, showing significant difference only between sites and not among training levels (Tab.3). Even if some minor differences among operators were obtained, these represent a random effect related to the accuracy of the method, as occurs in any visual census. However, the method appeared enough robust to distinguish assemblages between sites with similar habitats, few hundreds of meters apart, and EcoDivers are able to provide same results as skilled scientists.

Table 3. PERMANOVA test on differences among training levels (Tl, 3 levels, fixed) and between sites (Si, 2 levels, fixed) and their interactions (Tl × Si) (Bray-Curtis similarities abundance data)

| **Source** | **df** | **SS** | **MS** | **Pseudo-F** | ***P* (perm)** | **Unique perms** | ***P (MC)*** |
|---|---|---|---|---|---|---|---|
| Training level (Tl) | 2 | 3280 | 1639.9 | 1.544 | 0.2865 | 180 | 0.3049 |
| Site (Si) | 1 | 3429 | 3429.2 | 3.977 | 0.0045 | 9959 | 0.0127 |
| Tl × Si | 2 | 2125 | 1062.3 | 1.232 | 0.3213 | 9940 | 0.3151 |
| Res | 14 | 12072 | 862.3 | | | | |
| Total | 19 | 20999 | | | | | |

### 5.3.2 MedSens index applied to MPAs and monitoring zones

The MedSens index was calculated for all Italians MPA management zones (zone A: full protection, no dives allowed; zone B: partial protection, dives allowed and zone C: buffer zone, dives allowed) and No Take Zones (NTZ). According to the data available and the minimum requirements imposed, the index was calculated for 20 MPA management zones and 1 NTZ (see Supplementary material, S2).

As a first case study, we present the results of $MedSens_{tot}$ index for the management zones of Tavolara Capo Coda Cavallo MPA, Capo Gallo Femmine Island MPA, Tremiti Islands MPA and Tegnùe of Chioggia No-Take Zone (NTZ) (Fig. 2). Tavolara Capo Coda Cavallo MPA showed very high sensitive assemblages at zone B and moderate sensitivity at zone A (Fig. 2A). The assemblages living in the northern Adriatic coralligenous outcrops, included in the Tegnùe of Chioggia NTZ, are among the least sensitive of those found in Mediterranean protected areas (Fig. 2B). The Capo Gallo Femmine Island MPA presents moderate sensitivity of the assemblages (Fig. 2C), while the Tremiti Islands moderate sensitivity at zone C and very low sensitivity at zone B (Fig. 2D).

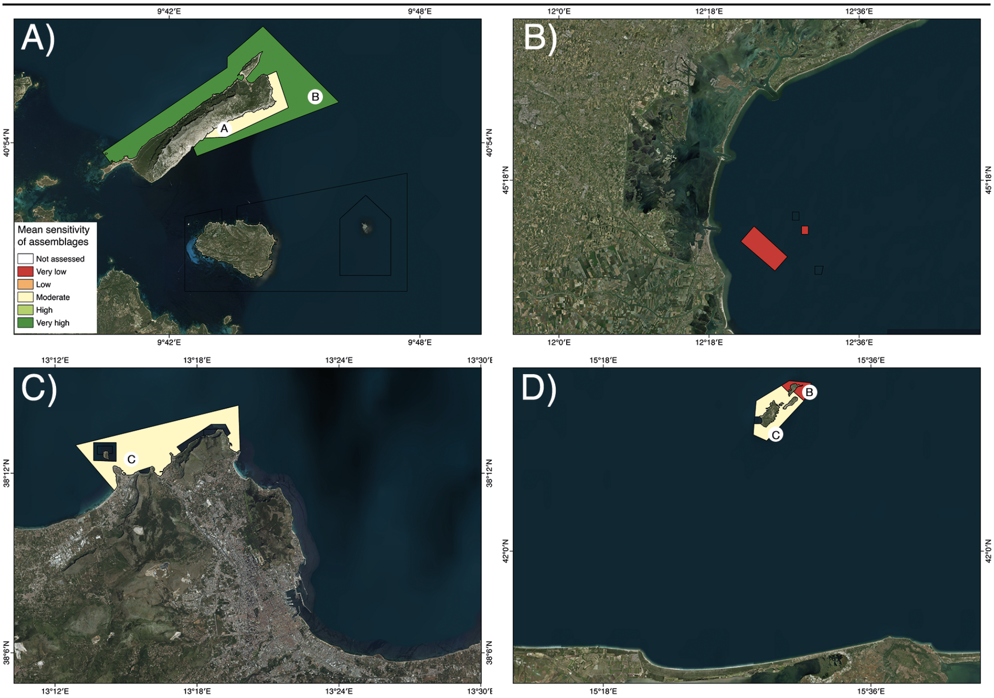


Figure 2. Overall sensitivity assessment of assemblages (RC *MedSens$_{tot}$* Index) living in Tavolara Capo Coda Cavallo MPA (A), Tegnùe of Chioggia NTZ (B), Capo Gallo Femmine Island MPA (C) and Tremiti Islands MPA (D). Circled letters indicate the level of protection in the management zone (Mercator projection, WGS84 datum).

