ISPRA
Istituto Superiore per la Protezione
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Sistema Nazionale
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Monitoring and assessment of the ecological status of coralligenous habitat. The coralligenous cliff

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MANUALI E LINEE GUIDA

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Backcover images: colonies of *Parazoanthus axinellae* (photo by Stefano Cellini); coralligenous cliff dominated by gorgonians (photo by A. Tommasi); red coral colonies (*Corallium rubrum*) (photo by M. Montefalcone).

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“After the magical moment when my eyes opened into the sea, it was no longer possible for me to see, think, live as before”

(Jacques-Yves Cousteau)

PREFACE

Italy has a unique biodiversity heritage in Europe, with a very high number of species and habitat types, many of which are distributed along the coastal strip. Italy is one of the countries with the greatest coastal development: almost 8000 km of coastline constitute a natural wealth and an economic resource that must be carefully preserved and managed with a view towards integrated and sustainable development. In fact, most of the human activities take place along the coasts, generating pressures that significantly alter both the emerged and submerged environments. At the same time, the marine-coastal strip hosts some of the most valuable natural habitats whose protection is to be considered a priority commitment for the conservation of biodiversity and, more generally, for the management and protection of the marine-coastal system.

Among the submerged coastal environments, coralligenous is one of the most important "hot spots" of Mediterranean biodiversity and one of the main marine ecosystems for distribution, biomass and role played in the carbonate cycle; but it is also one of the habitats most sensitive and vulnerable to environmental alterations, both on a local and global scale. Coralligenous cliff, in particular, is the most widespread type of coralligenous to the first 40 m of depth and thus more exposed to environmental alterations, especially those related to the anthropization of the coastal strip and climate change; therefore, it is considered a sensitive bioindicator of impact as well as a habitat under a high risk of degradation. The monitoring and assessment of the health status of the coralligenous cliff are hence important objectives to be considered in planning the protection and conservation programs of the marine-coastal strip, also considering the protection principles of marine environments expressed by European Community legislation and international treaties.

In this framework, in the last ten years several biotic indices have been developed to evaluate the coralligenous cliff quality, including the indices developed by the Italian experts authors of this manual. However, most of the indices are based on different approaches and metrics which often makes difficult to compare the results obtained with different methods. The same authors, over the years of scientific research carried out for the development of indices, have had to deal with the problem of comparability between different methods often applied in different marine areas, thus maturing the idea of guidelines "unifying" the different methodologies. The need for a comparison to find a common denominator of evaluation on a large space-time scale did the rest. After having intercalibrated the Italian indices, the authors focused on Mediterranean scientific production, analyzing and comparing methods, with the aim of integrating and standardizing in a single procedure the different approaches used in the Mediterranean to assess coralligenous cliff quality. Thus the STAR method (STAndaRdized coralligenous evaluation procedure) was born as a protocol that optimizes the balance between sampling effort and type of information obtained (data collection generally represents the most onerous part of monitoring plans, in terms of time and costs) allowing collection in a common database the ecological parameters considered up to now relevant for assessing the quality status of coralligenous cliff.

This document presents the STAR methodology and the particular cases of its application, consisting of the biotic indices developed by the Italian experts over the last years. The approach proposed by the authors was to provide not only general guidelines for monitoring and assessing the ecological status of coralligenous cliff, but also a theoretical-practical methodological manual to guide the operator step by step in the application of methods described in all its phases, from planning the field activities to final classification of the ecological quality status of coralligenous cliff.

For this purpose, the document has been divided into two parts: the Introduction and the Application part. The first initially describes the habitat investigated, the anthropogenic pressures threatening its integrity and the environmental policies concerning, more or less directly, this precious habitat; then, the summary of the methodologies used at the Mediterranean level is provided, introducing the STAR method and the Italian reference indices (ESCA, COARSE, ISLA) which represent its direct application. The Application part provides practical details of the application phases of methods described, from planning and carrying out field activities in scuba diving, to analyzing images in the laboratory, up to building the databases to calculate the ecological quality indices. The more descriptive practical sessions have been reported in 6 Sheets, in support of which there are 4 technical Annexes with tables, photographic cards for organism identification, examples of field images processed and spreadsheets pre-filled on the basis of field experiences. The application part ends with a paragraph discussing the results of the examples reported in the practical sessions (accompanied by a Box with a case study of intercomparison between indices) and the future perspectives of the applied methods.

The intent of the authors, in fact, is not to provide a "finished" product, but rather methodological guidelines which, although scientifically solid and validated on a large scale, may constitute a starting point for the development of increasingly accurate and effective tools that are, above all shared, used for monitoring and assessing the health status of Italian and Mediterranean coralligenous cliffs.
The authors' one is a first attempt at the Mediterranean level to respond to some important requests coming from European legislation and international agreements concerning the protection of the marine environment. These environmental policies all pursue the objective of protecting biodiversity and marine habitats through the creation of a conservation and protection action network shared across Mediterranean countries. To do this, the same environmental policies call for the development of monitoring and evaluation plans based on habitat-specific and standardized biological methods, in order to ensure the accuracy and comparability of the results on a large space-time scale. These plans, and the resulting marine strategies, must be based on the best available scientific knowledge and therefore be periodically updated.
The methodology presented in this manual is the result of ten years of studies carried out on the structure, ecology and way of response of coralligenous to anthropogenic impacts, the years during which dozens of publications in international and peer reviewed journals have been produced, as well as contributions in national and international conferences. It was devised for a particular type of coralligenous, the vertical cliff assemblages, and it is the result of interpolation of different methods in a standard “best sampling strategy” applicable on a large scale. Above all, it is the result of the effort carried out by the Italian researchers in the direction of an active dialogue not only with the Mediterranean partners, but also with all those Institutions which, in different ways, are engaged in the management and conservation of natural resources that make unique our coastal strip.
In this sense, ISPRA has given a very important contribution through the publication of this manual: ISPRA has taken an active part in the scientific research together with the Italian experts of coralligenous and it has played the role of "glue" between the world of academic scientific research and that of the applied environmental research more linked to institutional tasks. The Biology Unit of the CN-LAB (National Center for the National LABoratories network) of ISPRA, which is the promoter of this document, counts among its main tasks the development, validation and application of indexes and methodologies for ecological classification (declaratory of the CN-LAB Biology Unit, disposition n.1968/DG of 20/02/2017). Therefore, in line with its mandate, the research staff of the unit, thanks to the closer collaboration with colleagues from Italian universities and agency system, have developed, validated and applied the methodological guidelines object of this manual. This thick collaboration network created between the coralligenous experts belonging to different research structures is the most important added value of this work: a unifying effort aimed at contrasting the frequent disconnect between environmental policies and the technical-scientific world by virtue of principle, often recalled by European legislation, whereby these policies must never regardless from the best technical-scientific knowledge available. The aim of this manual is therefore to convey the most up-to-date scientific knowledge into unconventional, scientifically solid and standardized tools, useful to the managers of environmental issues in carrying out their respective institutional tasks, leaving them the decisions on the use of these tools in the appropriate legislative instruments.
It is my opinion that this manual should be considered a valid tool, useful to all those Administrations and Institutions involved in the protection and conservation programs of sensitive habitats, and also in relation to the address lines given by national and European development policies to protect marine-coastal environment and its resources.

Dr. Stefania Balzamo

ISPRA, CN-LAB Manager

PREMISE

Conservation and protection of habitats and species has been proposed in a strong manner at European level by the Habitats Directive (Council Directive 92/43 /EEC) which introduces for the first time, in the early 1990s, measures aimed at ensuring the maintenance and restoration of habitats and species of community importance according to a conservative approach of biodiversity protection. The advent of Water and Marine Strategy Framework Directives (WFD 2000/60/EC and MSFD 2008/56/EC) in the European legislative landscape represents the watershed of an environmental policy which, at the threshold of the 21st century, was dealing with a different world which posed new environmental problems compared to those faced in the previous century, first among all global changes. This required a new approach to environmental policies that found its maximum expression in the innovative "ecological" and "ecosystem" approach proposed by the WFD and the MSFD.
Since the moment the Framework Directives have opened the way to a new mode of monitoring and managing aquatic environments for their conservation and protection, a long way has been made and much more remains to be done. The coming years hence represent an important opportunity for continuing to develop, integrate and/or consolidate where necessary the legislative action plans, so as to make them increasingly responsive and effective with respect to the new environmental needs.
Preserving the ecological status of the coralligenous of our seas is thus only one of the many environmental challenges that the managers of these issues are called to face, in concert with the technical-scientific world, in full compliance with their respective roles and competence in the management of environmental policies. As also recalled by the same directives, the dynamism that characterizes the natural variability of marine systems, as well as the pressures and impacts as a function of the evolution of human activities and global changes, require that protection and conservation programs be flexible and adaptable to changing environmental needs and that take into account the latest scientific and technological developments. This is also in order to ensure the comparability of methods and actions at the Mediterranean level, with a view towards management and conservation of the *Mare Nostrum* that goes beyond the individual nation.
The periodic updating of the protection strategies for the marine environment, making use of the best technical-scientific knowledge available and the adoption of integrated and standardized methods on a national and Mediterranean scale, is therefore a winning key action in the environmental challenges awaiting us in the near future.
The aim of this manual is to give a first contribution in this direction, providing a useful and effective, scientifically solid and validated tool, for monitoring and assessing the ecological status of coralligenous cliff, which is the most widespread coralligenous type in the Mediterranean to the first 40 m of depth, and the most exposed to anthropogenic pressures and environmental alterations.
This manual is the result of multi-year scientific research on ecological indicators of response to anthropogenic pressures affecting coastal coralligenous, which then culminated in a process of integration and standardization of the methods used at the Mediterranean level in a single sampling and data collection procedure. This is carried out by employing scuba diver operators, in accordance with current safety procedures and following a non-destructive, simple but effective protocol, which allows to collect the most important ecological parameters through a single sampling effort and in respect of the habitat investigated.
By integrating the information obtained with the STAR method (STAndaRdized coralligenous evaluation procedure) on the coralligenous cliff with those collected by ROV on the deeper coralligenous, it is possible provide a complete picture of the health status of coralligenous habitat occurring along the marine-coastal strip.
The methodology presented here has been tested against pressures and validated at the Western Mediterranean sub-region scale; moreover, it is in line with indications of the Mediterranean Action Plan of the United Nations Environment Programme (UNEP/MAP) and with all the environmental policies that consider the sensitive habitats of coastal strip at the center of actions to protect the Mediterranean Sea.

Dr. Paolo Tomassetti

ISPRA, CN-LAB

CONTENTS

INTRODUCTION PART

1. CORALLIGENOUS HABITAT

1.1 Definition and ecological relevance of coralligenous

Definition and distribution

The word "coralligenous" was first used by Marion in 1883 to describe the hard bottoms in the Gulf of Marseilles between the infralittoral seagrass meadows of *Posidonia oceanica* and the coastal muddy of the circalittoral: a transitional zone located between 30 and 130 m deep. Later Pérès e Picard (1951, 1964) recognized coralligenous as a biocenosis. In May 2006, the RAC/SPA (Regional Activity Centre for Specially Protected Areas of the UNEP/MAP under the Barcellona Convention) proposed the following definition of coralligenous: "the coralligenous is considered as a typical Mediterranean underwater seascape comprising coralline algal frameworks that grow in dim light conditions and in relatively calm waters" (Ballesteros, 2006; UNEP/MAP-RAC/SPA, 2008). Coralligenous is thus recognized as a hard substrate of biogenic origin mainly produced by the accumulation of calcareous encrusting algae growing in peculiar environmental conditions and that is subsequently consolidated by the grow of calcareous animal organisms.

Coralligenous can developed between 25 e i 200 m of depth. The distribution of coralligenous is regulated by a combination of biotic and abiotic factors including light, nutrients, water circulation, temperature, sediment deposition and biological interactions. Due to their origin, coralligenous structures have very specific needs: reduced light, low and relatively constant temperature, clear waters with low resuspension and weak hydrodynamism.

Biocostruction and structure

Coralligenous habitat is characterized by bioconstruction resulting from the accumulation of carbonate skeletons produced by many plant and animal species which give rise to macroscopic structures that are maintained over time.

The calcareous red algae (Rhodophyta) belonging to the subclass of Corallinophycidae are the main builders of coralligenous; in particular, *Mesophyllum alternans* is the main algal builder developing in the shallow water of the North-Western Mediterranean. As the water deepens, other corallines such as *Lithophyllum frondosum*, *L. stictiforme* and *Neogoniolithon mamillosum* become important builders (Figure 1.1). The structures built by coralline algae are then strengthened by those organisms recognized as secondary bioconstructors, such as Peyssonneliaceae and various species of polychaetes, scleractinians (former madreporans) and bryozoans (Figures 1.2, 1.3). The coralligenous structures increase in size thanks to the progressive accumulation of the calcification product of successive generations of algae. The estimated growth rate for coralligenous concretions is about 0.006/0.83 mm $\cdot yr^{-1}$ (Ballesteros, 2006). The growth dynamics, however, is partly counterbalanced by mechanical demolition phenomena or by the action of bioeroders organisms which are able to pierce, crumble or dissolve calcium carbonate through different systems. Particularly important are the excavating sponges (e.g. genus *Cliona*) and molluscs (e.g. the "date of the sea", genus *Lithophaga*) (Figure 1.4). Three different types of eroding organisms can be distinguished: browsers, microborers and macroborers (Ballesteros, 2006).

Biodiversity and ecological value of coralligenous

Coralligenous communities constitute the most important "hot spot" of species diversity in the Mediterranean, together with *Posidonia oceanica* meadows (Boudouresque, 2004). However, in absence of previous estimates of the number of species that thrive in the coralligenous assemblages and considering their complex structure, they probably harbour more species than any other Mediterranean community (Ballesteros, 2006).

Bioconstruction leads to an increase in spatial heterogeneity, to greater structural complexity and so to an enrichment in microhabitat resulting in an increase in biodiversity (Figure 1.5). The cavernous structure of the coralligenous hosts a very complex community of organisms dominated by filter feeders (sponges, hydrozoans, anthozoans, bryozoans, serpulids, molluscs, tunicates), while inside the slits and crevices there is a very rich and diversified endofauna (polychaetes and crustaceans) (Cocito, 2004) (Figure 1.6).

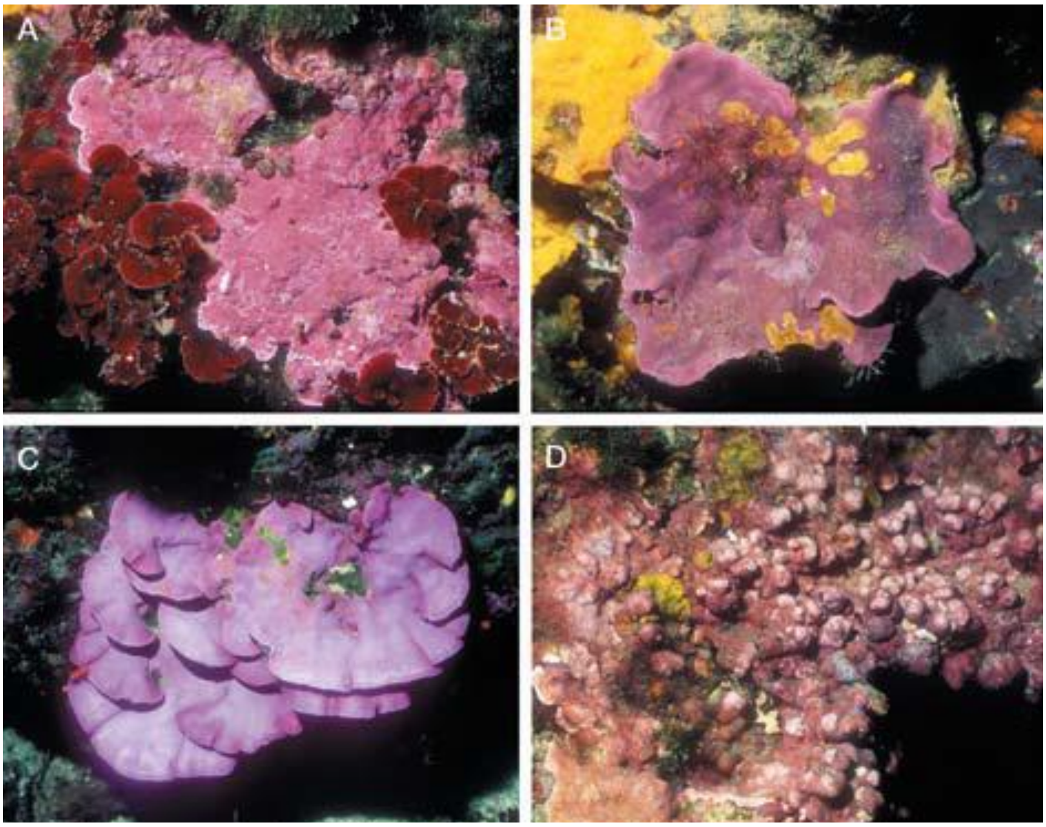

Figure 1.1 – Main red algal building species in coralligenous cliffs: A) *Mesophyllum alternans*; B) *Lithophyllum frondosum*; C) *Lithophyllum stictiforme*; D) *Neogoniolithon mamillosum* (photos by Ballesteros, 2006)

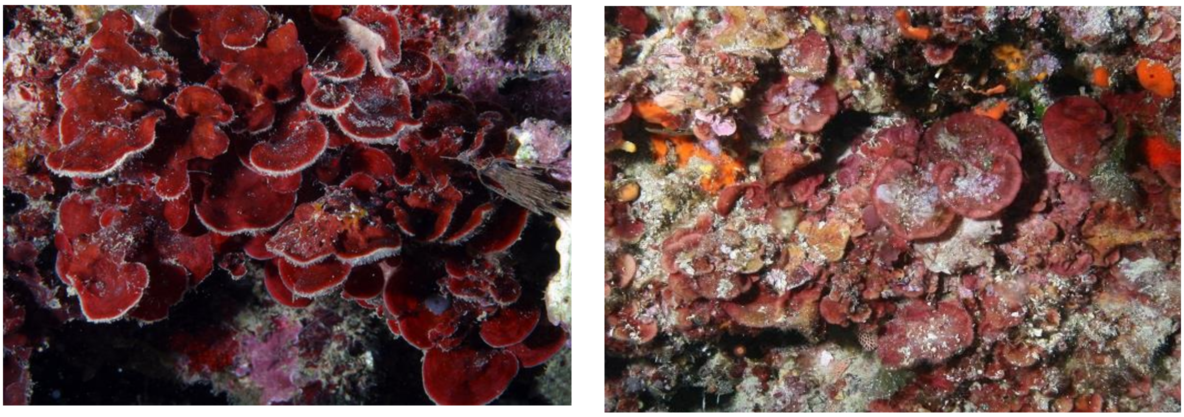

Figure 1.2 – Secondary building algal species (Peyssonneliaceae family) (photos by Andrea Lampis)

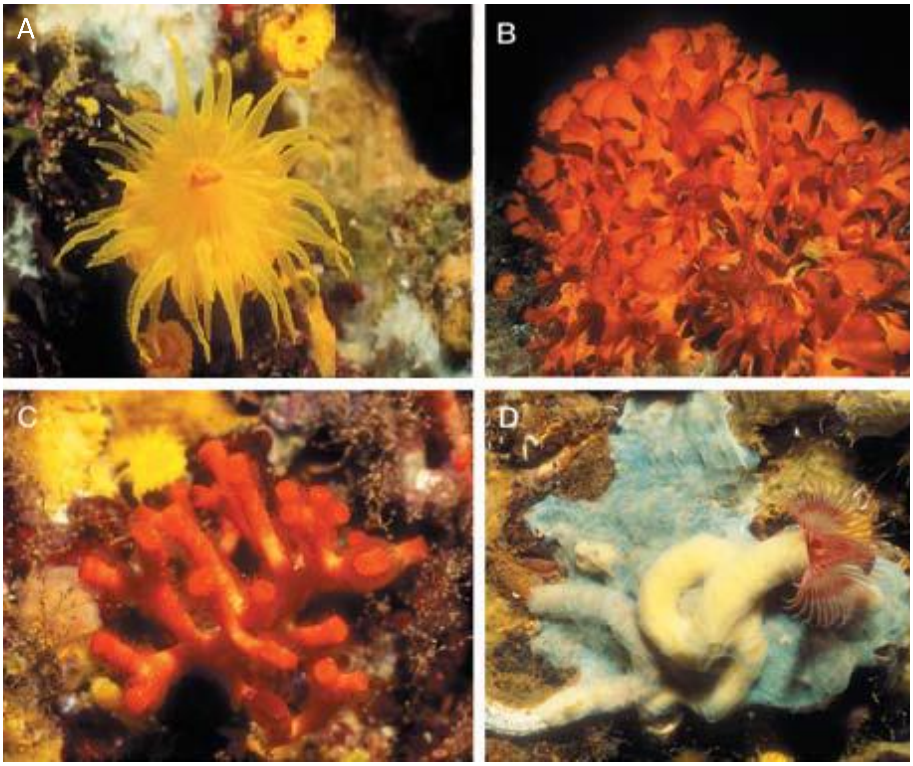

Figure 1.3 – Secondary building animal species. A) Scleractinians (former Madreporans) (*Leptopsammia pruvoti*); B and C) Bryozoans (*Pentapora fascialis* and *Myriapora truncata*); D) Polychaetes (*Serpula vermicularis*) (photos by Ballesteros, 2006)

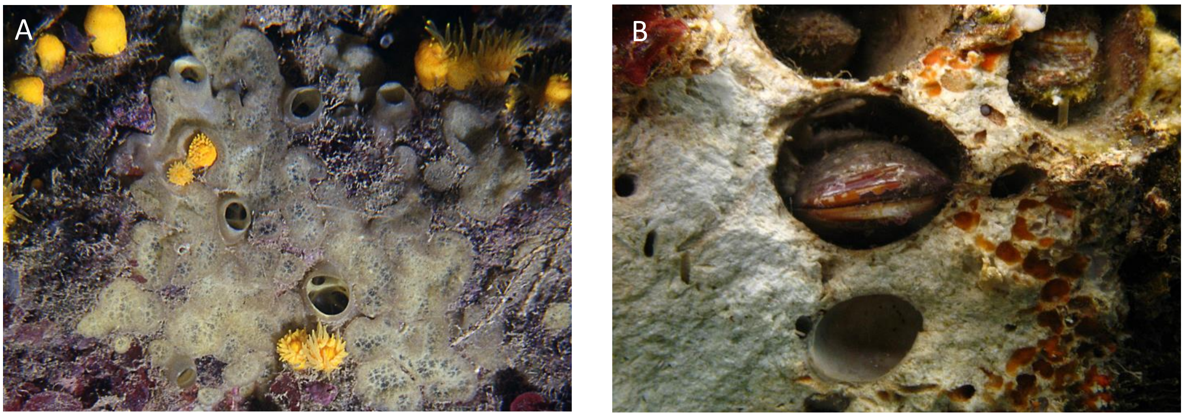

Figure 1.4 – Bioeroders animal organisms. A) Sponges (*Cliona viridis*) (photo by Simone Bava); B) Molluscs (*Lithophaga lithophaga*) (photo by Anthony Leydet)

In 1982 Hong proposed a classification based on four different categories of invertebrates which can be distinguished with respect to their position and ecological significance in the coralligenous structure (Hong, 1982):

1) Fauna contributing to build up, which help to develop and consolidate concretions created by the calcareous algae. Several bryozoans, polychaetes (serpulids), corals and sponges constitute this category. They include 24% of the total species number;
2) Cryptofauna colonising the small holes and crevices of the coralligenous structure. They represent around 7% of the species, including different molluscs;
3) Epifauna (living over the concretions) and endofauna (living inside the sediments retained by the buildup) which represent a great number of species (nearly 67%);
4) Eroding species, accounting for only around 1%.

Coralligenous assemblages are of enormus ecological and economic importance as: i) they are characterized by a great wealth of habitats and a high diversity of species; ii) represent an important economic resource due to the presence of many commercial species (e.g. lobsters, red coral); iii) are one of the most popular sites for recreational diving and so of great aesthetic and tourist value and iv) calcareous organisms are fundamental in the CO_2 balance.

Figure 1.5 – Structure and biodiversity of coralligenous habitat (photo by Alessandro Tommasi)

Figure 1.6 – Detail of a coralligenous cliff: richness in microhabitats and biodiversity (photo by Andrea Lampis)

1.2 Types of coralligenous

The morphology and inner structure of coralligenous mainly depend on depth, topography and the nature of prevailing algal builders (Laborel, 1987). Many types of coralligenous have been described (Bosence, 1983, 1985; Di Geronimo et al., 2001; Ballesteros, 2006; Bracchi et al., 2015), but the most used subdivision distinguishes coralligenous between cliffs or rims and platforms or banks (Pérès & Picard 1964; Ballesteros, 2006; SPA/RAC-UN Environment/MAP, 2019).

Coralligenous cliffs (Figures 1.7, 1.8) develop on coastal circalittoral rocky substrates mostly between 30 and 90 m, but they can be found between 15 and 130 m depending on environmental conditions (water turbidity, currents, exposure, bottom slope). The thickness of calcareous structures can vary from 20-25 cm to more than 2 m, usually increasing from shallow to deep waters. Two main forms of coralligenous cliff have been described: the rock wall coralligenous developing on the vertical or sub-vertical substrates with more or less thick concretions and the coralligenous concretion forming biogenous clumps even several meters thick out the horizontal rocky substrates (EUNIS, 2019).

Coralligenous platforms (Figure 1.9) are large tabular buildups developing on more or less horizontal bottoms of the continental shelf, mostly between 40 to 120 m of depth. They are mostly surrounded by sedimentary substrates, and may developed from the coalescence of rhodoliths or grow on rocky outcrops. The thickness of biogenic structures can vary between a few centimeters and several meters and the geometry of this habitat is really variable: from circular to ellipsoidal to sub rectangular shapes, more or less elongated, covering from a few square meters to tens of square kilometers (Bracchi et al, 2015).