As a second case study, based on the available data, it was possible to apply the MedSens index to a finer scale and to calculate the index for 12 out of 19 monitoring zones (see Supplementary material, S3) in the Portofino MPA (Fig.3). According to the *MedSens$_{tot}$* index results, 1 of the assessed monitoring zones had assemblages with very high sensitivity, 2 with high sensitivity, 8 with moderate sensitivity and 1 with low sensitivity (Fig. 3A). The MedSens index shows to discriminate well among the different source of disturbance pressures: toward the physical disturbs 1 monitoring zones had assemblages with high sensitivity, 8 with moderate and 3 with low (Fig. 3B); toward the chemical disturbs 1 monitoring zones had assemblages with high sensitivity, 2 with high, 4 with moderate, 4 with low and 1 with very low (Fig. 3C); toward the biological disturbs 3 monitoring zones had assemblages with high sensitivity, 7 with high and 2 with moderate  (Fig. 3D)

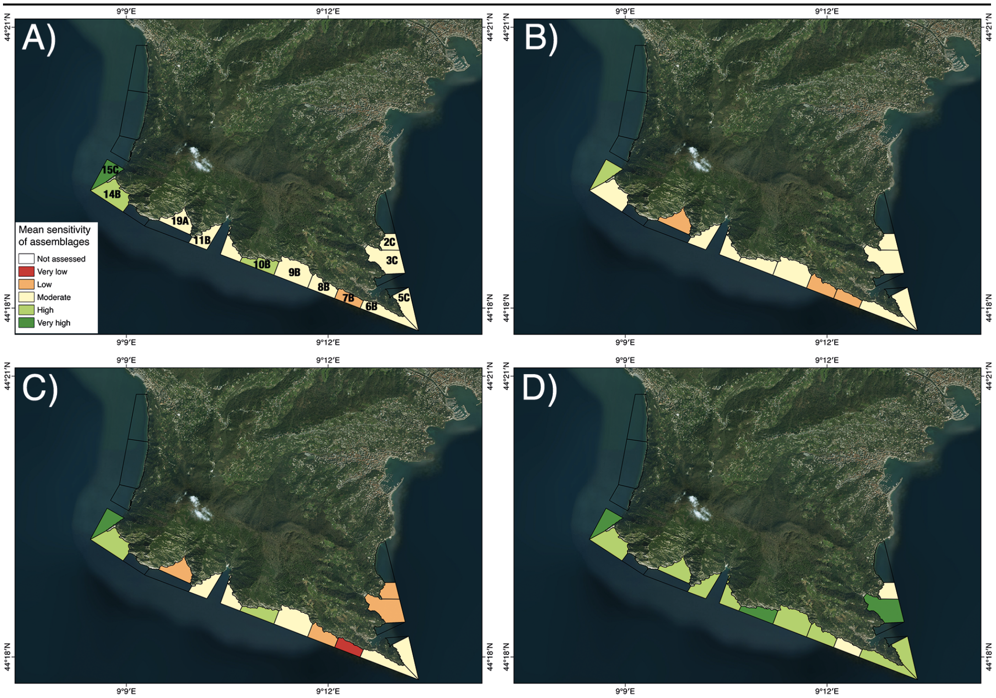


Figure 3. RC MedSens index applied on Portofino MPA monitoring zones towards the overall and the different source of disturbances: overall sensitivity of assemblages (A), sensitivity of assemblages towards physical disturbs (B), sensitivity of assemblages towards chemical disturbs (C) and sensitivity of assemblages towards biological disturbs (D) (Mercator projection, WGS84 datum).

## 5.4 | Discussions

Scuba divers volunteers may support researchers and managers in collecting data over spatial and temporal scales that would be impossible to cover otherwise. However, the data collected by volunteers are often effectively used in local studies (e.g. Foster-Smith & Evans 2003, Bramanti et al. 2011, Malpica-Cruz et al. 2016), but remain mostly unexploited for ecosystem-based management at regional and global scales (EMB; Hodgson 2001). The U-CEM protocol is a simple but effective visual census approach (Cerrano et al. 2017b). It is easy to teach and potentially may provide a timely updated large amount of geo-referred data along all the coasts of the Mediterranean Sea. Robust quality assessment and cross-validation of the data collected by lay people are of paramount importance. In this respect, the U-CEM protocol imply several automatic and manual data checks, and records not conforming to the required quality are discharged. If the recruitment of trained volunteers continues over time and the surveyed areas increase, the assessment and the monitoring of the Mediterranean Sea would be possible. Its wide replication in space and time opens up further study opportunities like on biogeography, species distribution and dynamics, and new perspectives for nature conservation and management.

Biotic indices may allow evaluations of the environmental quality status of coastal marine habitats (see Ponti et al. 2009 and reference therein). However, the effectiveness of the indices has to be tested in different condition and areas. The previously proposed biotic indices for the coralligenous habitats, like the Coralligenous Assemblage Index (CAI; Deter et al. 2012), the Coralligenous Assessment by Reef Scape Estimate index (COARSE; Gatti et al. 2015), the Ecological Status of Coralligenous Assemblages (ESCA; Cecchi et al. 2014) and the Index Coralligenous approach (INDEX-COR; Sartoretto et al. 2017) are intended only for specialists with academic training. However, well-trained volunteers, by applying non-destructive methods, may capture reliable information on the assemblages that can be used in the environmental quality assessment.