Although both types of coralligenous may be found within a larger bathymetric range, coralligenous cliffs developing mostly on vertical wall characterize coastal rocky systems until 40/50 m of depth, whereas platforms mainly occur on deeper continental shelfs (Ballesteros, 2006; Cánovas-Molina et al., 2016).

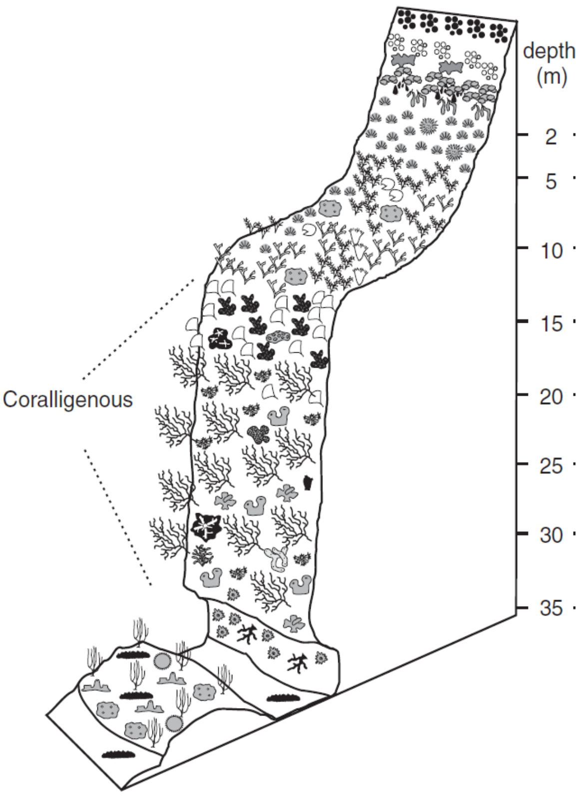

Figure 1.7 – Structure of coralligenous cliff developing on vertical rock wall (Ballesteros 2006, modified)

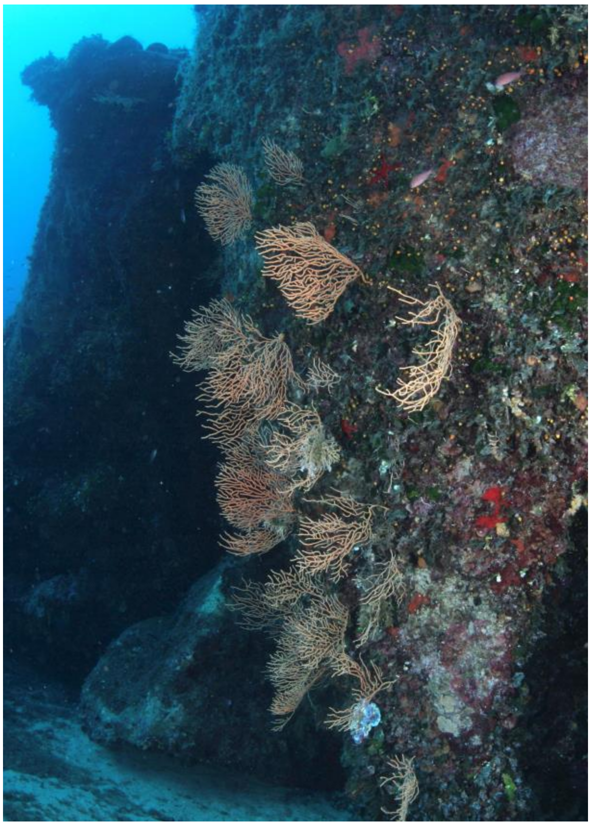

Figure 1.8 – Coralligenous cliff on vertical rock wall (photo by Edoardo Casoli)

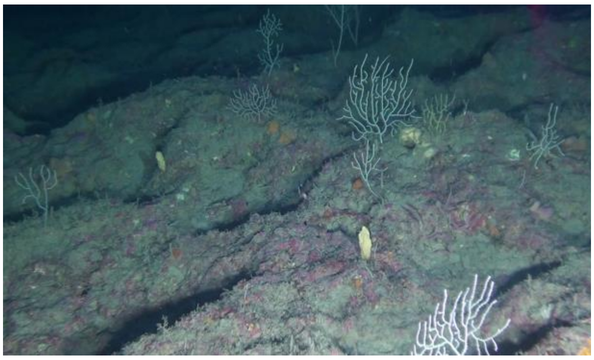

Figure 1.9 – Examples of coralligenous platforms (photos from ROV images)

1.3 Anthropogenic pressures

The coralligenous habitat is the result of a perfect but very delicate dynamic balance between bioconstruction and bioerosion. When this equilibrium is broken, demolition processes prevail and the coralligenous begins to degrade. The breakdown of the balance can be due to many causes, including natural ones, but more easily linked to anthropogenic pressures that act both locally and on a large scale. The coralligenous develops in particular and stable environmental conditions, any variation of these conditions can be lethal for organisms that are not adapted to change. Coralligenous is therefore a fragile ecosystem, particularly sensitive to environmental alterations and therefore threatened by climate change, mechanical destruction and modification of the water physical and chemical parameters (Piazzi et al., 2012; Gatti et al., 2015b; Cánovas Molina et al., 2016; Montefalcone et al., 2017).

Climate change and ocean acidification

Climate change can affect coralligenous assemblages in various ways. Increases in temperature for prolonged periods below the thermocline may cause the death of stenothermic organisms both directly and by favoring the proliferation of pathogens; large-scale die-offs of anthozoans and coralline algae have been repeatedly described in the recent years (Cerrano et al., 2000; Garrabou et al., 2001, 2009; Bramanti et al., 2005, 2013) (Figure 1.10A,B).
The increase in carbon dioxide in the atmosphere due to the use of fossil fuels and deforestation causes its increase also in marine waters. The CO_2 dissolved in the sea tends to react chemically with H_2O forming other compounds (H_2CO_3, HCO_3^-, H^+, CO_3^{2-}) which increase the acidity of the water. The consequent decrease in the pH value can have serious consequences for marine ecosystems as it can limit the availability of calcium carbonate ($CaCO_3$), a mineral essential for the composition of the skeletons and shells of many marine organisms, including coralline algae and other bioconstructor species of coralligenous. There is indeed a defined "saturation" limit beyond which $CaCO_3$ tends to dissolve, leading to a decrease in bioconstruction phenomena (Rodolfo-Metalpa et al., 2009; Lombardi et al., 2011).
The increase in temperature can favor the onset of phenomena such as the development of planktonic and benthic mucilages (Figure 1.10C). The latter, in particular, are produced by both native and introduced macroalgae (Phaeophyceae) and microalgae (Chrysophyceae), which cover all sessile organisms causing their death. The organisms most sensitive to this phenomenon are the gorgonians (Giuliani et al., 2005), but recently death of other organisms and of the coralline algae have been recorded (Schiaparelli et al., 2007; Piazzi et al., 2018a).

Invasive alien species

Some species introduced into the Mediterranean have become invasive (Boudouresque & Ribera, 1994; Boudouresque & Verlaque, 2002) and a number of them can thrive in, or are more or less adapted to, the coralligenous habitat. Currently, only introduced algal species are threatening the coralligenous community and then only in some areas of the Mediterranean. Probably the most dangerous alien species for the coralligenous community is the small red alga *Womersleyella setacea*, which is currently distributed along most of the Mediterranean basin: this species grows abundantly in coralligenous (and

other sublittoral) communities, forming a dense turf 1-2 cm thick over the encrusting corallines that constitute the calcareous concretion (Ballesteros, 2004) (Figures 1.10D, 1.11A). The dense turf of *W. setacea* decreases light availability to the encrusting corallines (avoiding or reducing photosynthesis and growth of these algae), increases sediment trapping (Airoldi et al., 1995), excludes other macroalgae by overgrowth and pre-emption (Piazzi et al., 2007a) and inhibits recruitment of corallines and other algal and animal species inhabiting the coralligenous community. Moreover, the species richness found in sites invaded by *W. setacea* is lower than that observed in non colonised sites (Piazzi et al., 2004).
Another alien alga developing dense turf is *Acrothamnion preissii*, a red alga able to grow in deep waters, although it was observed only in the coralligenous community of the Balearic Islands (Ballesteros, 2006) where it is never dominant in this environment and always grows together with *W. setacea* (Figures 1.10D, 1.11A).
Two other species that have been reported to act as invaders in the Mediterranean are *Asparagopsis taxiformis* (Ballesteros & Rodrìguez-Prieto 1996) and *Lophocladia lallemandii* (Patzner, 1998), which are becoming increasingly abundant both in shallow bottoms and deep waters around the Balearic Islands.
Caulerpa taxifolia, a green tropical alga belonging to the Caulerpaceae family, is another highly invasive alien species that can threaten coralligenous community. Although mainly found in relatively shallow waters (Meinesz & Hesse, 1991), it has been recorded down to a depth of 99 m and in some areas, such as Cap Martin (France), where it has totally invaded the coralligenous community (Belsher & Meinesz, 1995).
Undoubtedly more dangerous is the congeneric *Caulerpa cylindracea*, highly invasive in the coralligenous habitat with negative effects especially on sessile and encrusting organisms (Piazzi et al., 2007a; Piazzi & Balata, 2009; Gatti et al., 2015b, 2017) (Figure 1.11B). Most sessile organisms can partially or completely recover during the dormant period of *C. cylindracea* (Piazzi & Ceccherelli, 2006; Klein & Verlaque, 2009), but this period is not sufficient for coralline algae (Garrabou & Ballesteros, 2000) and this contributes to worsen the impact on coralligenous habitats (Piazzi et al., 2012).

Eutrophication

Urban, agricultural and industrial wastewater discharges cause an increase in nutrient concentration which in turn leads to a decrease in biodiversity and biomass of many sensitive organisms, a modification of the structure of assemblages and a decrease in the growth rate of coralligenous (Hong, 1983; Piazzi et al., 2011; Gatti et al., 2015a, 2015b). There are few data concerning the impact of pollutants on the growth of coralline algae, although it is known that orthophosphate ions inhibit their calcification (Simkiss, 1964). Furthermore, Hong (1980) observed that with increased pollution large thalli of *Mesophyllum alternans* are replaced by Peyssonneliaceae, which have a much lower building capacity (Sartoretto, 1996), and at the same time the bioeroders species increase their abundance.

Sedimentation

The increase of fine sediments in coastal areas due to deforestation, washout of land and human interventation on the shore line, may cause direct and indirect damage to coralligenous assemblages. The direct effects include the covering and suffocation of sessile organisms, the abrasion and damage of the most sensitive organisms to the advantage of the most opportunistic ones (Balata et al., 2005, 2007a, b) (Figure 1.10E). These mechanisms lead to a decrease in biodiversity and a structural change in assemblages which become dominated by opportunistic species (Balata et al., 2005, 2007). The main indirect effect is the increase in the turbidity of the water which causes the death of algae in the deeper areas and consequently a retreat towards the surface of the lower limit of the coralligenous (Piazzi et al., 2012).

Mechanical destruction

Many human activities can cause mechanical destruction of bioconstructed calcareous structures. Trawling is the fishing method considered to be the most destructive and is causing the degradation of large areas of coralligenous concretions (Boudouresque et al., 1990) by directly causing mechanical damage (breaking down the biostructure) and indirectly by negatively affecting the photosynthetic production of erect and encrusting algae as a consequence of increased turbidity and sedimentation rates. However, even artisanal and sport fishing can create significant damage to the most sensitive coralligenous organisms that can be damaged or removed by fishing gear such as lines and gillnets (Bavestrello et al., 1997; Ferrigno et al., 2018), both during the same fishing activity and outside of it,

as in the case of the involuntary abandonment of stranded or damaged fishing nets that constitute the so-called "ghost nets". Once they have remained on the bottom, they continue inexorably to damage the benthic and pelagic organisms that remain within it (Figure 1.10F).
Anchors destroy large portions of bio-built structure and remove organisms growing on them; damage repeated over time prevents the assemblages from recovering themselves, leading to a decrease in bioconstructions (Piazzi et al., 2012).
Even underwater activities, if poorly managed, can affect the more sensitive coralligenous organisms, such as gorgonians and erect bryozoans. Studies have found that the density and diameter of bryozoan colonies (especially *Pentapora fascialis*) are significantly reduced at sites highly frequented by divers (Garrabou et al., 1998; De la Nuez-Hernández et al., 2014).

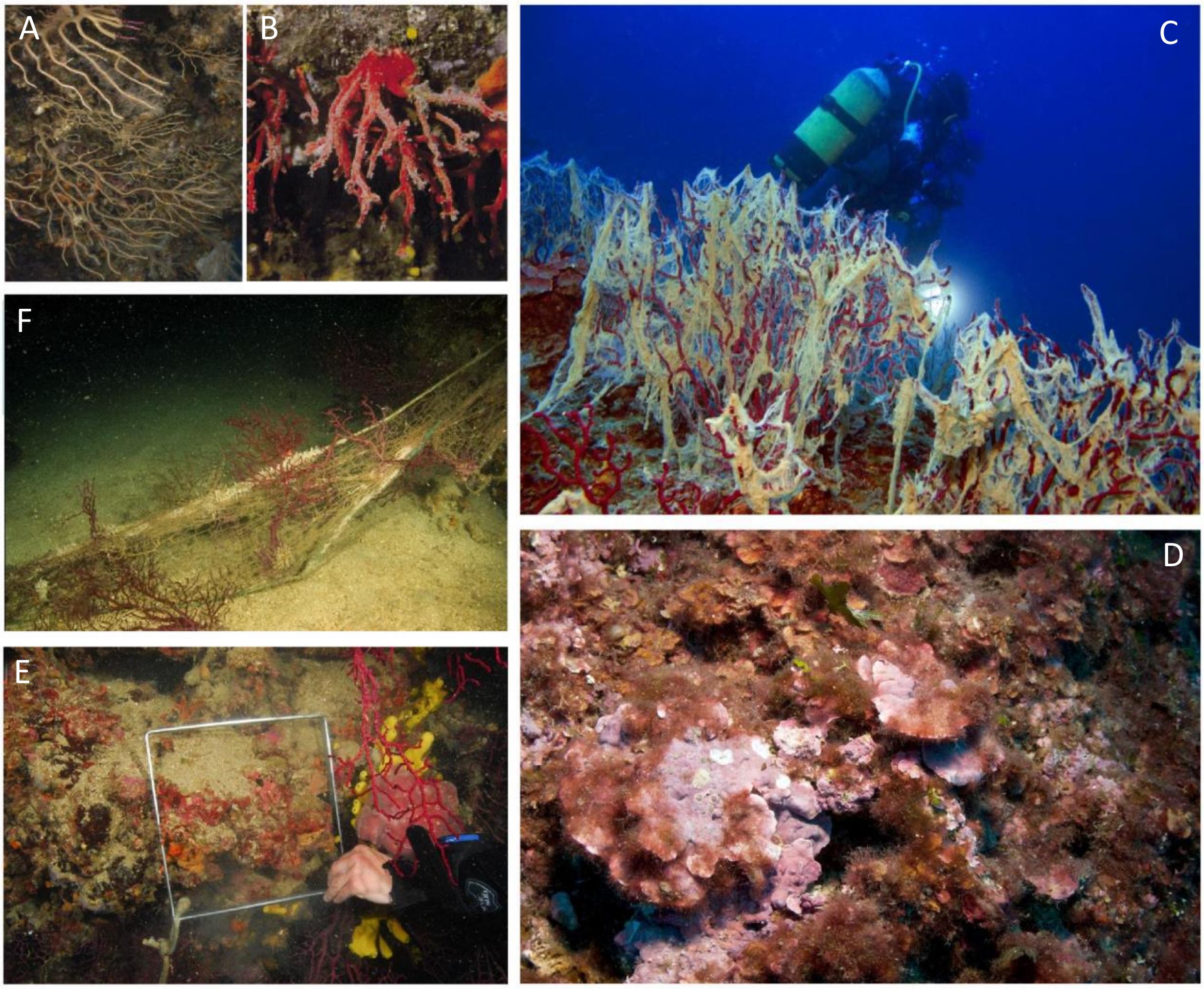

Figure 1.10 – Disturbances affecting coralligenous habitat (adopted from Garrabou et al., 2014, modified). Mass mortality outbreaks related to global climate change: A) naked branches (devoid of tissue) of the red gorgonian *Paramuricea clavata* (adopted from Garrabou et al., 2009), B) necrotic tissue (greyish in colour) of the precious red coral *Corallium rubrum* (adopted from Cerrano et al., 2000) and C) mucilaginous algal aggregates over gorgonian branches (photo by Sara Kaleb); D) invasive red turf algae *Womersleyella setacea* overgrowing coralligenous main builders, calcareous red algae (photo by Ante Žuljvić); E) sedimentation over coralligenous assemblages dominated by coralline algae; F) destructive impact of fishing nets (photo by Petar Kružić)

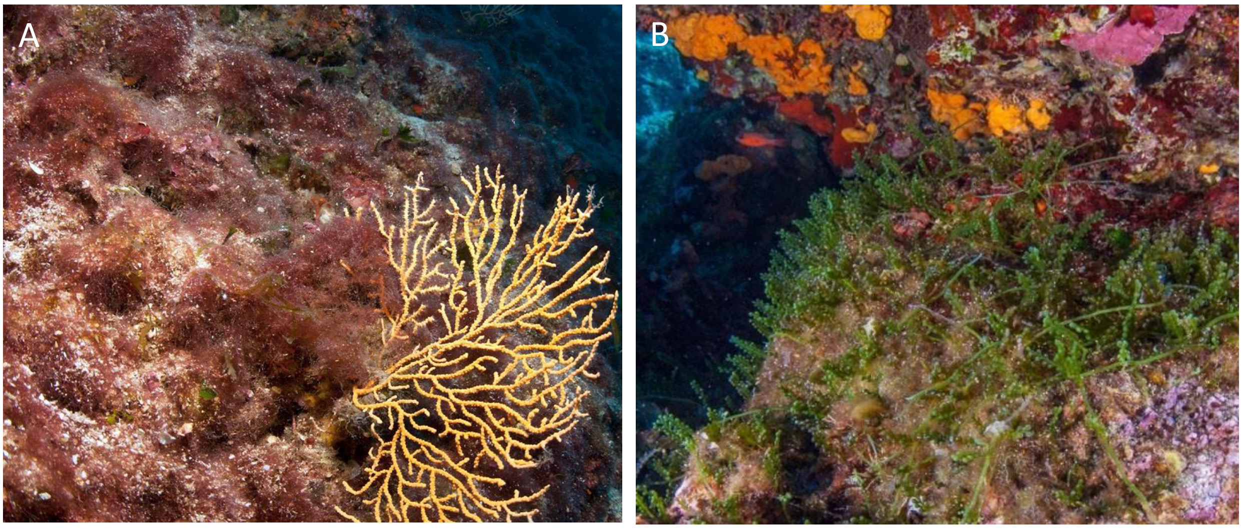

Figure 1.11 – Alien macroalgae invasion on coralligenous assemblages. A) *Womersleyella setacea* (photo by Ante Žuljvić) and B) *Caulerpa cylindracea* (photo by Yannis Issaris)

1.4 The coralligenous cliff: description and ecological value

Description

The coralligenous cliff usually shows a stratified structure: coralline algae create a secondary substrate that cover the rocky bottom, constituting in turn a substrate for the implantation of prostrate and erect species (Ballesteros, 2006). It can also develop different physiognomies in relation to biogeographical, environmental and biotic factors, which were have tried several times to classify in communities, associations or facies (True, 1970; Augier & Boudouresque, 1975; Giaccone, 2007).

Assemblages can indeed be dominated by animal or algal organisms in response to the dominant environmental factors. Among the animal ones, assemblages of *Paramuricea clavata*, *Eunicella cavolini*, *Eunicella singularis*, *Leptogorgia sarmentosa*, *Parazoanthus axinellae* and *Corallium rubrum* are considered (True, 1970; Augier & Boudouresque, 1975; Giaccone, 2007; Casas-Guell et al., 2015, 2016), but in some cases sponges (as in the Eastern Mediterranean, Pérès & Picard, 1968) or bryozoans (Zabala, 1986) may become dominant. Among the main macroalgal taxa we can mention the Fucales (*Cystoseira zosteroides*, *C. montagnei*, *C. usneoides*, *Sargassum hornschuchii*), the large brown algae with laminar thallus (*Laminaria rodriguezii*, *Phyllariopsis brevipes*), the red laminar algae (genera *Sebdenia*, *Kallymenia*, *Fauchea*, etc.) and *Halimeda tuna* (Ballesteros, 2006; Giaccone, 2007). In many cases, the erect layer consisting of large erect anthozoans is totally or partially absent and assemblages are mainly formed by macroalgae or bryozoans (Figures 1.12 and 1.13).

Coralligenous cliff as indicator of anthropogenic impact

Coralligenous cliffs and platforms are, for the most part, subject to different types of anthropic impact (Piazzi et al., 2019). Coralligenous platforms are exposed to pressures acting at greater depths, such as sediment and solid waste accumulation and trawling (Colloca et al., 2003; Watters et al., 2010; Mordecai et al., 2011; Ferrigno et al., 2018; Enrichetti et al., 2019). Conversely, the greater proximity to the coast of the coralligenous cliffs compared to platforms, makes the former more subject to environmental alterations linked to the anthropization of the coastal strip and to smaller depths, among which the increase in nutrients and sedimentation of terrigenous origin, water chemical pollution, underwater recreational diving, anchoring and damage from sport and artisanal fishing, the development of mucilages, the invasion of alien species and pathogens are the most common (Bavestrello et al. , 1997; Balata et al., 2005; Piazzi & Balata, 2011). For these reasons, coralligenous cliff is considered an excellent indicator of impacts related to anthropic activities affecting the adjacent coastal strip (Deter et al., 2012; Piazzi et al., 2012, 2017; Cecchi et al., 2014; Gatti et al., 2015a; Montefalcone et al., 2017; Sartoretto et al., 2017). Furthermore, given the smaller depth at which it develops, coralligenous cliff is particularly exposed to the effects of climate changes linked to the warming of surface waters (Cerrano et al., 2000; Giuliani et al., 2005; Linares et al., 2010; Gatti et al., 2015b, 2017; Bianchi et al., 2019a, b).

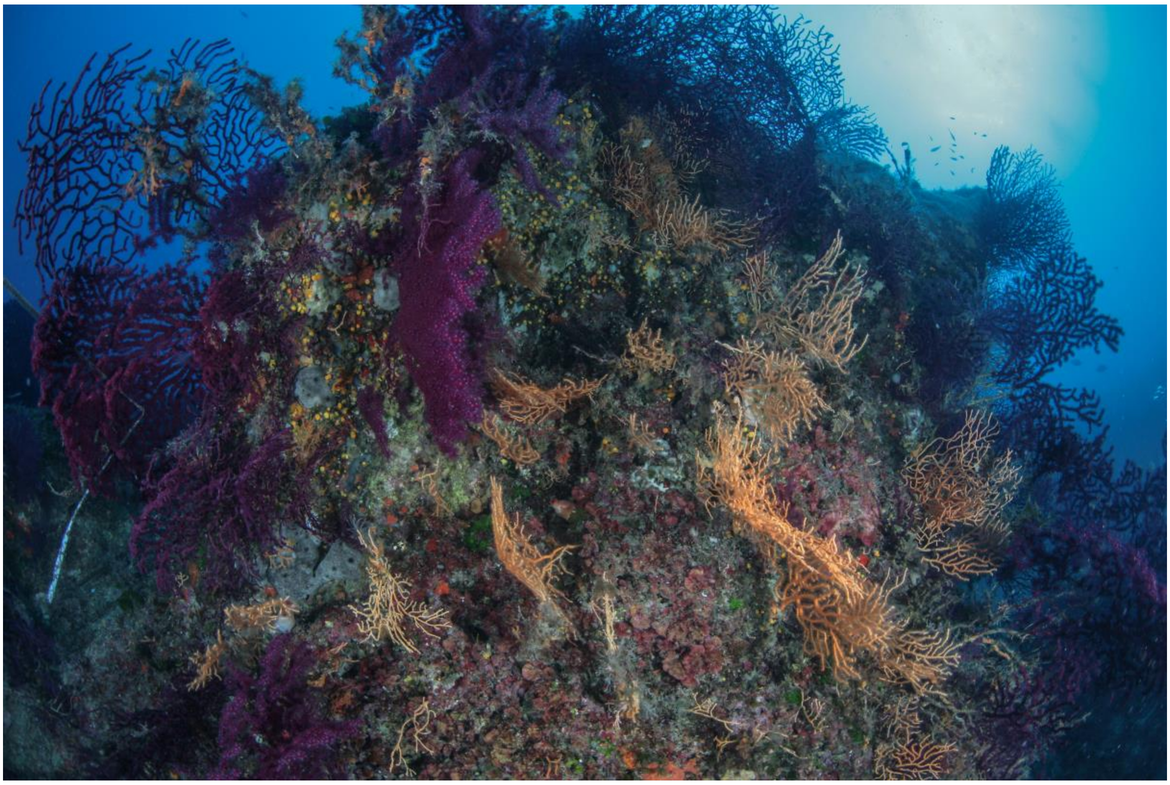

Figure 1.12 – Coralligenous cliff dominated by large erect anthozoans (photo by Edoardo Casoli)

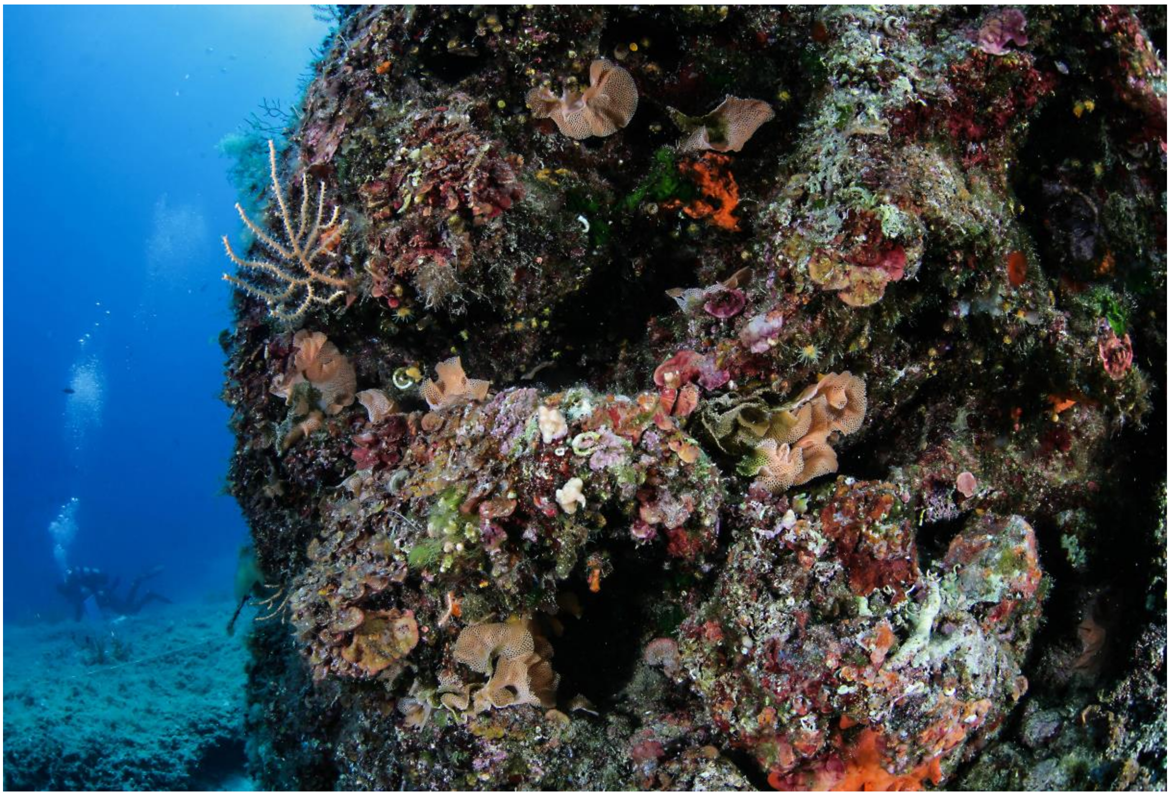

Figure 1.13 – Coralligenous cliff characterized by absence, or rare presence, of large erect anthozoans (photo by Edoardo Casoli)

1.5 Conservation and protection of coralligenous habitat: environmental policies and evolution of the legislative framework

Despite the importance of coralligenous communities and the high ecological value of many species therein present (some of which are legally protected such as *Savalia savaglia*, *Spongia officinalis*, etc.), many years had to pass before conservation and protection polices of the marine environment developed into specific legislative acts for this type of habitat.