The MedSens index represents the first attempt to derive a biotic index from data collected by volunteers in the Mediterranean Sea. It primarily provides a proxy of the mean sensitivity of the assemblages living in a zone toward the natural and anthropic pressures listed by MSFD. The higher the average sensitivity of the assemblages, the lower is the extent of the disturbances to which they are subjected, and therefore this testifies good environmental conditions.

The major weakness of MedSens index is the small number of species considered, which could be increased in future. However, the ranges of the biological and ecological features these species cover are wide. Furthermore, the high replication imposed for computation, improve the reliability of the results. According to data available, these indices may be applied and provide reliable results to administrative areas (i.e. MPA) and smaller areas (e.g. MPA monitoring subzones). The results of this index can supplement those obtained by specialists, often more limited in space and time.

The assessed management areas appear to be in a quite good condition although some multiple pressures may negatively affect the local assemblages. The overall high sensitivity of assemblages at Tavolara Capo Coda Cavallo MPA is in accordance with limited anthropic impacts in a well-managed area (see Bianchi et al. 2012; Fig.2A). While the lower mean sensitivity of assemblages showed at the outcrops in the North Adriatic Sea is in the agreement with the high anthropic disturb in the area, including several dystrophic crises (Zuschin & Stachowitsch 2009, Tomasovych et al. 2017) and intense trawling that may have limited the abundance of sensitive species (Ponti et al. 2011a, Falace et al. 2015, Melli et al. 2017; Fig. 2B). The moderate sensitivity of assemblages at Capo Gallo Femmine Island MPA appeared in accordance by the disturbances possibly due to the proximity to the city of Palermo, the largest city in Sicily with an important commercial port and intense coastal and nautical tourism (Bracciali et al. 2012; Fig. 2C). Although far away from mainland and major source of disturbances, Tremiti Islands MPA presented very low and moderate sensitive assemblages. These reduced ecological status could be related to high turbidity and sedimentation that affect the area (Cormaci & Furnari 1999, Cormaci et al. 2001) and to the invasion by the non-indigenous species *Womersleylla setacea* (Cormaci et al. 2000, Cormaci et al. 2001) and Caulerpa cylindracea within the MPA (Ponti & Cerrano 2016) (Fig. 2D).

MedSens index allows highlighting the areas that require management interventions, having the chance to assess the environmental quality status of coastal marine habitats at a finer scale and to identify the most likely disturbances acting in the study area, as represented in the second study case proposed. The Portofino MPA monitoring subzones appear to be in quite good and moderate conditions although some multiple disturb agents like high sedimentation (Mateos-Molina et al. 2015), intense nautical and diving tourism and increasing urbanisation (Mangialajo et al. 2007) may negatively affect the local assemblages (e.g. monitoring subzones 7B, 8B, 2C and 3C, Fig.3). Moreover, mass mortality events of high sensitive species, like gorgonians, where repeatedly recorded in the area (see the supplementary material in Turicchia et al. 2018). Maps showing the mean sensitivity of assemblages toward different sources of disturbances in each territorial unit (i.e. MPA monitoring subzones, Fig.3) can inform managers about the required enforcement of protection and intervention, which is an important step forward the implementation of EBM (Bianchi et al. 2012).

The need of many independent observations, in order to apply the indices in the selected zones and in time, may represent the occasion to raise public awareness and enhance the collaboration between coastal management authorities (e.g., MPA managers), dive centres (i.e. stakeholders) and researchers, through a participatory approach. The U-CEM protocol and the application of the RC MedSens index thus provide an effective strategy to achieve the objectives set by the European Union.

# Supplementary material Chapter 5

## S1 Taxa Mean Sensitivity Values (MSV)

Table 1. Taxa Mean Sensitivity Values (MSV) toward physical, chemical and biological pressures