The Council Directive 92/43/EEC (European Commission 1992, Habitats Directive transposed in the Italian national law through Presidential Decree n. 357/1997 and subsequent amendments) is the first European law that introduces measures aimed at ensuring the maintenance and restoration of habitats and species of community importance and for this reason it represents the milestone in the implementation of biodiversity conservation policies in Europe. The directive provides for the establishment of a European ecological network of legally protected sites, created by designating Special Areas of Conservation (SACs) for those habitats and species indicated as "priority" in its annexes. The SACs, together with the Special Protection Areas (SPAs) (Council Directive 79/409/EEC, Birds Directive), constitute the Natura 2000 network of protected natural areas established by European Community in order to protect the conservation status of species and habitats that require targeted protection actions. In this context, the only marine-coastal water habitat indicated as "priority" in the list of habitats of community importance is the *Posidonia oceanica* beds (Habitat 1120, Annex I, Habitats Directive). The coralligenous habitat, on the other hand, is not specifically mentioned, but is included in a more generic type of habitat called "Reefs" (Habitat 1170, Annex I, Habitats Directive). Consequently, during the years of the Directive 92/43/EEC implementation, one of the most important and sensitive habitats in the Mediterranean Sea remained substantially excluded from the list of Sites of Community Importance (SCIs) and thus from the designation of SAC for its protection and conservation. In many cases, however, a *de facto* protection was established, as an indirect protection of coralligenous assemblages occurred when they were present in areas designated for the conservation of other habitats or species regulated by the directive. However, since the legislation was not targeted to the coralligenous habitat, assemblages present outside the designated areas were excluded from monitoring and conservation plans while *ad hoc* monitoring plans were never applied for the coralligenous assemblages present in the same areas.

The last Commission Implementing Decision 2019/22 (EU, 2019), which adopts the twelfth update of the list of SCIs for the Mediterranean biogeographical region, extends this *de facto* protection to some of the most valuable coralligenous assemblages of the Mediterranean Sea. However, in line with the legislative framework of the directive, the protection constraint is not specific for coralligenous habitat itself, but rather related to its presence within the SIC.

A number of years later the first enactment of the Habitats Directive, coralligenous reefs were included among the priority protection habitats of the SPA/BD protocol (Specially Protected Areas and Biological Diversity in the Mediterranean) for which the Barcelona Convention required strict protection.

The Barcelona Convention, born in '76 as the "Convention for the Protection of the Mediterranean Sea against Pollution" under the aegis of UNEP (United Nations Environment Program), is the legal and operational instrument of the Mediterranean Action Plan (MAP) (Council Decision, 1977). Amended in 1995 to "Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean", it was ratified by Italy in 1999 (Law n.175 of May 27, 1999) and entered into force in 2004. MAP is an effort of regional cooperation involving 21 countries of the Mediterranean Sea and the European Union. Under the MAP agreement, the contracting parties to the Barcelona Convention and its Protocols undertake to face the challenges of protecting the marine-coastal environment, through integrated planning and management of coastal zones and by strengthening regional and national plans to implement sustainable development. The main obligations of the contracting parties refer to precautionary actions to prevent, fight and eliminate pollution of the Mediterranean Sea and to protect and enhance the marine environment; to this end, the convention has adopted 7 implementation protocols, including the SPA/BD Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean.

With the issuance of the European Framework Directives (on the Water, WFD 2000/60/EC and Marine Strategy, MSFD 2008/56/EC) a new scenario opens up in the European legislative panorama, which experiments for the first time with the revolutionary "ecological" and "ecosystem" approach introduced by these two directives. However, other ten years will pass from the enactment of the first Framework Directive before coralligenous becomes the object of specific and targeted legislation.

The Water Framework Directive 2000/60/EC (EC, 2000), transposed in the Italian national law through Legislative Decree n.152/2006, introduces an innovative approach in European legislation on water, both from an environmental point of view and administrative-management one. In fact, it establishes a reference framework for Community action on water matters for the purpose of protecting and managing water resources such as inland surface and ground water transitional and coastal water. The directive pursues ambitious objectives: to prevent qualitative and quantitative deterioration, to improve the status of waters and to ensure sustainable use, which should be based on the long-term protection of available water resources. To achieve these goals requires careful planning of short and long-term monitoring, based on the analysis of anthropogenic pressures, as well as programs of measures aimed at restoring the good ecological status of water bodies for which monitoring highlights deterioration

The WFD 2000/60/EC, however, does not change the legislative framework on the protection and conservation of coralligenous assemblages. In continuity with the previous Habitats Directive, the law on marine-coastal water refers to *Posidonia oceanica*, which is included among the benthic indicators of anthropogenic impact together with Macrozoobenthos of the soft bottoms and Macroalgae of superficial hard bottoms (from 0 to 3 m). All other marine communities of hard bottom developing below 3 m of depth are excluded from ecological quality status classification of water bodies, including the coralligenous communities of circalittoral rocks.

Thus, concrete protection actions (such as institution of new Marine Protected Areas aimed at conservation of coralligenous) are only recently urged through the adoption of the Action Plan for the Conservation of Coralligenous and Other Calcareous Bio-concretions in the Mediterranean Sea (UNEP/MAP 2008, 2017) under the Barcelona Convention, emphasizing the need to adopt standardized monitoring methods and programs. The Action Plan, however, indicates paths and actions to be taken for the protection and conservation of the coralligenous habitats, but it is not a legally binding program. So, by the end of the 2000s there was still no legislation targeted at coralligenous reefs and, consequently, specific monitoring and management plans for their conservation and protection were lacking.

A turning point in this sense was the Marine Strategy Framework Directive (MSFD 2008/56/EC), which introduces for the first time the legal obligation to assess the extent and condition of coralligenous habitats as part of the process defining the status of the “biodiversity” and “seafloor integrity” descriptors.

The MSFD 2008/56/EC (transposed in the Italian national law through Legislative Decree n. 190/2010 and subsequent amendments) is an innovative legislative tool for the protection of seas, as it constitutes the first binding legal act for Member States that considers the marine environment from a systemic perspective. To achieve MSFD 2008/56/EC and prevent degradation and restore the damaged ecosystems, each country must develop its own strategy, implementing measures necessary to achieve (or maintain) a "Good Environmental Status" (GES) of marine waters, referring to the status of marine environments that allows for the preservation of ecological diversity, the vitality of clean, healthy and productive seas and oceans, and ensures sustainable use of marine environment.

Compared to the previous directive, the MSFD broadens the normative horizons both in a spatial and methodological sense: it refers to both coastal and offshore environments, and it replaces the "deconstructive/structural" approach, based on the Biological Quality Elements (BQEs) defined on a water body scale, with the holistic/functional one for which a set of 11 Descriptors summarizes the environmental status of an entire system defined on a marine sub-region scale. In this context, the marine habitat as a whole, and no longer the single BQE, become subject of the quality monitoring and assessment for definition of the good ecological status.

Although based on opposite approaches, the two directives converge where assessment of the habitat condition under the MSFD necessarily passes through the analysis of the quality of biological communities. This can be done by applying habitat-specific and multi-metric indices already used under WFD 2000/60/EC or newly elaborated indices, as in the case of coastal marine habitats not regulated by the WDF 2000/60/EC (e.g. circalittoral rocky bottoms).

The integrated approach of the MSFD is one of the major strengths of this directive, which is also a candidate to become the environmental pillar of the future Integrated Maritime Policy (IMP) of the European Union (Casazza et al. 2007). The integration process proposed by the directive, in fact, does not only concern the ecosystem-based methodological approach, but also the normative framework, which requires that the application of Directive 2008/56/EC be conducted in a coordinated and synergistic way with the previous directives and in compliance with the commitments undertaken under the relevant international agreements on the protection of marine environment.

In particular, the MSFD requires an assessment of the condition and extent of special habitats, referring above all to those indicated in EU legislation (e.g. Habitats Directive) and in international conventions as habitats of high interest from a scientific and biodiversity point of view. Furthermore, in the juridical overlap area between the WDF and the MSFD (1 mile from the coast line), the creation of monitoring synergies that cover the entire range of sensitive habitats present in marine-coastal waters is required, in order to ensure complementarity between the two directives avoiding at the same time unnecessary overlaps. Finally, the convergence between criteria that define some descriptors of the MSFD and the key elements of the IMAP Guidelines (Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coast) (IMAP, 2016) allows further development of the ecosystem approach as an integrated strategy for implementation of the juridical framework of the Barcelona Convention and to do so in full compliance with the Marine Strategy Framework Directive, contributing to the harmonization process of measures and programs as required by the Convention and its protocols.
With the implementation of MSFD 2008/56/EC by the member states, an important process of European legislation is thus set up focused on the coralligenous habitat which, due to its high ecological value and sensitivity to anthropogenic pressures, is considered by this directive an important bioindicator for definition of some of its descriptors. This represents the first milestone of a long process which, among other things, will have the task of filling any gaps inherited from previous legislations.
The lack of legislation targeted on coralligenous has in fact had as direct consequence the lack of a standardized database at a national and the Mediterranean level, useful both as a cognitive and management tool for conservation of this sensitive habitat. This is one of the reasons why coralligenous was recently included in the European Red List of Habitats (IUNC, 2016; Gubbay et al., 2016) as one of the data-deficient marine habitats, thus confirming the urgent need for thorough investigations and accurate monitoring plans (Ballesteros, 2008) already highlighted during previous years by the UNEP/MAP Action Plan.
This need will be partially satisfied by the monitoring plans on coralligenous carried out under the MSFD 2008/56/EC implementation (La Mesa et al., 2019). In fact, European legislation requires the development of monitoring and assessment plans based on standardized methods, in order to guarantee comparability of results on a large spatial and time scale. Plans must also be developed on the basis of the best scientific knowledge available; thus, legislation requires the periodic updating of strategies for the marine environment on the basis of the evolution of technical and scientific knowledge.
In this context, the MSFD 2008/56/EC implementation into Italian national law has mainly focused on the coralligenous platform, a type of habitat that usually develops in deeper waters (> 40 m). Platforms are surveyed by remotely operated vehicles (ROVs), which are very effective and functional tools for this type of habitat as they work with no limits at depths prohibitive for the routine scientific diving. On the other hand, the instrumental limits intrinsic to the methodology may hinder its effective use on the rock walls of coralligenous cliffs, which are the most common type of habitat in more superficial waters (< 40 m). This habitat can be instead effectively surveyed in scuba diving, thus completing data picture provided by the ROV on the condition of coralligenous habitats occurring along the marine-coastal area (UNEP/MAP, 2017; 2019).

2. MONITORING AND ASSESSMENT OF THE ECOLOGICAL STATUS OF CORALLIGENOUS CLIFF

2.1 Methods used and ecological quality indices

Benthic ecosystems are crucial elements for WFD and for many MSFD descriptors (in particular, D1-Biological Diversity and D6-Seafloor Integrity), for surveillance under the Habitats Directive (Articles 11 and 17) and for IMAP guidelines of the Barcelona Convention.
In this context, the development of ecological quality indices is considered a fundamental tool for the implementation of European legislation (Birk et al., 2012), as monitoring and assessment of the health status of marine ecosystems are the first step in planning management strategies suitable for preventing further environmental deterioration.
With regard to the marine-coastal environments, many indices for the assessment of the ecological status of different ecosystems were developed in recent years, but only some of them have focused on coralligenous habitat.
Among these, the MAES (Mesophotic Assemblages Ecological Status index, Cánovas-Molina et al., 2016), the MACS (Mesophotic Assemblages Conservation Status, Enrichetti et al., 2019) and the CBQI (Coralligenous Bioconstructions Quality Index, Ferrigno et al., 2018) were developed to obtain information from the ROV images, so essentially used for deeper continental shelf coralligenous.
For coralligenous cliff, where scuba diving surveys can be effectively carried out, several indices have been proposed and used based on different approaches. ESCA (Ecological Status of Coralligenous Assemblages, Cecchi et al., 2014, Piazzi et al., 2017b), ISLA (Integrated Sensitivity Level of coralligenous Assemblages, Montefalcone et al., 2017) and CAI (Coralligenous Assessment Index, Deter et al., 2012) are based on a biocenotic approach that studies coralligenous assemblages in terms of composition and abundance of all species present for ESCA and ISLA, and abundance of three components (mud, bryozoans and limestone organisms) for CAI.
COARSE (COralligenous Assessment by Reef Scape Estimate, Gatti et al., 2012, 2015a) uses a landscape approach to provide information about the structure of coralligenous cliffs and their conservation status.
Although based on different approaches, all four indices use indicators that provide information on biodiversity and seafloor integrity, which are both descriptors of MSFD 2008/56/EC.
INDEX-COR (Sartoretto et al., 2014) integrates three components (sensitivity of taxa to organic matter and sedimentation, biodiversity, and structural complexity of assemblages) to define the health status of coralligenous assemblages.
Some indices which follow a more functional approach were built for other environments but were subsequently used also for coralligenous habitat, although not specifically developed for this ecosystem. For example, EBQI (Ecosystem-Based Quality Index, Ruitton et al., 2014) identifies the different functional components of the ecosystem and calculates a quality index for each of them; OCI (Overall Complexity Index, Paoli et al., 2016) instead combines measures of structural and functional complexity.
All the aforementioned indices proposed by the literature are considered valid for the purposes of monitoring and evaluating the coralligenous ecological status. However, they have been developed with different approaches and adopt distinct ecological descriptors and sampling techniques, thus hindering the comparison of data and results, unless an integration and intercalibration process is conducted to provide a standardized procedure.

2.2 STAR: an integrated and standardized procedure to evaluate ecological status of coralligenous cliffs

2.2.1 Reference framework and development of the STAR protocol

The latest UNEP/MAP report on the Action Plan for the Conservation of Coralligenous in the Mediterranean Sea (UNEP/MAP-RAC/SPA, 2017) recalls the European directives requirements and emphasizes the need to:

- develop standardized monitoring protocols to make data acquired with different methodologies comparable and to provide an estimate of the impact level of anthropogenic pressures as accurate as possible;
- develop ecological quality indices for the assessment of the coralligenous conservation status and carry out intercalibration activities of methods developed with the aim to provide a common reference framework to compare ecological status of coralligenous across different areas of the Mediterranean.

In doing so, the UNEP/MAP report also recommends to develop monitoring methods that take into account the different types and bathymetric distribution of coralligenous assemblages, employing divers within the safe operating depths (40 m) and recurring to the use of the ROV at higher depths (UNEP/MAP-RAC/SPA, 2014, 2017). This is also in line with the principle, highlighted several times in the literature, that biological monitoring methods and the related indices must be "habitat-specific" so they require different monitoring and evaluation procedures (Borja et al., 2010).
In this context, STAR (STAndaRdize coralligenous evaluation) was developed as an integrated and standardized monitoring procedure for the assessment of ecological status of coralligenous assemblages occur on vertical rock walls within 40 m of depth (Piazzi et al., 2019a, b; UNEP/MED 2019). In fact, different methods are used to define the ecological quality of the coralligenous cliffs, methods that are derived from different approaches and that use data obtained with different sampling methods and designs. This makes particularly difficult to compare the data and quality values obtained, as any differences found could be related to different procedure of data acquisition rather than real ability of each method to identify the effects of anthropogenic pressures. As recalled by UNEP, there is therefore the need to reach a standardized sampling procedure that allows for a real comparison between the coralligenous assessment methods.
The STAR procedure (Piazzi et al., 2019 a,b) was proposed with the aim of integrating and standardizing the approaches used so far to study coralligenous cliffs in order to obtain, through a single effort of sampling and data collection, the information useful for calculating most of the existing quality indices for this environment. By applying the STAR protocol it is indeed possible to build a single database complete with all parameters so far recognized as important from an ecological point of view and which can be used to apply the various methods currently in use (or to be developed) for assessing the conservation status of coralligenous habitat.

The STAR procedure was developed through the following subsequent steps:

- bibliographic research on methods used to define the ecological quality of coralligenous cliffs;
- selection of the most suitable ecological metrics or descriptors for definition of ecological quality;
- definition of a sampling method allowing to obtain useful information to calculate the largest possible number of quality indices used up to now;
- optimization of the sampling effort;
- development of a standardized procedure that integrates the requirements described above;
- testing of the new procedure along a gradient of anthropogenic pressures.

The final test showed that the use of STAR allows to assess accurately the effect of the anthropogenic pressures that characterized the study sites (Piazzi et al., 2019 a,b).

2.2.2 Application of the STAR method

The STAR procedure can be summarized in the following main points:

1. Surveys should be planned no more than once per year, in the April-June period.
2. Vertical substrate (85-90°) at around 35 m depth (±3 m) should be selected.
3. Sampling design needed to characterize a site (about 1 km cliff) should consist of three areas (plots) of 4 m^2 each, located tens of metres between each other.
4. 10 photographic samples of 0.2 m^2 each should be collected per each plot by scuba divers.

5. Thickness of the calcareous basal layer should be measured *in situ* through a hand-held penetrometer with a minimum of 6 replicated measures per each plot.
6. Maximum height of the upper layer (organisms sized >10 cm) for each species should be measured and the percentage of necrosis of erect anthozoans should be assessed *in situ* through a visual estimate.
7. Percentage cover of sediment, mucilages and cavities due to erosion processes should be estimated in each photografic sample.
8. Percentage cover of the conspicuous taxa/morphological groups should be evaluated for each photographic sample.
9. Sensibility level of each sample (Sensitivity Level, SL or Integrated Sensitivity Level, ISL) should be calculated by multiplyng the value of SL/ISL of each taxon/group for each class of abundance and then summing all values obtained for each taxon/group. The cover values should be classified in 8 classes of abundance: 1) 0< % ≤0.01; 2) 0.01< % ≤0.1; 3) 0.1< % ≤1; 4) 1< % ≤5; 5) 5< % ≤25; 6) 25< % ≤50; 7) 50< % ≤75; 8) 75< % ≤100).
10. Species richness (α-diversity) as the mean number of taxa/groups per photographic sample should be computed.
11. Homogeneity of assemblages (β-diversity) must be evaluated as the mean distance of centroids in a multivariate analysis performed with suitable software.

Sampling period

The coralligenous assemblages comprise mostly organisms with long life cycles and develop in rather stable environments for what concerns the abiotic factors (light, temperature, etc.); therefore, they do not show important seasonal variations (Abbiati et al., 2009) and the sampling frequency can be at least annual. However, structural variations have been observed for the macroalgal component, which reaches its maximum development between the end of spring and summer (Piazzi et al., 2004). Furthermore, the invasion of alien species which conversely reach their maximum cover in late summer-autumn (*Caulerpa cylindracea*) or in winter (*Asparagopsis taxiformis*), suggests to plan the monitoring activities between April and June in order to have the most structured native assemblage and with the least cover of alien species (Piazzi et al., 2019 a,b).

Sampling depth and slope of the rocky substrate

The bathymetric range of the coralligenous cliffs changes depending on latitude and transparency of the water. In addition, the structure of assemblages is deeply influenced by the slope of the substrate (Piazzi et al., 2004; Virgilio et al., 2006). In order to define a standardized sampling procedure suitable to collect comparable data, the range of sampling depth and the substrate slope were fixed at about 35 m depth (± 3 m) on a vertical surface (85-90°) (Piazzi et al., 2019 a,b). This condition ensures the presence of coralligenous assemblages at all latitudes and water characteristic investigated so far in the Mediterranean Sea. Furthermore, this is the bathymetric zone most sensitive to environmental alterations, it may be affected by the thermocline and is more exposed to the anthropic pressures acting on the marine-coastal strip; consequently, the assemblages that develop in this range of depth are considered excellent impact indicators (Piazzi & Balata, 2011).

Sampling design, sampling surface and number of replicas

Within the same biogeographical region, coralligenous assemblages are characterized by high variability at smaller spatial scale (from meters to tens of meters), while intermediate and larger scales (i.e. hundreds of metres to kilometres respectively) show a lower variability (Piazzi et al., 2016); this suggests increasing of replication at the first scale.
The coralligenous sampling surface has always been variable and linked to different approaches. Single samples range from 400 cm^2 for phytosociological studies (Piazzi et al., 2004; Falace et al., 2013) to 2 m^2 for landscape studies (Gatti et al., 2012, 2015a). The most used replicated sampling area is around 0.25 m^2 (Kipson et al., 2011; Deter, et al., 2012; Cecchi et al., 2014; Sartoretto et al., 2014; Piazzi et al., 2015, 2017a,b; Montefalcone et al., 2017). Regardless of the surface area of the single replica, the total sampling area to characterize a study site is much more comparable among different approaches and is approximately 5-6 m^2 (Kipson et al., 2011; Deter et al., 2012; Gatti et al., 2012, 2015a,b; Cecchi et al., 2014; Sartoretto et al., 2014; Piazzi et al., 2015, 2017a; Montefalcone et al., 2017).

Most of the study cases involve sampling along horizontal transepts, but it has been observed that, keeping the same total surface, there are no significant differences in the assemblages response by modifying the arrangement of the replicas (Piazzi et al., 2019 a,b).
In order to identify a sampling design that allows the application of indices deriving from different approaches, 3 plots of 4 m^2 each located tens meters away from each other should be sample within a study site, through a minimum of 10 photographic samples of 0.2 m^2 each per plot, for a total sampled area of 6 m^2 per site (Piazzi et al., 2019 a,b).

Sampling techniques

Coralligenous assemblages have been studied by destructive methods employing the total scraping of the substrate (Piazzi et al., 2004, 2007a; Kipson et al., 2009), by visual census techniques (Gatti et al., 2012, 2015a) and photographic methods associated with determination of taxa and/or morphological groups (Kipson et al., 2011, 2014; Deter et al., 2012; Teixidó et al., 2013; Cecchi et al., 2014; Sartoretto et al., 2014).
Destructive methods are not suitable to be used in sensitive and protected habitats; in addition, identification of organisms needs great taxonomic expertise and long time to analyse samples, making difficult to process the large number of replicas required for monitoring surveys (Balata et al., 2011).
Therefore, a mixed approach was proposed (Piazzi et al., 2019 a,b) which uses photographic techniques while integrating information through *in situ* collection of some variables (size of erect species, percentage of necrosis of the erect anthozoans colonies and consistency of the calcareous accretion) by means of the RVA method (Rapid Visual Assessment, Gatti et al., 2012).
The analysis of photographic samples can be performed by different methods (Dethier et al., 1993; Bianchi et al., 2004); among these, the use of a grid with at least 400 squares or manual contour of the organisms for the quantification of the covered surface through appropriate software (Trygonis & Sini, 2012; Cecchi et al., 2014) may be useful in order to reduce the subjectivity of the operator's estimate.

Metrics/Ecological descriptors

Sediment load

Coralligenous cliffs are particularly sensitive to increase of sedimentation rate which can led to changes in the structure of assemblages causing disappearance of sensitive organisms, such as photosynthesizing algae and filter-feeding invertebrates, facilitating the spread of more tolerant and opportunistic species, often introduced, and causing the reduction of both α- and β-diversity (Balata et al., 2005, 2007; Piazzi et al., 2012). The sediment cover estimated from photographic images was hence used by several authors as descriptor of ecological quality of coralligenous assemblages (Deter et al., 2012; Gatti et al., 2012, 2015a).