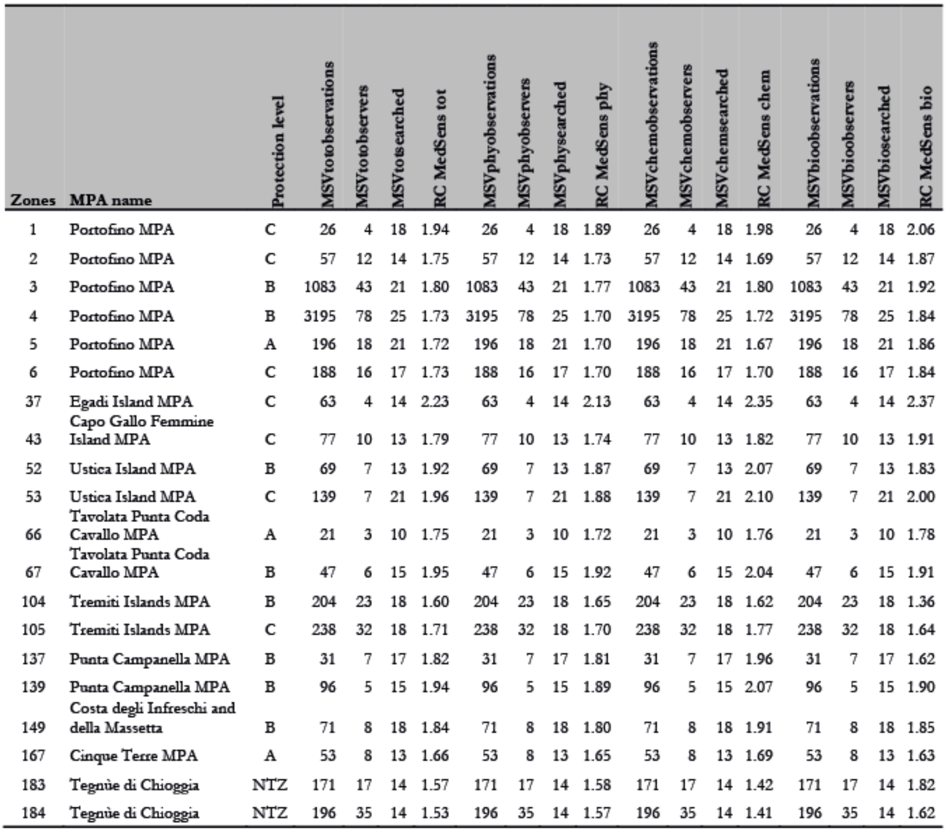
| Taxa | MSVtot | MSVphy | MSVchem | MSVbio |
|---|---|---|---|---|
| *Aplidium conicum* | NA | NA | NA | NA |
| *Aplidium tabarquensis* | NA | NA | NA | NA |
| *Aplysina* spp. | 1.261 | 1.538 | 0.714 | 1.333 |
| *Arca noae* | 1.696 | 1.308 | 2.167 | 2.250 |
| *Astroides calycularis* | 1.826 | 1.769 | 2.500 | 1.000 |
| *Axinella* spp. | 1.087 | 1.231 | 0.714 | 1.333 |
| *Balanophyllia europaea* | 1.864 | 1.769 | 2.333 | 1.333 |
| Gas bubbles | NA | NA | NA | NA |
| *Caulerpa cylindracea* | 0.520 | 0.643 | 0.571 | 0.000 |
| *Caulerpa taxifolia* | 0.560 | 0.643 | 0.571 | 0.250 |
| *Centrostephanus longispinus* | NA | NA | NA | NA |
| *Chlamys varia* | NA | NA | NA | NA |
| *Chromis chromis* | NA | NA | NA | NA |
| *Cladocora caespitosa* | 2.273 | 2.154 | 2.500 | 2.333 |
| *Conger conger* | NA | NA | NA | NA |
| *Corallium rubrum* | 2.409 | 2.308 | 2.333 | 3.000 |
| *Cornularia cornucopiae* | NA | NA | NA | NA |
| *Diplodus* spp. | 1.154 | 1.133 | 0.714 | 2.000 |
| *Epizoanthus* spp. | NA | NA | NA | NA |
| *Eunicella cavolini* | 2.522 | 2.462 | 2.500 | 2.750 |
| *Eunicella singularis* | 2.348 | 2.231 | 2.500 | 2.500 |
| *Eunicella verrucosa* | 2.043 | 1.692 | 2.333 | 2.750 |
| *Geodia cydonium* | 1.696 | 1.769 | 1.571 | 1.667 |
| *Hippocampus* spp. | 1.769 | 1.933 | 1.143 | 2.250 |
| *Homarus gammarus* | 1.640 | 1.214 | 1.857 | 2.750 |
| *Ircinia* spp. | NA | NA | NA | NA |
| *Leptopsammia pruvoti* | 1.727 | 1.769 | 2.000 | 1.000 |
| *Maasella edwardsi* | NA | NA | NA | NA |
| *Microcosmus* spp | NA | NA | NA | NA |
| *Ophidiaster ophidianus* | NA | NA | NA | NA |
| *Palinurus elephas* | 1.600 | 1.214 | 1.857 | 2.500 |
| *Paracentrotus lividus* | 1.583 | 1.462 | 1.429 | 2.250 |
| *Paramuricea clavata* | 2.565 | 2.462 | 2.667 | 2.750 |
| *Parazoanthus axinellae* | 1.636 | 1.769 | 1.833 | 0.667 |

| **Taxa** | **MSVtot** | **MSVphy** | **MSVchem** | **MSVbio** |
|---|---|---|---|---|
| *Patella ferruginea* | NA | NA | NA | NA |
| *Pecten jacobaeus* | NA | NA | NA | NA |
| *Pinna nobilis* | 1.957 | 1.923 | 1.500 | 2.750 |
| *Polycitor adriaticus* | NA | NA | NA | NA |
| *Rapana venosa* | NA | NA | NA | NA |
| *Savalia savaglia* | 2.217 | 2.385 | 2.000 | 2.000 |
| *Sciaena umbra* | 1.385 | 1.267 | 1.286 | 2.000 |
| *Scyllarides latus* | 1.625 | 1.231 | 1.857 | 2.500 |
| *Tethya* spp | NA | NA | NA | NA |
| *Trisopterus minutus* | NA | NA | NA | NA |

NA = Not Available

## S2 RC MedSens indices at italian MPA management zones

Table 2. RC MedSens indices calculated for italian MPA management zones

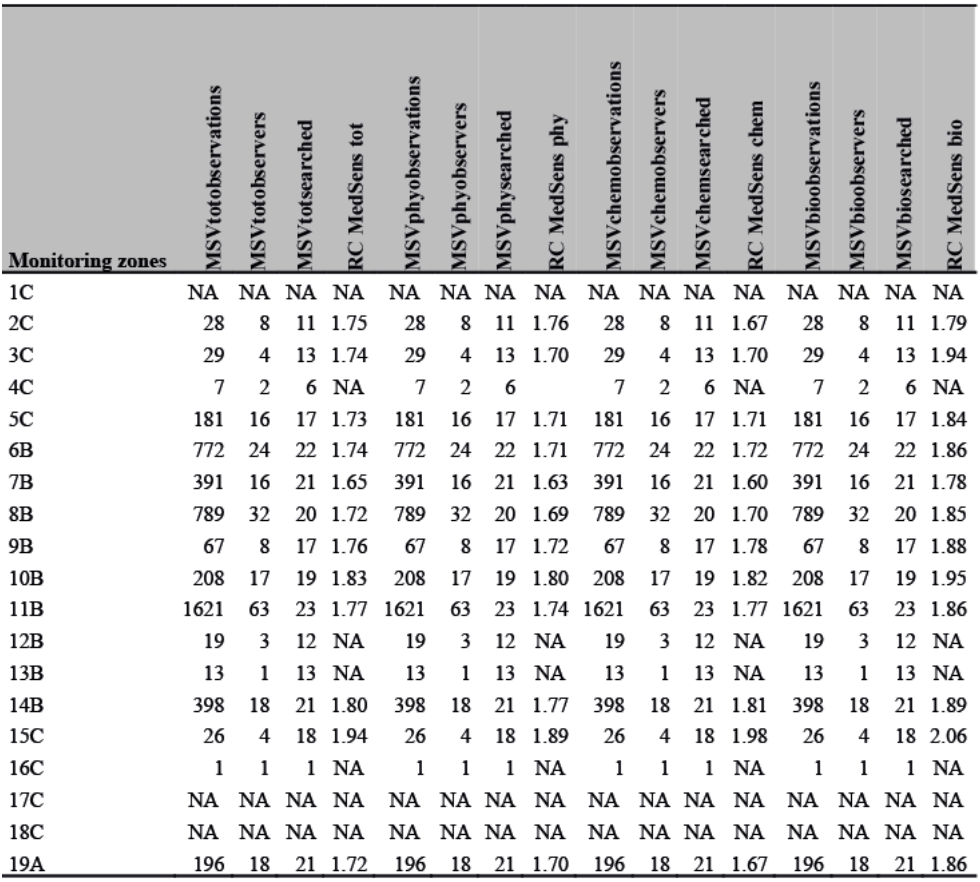


| Zones | MPA name | Protection level | MSVtotobservations | MSVtotobservers | MSVtotsearched | RC MedSens tot | MSVphyobservations | MSVphyobservers | MSVphysearched | RC MedSens phy | MSVchemobservations | MSVchemobservers | MSVchemsearched | RC MedSens chem | MSVbioobservations | MSVbioobservers | MSVbiosearched | RC MedSens bio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Portofino MPA | C | 26 | 4 | 18 | 1.94 | 26 | 4 | 18 | 1.89 | 26 | 4 | 18 | 1.98 | 26 | 4 | 18 | 2.06 |
| 2 | Portofino MPA | C | 57 | 12 | 14 | 1.75 | 57 | 12 | 14 | 1.73 | 57 | 12 | 14 | 1.69 | 57 | 12 | 14 | 1.87 |
| 3 | Portofino MPA | B | 1083 | 43 | 21 | 1.80 | 1083 | 43 | 21 | 1.77 | 1083 | 43 | 21 | 1.80 | 1083 | 43 | 21 | 1.92 |
| 4 | Portofino MPA | B | 3195 | 78 | 25 | 1.73 | 3195 | 78 | 25 | 1.70 | 3195 | 78 | 25 | 1.72 | 3195 | 78 | 25 | 1.84 |
| 5 | Portofino MPA | A | 196 | 18 | 21 | 1.72 | 196 | 18 | 21 | 1.70 | 196 | 18 | 21 | 1.67 | 196 | 18 | 21 | 1.86 |
| 6 | Portofino MPA | C | 188 | 16 | 17 | 1.73 | 188 | 16 | 17 | 1.70 | 188 | 16 | 17 | 1.70 | 188 | 16 | 17 | 1.84 |
| 37 | Egadi Island MPA | C | 63 | 4 | 14 | 2.23 | 63 | 4 | 14 | 2.13 | 63 | 4 | 14 | 2.35 | 63 | 4 | 14 | 2.37 |
| 43 | Capo Gallo Femmine Island MPA | C | 77 | 10 | 13 | 1.79 | 77 | 10 | 13 | 1.74 | 77 | 10 | 13 | 1.82 | 77 | 10 | 13 | 1.91 |
| 52 | Ustica Island MPA | B | 69 | 7 | 13 | 1.92 | 69 | 7 | 13 | 1.87 | 69 | 7 | 13 | 2.07 | 69 | 7 | 13 | 1.83 |
| 53 | Ustica Island MPA | C | 139 | 7 | 21 | 1.96 | 139 | 7 | 21 | 1.88 | 139 | 7 | 21 | 2.10 | 139 | 7 | 21 | 2.00 |
| 66 | Tavolata Punta Coda Cavallo MPA | A | 21 | 3 | 10 | 1.75 | 21 | 3 | 10 | 1.72 | 21 | 3 | 10 | 1.76 | 21 | 3 | 10 | 1.78 |
| 67 | Tavolata Punta Coda Cavallo MPA | B | 47 | 6 | 15 | 1.95 | 47 | 6 | 15 | 1.92 | 47 | 6 | 15 | 2.04 | 47 | 6 | 15 | 1.91 |
| 104 | Tremiti Islands MPA | B | 204 | 23 | 18 | 1.60 | 204 | 23 | 18 | 1.65 | 204 | 23 | 18 | 1.62 | 204 | 23 | 18 | 1.36 |
| 105 | Tremiti Islands MPA | C | 238 | 32 | 18 | 1.71 | 238 | 32 | 18 | 1.70 | 238 | 32 | 18 | 1.77 | 238 | 32 | 18 | 1.64 |
| 137 | Punta Campanella MPA | B | 31 | 7 | 17 | 1.82 | 31 | 7 | 17 | 1.81 | 31 | 7 | 17 | 1.96 | 31 | 7 | 17 | 1.62 |
| 139 | Punta Campanella MPA | B | 96 | 5 | 15 | 1.94 | 96 | 5 | 15 | 1.89 | 96 | 5 | 15 | 2.07 | 96 | 5 | 15 | 1.90 |
| 149 | Costa degli Infreschi and della Massetta | B | 71 | 8 | 18 | 1.84 | 71 | 8 | 18 | 1.80 | 71 | 8 | 18 | 1.91 | 71 | 8 | 18 | 1.85 |