Calcareous accretion

The calcareous accretion of coralligenous cliffs may be severely impaired by human-induced impacts (Sartoretto & Francour, 1997; Cerrano et al., 2001; Martin & Gattuso, 2009); thickness and consistency of the calcareous matrix can be hence considered an effective indicator of the status of bioconstruction processes. These can be measured *in situ* through a hand-held penetrometer: a null penetration indicates the absence of bioconstruction, a millimetric penetration indicates the presence of active bioconstruction while a penetration greater than one centimeter reveals ongoing bioerosion processes (Gatti et al., 2012). The calculation of an average of 6 replicas performed for each plot investigated provides a suitable measure of the bio concretion status (Piazzi et al., 2019 a,b).

Erect anthozoans

Erect anthozoans are organisms particularly sensitive to many anthropic pressures ranging from climate change to the proliferation of mucilages and to mechanical damage by fishing tools or anchors (Bavestrello et al., 1997; Cerrano et al., 2000; Giuliani et al., 2005; Linares et al., 2010; Ponti et al., 2014; Gatti et al., 2015b; Montefalcone et al., 2017). Although their presence and abundance are not necessarily related to environmental quality (Virgilio et al., 2006; Casas-Güell et al., 2015), where erect anthozoans became structuring elements their development and health status are considered suitable descriptors of ecological quality (Deter et al., 2012; Gatti et al., 2012, 2015a; Cerrano et al., 2014; Kipson et al., 2014). Thus, for those coralligenous cliffs characterized by gorgonian *facies*, information about the maximum height of colonies and percentage of necrosis should be collected (Piazzi et al., 2019 a,b).

Alpha biodiversity and strucuture of assemblages

The structure of assemblages can vary as a result of different anthropic pressures, such as e.g. the increase of nutrients or sedimentation, invasion by alien species and development of mucilages (Balata et al., 2005, 2007; Piazzi et al. al., 2011, 2012, 2019a, b; Gatti et al., 2015b, 2017). The main effects of such human dsturbances are reduction in alpha biodiversity, disappearance or reduction of sensitive species such as erect bryozoans (Sala et al., 1996; Garrabou et al., 1998; Deter et al., 2012; De la Nuez-Hernández et al., 2014; Gatti et al., 2012, 2015a), erect anthozoans (Deter et al., 2012; Cerrano et al., 2014; Kipson et al., 2014; Gatti et al., 2015a) and sensitive macroalgae (Balata et al., 2011; Cecchi et al., 2014; Piazzi et al., 2017b), and the increase in opportunistic species such as algal turf (Balata et al., 2005, 2007; Piazzi et al., 2011, 2012). The evaluation of number of taxa/groups per sample and the abundance of each taxon/group are therefore fundamental indicators to define ecological quality of coralligenous (Piazzi et al., 2019 a,b).

Beta biodiversity and spatial heterogeneity of assemblages

Coralligenous assemblages are characterized by a "patchy" distribution of organisms due to biotic interactions that usually regulate the communities (Ferdeghini et al., 2000; Piazzi et al., 2004, 2015; Balata et al., 2005; Abbiati et al., 2000) al., 2009; Ponti et al., 2014). One of the main effects of human disturbance such as pollution, sedimentation or invasion by alien species is the loss of this peculiar spatial distribution and the reduction of natural heterogeneity of assemblages (Balata et al., 2007; Piazzi & Balata, 2008; Piazzi et al., 2016). Beta diversity is therefore considered an excellent descriptor of the ecological quality of coralligenous (Piazzi et al., 2019 a,b) and can be estimated as measure of the dispersion value of replicas with respect to centroid in a multivariate analysis (Anderson, 2006).

2.3 Application of the STAR method in Italy: the ESCA, COARSE and ISLA indices

As reported in the previous paragraph, the STAR protocol is a univocal and standardized method to collect data useful for the evaluation of the ecological status of coralligenous cliff; the information collected by applying the STAR method can be processed in different ways, depending on the objectives of the work. The calculation of indices for the assessment of ecological status of coralligenous cliff hence represents a particular case of application of the STAR method. In Italy, the ecological quality indices elaborated for this type of evaluation are three and are described below.

2.3.1 ESCA index (Ecological Status of Coralligenous Assemblages)

Description and development of the index

The ESCA index is a method for assessing the ecological status of coralligenous based on the structural and functional analysis of the assemblages carried out on photographic images collected in underwater scuba diving. This sampling method is inexpensive and non-destructive, and is therefore in line with the spirit of the European legislation for the protection of sensitive habitats; this allows the use of the ESCA index even in marine protected areas.

The ESCA index was initially proposed to assess the ecological quality of circalittoral macroalgal assemblages as part of the implementation works of the WFD 2000/60/EC, which took into account only the superficial assemblages of the infralittoral fringe (Cecchi et al., 2014).
In this first phase, the ESCA index was developed through manipulative experiments aimed at evaluating effect of the anthropic impact on macroalgal community; three pressure indicators were selected:

- biological invasion by *Caulerpa cylindracea (*former *racemosa)*
- increase in sedimentation rate
- nutrient enrichment

The different conditions were reproduced in the field at three different levels of stress (low, moderate and high) and the data collected were subsequently used to identify the most suitable indicators of ecological status for the investigated community. These experimental studies have allowed to select the following metrics describing changes in the structure of coralligenous assemblages subject to pressures:

- Presence/absence and abundance of sensitive taxa (Sensitivity Level, SL)
- Diversity of assemblages (α-diversity)
- Hetereogeneity of assemblages (β-diversity)

These three parameters were then combined with each other in order to obtain a final value of EQR (Ecological Quality Ratio) calculated as the ratio between the ESCA values measured at the study site and those obtained in the reference areas.

Following its first proposal (Cecchi et al., 2014), ESCA was tested and validated through the study of the index response to a gradient of anthropogenic pressures (Piazzi et al., 2015). In this and in another study (Piazzi et al., 2014b), the robustness of the photographic method was also tested compared to the destructive one, which is surely much more accurate but not practicable in a sensitive habitat such as coralligenous. In order to optimize the operational effort in applying the ESCA index, the effectiveness of different sampling designs in providing the most accurate possible quality assessment was also tested. This led to definition of the optimal sampling design (in terms of number of replicas at different spatial scales) to evaluate the ecological status of coralligenous in an effectively way but with the minimum sampling effort (Piazzi et al., 2015).

Finally, in its latest version (Piazzi et al., 2017b), the ESCA index has been integrated with the animal component of macro-megazoobenthos thanks to the collaboration and sharing of the contents of the first ESCA study with the researching group of the University of Genoa.

ESCA is the first ecological quality index used to assess the status of coastal waters on the basis of a biocenotic study of both animal and plant assemblages. It is a multi-metric index that provides information on biodiversity alterations at different scales of assemblages (alpha and beta) in response to anthropogenic pressures. Therefore, the ESCA index synthesizes complex information in a simple and reliable way and may represent an effective tool to be used in monitoring programs and environmental impact assessment studies (Cecchi et al., 2014; Piazzi et al., 2015, 2017b).

Application and validation of the ESCA index

The use of the ESCA index in coastal marine monitoring plans began in 2008 and in ten years it has been applied in 44 locations scattered in the Italian regions of Liguria, Tuscany, Lazio, Sardinia and the in the French Riviera (France), for a total of more than 120 sites.

The ESCA index was used in Tuscany and Sardinia in application of the non-mandatory surveys foreseen by the Italian national plan in the first monitoring cycle of the MSFD 2008/56/EC (MATTM-ISPRA, 2019).

ESCA was also used in the monitoring and impact assessment plans following the shipwreck of the cruise ship "Costa Concordia" at the Giglio Island in the Tuscan Archipelago National Park (Penna et al., 2017).

The index is used in monitoring the marine protected areas of Capo Carbonara (Piazzi et al., 2018a), the Asinara Island (Sardinia) and the Meloria Shoals (Tuscany), and in the Tuscan Archipelago National Park.

The ESCA index is therefore tested and validated on a large space-time scale, consisting of a database of 10 years of data collected across an area representative of the Western Mediterranean (Piazzi et al., 2021). However, the spatial scale should be extended to other marine sub-regions such as the Ionian Sea, the Central Mediterranean and the Adriatic Sea. The latter is represented for Italy only by the Apulian coasts, since the rest of the Adriatic is substantially devoid of coralligenous cliffs. The final goal is thus to expand the validation dataset and above all to test, and recalibrate where necessary, the reference values currently calculated on the Montecristo Island conditions.

2.3.2 COARSE index (COralligenous Assemblages by Reef Scape Estimate)

Description and development of the index

The COARSE index was first proposed by Gatti et al. (2012) as part of the monitoring of the status of coralligenous assemblages following the extension works of the Vado Ligure commercial port (Savona). It was later modified and integrated by testing it on a gradient of anthropogenic pressures along the French coasts (Gatti et al., 2015a). The index is based on the RVA (Rapid Visual Assessment) visual detection technique, i.e. on measurements and direct observations carried out *in situ* by scuba divers aimed at collecting the information for the ecological status assessment of coralligenous habitat, which is included in the MSFD 2008/56/EC as indicator of biodiversity and seafloor integrity. COARSE is the only index that, in addition to species richness and biotic cover, takes into account the stratified structure of coralligenous, which is typically composed of three layers (Figure 2.1):

1. Basal Layer (BL): consisting of encrusting or small size organisms (<1 cm);
2. Intermediate Layer (IL): organisms characterized by moderate height growth (from 1 to 10 cm);
3. Upper Layer (UL): composed of massive or arborescent organisms with appreciable height growth (> 10 cm).

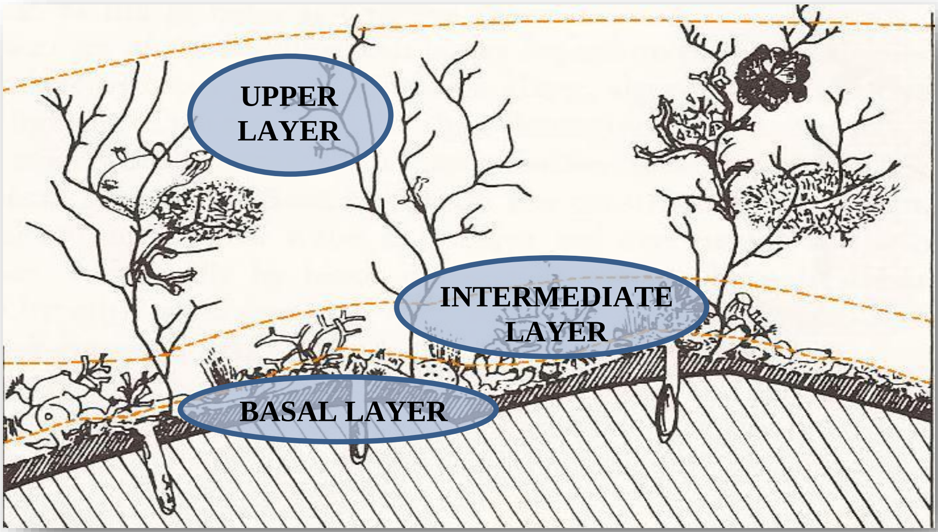

Figure 2.1 – Stratified structure of coralligenous (by Ros et al., 1985, modified)

For each layer 3 ecological descriptors are calculated: for the Basal Layer 1) % cover of Benthic Categories (BCs), 2) frequency of perforating organisms (PERforating, PER) and 3) thickness and consistency of calcareous matrix (PENetrometry, PEN); for the Intermediate Layer (IL) 1) presence/absence (Species richness, S), 2) number of Erect Calcified Organisms (ECO) and 3) presence of Sensitive Bryozoans (SB); for the Upper Layer 1) cover of taxa/groups (%), 2) Necrosis (N) and 3) Maximum Height measured for the erect species (MH). A score is assigned to the value of each descriptor, and the means obtained for each descriptor and for each layer are then integrated into the final COARSE formula that provides the Quality (Q) value for the investigated site. The calculation of the COARSE index can thus also be carried out for a single layer, and this allows to evaluate impacts acting differently on organisms adhering to the substrate or on erect ones of different sizes (Gatti et al., 2015a).

The aggregation methods for "joining" the 3 descriptors of each layer can be different: the formula of STORIE (Gatti et al., 2012, 2015a), the geometric mean and the arithmetic mean (respectively from the most "severe" and conservative to less severe); moreover, the classification scale in 5 quality classes can be constructed at homogeneous or non-homogeneous intervals. Recent index validation studies carried out in over 60 sites throughout Italy led to a revision of the original proposal (which included, among other things, only 3 quality classes), suggesting the geometric mean as aggregation method of descriptors and 5 non-homogeneous class intervals, i.e. defined on the basis of the frequency distribution of values obtained in the sites analyzed. This proposal allows to obtain classification values closer to local realities and to more effectively intercalibrate the COARSE index with other ecological quality indices [1].

Unlike other approaches (e.g. ESCA or CAI indices) which employ exclusively the photographic sampling, the RVA visual technique used in COARSE shows several advantages, including the possibility of measuring and sampling some parameters that can be detected only *in situ*. Furthermore, the simultaneous evaluation of the stratified structure and composition of the coralligenous confirmed that this particular habitat is very vulnerable to physical stresses, such as the increase in temperature, marked sedimentation and mechanical damage caused by anchoring. This approach therefore represents an effective tool for assessing also the seafloor integrity.

Application and validation of the COARSE index

The COARSE index has been used in the assessment of the environmental quality of coralligenous since 2010. To date it has been used in 46 locations scattered in the Italian regions of Liguria, Tuscany, Lazio, Sicily, Sardinia and the French Riviera (France), for a total of about 90 sites.

COARSE was also used in the last phase of the monitoring and impact assessment plan following the shipwreck of the cruise ship "Costa Concordia" at the Giglio Island in the Tuscan Archipelago National Park (Casoli et al., 2017).

The index is used in monitoring the marine protected areas of Capo Carbonara (Piazzi et al., 2018a), Asinara Island (Sardinia) and in the Tuscan Archipelago National Park. The COARSE index was therefore validated on a large spatial scale, however representative of the Western Mediterranean only. It would therefore be necessary to expand the application geographical area to test its validity in those marine sub-regions not yet investigated.

2.3.3 ISLA index (Integrated Sensitivity Level of coralligenous Assemblages)

Description and development of the index

The existing ecological indices for assessing the quality status of coralligenous based on a biocenotic approach have adopted, as descriptor of ecological quality, the sensitivity of coralligenous species (e.g. Sensitivity Level) to an environmental stress induced by the water quality alteration. The ISLA index (Integrated Sensitivity Level of coralligenous Assemblages, Montefalcone et al., 2017) instead aims to distinguish and measure the sensitivity to stress and sensitivity to disturbance of the main sessile organisms characterizing coralligenous assemblages. The disturbance is mainly linked to the loss of biomass, so it become evident with death or damage of individuals or colonies; stress is linked to the reduction in productivity and it reveals itself through alterations of organisms or assemblages in response to factors acting at a lower level of intensity than which causes their death (Montefalcone et al., 2011). ISLA is therefore an integrated ecological index based on a biocenotic approach, which however combines the two components of sensitivity to assess the ecological status of coralligenous habitat.

Based on a combined approach among experiences reported in the literature and expert judgment, a list of the main taxa or morphological groups of sessile organisms (macroalgae and macro-invertebrates) of the coralligenous was produced. To each taxon/group a Disturbed Sensitivity Level (DSL) score was assigned, defined by analyzing a series of biological traits (i.e. growth form, reproductive mode, size, growth rate, potential of bioconstruction and generational time). A Stress Sensitivity Level (SSL) score of the taxa/groups was also defined, on the basis of expert judgment. The sensitivity to disturbance and sensitivity to stress were then combined into an Integrated Sensitivity Level (ISL) value for each taxon/group as a function of the anthropogenic pressures (see Montefalcone et al., 2011).

Note:

[1] The STORIE aggregation method tends to be very severe and conservative, as it brings out more the impact situations present: only one negative value out of three is enough to significantly lower the total quality of the layer. On the other hand, the use of the arithmetic mean would be much less conservative, since it would tend to make uniform the different situations detected by the three descriptors, highlighting only the average conditions. The use of the geometric mean therefore seems to be a good compromise between the two previous aggregation methods, since while bringing out any impact situations, it is also able to enhance the situation detected by the other descriptors

As for the ESCA index, the integrated sensitivity values are combined with the presence/absence and abundance data of sensitive taxa/groups; the ISLA index is finally obtained from the sum of the integrated values of the sensitivity level of all species present, and then used to assess the ecological status of coralligenous assemblages.

Application of the ISLA index

The ISLA index was only recently proposed (2017) and has been tested and applied in the locations already studied through the ESCA method. In relation to its recent development, the index has yet to be tested on gradients of anthropogenic pressures and validated on a wider space-time scale. The preliminary tests showed a good response to changes of environmental conditions (Montefalcone et al., 2017; Piazzi et al., 2018b). Furthermore, the possibility of separating the effects related to stress from those related to disturbance can allow information on the effects of specific pressures to be obtained.

APPLICATION PART

3. PLANNING AND SEA SAMPLING ACTIVITIES

3.1 Sampling design

The proposed sampling design is based on studies carried out at the main spatial variability scales of coralligenous reported in the literature (Piazzi et al., 2014a, 2015, 2016; Gatti et al., 2015a; Piazzi et al., 2019b). As much of the scientific literature focuses on the assessment of the habitat condition in relation to implementation of the MSFD 2008/56/EC, the scale of the proposed spatial replicas has been designed in such a way as to ensure compatibility between the study activities carried out on the coralligenous cliff for different purposes and the conventional ones foreseen by the national monitoring plan performed in Italy with the ROV (MATTM-ISPRA, 2020). However, the proposed sampling design is was devised to be extremely versatile and applicable to a wide range of situations, by virtue of the different spatial scales considered. It therefore responds to various monitoring objectives (impact assessment, conservation status, etc.) and is in line with the indications given at the Mediterranean level for the protection of coralligenous habitats.

The study areas should be selected on the basis of direct observations and/or bathy-morphological and extension data of the cliff habitat acquired through suitable tools (Multibeam, Side Scan Sonar, ROV); after that, 3 Sites of survey for each area should be identified, possibly not less than 500 m apart from each other, and 3 Plots (P1, P2, P3) for each site shoul be selected for photographic sampling, some tens of meters away from each other. Each plot consists of a 4 m^2 cliff surface within which 10 photographic samples (replicas) of 0.2 m^2 each (about 0.5 m × 0.4 m) are acquired for a total of 30 photos per site. At the same time as the photographic sampling, data related to the parameters measured *in situ* are also acquired within each plot.

By way of example, the sampling design applied by the Italian Regional Agency for the Environmental Protection of Tuscany during the routine monitoring is reported, with the following sampling areas:

1) Argentario - promontory with island characteristics albeit being part of the mainland coast of Tuscany. In fact, the two Feniglia and Giannella tombolos connect the Argentario Mount to the "mainland" (generic geographic reference: Lat 42°23,343'N; Lon 011°06,543'E). The Argentario Mount is not subject to any protection restrictions. Selected sites: Scoglio del Corallo, Argentarola, Secca di Capo d'Uomo.

2) Romito - stretch of coast south of the Livorno town, so partially affected by the presence of human activities such as maritime traffic, fishing and tourism (generic geographic reference: Lat 43° 30,960'N; Lon 010°19,864'E). There are no protection restrictions along the Romito coasts. Selected sites: Sassoscritto, Boccale, Calignaia.

3) Capraia - the cliffs of Capraia island are typical of the isolated areas and thus free from direct anthropogenic impacts (generic geographic reference: Lat 43°02,056'N; Lon 009°50,614'E). However, they differ from those of the other Archipelago islands as the coralligenous communities completely lack the most consistent upper layer (gorgonians). Capraia is subject to protection restrictions for about 80% of the island territory and 90% of marine waters (the port area and the surrounding areas are excluded). As regards coastal waters, the new protection zoning (resolution no.47 of 11 July 2017) provides for an A zone (about 10%), a B zone (about 55%) and C and D zones (in equal parts). Furthermore, following islets are also protected as A zone: La Peraiola, Le Formiche, Lo Scoglione, the Scoglio del Gatto and the Scoglio della Manza. Selected sites: Lo Scoglione, Civitata, Punta del Capo.

4) Pianosa - the cliffs of Pianosa island are typical of the isolated areas and thus free from direct anthropogenic impacts (generic geographic reference: Lat 42°34,599'N; Lon 10°04,600'E). The island is subject to protection restrictions (zone A) for 100% of the island territory (including La Scarpa and La Scola islets) and diving is allowed only through authorized diving centers. Selected sites: Secca del Marchese, Cala dell'Alga, La Scola.

The standard data processing format attached to this manual (Annex D) will therefore be based on the practical experience carried out by the Tuscany ARPA.

3.2 Planning and carrying out of scuba diving activities

The underwater sampling must be carried out by at least two scuba diving operators working in pairs, with methods and times provided by the common safety procedures indicated for the scientific diving investigations (Flemming&Max, 1996; Joiner, 2001; BPAS, 2013).

Depth and slope

The optimal sampling depth is 35 m on vertical cliff with a slope of 85-90°. If substrate morphology of the sampling site does not allow to exactly respect this depth, a tolerance range of ± 3 m can be foreseen.

Work equipment

The sampling in scuba diving should be carried out using a digital camera, equipped with flash or illuminators, with a minimum resolution of 6-8 megapixels (which correspond to a digital photo of about 2-3MB). The camera must be equipped with a frame that allows to photograph an area of 50 cm × 40 cm. The frame is a structure (in PVC or stainless steel) assembled on the camera that allows to standardize the photographic images captured by fixing the minimum sampling surface (0.2 m^2) and the distance from the cliff at the same time. Examples of frames and their use are shown in Figures 3.1 and 3.2.

Figure 3.1 – Example of a pvc frame with fixed surface and spacer (A) and its use in the field (B)

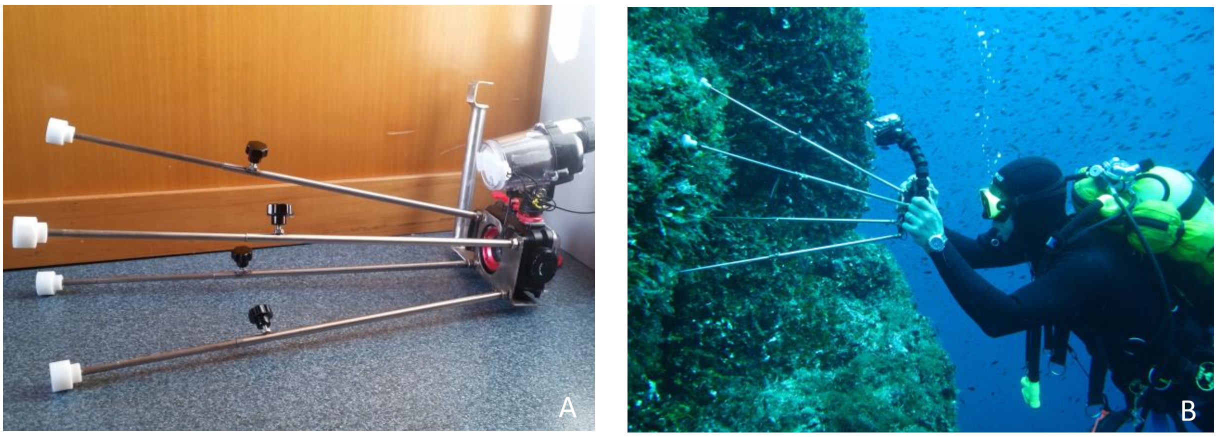

Figure 3.2 – Example of a stainless steel frame with adjustable surface and spacer (A) and its use in the field (B)

The choice of the frame type depends on subjective factors related to manual skills, costs, etc., so what really matters is to ensure the minimum sampling area for all photos. From a practical point of view, a frame like that represented in Figure 3.1 has the advantage of providing a fixed surface visually delimited by the PVC frame, thus facilitating the framing during the sampling phase and the subsequent image processing. On the other hand, the structure is more bulky and consequently less handy, especially on cliffs characterized by a cospicuous erect layer (large branched gorgonians can hinder the positioning of the frame on the rock wall). Moreover, the structure is fixed and calibrated on the basis of the camera settings for which it was built, so if the camera chenge it may be necessary to change also the frame. This problem does not arise if a frame like that represented in Figure 3.2 is used. It consists of 3/4 telescopic feets, welded to an *ad hoc* modeled structure fixed to the camera body, which allow to adjust the distance of the lens from the photographed surface as needed. This type of frame is thus not only extremely versatile, but also very practical and handy to use on all types of cliffs, in particular those rich in branched colonies of gorgonians. However, its use requires particular attention in the preliminary setting of the camera, as the absence of a visible frame imposes to check each time, before each sampling, that the framed surface is at least equal to the minimum reference surface (0.2 m^2). The settings of digital cameras, in fact, often do not allow to set a precise 50 cm × 40 cm frame; therefore, it is necessary to find the right combination of settings giving as final result a surface value that includes the minimum reference value. One method could be to fix always the lower side (40 cm) leaving the other side accordingly, so as to obtain a surface that could result slightly higher, but still comparable to 0.2 m^2.
As an alternative to the aforementioned types of frames, it is possible to use a simple frame, held in position by the second diver, which acts as squaring frame allowing to photograph always the same surface (Figure 3.3); in this case, however, it is necessary to equip the camera with a rigid spacer so as to ensure the parallelism of the camera sensor with respect to the sampled surface.

Figure 3.3 – Squaring frame of the sampling surface

In addition to devices and safety Personal Protection Equipment (PPE) required by the general guidelines for diving safety (Flemming & Max, 1996; Joiner, 2001; BPAS, 2013), each pair of operators should have a clinometer for the slope measurement of coralligenous cliff, a hand-held penetrometer to measure the calcareous accretion status of the basal layer and a metric rope to measure the maximum height of the upper layer. A clinometer consists of a weight and a semi-circumference indicating the degrees and can also be made directly on the underwater slate. A penetrometer can be obtained using an underwater knife with a blunt tip and marked at a distance of one centimeter from the apex (Figure 3.4). Alternatively, a flat-blade screwdriver marked to the centimeter can be used.