| 167 | Cinque Terre MPA | A | 53 | 8 | 13 | 1.66 | 53 | 8 | 13 | 1.65 | 53 | 8 | 13 | 1.69 | 53 | 8 | 13 | 1.63 |
| 183 | Tegnùe di Chioggia | NTZ | 171 | 17 | 14 | 1.57 | 171 | 17 | 14 | 1.58 | 171 | 17 | 14 | 1.42 | 171 | 17 | 14 | 1.82 |
| 184 | Tegnùe di Chioggia | NTZ | 196 | 35 | 14 | 1.53 | 196 | 35 | 14 | 1.57 | 196 | 35 | 14 | 1.41 | 196 | 35 | 14 | 1.62 |

A = integral protection, B = partial protection, C = buffer zone; NTZ = no take zone

## S3 RC MedSens indices at Portofino MPA monitoring zones

Table 3. RC MedSens indices calculated for Portofino MPA monitoring zones



| Monitoring zones | MSVtotobservations | MSVtotobservers | MSVtotsearched | RC MedSens tot | MSVphyobservations | MSVphyobservers | MSVphysearched | RC MedSens phy | MSVchemobservations | MSVchemobservers | MSVchemsearched | RC MedSens chem | MSVbioobservations | MSVbioobservers | MSVbiosearched | RC MedSens bio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1C | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 2C | 28 | 8 | 11 | 1.75 | 28 | 8 | 11 | 1.76 | 28 | 8 | 11 | 1.67 | 28 | 8 | 11 | 1.79 |
| 3C | 29 | 4 | 13 | 1.74 | 29 | 4 | 13 | 1.70 | 29 | 4 | 13 | 1.70 | 29 | 4 | 13 | 1.94 |
| 4C | 7 | 2 | 6 | NA | 7 | 2 | 6 |  | 7 | 2 | 6 | NA | 7 | 2 | 6 | NA |
| 5C | 181 | 16 | 17 | 1.73 | 181 | 16 | 17 | 1.71 | 181 | 16 | 17 | 1.71 | 181 | 16 | 17 | 1.84 |
| 6B | 772 | 24 | 22 | 1.74 | 772 | 24 | 22 | 1.71 | 772 | 24 | 22 | 1.72 | 772 | 24 | 22 | 1.86 |
| 7B | 391 | 16 | 21 | 1.65 | 391 | 16 | 21 | 1.63 | 391 | 16 | 21 | 1.60 | 391 | 16 | 21 | 1.78 |
| 8B | 789 | 32 | 20 | 1.72 | 789 | 32 | 20 | 1.69 | 789 | 32 | 20 | 1.70 | 789 | 32 | 20 | 1.85 |
| 9B | 67 | 8 | 17 | 1.76 | 67 | 8 | 17 | 1.72 | 67 | 8 | 17 | 1.78 | 67 | 8 | 17 | 1.88 |
| 10B | 208 | 17 | 19 | 1.83 | 208 | 17 | 19 | 1.80 | 208 | 17 | 19 | 1.82 | 208 | 17 | 19 | 1.95 |
| 11B | 1621 | 63 | 23 | 1.77 | 1621 | 63 | 23 | 1.74 | 1621 | 63 | 23 | 1.77 | 1621 | 63 | 23 | 1.86 |
| 12B | 19 | 3 | 12 | NA | 19 | 3 | 12 | NA | 19 | 3 | 12 | NA | 19 | 3 | 12 | NA |
| 13B | 13 | 1 | 13 | NA | 13 | 1 | 13 | NA | 13 | 1 | 13 | NA | 13 | 1 | 13 | NA |
| 14B | 398 | 18 | 21 | 1.80 | 398 | 18 | 21 | 1.77 | 398 | 18 | 21 | 1.81 | 398 | 18 | 21 | 1.89 |
| 15C | 26 | 4 | 18 | 1.94 | 26 | 4 | 18 | 1.89 | 26 | 4 | 18 | 1.98 | 26 | 4 | 18 | 2.06 |
| 16C | 1 | 1 | 1 | NA | 1 | 1 | 1 | NA | 1 | 1 | 1 | NA | 1 | 1 | 1 | NA |
| 17C | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 18C | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 19A | 196 | 18 | 21 | 1.72 | 196 | 18 | 21 | 1.70 | 196 | 18 | 21 | 1.67 | 196 | 18 | 21 | 1.86 |