For the measurement of other useful parameters (depth, morphology and exposure of the substrate), the diver will refer to the common equipment supplied (in particular, dive computer/depth gauge, underwater slate, compass).

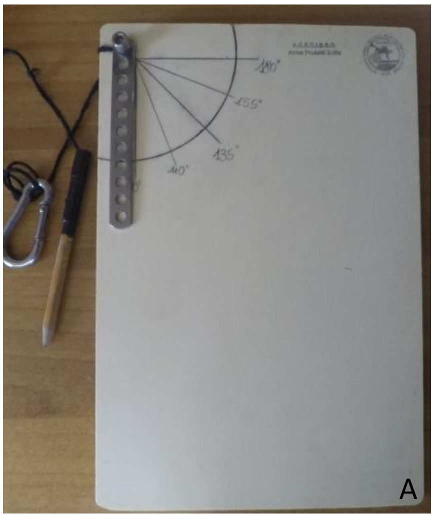

Figure 3.4 – Example of clinometer (A) and penetrometer (B)

The plots should be randomly chosen among those being at the established depth and showing a slope as close as possible to 90° with respect to the bottom.
Where the morphology of the substrate is suitable, squares of 2 m × 2 m should be selected; otherwise, rectangles can also be choosen as long as they have a 4 m^2 surface (Figure 3.5).

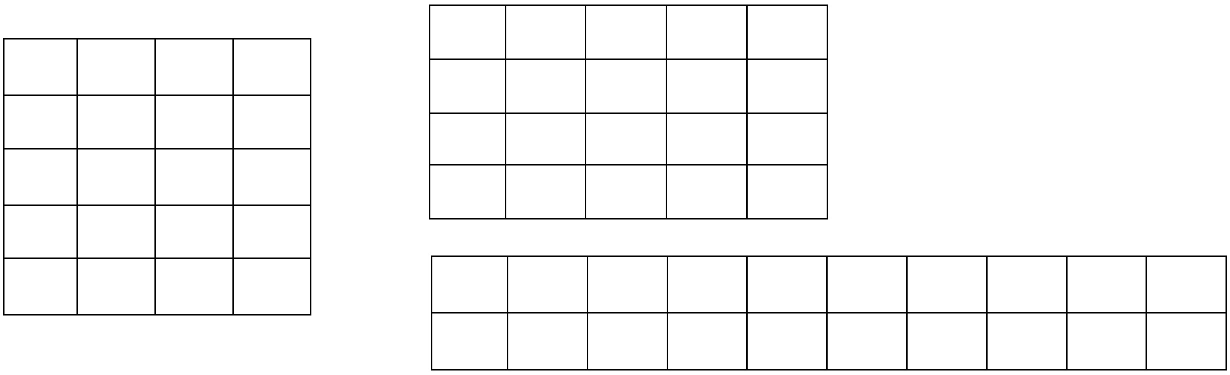

Figure 3.5 – Examples of photographic sampling plots of 4m^2 total surface

Surveys to be performed in each sampling plot

For each sampling plot the following measurements (additional parameters) should be taken: depth, substrate morphology, slope and exposure.

- <u>Depth</u>
  Depth must be taken in the center of the plot by depth gauge or dive computer.

- <u>Morphotype of substrate on which coralligenous grows</u>
  The geomorphological conformation of the sampling plot should be noted on the basis of the seafloor topography, i.e. whether it is located on cliff (C), blocks (B), landslides (L) or biogenic formations (BF) such as entirely bioconstructed structures.

- Slope

  The inclination of the central point of the plot should be measured using a clinometer and noted on the slate.

- Exposure

  The orientation of the rock wall should be measured using a compass. The operator should perform the measurement by positioning himself at the center of the sampling plot and marking the compass degrees. The exposure of the wall will be given by the compass degrees opposite to those indicated by pointing the instrument at the center of the rock wall.

The following parameters should also be noted: consistency of the calcareous matrix, maximum height of the structuring species (massive or arborescent) forming the upper layer and percentage of necrosis on the gorgonians colonies.

- Consistency of calcareous matrix

  The consistency of calcareous matrix can be measured using a hand-held penetrometer. The operator pushes the penetrometer into the calcareous substrate and detects if penetration is 1) zero, 2) less than one centimeter, 3) greater than one centimeter. For each plot 6 measurements should be made.

- Maximum height of the upper layer

  The maximum height of the structuring, massive or arborescent species of the upper layer (organisms greater than 10 cm in height), if they are present within the plot, is measured by means of a metric rope taking the distance from the substrate to the apex of the larger organism or colony.

- Necrosis

  The presence of necrosis on the gorgonians colonies (if these last are present) should be visually estimated as total percentage of cover on the all colonies of the plot considered. Both dead parts of colony that appear "naked" as lacking in tissue and those affected by epibiosis are considered necrosis, since epibionts colonize only the dead parts of the colony (Sheet 1).

Photographic sampling

Ten surfaces (50 cm × 40 cm each) should be photographed chosen randomly from the 20 available within the sampling plot. The random selection should be made before the dive through the table of random numbers and the surfaces to be sampled shown above the slate. For example:

Numbers drawn: 1, 4, 6, 8, 9, 11, 13, 16, 18, 19

1			4
	6		8
9		11	
13			16
	18	19	

If the camera is equipped with a frame with spacer structure, the photographic sampling can be carried out by a single operator so that the second one can measure parameters to be detected *in situ*. Conversely, if a simple frame is used, one operator places the frame on the surfaces to be sampled and the other takes the photos ensuring the parallelism of the camera sensor with respect to the bottom by means of a rigid spacer (1).

Note:

(1) It is advisable to take more than 10 photos per plot in order to replace any blurry, dark or other photos unsuitable for image processing. During scuba diving, pay attention to the positioning of the flash/ illuminators and to not overlap the images. It is also advisable to separate or identify in some way the groups of 10 replicas collected in each plot, by clicking e.g. an empty photo between one group and the other or by indicating with the hand the number of the plot (1, 2 or 3) for each group.

It is advisable to check, and eventually reset, the camera settings before each sampling survey in order to ensure that all photos have the same surface, especially if a frame with telescopic feets is used.
The minimum reference surface is indeed standardized at 2000 cm^2 (50 cm × 40 cm) but in practice it is not always possible to set it precisely on the camera; in this case, it is recommended to fix the lower side (40 cm) leaving the other side accordingly in such a way as to obtain a slightly larger surface (e.g. 53 cm × 40 cm, equal to 2120 cm^2) which however include the minimum reference surface.
Generally, the most important thing is to fix the reference surface for all the photos taken in a given survey campaign and then consider the same surface in the next phase of image analysis and subsequent elaboration of data.

SHEET 1
CONSERVATION STATUS OF GORGONIANS: NECROSIS/EPIBIOSIS

The visual census of health status of the gorgonians is carried out by positioning itself at the centre of the sampling plot and making a visual measurement of the percentage cover of the "dead zones" of gorgonians on the all colonies colonizing the 2 m x 2 m plot.
For example: if the gorgonians in the plot are all alive and free from necrosis, a percentage of 0% will be recorded; if they are all dead it will be 100%; if in the plot there are 10 colonies of which 5 show necrosis/epibiosis, the total cover surface of the dead zones of the 5 colonies must be evaluated and then reported as % cover referred to the 10 colonies present in the plot.

A colony is considered to be affected by necrosis when the dead portion exceeds or is equal to 10% of its total surface (Figure 1).

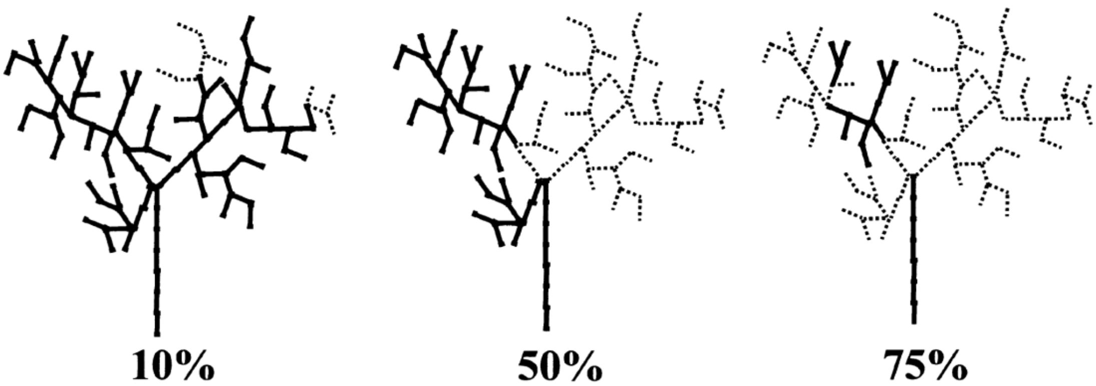

***Figure 1** – Estimation of the colony's extent of injury (adopted from Perez et al., 2000). According to Garrabou et al., 2014, colonies with >10% injured surface are considered as affected.*

The "dead zones" are parts of the colony that appear naked and /or covered by epibionts: it is not possible to distinguish the two conditions, also because epibiosis are most often consequence of the colony necrotization. Therefore, the Necrosis parameter should always be evaluated as the % cover of necrosis/epibiosis considered as a whole.

For the affected colonies it should also be noted whether necrosis are recent (presence of denuded axis or axis colonized by pioneering species such as hydrozoans), old (axis covered by long-lived species such as bryozoans, calcareous algae) or if both types of necrosis are present (Figure 2).
However, for the purposes of the method here presented it is enough to record the % of necrosis of the affected colonies present on the sampled surface of 4 m^2.

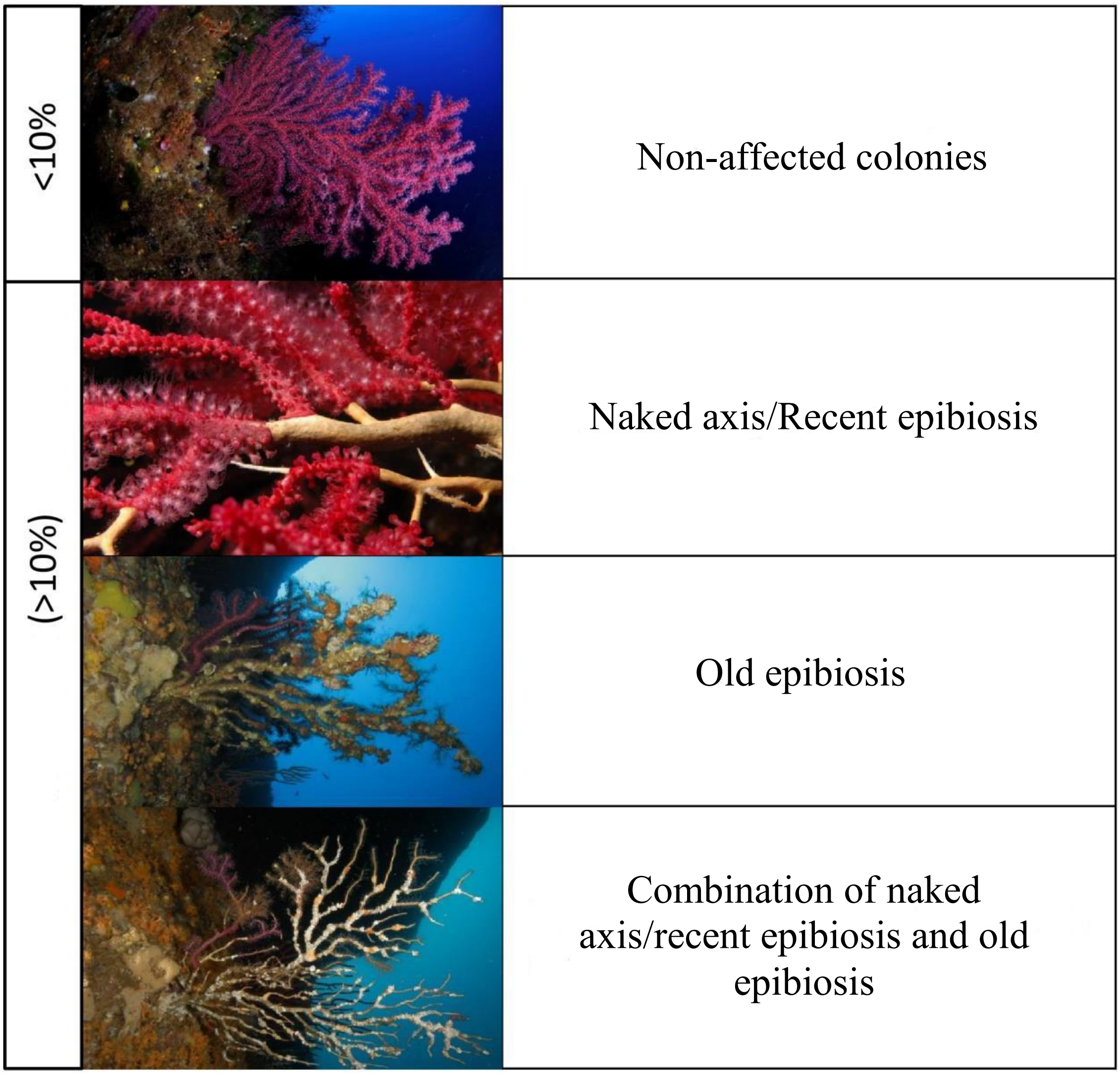

Figure 2 *– Gorgonian colonies non-affected and affected by different types of necrosis/epibiosis (by Garrabou et al., 2014, modified)*

3.3 Parameters gettable from *in situ* surveys

Beyond the supplementary parameters (depth, morphotype of substrate, slope and exposure of the cliff), which do not enter into the indices calculation but are still useful information for the interpretation of the results, the parameters obtainable from the surveys carried out in the field are:

- Consistency of the calcareous matrix: the results of the penetrometric tests should be reported, expressed in the three categories 1 = null, 2 = greater than one centimeter, 3 = less than or equal to one centimeter;
- Structure of large megabenthic assemblages (species morphometry): the maximum height (in cm) of the species forming the upper layer should be reported;
- Presence of necrosis on colonies of erect anthozoans (gorgonians): should be reported as the total % cover on the all colonies present in the sampling plot, expressed in one of the three categories 1 = > 75%, 2 = 10% ≤ N ≤ 75%, 3 = < 10%

4. IMAGE ANALYSIS IN THE LABORATORY

Each photographic sample should be subject to image analysis in order to assess presence and cover percentage of the main taxa and/or morphotypes (morphological groups) of plants (macroalgae) and animal. If there are elements "strangers" to the coralligenous assemblages (sediment, mucilages and/or cavities), their percentage cover should also be estimated and recorded in a separate calculation column. If overlap of cover occurs, whether between taxa/groups or between these and the sediment or mucilages, the general rule to apply is to report data relating to the most evident element. For example, if an image shows a layer of algal turf covering *Peyssonnelia* spp. but this last is still visible, the reported value will be the cover of *Peyssonnelia* spp.; if the algal turf is so dense as to hide the underlying taxon/group from the view, then turf value will be reported on the spreadsheet. The same goes for the strangers elements: if a taxon/group is covered by a veil of sediment or mucilages through which it is still possible to identify it, then the percentage of the taxon/group cover will be reported. Conversely, if the sediment or mucilages layer is so dense as to prevent identification, then the cover of the strangers element will be reported. Instead, the cavities, as well as other strangers elements (e.g. blurred parts, frame elements photographed accidentally etc.) that do not allow the identification of organisms, will be recorded as a percentage of unreadable cover observed on the photographic image.
Once covers of sediment, mucilages and/or cavities have been recorded, these data will be treated differently depending on the index applied (see the following paragraphs).
In any case, when mucilages and/or cavities occupy an image surface >25%, the photo is considered an invalid sample and is discarded.
On the other hand, when the sediment occupies a surface >25%, the photo is discarded if the aim is to proceed with the calculation of the ESCA and ISLA indices while it is considered a valid sample if the COARSE index is applied (see the following paragraphs).
However, it is always important to record the cover data relating to mucilages and/or sediment as their presence (or absence) provides anyway important ecological information contributing to the global assessment of the coralligenous habitat condition.

4.1 Categories of organisms

Some organisms can be easily recognized at the species or genus level; for all the others the morphological groups can be used. For the identification of the categories of organisms, the photo cards of the main taxa/groups are shown in the Annex B; in support of these, images showed in the books for underwater observations (e.g. Mojetta & Ghisotti, 1994; Trainito, 2004; Trainito & Baldaconi, 2013, 2016; Notari & Fossati 2015; Rodriguez-Prieto et al. 2015; Bertolino & Ferranti, 2019 etc.) can be used as reference. However, it is always better to stop at a higher taxonomic level than to provide an incorrect determination.
The list of the main taxa/morphological groups of plant and animal organisms that can be recognized by image analysis is shown below in the Sheet 2 (Parravicini et al., 2010; Cecchi et al., 2014; Piazzi et al., 2014a, 2017b).
When a species (or genus) within a morphological group is recognizably with certainty, this information can be reported in brackets: for example, for the group "Siphonal Chlorophyta with vesicle thallus" the genus *Codium* spp. can be indicated in brackets since the morphology of its thallus is always well distinguishable from the genus *Valonia* spp. If a species or genus can be only partially recognized within a category, both items should be reported. Example:

- Erect cylindrical Rodophyta
- Erect cylindrical Rodophyta (*Osmundea pelagosae*)

For taxa/groups classified as morphological groups or forms of growth, the examples of species (or genera) provided in brackets are only indicative and useful to provide the operator with an idea of which organisms may represent a particular category. In fact, these species (or genera) are not always detectable from photographic images, reason for which "morphological groups" are used.

Moreover, some species potentially detectable from the images, could take different forms in relation to the environmental growing conditions; in the case of sponges, for example, a species that under natural conditions develops a prostrate form could take on a more massive or even bushy form in conditions of increased sedimentation.

To each taxon/group is assigned a degree of the base colour coded by way of example in the Sheet 2 for each category of belonging.
To the alien species and "rare" groups for which colour is not indicated, one at discretion of the operator can be assigned.

SHEET 2
CATEGORIES OF ORGANISMS (TAXA/GROUPS)

MACROALGHE

Alien species [e.g. *Caulerpa cylindracea* Sonder, 1845; *Caulerpa taxifolia* (M. Vahal) C. Agardh, 1817; *Asparagopsis* spp.]

Algal turf [(1)] (colour: purple) [e.g. *Lophosiphonia* spp., *Polysiphonia* spp., *Sphacelaria* spp.]

Red algae (colour: red)

Encrusting calcified Rhodophyta [e.g. *Mesophyllum* spp., *Lithophyllum* spp., *Neogoniolithon* spp.]
Articulated calcified Rhodophyta [e.g. *Amphiroa* spp., *Tricleocarpa fragilis* (Linnaeus) Huisman et R.A. Towsend, 1993]
Peyssonnelia spp.
Erect cylindrical Rodophyta [(2)] [e.g. *Botryocladia* spp., *Osmundea pelagosae* (Shiffner) K.W. Nam, 1994]
Erect flattened Rhodophyta [e.g. *Kallymenia* spp., *Halymenia* spp., *Phyllophora* spp., *Meredithia microphylla* (J.Agardh) J.Agardh, 1892, *Acrodiscus vidovichii* (Meneghini) Zanardini, 1868]

Green algae (colour: green)

Siphonal/siphonocladal Chlorophyta with separate filaments [(3)] [e.g. *Pseudochlorodesmis* spp.; *Cladophora* spp., *Bryopsis* spp.]
Siphonal Chlorophyta with vesicle thallus [*Valonia* spp., *Codium* spp.]
Flabellia petiolata Turra (Nizamuddin), 1987
Palmophyllum crassum (Naccari) Rabenhorst, 1868
Halimeda tuna (J. Ellis et Solander) J.V. Lamouroux, 1816

Brown algae (colour: brown)

Dictyotales [e.g. *Dictyota* spp., *Dictyopteris* spp.]
Erect cylindrical Ochrophyta [e.g. *Halopteris* spp., *Sporochnus* spp.]
Encrusting Ochrophyta [e.g. *Aglaozonia* spp., *Zanardinia typus* (Nardo) P.C. Silva, 2000]
Erect flattened Ochrophyta [e.g. *Laminaria* spp., *Phyllariopsis* spp.]
Fucales [e.g. *Cystoseira* spp., *Sargassum* spp.]

Note:

(1) Algae belonging to various taxa characterized by filamentous thallus consisting of uniseriate or pluriseriate filaments (respectively one or more rows of cells) that form a sort of "turf".
(2) "Cylindrical" is the term used in botany to describe a circular cross section (or distorted circle) with a single surface (a layer of cells for the algae) surrounding it. This type of section is the opposite of the "laminar" cross section which has an upper surface distinct from the lower one. The cross section of a branch in a tree, for example, is quite round so the branch is cylindrical while a leaf is thin and elongated with distinct upper and lower surfaces, so it is laminar. However, the fleshy leaves of succulents are sometimes cylindrical.
(3) The siphonal and siphonocladal organization of the thallus provides filaments with plurinuclear cells respectively without septa or setted.

MACRO-MEGA ZOOBENTHOS

Hydrozoans (colour: white)

Small hydroids [e.g. *Sertularella* spp.]
Large hydroids [e.g. *Eudendrium* spp.]

Sponges (colour: pink)

Perforating sponges [e.g. *Cliona* spp.]
Encrusting sponges [e.g. *Phorbas* spp., *Spirastrella cunctatrix* Schmidt, 1868, *Crambe crambe* (Schmidt, 1862)]
Prostrate/hemispherical sponges [(1)] [e.g. *Ircinia* spp., *Chondrosia reniformis* Nardo, 1847, *Petrosia ficiformis* (Poiret, 1789); *Agelas oroides* (Schmidt, 1864)]
Arborescent/massive sponges [(2)] [e.g. *Axinella polypoides* Schmidt, 1862, *A. cannabina* (Esper, 1794); *Spongia* spp., *Sarcotragus* spp.]
Bushy sponges [(3)] [e.g. *Aplysina* spp., *Axinella damicornis* (Esper, 1794), *A. verrucosa* (Esper, 1794), *Acanthella acuta* Schmidt, 1862]

Bryozoans (colour: blue)

Encrusting bryozoans [e.g. *Schizoporella* spp., *Schizomavella* spp.]
Ramified bryozoans [e.g. *Cellaria fistulosa* (Linnaeus, 1758), *Caberea boryi* (Audouin, 1826)]
Myriapora truncata (Pallas, 1766)
Turbicellepora avicularis (Hincks, 1860)
Pentapora fascialis (Pallas, 1766)
Reteporella grimaldii (ex *Sertella*) (Jullien, 1903)
Adeonella calveti (Canu & Bassler, 1930), *Smittina cervicornis* (Pallas, 1766),

Ascidians (colour: lilac)

Encrusting ascidians (also epibionts) [e.g. *Diplosoma* spp., *Botryllus* spp., *Didemnum* spp.]
Erect ascidians [e.g. *Halocynthia papillosa* (Linnaeus, 1767)]

Anthozoans (colour: yellow)

Parazoanthus axinellae (Smidt, 1862)
Leptogorgia sarmentosa (Esper, 1789)
Azooxantellate (former Madreporans) solitary scleractinians [e.g. *Leptopsammia pruvoti* Lacaze-Duthiers, 1897]
Azooxantellate colonial scleractinians [e.g. *Phyllangia americana mouchezii* Lacaze-Duthiers, 1897; *Polycyathus muellerae* (Abel, 1959)]
Eunicella verrucosa (Pallas, 1766)
Alcyonium acaule Marion, 1878
Corallium rubrum (Linnaeus, 1758)
Paramuricea clavata (Risso, 1826)
Alcyonium coralloides (Pallas, 1766)
Zooxantellate solitary scleractinians [e.g. *Balanophyllia europaea* (Risso, 1826)]
Zooxantellate colonial scleractinians [e.g. *Cladocora caespitosa* (Linnaeus, 1767), *Madracis pharensis* (Heller, 1868)]
Eunicella cavolini (Koch, 1887)
Eunicella singularis (Esper, 1791)
Savalia savaglia (Bertoloni, 1819)

Polychaetes (colour: grey)

Large serpulids [e.g. *Protula intestinum* (Lamarck, 1818), *Serpula vermicularis* Linnaeus, 1767)]
Salmacina-Filograna complex

Macroforaminifera [e.g. *Miniacina miniacea* (Pallas, 1766)]

Stolonifera [e.g. *Sarcodictyon catenatum* Forbes, 1847)]

Bivalve molluscs [e.g. *Lithophaga lithophaga* Linnaeus, 1758, *Arca barbata* Linnaeus, 1758, *Pteria hirundo* Linnaeus, 1758]

Actinians [e.g. *Cribrinopsis crassa* Andrés, 1881]

Vermetids [e.g. *Thylacodes arenarius* Linnaeus, 1758]

Note:
(1) Generally small in size, growing mainly in plane than in height (r>h) without a specific and regular shape or with a determined hemispherical shape (r = h), usually attached to the substrate along most of the basal area
(2) Large in size, with an erect and branched (h >> r) or massive (r >> h) habit
(3) Generally small and with erect habit, forming low bushes, usually regularly branched and with a restricted area of attachment to the substrate

4.2 Software and devices for image analysis

For the image analysis, it is recommended to use software able to mark in a different way the covers of taxa/groups and to calculate the percentage of covered area respect to the photographed one. There are many commercially available software, but also some open source or free software downloadable on line, such as ImageJ or photoQuad, perfectly meet the purpose (Trygonis & Sini, 2012).
This manual presents, by way of example, the use of the ImageJ program (1) in the analysis of images collected during a photographic sampling carried out through a frame with telescopic feets. The program can be free downloaded online (http://imagej.nih.gov/ij/download.html) and the reference sheet (Sheet 3) is only a quick guide to use the software; for further information, please refer to the user manual available on line (https://imagej.nih.gov/ij/docs/) or on the ImageJ toolbar (*Help>Documentation*).
The ImageJ program allows to analyze photographic data by means of the "patches" mosaic method. The software, originally designed by NIH Image for Macintosh, offers the advantages of portability on different platforms from the Java language. Each taxon or group is outlined and the area obtained is filled with a specific colour. The result of this operation generates a heterogeneous mosaic with patches of different size and colours which allow to discriminate among various taxa/groups occur on the photographed surface. The software then calculates the surface of each patch allowing to obtain the cover in cm^2 of each taxon/group by summing all the patches.
As shown in the example, image processing can be performed directly on the computer screen using the mouse and keyboard. However, the pen graphic tablets commercially available can make the same processing much faster and more accurate. These devices are connected to the computer via USB or Bluetooth and allow to create the mosaic of coloured patches on the image using the pen on the tablet instead of the mouse.
Practical examples of images processed with ImageJ software are reported in Annex C starting from field photos taken in three different environmental conditions (high, moderate and low impact).