NA = not available

# Chapter 6

## General discussion and future directions

The coralligenous biogenic reefs are among the richest, the more diverse and threatened habitats in the Mediterranean Sea. They harbour long-lived algae and sessile invertebrates, which belong to different taxonomic groups, and show low population dynamics (Ballesteros 2006, Ingrosso et al. 2018). They are characterised by high spatial variability and limited temporal changes (Ponti et al. 2011a, Teixido et al. 2011), unless anthropogenic disturbances (Bavestrello et al. 1997) or anomalous natural events (Teixido et al. 2013) occur. Several pressures affect the coralligenous assemblages like nutrient enrichment, increase of sedimentation, mechanical impact and invasive species (Micheli et al. 2013). However, despite their importance, knowledge of the ecological processes that underlie the formation and resilience of these reefs are still little known. This thesis aims at improving the knowledge on the coralligenous habitats providing information on the ecological processes controlling their development and tools for their ecological status assessment.

Among the services gorgonian forests provide, they offer refuges to several invertebrates compared to no-forested seabed and improve the benthic-pelagic coupling facilitating the capture of planktonic preys by suspension feeders by decreasing the speed and increasing the turbulence of water flow (Shashar et al. 1996, Valisano et al. 2016). Moreover, gorgonians affect the structures of sessile and vagile communities (Ponti et al. 2014, Ponti et al. 2016), while providing the condition for the settlement and growth of the encrusting calcareous rhodophytes, that are the main builders of the coralligenous bioconstructions (Ponti et al. 2014, Ponti et al. 2018).

The first Chapter of this thesis addresses the abundance and composition of epibenthic assemblages inside and outside the *Paramuricea clavata* forests. On overall red gorgonian forests may enhance bioconstruction processes and increase resistance and resilience of the benthic assemblages in the Mediterranean coralligenous habitats. This species and its forests, together with their understoreys, should be considered as essential elements of the ecology of the Mediterranean Sea, and therefore worthy of specific and effective protection measures. Conservation strategies should reduce the risk of mechanical damage by regulating fishing activities, anchorages, and scuba diving where gorgonian forests are present. Moreover, when evident alterations are documented, restoration actions should be implemented to recover the integrity of gorgonian forests.

Gorgonian populations of *P. clavata*, *Eunicella cavolini* and *Eunicella singularis* are affected by several threats. Local threats could be identified in physical damages due to fishing lines and nets, anchor and recreational divers (Bavestrello et al. 1997, Linares & Doak 2010), suffocation by mucilaginous aggregates (Mistri & Ceccherelli 1996a, Giuliani et al. 2005), invasion from non-indigenous species (Cebrian et al. 2012) and increase in pollution, water turbidity and sedimentation rates (Mateos-Molina et al. 2015), while both regional and local threats could be identified in mass mortality events induced by positive seawater temperature anomalies (Cerrano et al. 2000, Cerrano & Bavestrello

2008, Linares & Doak 2010). In the Mediterranean Sea, mass mortality events date back to the 1980s (Harmelin & Marinopoulos 1994), and nowadays the frequency is increasing (Coma et al. 2009). During the regional scale events of mass mortality in 1999 and 2003, the mortality of gorgonians reached values of 50-80% over ~1000 km along the north western Mediterranean coast (Cerrano et al. 2000, Linares et al. 2005, Coma et al. 2006b, Cupido et al. 2008, Garrabou et al. 2009, Calvo et al. 2011). In these two events, the positive temperature anomalies reached 3-4°C above the climatic average, and they were detected down to 25 m (in 2003) and 40 m depth (in 1999) (Cerrano et al. 2000, Cupido et al. 2008, Bensoussan et al. 2010, Calvo et al. 2011, Crisci et al. 2011, Sini et al. 2015). The main effects of mass mortality events on gorgonian populations are biomass loss, density reduction, and shift towards smaller colonies (Cerrano et al. 2005, Linares et al. 2005, Cupido et al. 2008, Linares & Doak 2010), as well as a negative impact on the reproduction (Linares et al. 2008b, Cupido et al. 2012, Tsounis et al. 2012).