Note:

(1) The description refers to execution of the ImageJ version available with Java 8 on the Windows 10 operative system; it may therefore undergo variations among different versions

SHEET 3
IMAGE ANALYSIS BY *ImageJ*: QUICK GUIDE

1) Start the programme ImageJ, on the toolbar click ***File>Open*** *and open the photo (.jpg format) to be processed (Figure 1). The photographed surface is expressed in pixels (indicated at the top left, e.g. 4224x3168) and it should be converted into* cm^2 *so* ***Analyze>Set scale****: enter the parameters of the smaller side of the photo, i.e. the second number in* Distance in pixel *(3168) and the height in cm in* Known distance *(40) so as to obtain the reference surface for image processing (in the example, 53 cm x 40 cm equal to 2120* cm^2*), then* Unit of lenght = cm *finally tick* Global → *OK*

2) ***Analyze>Set measurement****: tick only* Area *and* Decimal places = 2 → *OK*

3) Before proceeding with selection and calculation of the coloured areas for the single taxa/groups, we reccomended to identify one taxon/group to be excluded a priori *from the image processing, in order to speed up the photo analysis. The choice generally falls on the most evident taxon/group, i.e. the one showing greater cover area than others (usually the Encrusting calcified Rhodophyta or* Peyssonnelia *spp.). This taxon/group will not be selected with the software colour palette and its cover percentage will be calculated by subtraction in the data processing phase (see paragraph 5.1).*

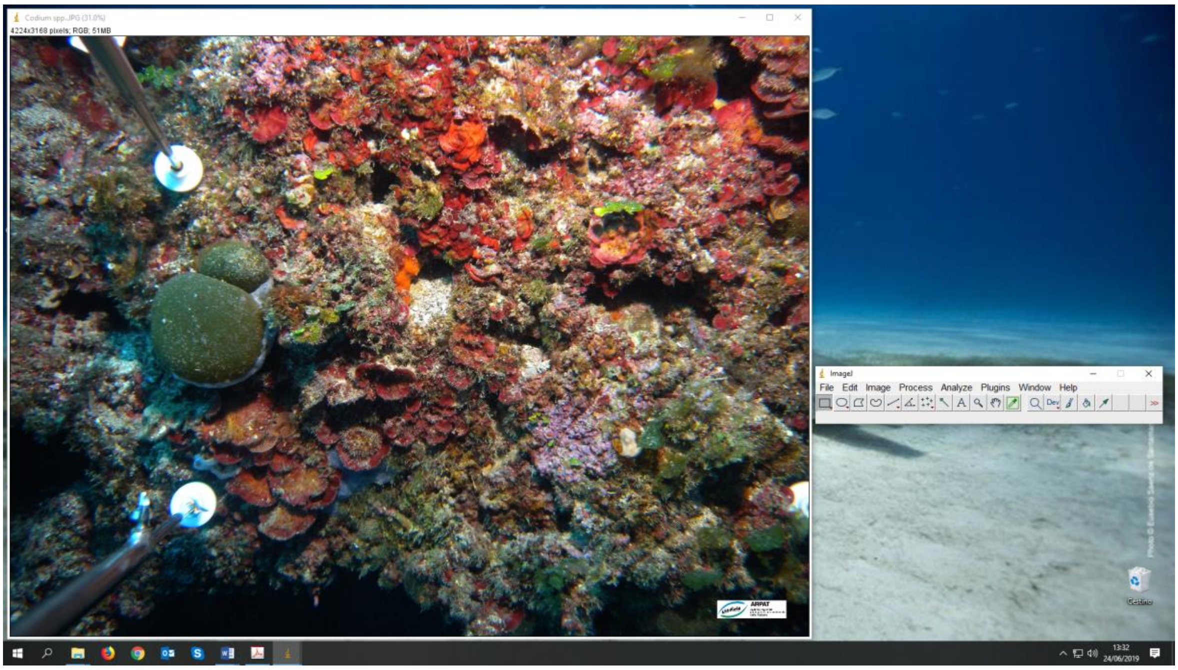

Figure 1 *– Screenshot example showing a photo to be processed (left) and the toolbar of ImageJ (right)*

4) On the toolbar, click the Freehand selection *command ("heart" symbol) and, holding down the lowercase key on the keyboard, select (by surrounding them) all the organisms belonging to the first taxon/group. At the end of the operation, fill the selection with the colour coded in Sheet 2 for that taxon/group → double click on the* Color picker *command ("dropper" symbol), choose the colour for the background B (rectangle placed at the bottom) and click* X *key on the keyboard.*

The selected taxon/group should be filled immediately and only then, once the colour has been filled, you can move on to the next group selection.
Before moving on to the next taxon/group, the previous one must be deselected.
To undo the last filling operation: ***Edit>Undo***
To undo all fills: ***File>Revert***

To change the colour/thickness of the selection border: ***Edit >Selection>Properties****, insert colour in* Stroke color *and thickness in* Width *→ OK.*
To zoom and process the image: click on the Magnifying glass *command ("magnifying glass" symbol) and then on the photo, each click zooms the image and the reference square appears at the top left. Select the taxon/group by moving from time to time the reference square with the* Scrolling tool *command ("hand" symbol) in order to select all the organisms present in the photo. Once the selection operation is completed, double click on the magnifying glass command to return to the original size and colour fill.*
It is recommended not to zoom more than 2 times, as a correct image processing resulted from the right compromise between detail on the taxon/group and speed of the image reading.
When organisms such as corals/bryozoans show small branched colonies scattered over the entire surface of the image (rather than large colonies concentrated in a few points) a global quick visual estimate should be made rather than selecting the individual colonies one by one. One method may be to estimate the area occupied by all the small colonies by selecting a rectangle (or other geometric shape) whose surface is equal to the sum of the area occupied by all the individual colonies scattered throughout the photographic image.
If the image shows shaded zones that hinder identification, select the area in question with the free hand command then ***Image>Adjust>Britness/Contrast*** *or* ***Color Balance*** *to work on light and colour. Generally, any of the operations described above can only be undone once.*

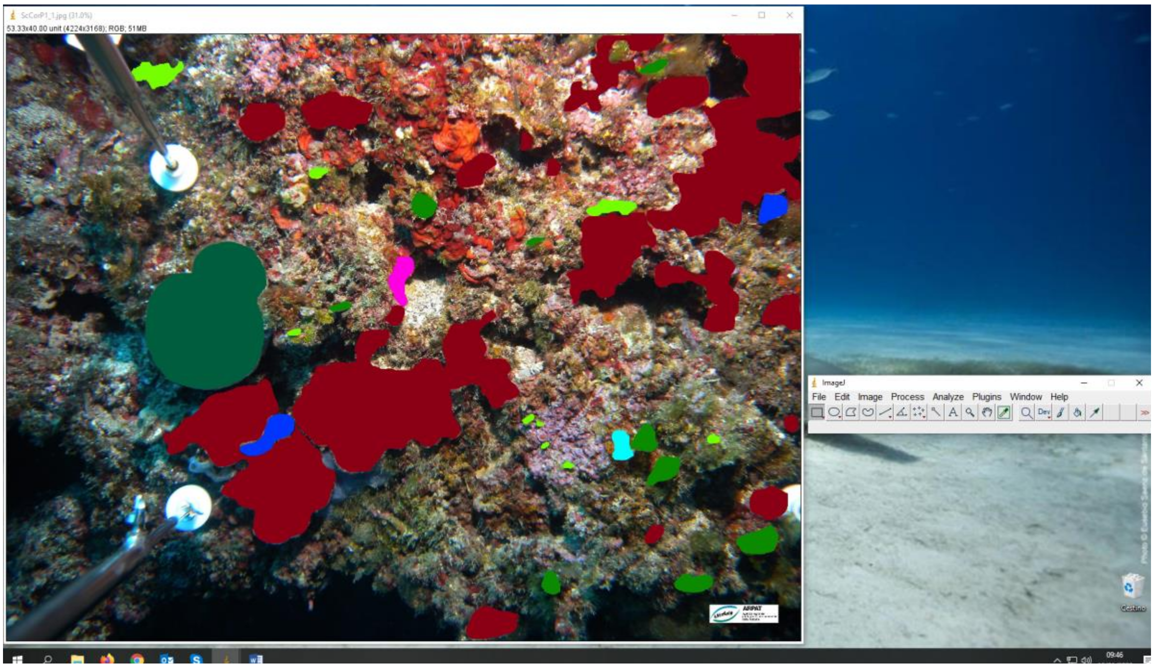

Figure 2 *– Screenshot example showing a photo being processed*

5) To calculate the total cover of each taxon/group (the cm^2 value of the photographed area covered by the taxon/group): Wand (tracing) tool *command ("star pointer" symbol), click on the first coloured patch to highlight it and, holding down the uppercase key, highlight all other patches of the same colour →* ***Analyze>Measure****. The open window provides the cover value of the taxon/group; to save it, click on the toolbar of the same window* ***File>Save as****, name the file with the taxon/group (e.g. Encrusting bryozoans) and the cover will be saved as an excel file.*
It is recommended to save taxa/groups processed (one for each excel file) in a folder with the name of the reference photo, in order to store in the same folder the original photo, the processed one and all the excel files relating to the taxa/groups identified in the same photo.

6) On the main toolbar, click ***File>Save as>Jpg****: save the processing with a detectable name, so as to archive the processed photo together with the original one.*

4.3 Calculation of abundance and spreadsheets

Cover is the percentage of sampling surface occupied by the taxon or group and is calculated: % cover = $cm^2/2000 \times 100$. The abundance of plant taxa (macroalgae) is always calculated as % cover while the abundance of conspicuous megabenthic taxa (in particular massive and arborescent structuring species of the upper layer) should be reported both as % cover and n° of colonies occur on m^2. The cover data of each taxon/group and/or number of colonies should be entered in a specially prepared excel file, according to the objectives of the work.

4.4 Parameters gettable from the photographic image analysis

The analysis of the photographic images can provide for an estimate of the variables useful for calculating the ecological quality indices, i.e.:

- percentage cover, with respect to the photographic sample surface, of each taxon, morphological group and/or sediment present;
- species richness: number of taxa/groups observed in the photographic sample.

It also provide for an estimate of the "accessories" variables, ie. those ones that do not enter into the calculation of the indices presented, but which constitute useful data to complete the picture on the quality status of coralligenous habitats, i.e.:

- n° of colonies per m^2 of conspicuous megabenthic taxa of the upper layer (massive and arborescent structuring species);
- abundance (density per m^2) and type of anthropogenic waste observed.

5. DATA ANALYSIS: DATABASE BUILDING AND ECOLOGICAL QUALITY INDICES CALCULATION

The STAR procedure described above is a standardized method that integrates, in a robust and effective way, the different approaches available in the literature for assessment condition of the coralligenous habitats developing on the vertical cliff in the bathymetric range between 30 and 40 m depth.
It therefore allows to collect as much information as possible by optimizing the sampling effort and providing at the same time data that can be used in different ways depending on the objectives of the work. In particular, by applying the STAR method it is possible to build a single dataset (called STAR database) from which a series of ecological quality indices reported in the literature can be calculated. This dataset is built by reporting on an excel file the field parameters (calcareous matrix consistency, maximum height of the upper layer, necrosis) and those obtained in the laboratory from the photographic image analysis. Once the STAR database has been compiled, it can be used to calculate various ecological quality indices, which are therefore considered a special case of application of the STAR method. In this manual, only the indices developed in Italy, already described in the introduction part, will be dealt with.
The first format to build is the "STAR database", the excel format in which the parameters recorded in the field are reported, the "raw" cover data captured by the photographic images (expressed in cm^2 for each taxon/group created by the ImageJ program on the .xlsx file) and data relating to the supplementary parameters which do not enter directly into the calculation of the indices, but which are however useful for an overall assessment of the condition of the habitat under investigation. In the same file, the raw data will then be transformed into cover percentages, which are data suited to calculate the ecological quality indices. The STAR database constitutes hence the starting point for description of calculation of all the three indices presented in this manual.
Once the STAR database has been set, the operator can proceed with the construction of the format relating to the specific index that will be applied (ESCA, COARSE, ISLA).
The following paragraph shows the detailed procedure for setting up the STAR database. For the building of the ESCA, COARSE and ISLA indices format, reference will be made to Sheets 4, 5 and 6 respectively. The standardized spreadsheets are available in the Annex D of this manual.

5.1 Setting up the spreadsheets and STAR database building

This paragraph describes how to build the basic dataset "STAR database" containing data collected *in situ* and those obtained in the laboratory from photographic images, i.e. covers of all the taxa/groups needed to calculate the indices proposed in this manual. The supplementary parameters (depth, substrate morphotype, slope and exposure of the cliff) recorded in the field are also reported in this file.
The file is structured in a way to appropriately consider also covers of the "strangers" elements that will be analyzed, such as natural cavities [(1)], mucilages and sediment. In fact, small cavities (especially if deep) naturally occur in the coralligenous rock wall may escape the light beam of the illuminator resulting in "shadow areas" which subtract cm^2 from the photographed surface. Even the mucilages and the fine sediment deposited on the organisms are "strangers" elements and therefore their cover should be considered separately in the format that is going to built. However, since the sediment is considered differently depending on the method applied (see Sheets 4, 5 and 6), when the sediment becomes a component of the samples to be analyzed, it is necessary to decide in advance the index to be applied so as to appropriately consider the sediment variable in the construction of the format.
The “STAR database” format is composed of several worksheets, each of which should be built and named as follows (in progression):

I) <u>Taxa/groups calculation by difference</u>: starting from the first row/column, the sampling sites are entered, i.e. Scoglio del Corallo (ScoCor), Argentarola (Argent) and Secca di Capo d'Uomo (CapoUo) which are the three sites selected for the Argentario area, then the three Plots (P1, P2, P3), that are the spatial replicas of the Site, and finally the photographic replicas for each plot (1-10). With the same approach, the other three Areas of the example (Romito, Capraia and Pianosa) are reported.

Note:

[(1)] The unreadable parts of the photo, such as e.g. blurred areas, elements of the frame photographed accidentally etc. fall in this category

Starting from column F and scrolling horizontally, columns will be reported as follows: cm^2 ref, i.e. the minimum reference surface in cm^2 which is equal to the photo surface (about 2000 cm^2, in absence of "strangers" elements), then "Cavities", "Mucilages" and "Sediment" and finally cm^2, where the reference surface is recalculated when strangers elements are present. Then the morphological groups are listed column by column, the macroalgae first followed by the animals (columns K-BL), proceeding from the most tolerant to the most sensitive (Tables 1 and 3, Annex A).
Where cavities, mucilagse and/or sediment are present, it is necessary to first calculate the surface occupied by these elements, reporting it in the dedicated columns (G, H, I) and then processing data accordingly, based on the applied index. If ESCA and ISLA indices will be calculated, the area obtained for all three elements should be subtracted from the total sampling area (2000 cm^2) and then proceed with the calculation of the area occupied by the various taxa in that photo based on the new surface obtained. The new surface is automatically calculated in column J and should then be reported as a value in column F. For example, if cavities + mucilages + sediment surface occupies 200 cm^2, i.e. 10% of the photographed surface, the cover of the various taxa/groups will be calculated on a total area of 2000-200 cm^2 = 1800 cm^2. On the other hand, if the COARSE index will be calculated, the percentage of sediment cover will not be subtracted but considered together with the algal turf as a unique group (TURF/SED) in the calculation of the index (see Sheet 5). The elaboration of the data for cavities and mucilages remains unchanged (subtraction and recalculation of the reference surface).
At this point, columns of the taxa/groups will be filled in reporting their cover value calculated by ImageJ in the corresponding cells. All the cells will be filled with the exception of the taxon/group excluded *a priori* from the ImageJ analysis (in the example format, the Encrusting calcified Rhodophyta, column O) which is highlighted in BLUE. Once all the values have been entered, the total area occupied by the taxa/groups (539) has already been calculated in the BM column as well as the cover value of the taxon/group (Enc.Cal.Rh., 1461) alongside (column BN), which is calculated by difference (F3-BM3) between the value of the useful surface photographed 2000 (F3) and that occupied by all the other taxa/groups (BM3, i.e. 539).
The values obtained by difference are then reported in the N column (Encrusting calcified Rhodophyta) of the worksheet II.

II) <u>Raw data:</u> is a copy of the worksheet I reporting the values of taxa/groups calculated by difference (Encrusting calcified Rhodophyta, column N) obtained in worksheet I. At the end of the list of algae and animal taxa/groups (column BL) the total area is calculated as sum from J3-BK3, i.e. the sum of all the partial areas covered by each taxon/group with respect to the total reference area (2000 cm^2 or less).

III) <u>Complete database (cm^2 e %)</u>: is a copy of worksheet II where the percentage cover is calculated starting from the cm^2 values. In the second part of the worksheet (columns BN-DO) the same taxa/groups are listed horizontally, this time calculating the % cover with respect to the reference surface. To do this, enter the value 100 in the BM column and enter the conversion formula from cm^2 to % (100 * taxa/groups cover in cm^2/2000) in all the following cells. For example, in the first column (BN, Alien species) enter the formula (BM3*J3/F3) and so on, obtaining all the surfaces covered by each taxon/group in partial % with respect to the reference surface.
Finally, an additional column (DP, % tot) is reported in which to insert the formula of the sum of the % values (BN3: DO3) which should always give 100 (useful to check for any errors).

IV) <u>General database</u>: is a copy of worksheet III in which only the percentage cover data of the taxa/groups are reported. This is the general database from which to proceed with the calculation of the three indices. It is buildt by copying the % cover values of the taxa/groups reported in the J-BK block of the worksheet III and pasting them on the worksheet IV (copy> paste special > values).

V) <u>Field data</u>: data collected directly in the field are reported in this worksheet, ie: i) the six measurements for each plot obtained with the penetrometer (Penetrometric measurements, in cm) and their calculated mean (PEN, column G); ii) the total % of necrosis (N) with respect to all the colonies of the plot considered (reported as mean cover % per plot); iii) the height measurement of the "highest" erected organism (Maximum Height, MH), in this case *Paramuricea clavata* and *Eunicella cavolini*.

VI) <u>Supplementary parameters</u>: the data collected in the field relating to supplementary parameters (depth, morphotype, slope and exposure of the cliff) are reported.

The reference sheets for building the calculation format of the three indices are reported below. For each index, a dedicated excel file is prepared, named "ESCA (or COARSE or ISLA) format" and organized by worksheets as described in each of the reference sheet.

SHEET 4
ESCA CALCULATION FORMAT

To calculate the ESCA index the three metrics SL, α and β-diversity should be computed. For easier practical execution, the first worksheet of this format reports the cover percentages of taxa/groups which represent the starting point for subsequent calculations.

1) General database: is the first worksheet and is a copy of worksheet IV of the STAR database format described in paragraph 5.1; the taxa/groups highlighted in red are those not considered in the ESCA index (they will instead be part of the ISLA index calculation). These groups will therefore be eliminated or merged, depending on the species, in the next worksheet. The three "strangers" elements not included in the index calculation (Cavities, Mucilages and Sediment) are also highlighted in red and they will be deleted from the ESCA database, after the subtraction and recalculation of the new reference surface.

2) ESCA database: is the % cover database used to calculate the ESCA index metrics. It is obtained by creating a copy of worksheet 1 without the following taxa/groups: Alien species, Macroforaminifera, Stolonifera, Bivalve molluscs, Actinias, Vermetids. Instead, following taxa/groups are merged, and thus considered as a single category of organisms in the identification phase: Small+Large hydroids (Hydrozoans), Azooxantellate scleractinians (solitary+colonial), Zooxantellate scleractinians (solitary+colonial). The strangers elements are also deleted from the database after recalculation of the minimum reference surface.

3) % means calculation: set up the worksheet with name of the Areas (Argentario, Romito, Capraia and Pianosa) and Sites (Scoglio del Corallo, etc.). Copy and paste from worksheet 2 the % cover values of the taxa/groups and calculate the mean of % cover of each taxon/group for each Site/Area, depending on the objective of the study which defines the spatial scale to be considered (in this example calculation per Site was performed). The means values can be also calculated through multivariate statistical analysis software (R, PERMANOVA$^+$ for PRIMER, etc.) starting from the dedicated matrix (see point 6).

4) % means - Abundance classes: report all means calculated in worksheet 3 (copy> paste special > values), obtaining a row with the columns of taxa/groups for each Site (C-AU). Below the grid, the cover ranges corresponding to different abundance classes are shown, that are: 1) >0-≤0.01%; 2) >0.01-≤0.1%; 3) >0.1-≤1%; 4) >1-≤5%; 5) >5-≤25%; 6) >25-≤50%; 7) >50-≤75%; 8) >75-≤100%. In the rows AW-CO, calculation of the belonging abundance classes for each group is reported [formula: =SE(C3>75;8;SE(C3>50;7;SE(C3>25;6;SE(C3>5;5;SE(C3>1;4;SE(C3>0.1;3; SE(C3>0.01;2;SE (C3>0;1;0))))))))].

*5) EQV_{SL} calculation: copy the values of abundance classes calculated in worksheet 3 on this new worksheet, then near each group add a column where the SL × AC value is calculated, i.e. the value obtained by multiplying the Sensitivity Level of each taxon/group (SL, reported in Table 1-Annex A and in this worksheet at the top of each group) by the Abundance Class (AC, from 1 to 8) corresponding to the cover value of the same taxon/group (formula: =(C3*C1)). Repeat the same for all the other groups and then calculate the EQV_{SL} value (per Site) as the sum of all the SL × AC row values obtained for each taxon/group.*

6) R matrix: in this worksheet the % abundance matrix needed to calculate the β-diversity through the R software is reported. The analysis are performed on an untransformed data matrix.

Processing with R requires a matrix set up as indicated in the worksheet (first row is header dedicated, factors and variables as columns, observations as rows) and without applying any type of formatting (e.g. coloured characters, backgrounds, cell borders, etc.).
The matrix should then be saved in a dedicated excel file (Diversity with R, acronym DivR) in ".csv" format, since this is the file that will be loaded by the R program at time of processing. If "free" mutivariate statistical software other than R is used or if license of commonly used paid software is available (e.g. PERMANOVA$^+$ for PRIMER, PP6$^+$), meet the language of the program used is needed, setting up the matrix as required by the specific software. By way of example, the matrix for data processing required by the PP6$^+$ package, a very popular software for multivariate analysis of ecological data, will be shown in the next worksheet.

7) <u>PP6$^+$ matrix</u>: if PP6$^+$ license is available, this worksheet can be the source of % abundance matrix needed for calculation of β-diversity via PP6$^+$ package. Also in this case, the analysis should be carried out on the untransformed data matrix. To build the matrix, copy the abundance % of the worksheet 2 (copy> paste special > values), then leaving an empty column and reporting in the last three columns (AV, AW, AX) the determining factors (Area, Site and Plot).

8) <u>S calculation (alpha diversity)</u>: in this worksheet the number of taxa/groups of each photo is reported and then the mean number of taxa/groups for each sampling site is calculated (S = mean value of n° taxa/groups calculated on 30 photos). If the considered scale is the Area, the calculation will be made as the mean value of taxa/groups by Area (S = mean value of n° taxa/groups calculated on 90 photos). The calculation of the α-diversity at the selected scale can also be performed using R or other software, starting from the corresponding matrix (the same used for the calculation of β-diversity).

9) <u>EQV alpha div:</u> the summary of the mean values calculated in the previous worksheet for each Site (or Plot/Area according to considered spatial scale) is reported.

10) <u>EQV beta diversity.</u>: the multivariate analysis of the groups dispersion (replicas) with respect to centroid (dispersion homogeneity test) is performed on the matrix set up in the worksheet 6. After loading the matrix (file DivR.csv) on the R program, create an object that includes only the cover values (DivR1 file) and install the "vegan" package containing the function requested for the analysis ("betadisper"). By applying the "vegdist" function ("bray" method), the distance matrix is built (Bray-Curtis dissimilarity matrix) and on the newly created object dissDivR = vegdist ("DivR1", method = bray) apply the "betadisper" function, specifying the spatial scale of the DivR2 = betadisper (dissDivR, DivR$Sito, type "centroid") grouping. The EQV_{beta} is provided by the program as the result output "Average distance to centroid" (0.114; 0.155; etc.).
If the PP6$^+$ program is used, a PERMDISP analysis on the matrix set up in the worksheet 7 will be performed. Proceed by creating: – the PERMANOVA design with all the factors (Area, fixed, 3 levels; Site, random nested in Area; 3 levels; Plot, random nested in Site, 3 levels) – the resemblance matrix (Bray-Curtis similarity) and running the PERMIDSP (untransformed matrix) after setting the centroid on the factor of interest (in this case the Site, 30 photos, n° perm = 9999). The EQV_{beta} value is provided by the output of the program "Average" (11.4; 15.5; etc.).

11) <u>Final EQR'</u>: to perform the ESCA final calculation, the EQV_{SL}, EQV_{alpha} and EQV_{beta} values obtained in the previous worksheets are arranged in alternating columns (C, F, I). Each value is divided by the Montecristo reference value (in bold), respectively EQV_{SL} = 540, EQV_{α} = 15 and EQV_{β} = 0.20, obtaining the values of EQR_{SL}, EQR_{α} and EQR_{β} for each site (in blue). The mean of these three values provides the final value of EQR' which will be used for the classification of the Site/Area according to the following scale of values: i) High ($EQR \geq 0.8$); ii) Good ($0.6 \leq EQR < 0.8$); iii) Moderate ($0.4 \leq EQR < 0.6$); iv) Poor ($0.2 \leq EQR < 0.4$) and v) Bad ($EQR < 0.2$).

SHEET 5
COARSE CALCULATION FORMAT

To calculate the COARSE index, the three ecological descriptors for each layer (basal, intermediate and upper) should be computed.
For easier practical execution, the first worksheet of this format reports the cover percentages (worksheet IV of the STAR database file) requested to perform subsequent calculations; unlike ESCA, calculations are carried out for single Plot and thus for each of the three groups of 10 photos constituting the replicas of the Site. The next worksheet will instead report the measurements/observations made in the field (Field data). At this point, all the elements needed to proceed with calculation of the three descriptors and COARSE index are available.

1) General database: is the first worksheet and is a copy of worksheet IV of the STAR database format; the taxa/groups highlighted in red will not be considered in the COARSE index (while they will enter in the calculation of the ISLA index). These groups will thus be eliminated or merged, depending on the species, in elaborating the three different layers (basal, intermediate, upper) as described in the subsequent worksheets. The groups eliminated from the general database are: Alien species, Macroforaminifera, Stolonifera, Bivalve molluscs, Actinias and Vermetids; the Small + Large hydroids (Hydrozoans) are instead merged. The taxon/group of Perforating sponges is highlighted in blue since in the next worksheets the % cover will be converted into presence/absence values of these organisms per sampled plot (n° borer marks/2m^2, total value of the photographed surface). The strangers elements not considered in the index calculation (Cavities and Mucilages) are also highlighted in red; once the new reference surface has been subtracted and recalculated, they will be deleted from the COARSE database. Conversely, the percentage of sediment cover is maintained in the database as it will enter into the calculation of the cover of one of the basal layer benthic categories (algal TURF/SEDiment).

2) Field data: is a copy of the worksheet V of the STAR database and reports data collected in the field which directly enter into the calculation of the COARSE index.

3) Basal Layer (BL): is a copy of the worksheet 1 in which, however, only taxa/groups belonging to the basal layer are reported (encrusting or small size organisms <1cm). In the last columns (R, S, T, U) the four benthic categories forming the Basal Layer are constructed as the sum of the % cover, i.e.: i) TURF/SED (Sediment+Algal turf+Siphonal/siphonocladal Chlorophyta with separate filaments); ii) ECR (Encrusting Calcified Rhodophyta); iii) NCEA (Non Calcified Encrusting Algae, i.e. Peyssonnelia *spp.+Encrusting Ochrophyta+*Palmophyllum crassum*); and iv) EA (Encrusting Animals, i.e. Sponges+Bryozoans+Encrusting ascidians). In the last column (V) frequency of perforating sponges observed for each photo as n° borer marks/replica is reported.*

4) BL means: cover values of the categories obtained in the worksheet 3 are reported and mean of the % cover for each Plot (P1, P2, P3) is calculated, i.e. mean of 10 photographic replicas. Furthermore, the frequency of perforating sponges per sampling plot is calculated as the mean value obtained on 10 photos, expressed in n° borer marks/2m^2.

*5) BL descriptors: in this worksheet the three descriptors for the Basal Layer are calculated which are: 1) % cover of Benthic Categories (BCs); 2) frequency of perforating organisms (PERforating, PER); 3) thickness and consistency of the calcareous matrix (PENetrometry, PEN). For calculation of the three descriptors, first of all the mean cover values obtained in the previous worksheet for the four benthic categories (TURF/SED, NCEA, EA, ECR), the frequency values of perforating organisms (PER) and values of penetrometry (PEN) are reported. A score is associated with the mean value of each group, as reported in Table a of the same worksheet (see also Table 2-Annex A). The final value of the BC descriptor for each category (L-O columns) is obtained by applying the formula (mean value*score taxon/group)/100, in the example E4*1/100) while the Total BC as the sum of the five scores obtained for single category (column P).*

6) <u>Intermediate Layer (IL)</u>: is a copy of the worksheet 1 where only taxa/groups of organisms with average height between 1 and 10 cm are reported. First of all, presence (1) or absence (0) for each group is detected for each Plot (P1, P2, P3), then in the last three columns (AI, AJ, AK) the three descriptors of Intermediate Layer are reported: 1) Species richness (S, as the sum of the presence/absence values); 2) Erect Calcified Organisms (ECO, as the number of calcified organisms present; they are highlighted with an asterisk on the name of the taxon/group); 3) Sensitive Bryozoans (SB, the most sensitive bryozoans species detected according to Table 2-Annex A, which lists the organisms according to increasing sensitivity from 1, least sensitivity, to 3 most sensitive).

7) <u>IL descriptors</u>: in this worksheet, values of the three descriptors S, ECO and SB that define the Intermediate Layer are calculated. These values are obtained by associating the score corresponding to each category as reported in Table b of the same worksheet (see also Table 2-Annex A).

8) <u>Upper Layer (UL)</u>: is a copy of the worksheet 1 from which the taxa/groups of massive or arborescent organisms with a height > 10 cm are extrapolated. For each taxon/group, the mean % cover for each Plot (10 photos) is first calculated and mean values of percentages cover of all taxa/groups are summed in the last column (P).

9) <u>UL descriptors</u>: in this worksheet, values of the three descriptors that define the Upper Layer are calculated: 1) total % cover of taxa/groups; 2) Necrosis (N); 3) Maximum Height (MH), measured for each species on the "tallest" organism observed in the Plot. The final value of each descriptor is obtained by associating the score corresponding to each parameter, as reported in Table c (see also Table 2-Annex A).

10) <u>COARSE calculation</u> [(1)]: values of the three descriptors obtained in the previous worksheets for each layer are reported on this worksheet (columns F-N). For each layer, each descriptor is averaged over the three sampling plots (P-X columns). The value of the COARSE Quality index is calculated for each layer as Q_L, which is obtained by applying the formula $Q_L=(X_L*Y_L*Z_L)$^(1/n) where L is the layer (basal, intermediate, upper), X-Y-Z the value of the descriptor per layer and n the number of the layers (3 in this case). The value of quality per site (Q_O) is obtained from the mean of the values of the three layers Q_{BL}, Q_{IL} and Q_{UL}.
Based on the Q values obtained, each stratum/site is classified into: i) High ($2.55<Q \leq 3$); ii) Good ($2.35 < Q \leq 2.55$); iii) Moderate ($2.05 <Q \leq 2.35$); iv) Poor ($1.55 < Q \leq 2.05$) and v) Bad ($Q \leq 1.55$).

Note:
[(1)] Calculation formula of the COARSE Quality index and classification scale updated with respect to the original proposal reported in Gatti et al. 2015a

SHEET 6
ISLA CALCULATION FORMAT

To calculate the ISLA index, the Integrated Sensitivity Level (ISL) metric should be computed in order to obtain the final EQR value of the index. Therefore, in the first worksheet of this format the cover percentages (General database) needed to perform the subsequent calculations are reported.

1) General database: is the first worksheet and is a copy of the worksheet IV of the STAR database format described in the paragraph 5.1. The elements not foreseen in the index calculation are highlighted in red (Cavities, Mucilages and Sediment); once the new reference surface has been subtracted and recalculated, they will be deleted from the ISLA database.

2) ISLA database: is the % covers database needed to calculate the ISLA index metric. It is obtained by creating a copy of worksheet 1 and by eliminating the "strangers" elements (after recalculation of the reference surface).

3) % means calculation: the worksheet is set up reporting the name of the Area (Argentario, Romito, Capraia and Pianosa) and Sites (Scoglio del Corallo, etc.). Copy and paste from the worksheet 2 the % cover values of the taxa/groups and calculate the mean % cover for each group and for each Site/Area, depending on the objective of the study and thus on the spatial scale considered (Site, in this case).

4) % means - Abundance classes: the values of all means calculated in the previous worksheet are reported on this worksheet, obtaining a row with the columns of taxa/groups for each Site (C-BD). The cover intervals corresponding to different abundance classes are shown below the grid, i.e.: 1) >0-≤0.01%; 2) >0.01-≤0.1%; 3) >0.1-≤1%; 4) >1-≤5%; 5) >5-≤25%; 6) >25-≤50%; 7) >50-≤75%; 8) >75-≤100%.
The BF-DG rows show the calculation of the belonging abundance classes for each group applying the following formula:
=SE(C3>75;8;SE(C3>50;7;SE(C3>25;6;SE(C3>5;5;SE(C3>1;4;SE(C3>0.1;3;SE(C3>0.01;2;SE(C3>0;1;0))))))))

*5) EQV_{ISL} calculation: in this worksheet, values of abundance classes calculated in the worksheet 4 are copied and near to each group a column is added in which the ISL × AC value is calculated by multiplying the Integrated Sensitivity Level value of each taxon/group (ISL, reported in Table 3-Annex A and in the same worksheet at the top of each group) by the Abundance Class (AC, from 1 to 8) corresponding to the cover value of the same taxon/group (formula: =(C3*C1). The same is done for all the other groups and then the EQV_{ISL} value (per Site/Area) is calculated in the last column (DG) as the sum of all the ISL × AC row values obtained for each taxon/group.*

6) Final EQR': to perform the final ISLA calculation, the EQV_{ISL} values calculated in the previous worksheet are reported and each value is divided by the Montecristo reference one (in bold), that is EQV_{ISL} = 370. The final EQR value will provide the classification of the Site (or Area), according to the following scale of values: i) High (EQR ≥ 0.8); ii) Good (0.6 ≤ EQR < 0.8); iii) Moderate (0.4 ≤ EQR < 0.6); iv) Poor (0.2 ≤ EQR < 0.4) and v) Bad (EQR < 0.2).

6. INDEX COMPARISON AND INTEGRATED APPLICATION

The practical exercise carried out, by way of example, with the ESCA, COARSE and ISLA format, shows that all the three applied indices have allowed to assess a High/Good ecological quality status in the Pianosa island, a Good one in the Argentario Mount and a Moderate in Livorno, while discordant values were obtained for the Capraia island (Figure 6.1). In fact, the ESCA and ISLA indices classify Capraia island in a Good ecological quality status albeit with different EQR values, while COARSE detects a Moderate quality status. The differences obtained are coherent and intrinsically linked to the different approaches with which the indices were developed, so the discrepancy can be attributed to the quite peculiar natural characteristics of the Capraia coralligenous assemblages. In fact, the Capraia assemblages totally devoid of the upper layer formed by gorgonians so the assessment of this type of coralligenous can be strongly penalized by an index such as COARSE, which provides for the integration of all the three coralligenous stratocenoses. A correct assessment of the ecological status of coralligenous cliff should therefore take into account the structure of assemblages naturally occurring in the investigated area, applying the indices in a coherent and integrated way, so as to obtain as much information as possible while avoiding underestimation of real values of the ecological quality.

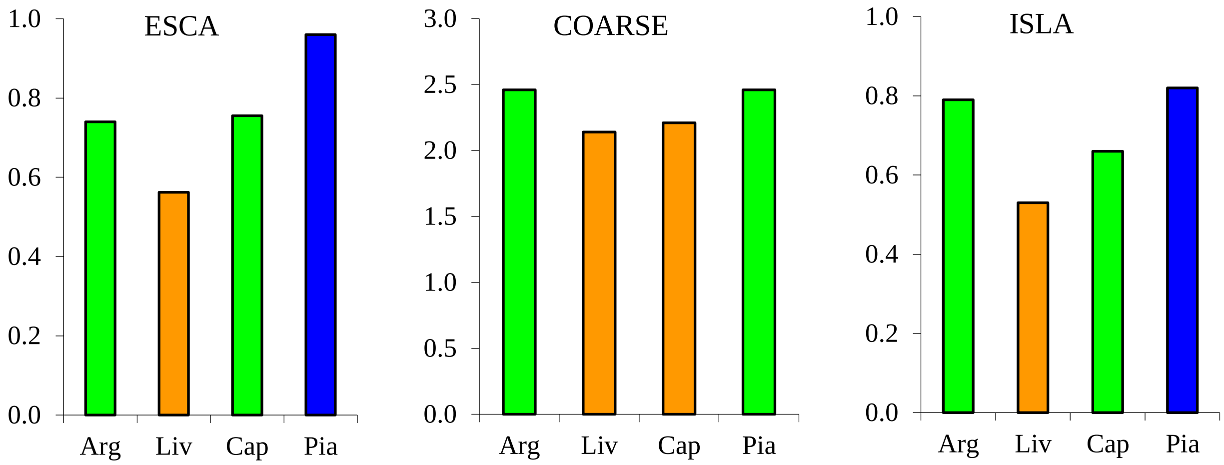

Figure 6.1 – Ecological quality values (EQR' and Q) obtained through application of the three indices. Arg=Argentario, Liv=Livorno, Cap=Capraia, Pia=Pianosa

BOX 1

ESCA *vs* COARSE INTERCOMPARISON: CASE STUDY OF APPLICATION ALONG THE WESTERN MEDITERRANEAN COASTS

The ESCA and COARSE indices were used simultaneously in about fifty sites. Part of this study was published demonstrating the ability of both indices to detect changes in the ecological quality of coralligenous assemblages along gradient of anthropogenic pressures (Piazzi et al., 2017a). However, differences were found in the classification of coralligenous quality status provided by the two indices, essentially due to the different approach used for their developing.

Study area, conditions and intercomparison sites

The published study was carried out in the Western Mediterranean basin, in 24 sites selected along the coasts of France, Liguria, Tuscany, Lazio and Sardinia, according to the level of human pressures they experience (Figure 1). Eight sites were selected within Marine Protected Areas (MPAs) where conservation plans are well enforced (Portofino-Altare, Portofino-Secca Gonzatti, Tavolara-Secca del Papa, Tavolara-Occhio di Dio, Asinara-Punta Tumbarino, Asinara-Pedra Bianca, Capo Carbonara-Cavoli, Capo Carbonara-Serpentara); eight sites were selected in unprotected but with low levels of human pressures areas (low pressures, LP)(Les Deux Frères, Bec de l'Aigle, Ile du Planier, Elba-Picchi di Pablo, Elba-Punta Galera, Costa Paradiso-West, Costa Paradiso-East, Torre delle Stelle); finally, eight sites were located in areas subject to high levels of human pressures (high pressures, HP) (Sêche des Pêcheurs, Figuerolle, Large Oursinière, Livorno-Boccale, Livorno-Calignaia, Civitavecchia, Sant'Agostino, Santa Marinella) (Figure 1). The level of human pressures in each study site was evaluated through the use of the anthropization index, which was defined as the sum of nine impact factors affecting coralligenous cliffs (i.e. urbanization and urban waste, ports, tourism, industrial activity, sediment load, aquaculture, agriculture waste, fishing, and anchoring) (Piazzi et al., 2015). Each impact factor was classified from 0 (no impact) to 2 (strong impact), according to presence and type of human pressure and to distance of the site from the source of impact (Gatti et al., 2015a; Piazzi et al., 2015, 2017a). In this study, differences between unprotected sites with a low level of human pressures and the selected MPAs are mostly due to the lack of impacts, such as anchoring and fishing, in these latter.

Sampling methods, data analysis and ecological quality indices calculation

In each site, photographic sampling and in situ *surveys were carried out according to the STAR protocol described in the previous paragraphs. Data collected were processed with multivariate statistical methods to highlight differences in the structure of assemblages observed under the three different pressure levels (MPAs, low and high anthropic impact) and spatial scale (Site and Plot).*
The two ESCA and COARSE indices were then calculated for each site, according to the procedures already described. The quality values obtained for each index, as well as the values of the metrics used by ESCA (SL, α and β-diversity) and of the strata used in the COARSE (basal, intermediate and upper layers) were analyzed by univariate statistical techniques in order to compare the effectiveness of the two indices in discriminating the quality status of the coralligenous subject to different pressure levels. Finally, the response of each metric to different environmental conditions was tested using ternary diagrams, which discriminate the position of each group of sites (MPA, low and high impact) on the basis of the metric used.

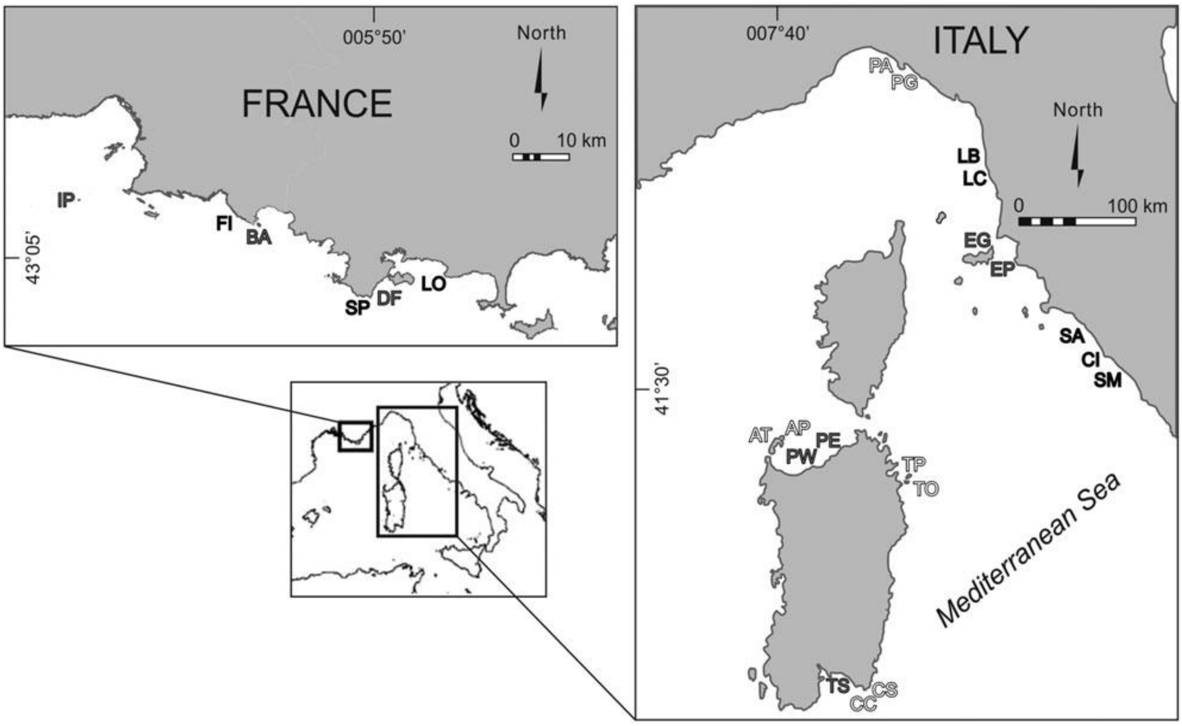

Figure 1 *– Location of the 24 sites investigated. Marine Protected Ares (MPAs): Portofino-Altare (PA), Portofino-Secca Gonzatti (PG), Tavolara-Secca del Papa (TP), Tavolara-Occhio di Dio (TO), Asinara-Punta Tumbarino (AT), Asinara-Pedra Bianca(AP), Capo Carbonara-Cavoli (CC), Capo Carbonara-Serpentara (CS); unprotected with Low level of human Pressure(LP): Les Deux Frères (DF), Bec de l'Aigle (BA), Ile du Planier (IP), Elba-Picchi di Pablo(EP), Elba-Punta Galera (EG), Costa Paradiso-West (PW), Costa Paradiso-East (PE), Torre delle Stelle (TS); unprotected with High level of human Pressure (HP): Sêche des Pêcheurs (SP), Figuerolle (FI), Large Oursinière (LO), Livorno-Boccale (LB), Livorno-Calignaia (LC), Civitavecchia (CI), Sant'Agostino (SA), Santa Marinella (SM). Sites located in MPAs, in LP and in HP areas are reported in white, grey and black letters respectively*

Results

The multivariate analysis detected significant differences in the assemblages structure between the HP sites and all the others, while assemblages observed in the LP sites and MPAs are quite similar. The ESCA index highlighted a quality status ranging from good to high in the MPAs and in most of the LP sites, while the HP sites are classified in a moderate quality status (Figure 2a). COARSE, on the other hand, classifies MPAs and LP sites in a quality status ranging from good to moderate while the quality of HP sites varies between moderate and bad, depending on the site considered (Figure 2b).

The univariate analysis performed on the ESCA and COARSE values shows that, despite evident differences of classification, both indices well discriminate the real environmental conditions to which coralligenous cliffs are subjected (HP vs MPAs, LP).

The ternary diagrams show that the metrics used for ESCA are effective in discriminating different environmental conditions only when they are combined together; if they are used individually, the same metrics are not sensitive to different pressure levels. In contrast, the metrics used for COARSE are sensitive to different levels of anthropogenic pressure even when they are applied separately.

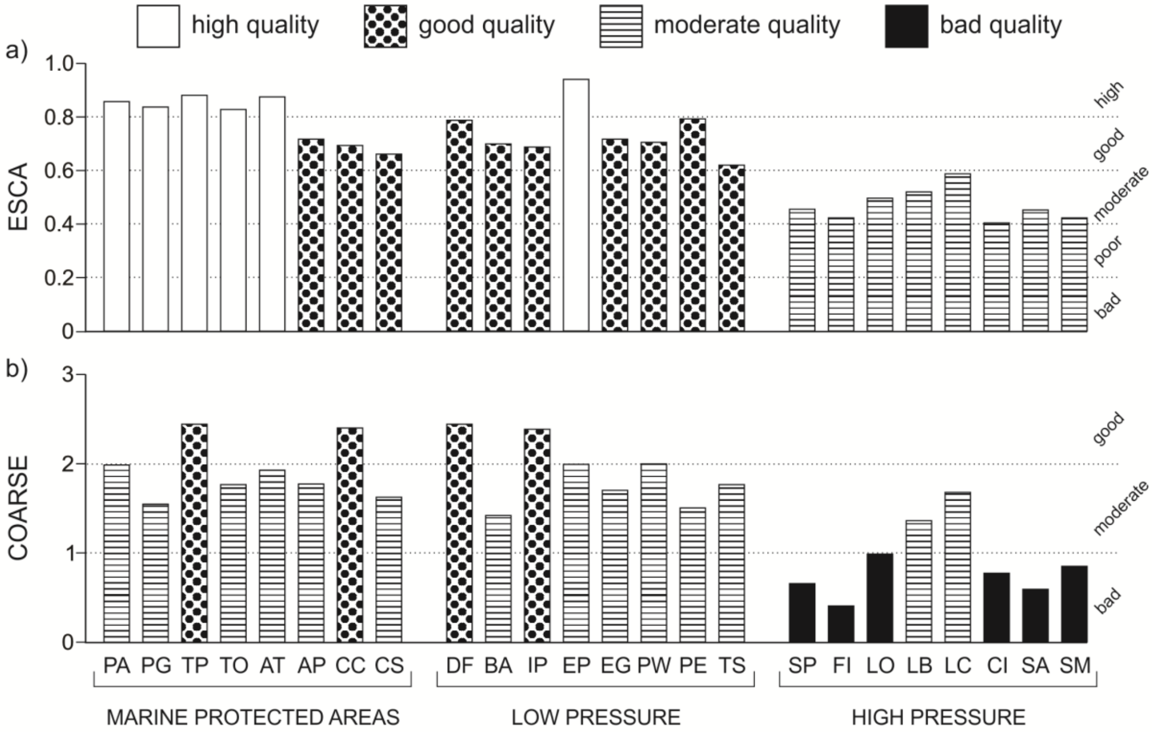

Figure 2 *– Values of ecological quality calculated by applying the ESCA (a) and COARSE (b) indices in the investigated sites (Marine Protected Areas, MPAs: PA=Portofino-Altare, PG=Portofino-Secca Gonzatti, TP=Tavolara-Secca del Papa, TO=Tavolara-Occhio di Dio, AT=Asinara-Punta Tumbarino, AP=Asinara-Pedra Bianca, CC=Capo Carbonara-Cavoli, CS=Capo Carbonara-Serpentara; Low Pressure, LP: DF=Les Deux Frères, BA=Bec de l'Aigle, IP=Île du Planier, EP=Elba-Picchi di Pablo, EG=Elba-Punta Galera, PW=Costa Paradiso-West, PE=Costa Paradiso-East, TS=Torre delle Stelle; High Pressure, HP: SP=Sêche des Pêcheurs, FI=Figuerolle, LO=Large Oursinière, LB=Livorno-Boccale, LC=Livorno-Calignaia, CI=Civitavecchia, SA=Sant'Agostino, SM=Santa Marinella).*

Discussion

This study is a first attempt to evaluate the ecological quality of coralligenous cliffs through the concurrent use of two indices based on two different and fully independent approaches: a biocenotic approach considering species composition and biodiversity, assessed at multiple levels (ESCA), and a landscape approach that also considers the structure and tridimensional feature of the habitat (COARSE). These two approaches provide different but complementary information to assess the ecological quality of coralligenous cliffs and to detect effects of anthropogenic pressures on the associated assemblages.

Each of the two indices indeed integrates different metrics by working at different levels of ecosystem complexity, in relation to the approach followed in developing the index. Both indices are sensitive to different levels of anthropogenic pressure and highlight high impact conditions in a similar way. However, ESCA and COARSE show a different sensitivity to conditions that are not or very low affected; in particular, COARSE attributes a "lower" classification value compared to ESCA to some MPA and LP sites. This different sensitivity is a direct result of the different approach used by the two indices.