Thus thermal stress plays an essential role in the onset of gorgonian and, in general, of filter feeding invertebrates mass mortality events. These events profoundly affect the coralligenous biogenic reef by decreasing habitat complexity and heterogeneity. By utilising a combination of high-resolution oceanographic analysis, oceanographic models and citizen science initiatives, we propose that an early warning system for the concomitance of heat waves and mortality events can be put in place. A temperature-based coral disease surveillance tool could then be established for the entire Mediterranean Sea. Such a tool would allow for the timely study of mass mortality phenomena and the implementation of prompt mitigation and restoration initiatives.

The coralligenous reefs are the result of the interplay between building activities and physical and biological eroding processes and are characterised by low temporal variability and high spatial heterogeneity. The study of the microscale processes, driving the dynamic balance leading to the formation of coralligenous banks, requires sophisticated investigation techniques such as the High resolution Computed Tomography (CT). In this thesis, this technique has been used to analyse short and long-term (i.e., 3 and 12 years) bio-construction and erosion processes occurring in experiment travertine tiles deployed on different typologies of mesophotic biogenic reefs in the northern Adriatic Sea. CT offers new perspectives and approaches in the analysis, understanding and representation of ecological processes. These are especially valuable for ecological processes less evident due to the spatiotemporal scales or the places where they occur. The experimental data obtained by applying new technologies allow exploring the mechanisms that underlies the formation and maintenance of the species diversity of coralligenous reefs. The experimental and innovative approach used could lead towards the implementation of appropriate conservation strategies of mesophotic coralligenous habitats in the Mediterranean Sea.

Marine Citizen Science (CS) projects play a relevant role in environmental education and in increasing people awareness, which lies at the basis of nature conservation. Since 2006, trained scuba diver volunteers are collecting data on the distribution and abundance of 43 easily identifiable selected key marine species along the coasts of the Mediterranean Sea (www.reefcheckmed.org). The proposed Reef Check Mediterranean Species Sensitivity (MedSens) indices are based on these species distribution data and the sensitivities of the selected species toward physical, chemical and biological pressures, as indicated by the Marine Strategy Framework Directive (MSFD, 2008/56/EC). These indices are useful for the evaluation of ecological quality status and temporal changes in coralligenous assemblages, allowing the identification of disturbances on the monitored species. Consequently, these indices could be adopted to integrate specialised studies in order to fulfil the goal of MSFD to establish innovative, cost-effective monitoring programs and protocols for status assessment, effective management and protection measures of coralligenous habitats.

Coralligenous bioconstructions are vulnerable habitats threatened by multiple stressors, both al local and global scale, in need of conservation measures (Fraschetti et al. 2009). Coralligenous reefs required millennia for their building, and recovery after impacts may requires long time (Sartoretto et al. 1996, Ballesteros 2006). Concern for the loss of these habitats and the related consequences (e.g. impairment of fertilisation and larval connectivity, shifts in the structure of benthic assemblages and the related ecosystem functioning) are rising. 'Acting to change' is the core of marine conservation that asks for the application of scientific knowledge to advance the protection of the marine environment.

Given the vulnerability of gorgonian forests, it is urgent to map their distribution, density and extensions, which are the knowledge needed for any ecosystem-based conservation action (Ponti et al. 2019). Filling this knowledge gap is possible by a multiple-source approach that combines data from scientific literature with information collected by citizen scientists, opportunely informed and trained, or gathered by interviewing scuba divers (Local Ecological Knowledge, LEK; Anadon et al. 2009) and retrieved from social networks and webpages (Web Ecological Knowledge, WEK; Di Camillo et al. 2018). Having information on the distribution patterns, resistance and sensitivity of the species as well as risk of occurrence of mass mortality events may help improving restoration protocols and prompting mitigation actions. Temperature-based surveillance tools should be enforced for promptly alert in case of temperature anomalies and improve marine ecosystem conservation. Being present during a mass mortality event could allow to study the organisms affected and to learn the physiological mechanisms that lead to such a devastation and implement actions to prevent or at least mitigate it.

The understanding of the processes at the basis of the resistance and resilience of coralligenous reef is challenge that needs new approaches technologies (e.g. see

Hofmann & Gaines 2008, Bicknell et al. 2016, Griffin et al. 2017, Palma et al. 2017, Palma et al. 2018). The use of no-destructive technologies allows studying the samples without damaging them. Computed Tomography is one of this technology that allows to "see inside" the objects offering a new perspective on bioerosion patterns.

The knowledge on the ecological processes and the new investigating and assessment tools developed in this thesis should provide an effective contribution and a step forward to the conservation of the Mediterranean coralligenous habitats.

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