In fact, by integrating all three coralligenous layers in the final quality value, the COARSE assigns lower values to coralligenous assemblages which partially or totally lack the upper layer (e.g. absence of anthozoans and arborescent sponges), regardless of the causes which determine the absence or the rarefaction of this layer (human impact or natural original conformation). This means that the index will underestimate the quality not only of those coralligenous cliffs that are really subject to stress and hence impacted, but also of cliffs that are naturally devoid of the upper layer (see for example Costa Paradiso, Asinara and Occhio di Dio).
On the other hand, ESCA does not distinguish the layers but is based on species composition and their abundance, regardless of the landscape conformation of the assemblage.
More generally, we can state that differences between ESCA and COARSE are the result of the different "history" of the two indices. In fact, the ESCA index was developed according to a "bottom-up" reasoning, i.e. starting from data collected in the field studies for the evaluation of impact on the Tyrrhenian coralligenous cliffs (Balata et al., 2005, 2007a, 2007b; Piazzi & Balata, 2009; Gennaro & Piazzi, 2011; Piazzi et al., 2011). Consequently, the metrics used to construct ESCA index were selected on the basis of their response to local anthropogenic stresses (Piazzi et al., 2012; Cecchi et al., 2014; Piazzi et al., 2015, 2016). Conversely, development of the COARSE index followed a "top-down" logic, starting from the theoretical foundations of literature and testing its robustness in the field. Therefore, descriptors used were selected on the basis of a concept of "intrinsic" quality of coralligenous cliffs, regardless of any local anthropogenic pressure (Gatti et al., 2015a). By applying the COARSE index, it is possible to detect the presence of impacts affecting calcareous structures and erect species, as well as the sub lethal effects acting at different levels; in fact, the presence of necrosis on the erect organisms or the loss of consistency of the calcareous matrix of the basal layer represent important signs of stress (Coma et al., 2004; Teixidó et al., 2013) which cannot be highlighted by applying the ESCA index. More generally, the ESCA index, by focusing on the ecological value of taxa/groups and on biodiversity, better illustrates the relationship between coralligenous assemblages and water quality while COARSE, by focusing more on the topological aspect (vertical heterogeneity) of the coralligenous landscape, better describes the seafloor integrity.
The three metrics used by ESCA are complementary and none of them, taken individually, provide additional information to what is detected by the index itself. On the contrary, the three COARSE descriptors, taken individually, can provide different information, that may be partly lost upon their integration into the final calculation of the index. For this reason, when applying the COARSE index for assessing the quality of a site, it is often advisable to keep layers separate, so as to better identify factors acting on the individual layers and to better calibrate the actions to be taken to bring back the system to the good ecological status.

6.1 Conclusions and future perspectives

Since the moment the European Framework Directives became part of the environmental legislative framework, many ecological quality indices have been developed for the assessment of ecological status of benthic marine habitats. However, examples of intercomparison and intercalibration between indices are currently very rare: the only intercalibration exercise carried out at the Mediterranean level in the framework of European directives was relating to indices developed for *Posidonia oceanica* and soft bottoms habitats (Macrozoobenthos) and hard bottoms of the upper infralittoral (Macroalgae) under the WFD 2000/60/CE implementation.
The use of different indices to test ecological quality of coralligenous assemblages at the same time has therefore been carried out in an experimental way up to now; however, it represents an important practice that should be considered in monitoring programs as the indices described so far are based on different approaches (ESCA and ISLA are built starting from a biocenotic approach while COARSE from a landscape one) and they partly use different metrics, thus providing complementary information on the effects of different anthropogenic pressures.
In particular, ESCA uses different metrics ranging from the organism sensitivity level to the alpha and beta diversity patterns, thus providing information on the alterations affecting different aspects of biodiversity at different levels of biological organization.

ISLA is based only on the level of organism sensitivity, but succeeds to separate sensitivity to disturbance from sensitivity to stress.
Finally, COARSE analyzes separately the three layers of coralligenous assemblages, indirectly providing information on which layer is mainly affected from pressures acting in the investigated area.
The various indices can thus highlight effects of stress and disturbances of different origins and at different levels of ecosystemic complexity, so their simultaneous use allows for a more complete assessment of coralligenous ecological quality in relation to different anthropogenic pressures.
The use of multiple descriptors and the integration of information from multiple ecosystem levels (from species to landscape) is considered a valid approach to identify changes in ecosystem quality (Borja et al., 2009; Martinez-Crego et al., 2010). In addition, the simultaneous use of different descriptors allows detection of the responses of assemblages to specific impacts as well as better addressing intervention measures and conservation plans. This is very important considering that the European legislative framework on environmental issues requires not only the monitoring of marine ecosystems, but also the planning of concrete intervention measures aimed at restoring the good ecological and environmental status where these have been lost.
The approach of intercomparison/intercalibration between indices in the assessment of ecological and environmental status of marine environments should therefore became increasingly widespread, in order to identify their strengths and weaknesses so as to develop integrated and effective methods useful to the legislator for responding in an ever more suitable way to the requests of European directives.
Another important aspect to consider in applying the ecological quality indices is related to the reference conditions. In accordance with concepts introduced by the European Framework Directives, the first fundamental step in development of a method is to establish the Reference Conditions (RC) of the marine assemblages that are the "blank" conditions (absence or very low anthropic impact) versus which the different conditions observed in the field should be compared from time to time. The choice of RC must hence be made carefully, both in terms of site and spatial scale of reference. Marine assemblages are subject to a certain natural variability especially in relation to the spatial scale considered; from this point of view, coralligenous is one of the least variable assemblages since it develops in relatively stable environmental conditions (dim light, poor hydrodynamism and low temperatures), which occur quite homogeneously across different areas of the Mediterranean Sea.
The method presented in this manual has been tested and validated on a database of 50 representative sites of the Western Mediterranean Sea (France, Liguria, Tuscany, Sardinia, Lazio), selected according to 3 types of environmental conditions: Marine Protected Areas (MPAs), marine not protected areas characterized by low anthropic impact (Low Pressure, LP) and marine areas under a high anthropic impact (High Pressure, HP). This was done for the purpose of field testing the effectiveness of the STAR method along the spectrum of possible environmental conditions. The results obtained showed that the reference conditions selected under the STAR method (MPA of Montecristo Island, Tuscan Archipelago) are suitable for classifying the quality status of coralligenous assemblages of all sites located along the Western Mediterranean coasts, from France to the Italian coasts of Lazio and Sardinia; therefore, these RC were assumed to be representative of the Western Mediterranean Sea sub-region.
By extending the spatial scale to the Mediterranean basin, there are no concrete elements yet to state with certainty that the same reference conditions are suitable for defining the ecological status of coralligenous communities occurring in the other marine sub-regions (Central Mediterranean Sea, Ionian Sea, Adriatic Sea and Eastern Aegean Sea). In particular, due to its "closed sea" characteristics, the Adriatic Sea shows marine assemblages often different from those typical of the Western Mediterranean, which could require a substantial revision of the reference conditions currently used, as happened in the past also for other methods adopted at the national level by the Mediterranean countries.
In conclusion, a correct assessment of the ecological status of coralligenous cliffs on a Mediterranean scale through the STAR method, will necessarily require its validation at the not yet tested spatial scales. To this scope, it is first necessary to verify the suitability of the current reference conditions at the new spatial scale, establishing new ones where necessary and recalibrating the method on the new RC in different environmental conditions (absence/presence of pressures).
Intercomparison between indices and validation on a Mediterranean scale hence represent important future goals of a method that has already shown good potential on a large scale and which could thus become a useful tool to be employed in national monitoring plans of the countries overlooking the Mediterranean Sea.

REFERENCES

Abbiati M., Airoldi L., Costantini F., Fava F., Ponti M., Virgilio M. (2009). Spatial and temporal variation of assemblages in Mediterranean coralligenous reefs. In: C. Pergent-Martini, Brichet M. (Eds.), Proceedings of the first symposium on the coralligenous and other calcareous bio-concretions of the Mediterranean Sea, Tabarka, Tunis, 15-16 January 2009 (pp. 34-39). Tunis, Tunisia: UNEP/MAP-RAC/SPA.

Aguado-Gimenez F., Ruiz-Fernandez J.M. (2012). Influence of an experimental fish farm on the spatio-temporal dynamic of a Mediterranean maerl algae community. Marine Environmental Research 74, 47-55.

Airoldi L., Rindi F., Cinelli F. (1995). Structure, seasonal dynamics and reproductive phenology of a filamentous turf assemblage on a sediment influenced, rocky subtidal shore. Botanica Marina 38, 227-237.

Anderson M.J. (2006). Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245-253.

Augier H., Boudouresque C.F. (1975). Dix ans de recherches dans la zone marine du Parc National de Port-Cros (France). Troisième partie. Annales de la Societé des Sciences Naturelles et d'Archéologie de Toulon et du Var 27, 131-170.

Balata D., Piazzi L., Cecchi E., Cinelli F. (2005). Variability of Mediterranean coralligenous assemblages subject to local variation in sediment deposits. Marine Environmental Research 60, 403-421.

Balata D., Piazzi L., Benedetti-Cecchi L. (2007a). Sediment disturbance and loss of beta diversity on subtidal rocky reefs. Ecology 8, 2455-2461.

Balata D., Piazzi L., Cinelli F. (2007b). Increase of sedimentation in a subtidal system: effects on the structure and diversity of macroalgal assemblages. Journal of Experimental Marine Biology and Ecology 351, 73-82.

Balata D., Piazzi L., Rindi F. (2011). Testing a new classification of morphological functional groups of marine macroalgae for the detection or responses to disturbance. Marine Biology 158, 2459-2469.

Ballesteros, E. (2008). Action plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea. Tunis, Tunisia: UNEP/MAP–RAC/SPA

Ballesteros E. (2006). Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanography and Marine Biology: an Annual Review 44, 123-195.

Ballesteros, E., Rodríguez-Prieto C. (1996). Presència d'*Asparagopsis taxiformis* (Delile) Trevisan a Balears. Bolletí de la Societat d'Història Natural de les Balears 39, 135-138.

Bavestrello G., Cerrano C., Zanzi D., CattaneoVietti R. (1997). Damage by fishing activities in the Gorgonian coral *Paramuricea clavata* in the Ligurian Sea. Aquatic Conservation Marine Freshwater Ecosystem 7 (3), 253-262.

Belsher T., Meinesz A. (1995). Deep-water dispersal of the tropical alga *Caulerpa taxifolia* introduced into the Mediterranean. Aquatic Botany 51, 163-169.

Bertolino M., Ferranti M.P. (2018). Pinneggiando nei mari italiani. Atlante della flora e della fauna. Hoepli (Eds.).

Bianchi C.N., Pronzato R., Cattaneo-Vietti R., Benedetti-Cocchi L., Morri C., Pansini M., Chemello R., Milazzo M., Fraschetti S., Terlizzi A., Peirano A., Salvati E., Benzoni F., Calcinai B., Cerrano C., Bavestrello G. (2004). Mediterranean marine benthos: a manual of methods for its sampling and study. 6: Hard bottoms. Biologia Marina Mediterranea 11(suppl. 1), 185-215.

Bianchi C.N., Azzola A., Bertolino M., Betti F., Bo M., Cattaneo-Vietti R., Cocito S., Montefalcone M., Morri C., Oprandi A., Peirano A., Bavestrello G. (2019a). Consequences of the marine climate and ecosystem shift of the 1980-90s on the Ligurian Sea biodiversity (NW Mediterranean). The European Journal of Zoology 86 (1), 458-487.

Bianchi C.N., Azzola A., Parravicini V., Peirano A., Morri C., Montefalcone M. (2019b). Abrupt change in a subtidal rocky reef community coincided with a rapid acceleration of sea water warming. Diversity 11, 215.

Birk S., Bonne W., Borja A., Brucet S., Courrat A., Poikane S., Solimini A.G., van de Bund W., Zampoukas N., Hering D. (2012). Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators 18, 31-41.

Borja A., Ranasinghe A., Weisberg S.B. (2009). Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: challenges for the future. Marine Pollution Bulletin 59, 1-4.
Borja, A., Elliott, M., Carstensen, J., Heiskanen, A.S. & Van De Bund, W. (2010). Marine management – towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. Marine Pollution Bulletin 60, 2175-2186.
Bosence D.W.J. (1983). Coralline algal reef frameworks: Cenozoic carbonates. Journal of the Geological Society 140(3), 365-376.
Bosence D.W.J. (1985). The "Coralligene" of the Mediterranean – a Recent Analog for the Tertiary Coralline Algal Limestones. In: Toomey D.E., Nitecki M.H. (Eds.), Paleoalgology: Contemporary Research and Applications 216-225.
Boudouresque C.F. (2004). Marine biodiversity in the Mediterranean: status of species, populations and communities. Scientific Reports of Port-Cros National Park 20, 97–146.
Boudouresque C.F., Ribera M.A. (1994). Les introductions d'espèces végétales et animales en milieu marin–conséquences écologiques et économiques et problèmes législatifs. In: Boudouresque C.F., Meinesz A., Gravez V. (Eds.), First International Workshop on *Caulerpa taxifolia*, (pp 29-102), GIS Posidonie, Marseille.
Boudouresque C.F., Verlaque M. (2002). Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. Marine Pollution Bulletin 44, 32-38.
Boudouresque CF, Meinesz A., Ballesteros E., Ben Maiz N., Boisset F., Cinelli F., Cirik S., Cormaci M., Jeudy de Grissac A., Laborel J., Lanfranco E., Lundberg B., Mayhoub H., Panayotidis P., Semroud R., Sinnassamy J.M., Span A. (1990). Livre Rouge "Gérard Vuignier" des végétaux, peuplements et paysages marins menacés de Méditerranée. MAP Technical Report Series, 43. Athens: UNEP/IUCN/GIS Posidonie, 1-250
Bracchi V.A., Savini A., Basso D., Marchese F., Corselli C. (2015). Coralligenous habitat in the Mediterranean Sea: a geomorphological description from remote data. Italian Journal of Geoscience 134, 32-40.
Bramanti L., Magagnini G., De Maio L., Santangelo G. (2005). Recruitment, early survival and growth of the Mediterranean red coral *Corallium rubrum* (L. 1758), a 4-year study. Journal of Experimental Marine Biology and Ecology 314, 69-78.
Bramanti L., Movilla J., Guron M., Calvo E., Gori A., Dominguez-Carrió C., Grinyò J., Lopez-Sanz A., Martinez-Quintana A., Pelejero C., Ziveri P., Rossi S. (2013). Detrimental effects of ocean acidification on the economically important Mediterranean red coral (*Corallium rubrum*). Global Change Biology 19 (6), 1897-1908.
BPAS (2013). "Buone prassi per lo svolgimento in sicurezza delle attività subacquee di ISPRA e delle Agenzie Ambientali". ISPRA Manuali e Linee Guida 94/2013. Cap. 11 "Attrezzature, Dispositivi di sicurezza e Protezione Individuale".
Cánovas-Molina A., Montefalcone M., Vassallo P., Morri C., Bianchi, C.N., Bavestrello G. (2016). Combining literature review, acoustic mapping and *in situ* observations: an overview of coralligenous assemblages in Liguria (NW Mediterranean Sea). Scientia Marina 80, 7-16.
Casas-Güell E., Teixidó N., Garrabou J., Cebrian E. (2015). Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. Marine Biology 162, 901-912.
Casas-Güell E., Cebrian E., Garrabou J., Ledoux J.B., Linares C., Teixidó N. (2016). Structure and biodiversity of coralligenous assemblages dominated by the precious red coral *Corallium rubrum* over broad spatial scales. Scientific Reports 6, 36535.
Casazza G., Lopez y Royo C., Dalù M., Saccomandi F., Spada E., Silvestri C. (2007). Recent policies tools for ecological evaluation of Mediterranean Sea. In: Özhan E. (ed), MEDCOAST 07. Proceedings of the Eight International Conference on the Mediterranean Coastal Environment. Alexandria, Egypt. Vol. 1. MEDCOAST Secretariat, Turkey: 129-140.
Casoli E., Ventura D., Cutroneo L., Capello M., Jona-Lasinio G., Rinaldi R., Criscoli A., Belluscio A., Ardizzone G.D. (2017). Assessment of the impact of salvaging the Costa Concordia wreck on the deep coralligenous habitats. Ecological Indicators, 80, 124-134.
Cecchi E., Gennaro P., Piazzi L., Ricevuto E., Serena F. (2014). Development of a new biotic index for ecological status assessment of Italian coastal waters based on coralligenous macroalgal assemblages. European Journal of Phycology 16, 1709-1717.
Cerrano C., Bavestrello G., Bianchi C.N., Cattaneo-Vietti R., Bava S., Morganti C., Morri C., Picco P., Sara G., Schiaparelli S., Siccardi A., Sponga F. (2000). A catastrophic mass-mortality episode of

gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecology Letters 3, 284-293.
Cerrano C., Bavestrello G., Bianchi C. N., Calcinai B., Cattaneo-Vietti R., Morri C., Sarà M. (2001). The role of sponge bioerosion in Mediterranean coralligenous accretion. In: Faranda F.M., Guglielmo L., Spezie G. (Eds.), Mediterranean ecosystems: Structures and process (pp. 235-240). Milan, Italy: Springer-Verlag.
Cerrano C., Bertolotto R., Coppo S., Palma M., Pantaleo U., Valisano L., Bavestrello G., Ponti M. (2014). Assessment of coralligenous assemblages status in the Ligurian Sea. In: Bouafif C., Langar H., Ouerghi A. (Eds.), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29-30 October 2014 (pp. 61-65). UNEP/MAP–RAC/SPA: Tunis, Tunisia.
Cocito S. (2004). Bioconstruction and biodiversity: their mutual influence. Scientia Marina 68, 137-144.
Colloca F., Cardinale M., Belluscio A., Ardizzone G. (2003). Patternof distribution and diversity of demersal assemblages in the central Mediterranean sea. Estuarine, Coastal and Shelf Science 56, 469-480.
Coma R., Pola E., Ribes M., Zabala M. (2004). Long-term assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecological Applications 14, 1466-1478.
Commissione Europea (1979). Direttiva 79/409/CEE del Consiglio del 2 Aprile 1979 concernente la conservazione degli uccelli selvatici. Gazzetta Ufficiale dell'Unione Europea, L103, 1-27.
Commissione Europea (2000). Direttiva 2000/60/CE del Parlamento europeo e del Consiglio, del 23 ottobre 2000, che istituisce un quadro per l'azione comunitaria in materia di acque. Gazzetta Ufficiale delle Comunità Europee, L327, 1-72.
Commissione Europea (2008). Direttiva 2008/56/CE del Parlamento Europeo e del Consiglio del 17 Giugno 2008 che istitutisce un quadro per l'azione comunitaria nel campo della politica per l'ambiente marino (Direttiva Quadro sulla Strategia per l'ambiente Marino). Gazzetta Ufficiale dell'Unione Europea, L164, 19-40.
Commissione Europea (2019). Decisione di Esecuzione (UE) 2019/22 della Commissione del 14 Dicembre 2018 che adotta il dodicesimo aggiornamento dell'elenco dei Siti di Importanza Comunitaria per la regione biogeografica Mediterranea. Gazzetta Ufficiale dell'Unione Europea, L7, 522-611.
Consiglio dell'Unione Europea (1977). Decisione del Consiglio 77/585/CEE del 25 Luglio 1977 relativa alla conclusione della convenzione per la protezione del Mare Mediterraneo dall'inquinamento e del protocollo sulla prevenzione dell'inquinamento del Mare Mediterraneo dovuto allo scarico di rifiuti da parte di navi e di aeromobili. Gazzetta Ufficiale delle Comunità Europee, L240, 1-44.
Consiglio dell'Unione Europea (1992). Direttiva del Consiglio 92/43/CEE del 21 Maggio1992 sulla conservazione degli habitat naturali e della fauna e flora selvatica. Gazzetta Ufficiale dell'Unione Europea, L 206, 7-50.
De la Nuez-Hernández D., Valle C., Forcada A., González Correa J.M., Fernández Torquemada Y. (2014). Assessing the erect bryozoan *Myriapora truncata* (Pallas, 1766) as indicator of recreational diving impact on coralligenous reef communities. Ecological Indicators 46, 193-200.
Deter J., Descamp P., Boissery P., Ballesta L., Holon F. (2012). A rapid photographic method detects depth gradient in coralligenous assemblages. Journal of Experimental Marine Biology and Ecology 418-419, 75-82.
Dethier M.N., Graham E.S., Cohen S., Tear L.M. (1993). Visual versus random-point percent cover estimations: 'Objective' is not always better. Marine Ecology Progress Series 110, 9-18.
Di Geronimo I., Di Geronimo R., Rosso A., Sanfilippo R. (2001). Structural and taphonomic analysis of a columnar coralline algal build-up from SE Sicily. Geobios Mémorie Spécial 35, 86-95.
Hong J.S. (1983). Impact of pollution on the benthic community. Environmental impact of the pollution on the benthic coralligenous community in the Gulf of Fos, north-western Mediterranean. Bulletin of Korean Fishery Society 16, 273-290.
Enrichetti F., Bo M., Morri C., Montefalcone M., Toma M., Bavestrello G., Tunesi L., Canese S., Giusti M., Salvati E., Bianchi C.N. (2019). Criteria to assess the environmental status of temperate mesophotic reefs. Ecological Indicators 102, 218-229.
EUNIS, 2019. EUNIS Habitat Classification. European Environment Agency. https://www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification

Falace A., Kaleb S., Curiel D., Miotti C., Galli G., Querin S., … Bandelj V. (2015). Calcareous bio-concretions in the northern Adriatic Sea: Habitat types, environmental factors that influence habitat distributions, and predictive modeling. PLoS ONE 10, e0140931.

Ferdeghini F., Acunto S., Cocito S., Cinelli F. (2000). Variability at different spatial scales of a coralligenous assemblage at Giannutri Island (Tuscan Archipelago, northwestern Mediterranean). Hydrobiologia 440, 27-36.

Ferrigno F., Apolloni L., Russo G.F., Sandulli R. (2018). Impact of fishing activities on different coralligenous assemblages of Gulf of Naples (Italy). Journal of the Marine Biological Association of the United Kingdom 98 (1), 41-50.

Flemming N.C., Max M.D. (1996). Scientific Diving: A General Code of Practice. Unesco, 2nd edition. Scientific Committee of the Confederation Mondiale des Activites Subaquatiques (CMAS).

Garrabou J., Ballesteros E. (2000). Growth of *Mesophyllum alternans* and *Lithophyllum frondosum* (Corallinales, Rhodophyta) in the northwestern Mediterranean. European Journal of Phycology 35, 1-10.

Garrabou J., Sala E., Arcas A., Zabala M. (1998). The impact of diving on rocky sub-littoral communities: a case study of a bryozoan population. Conservation Biology 12 (2), 302-312.

Garrabou J., Perez T., Sartoretto S., Harmelin J.G. (2001). Mass mortality event in red coral *Corallium rubrum* populations in Provence region (France, NW Mediterranean). Marine Ecology Progress Series 217, 263-272.

Garrabou J., Coma R., Bensoussan N., Bally M., Chevaldonné P., Cigliano M., Diaz D., Harmelin J.G., Gambi M.C., Kersting D.K., Ledoux B., Lejeusne C., Linares C., Marschal C., Perez T., Ribes M., Romano J.C., Serrano E., Teixidó N., Torrents O., Zabala M., Zuberer F., Cerrano C. (2009). Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biology 15, 1090-1103.

Garrabou J., Kipson S., Kaleb S., Kruzic P., Jaklin A., Zuljevic A., Rajkovic Z., Rodic P., Jelic K., Zupan D. (2014): Monitoring Protocol for Reefs - Coralligenous Community, MedMPAnet Project

Gatti G., Montefalcone M., Rovere A., Parravicini V., Morri C., Albertelli G., Bianchi C.N. (2012). Seafloor integrity down the harbor waterfront: the coralligenous shoals off Vado Ligure (NW Mediterranean). Advances in Oceanography and Limnology 3, 51-67.

Gatti G., Bianchi C.N., Morri C., Montefalcone M., Santoretto S. (2015a). Coralligenous reefs state along anthropized coasts: application and validation of the COARSE index, based on a rapid visual assessment (RVA) approach. Ecological Indicators 52, 567-576.

Gatti G., Bianchi C.N., Parravicini V., Rovere A., Peirano A., Montefalcone M., Massa F., Morri C. (2015b). Ecological change, sliding baselines and the importance of historical data: lessons from combining observational and quantitative data on a temperate reef over 70 years. PLoS ONE, 10, e0118581

Gatti G., Bianchi C.N., Montefalcone M., Venturini S., Diviacco G., Morri C. (2017). Observational information on a temperate reef community helps understanding the marine climate and ecosystem shift of the 1980-90s. Marine Pollution Bulletin 114, 528-538.

Gennaro P., Piazzi L. (2011). Synergism between two anthropic impacts: invasion of macroalga *Caulerpa racemosa* var. *cylindracea* and seawater nutrient enrichment. Marine Ecology Progress Series 427, 59-70.

Giaccone G. (2007). Il coralligeno come paesaggio marino sommerso: distribuzione sulle coste italiane. Biologia Marina Mediterranea 14 (2), 126-143.

Giuliani S., Lamberti C.V., Sonni C., Pellegrini D. (2005). Mucilage impact on gorgonians in the Tyrrhenian sea. Science of the Total Environment, 353, 340-349.

Gubbay S., Sanders N., Haynes T., Janssen J. A. M., Rodwell J. R., Nieto A., … Calix M. (2016). European Red List of habitats. Part 1. Marine habitats. Luxembourg City, Luxembourg: European Union Publications Office.

Hong J.S. (1980). Étude faunistique d’un fond de concrétionnement de type coralligène soumis à un gradient de pollution en Méditerranée nord-occidentale (Golfe de Fos). Thèse de Doctorat. Université d’Aix-Marseille II.

Hong J.S. (1982). Contribution à l’étude des assemblages d’un fond coralligène dans la région marseillaise en Méditerranée Nord-Occidentale. Bulletin of Korea Ocean Research and Development Institute 4, 27-51.

IMAP (2016). Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria UNEP/MAP Athens, Greece 2016.

IUCN (2016). Guidelines for the application of IUCN Red List of Ecosystems Categories and Criteria. Version 1.0. In: Bland L.M., Keith D.A., Murray N.J., Miller R., Rodriguez, J.P. (Eds.). International Union for Conservation of Nature (IUCN), Gland, Switzerland. ix + 93pp.

Joiner J.T. (2001). NOAA diving manual: Diving for science and technology. Forth Edition. National Oceanic and Atmospheric Administration, Office of Oceanic and Atmospheric Research, National Undersea Research Program, Office of Marine and Aviation Operations, NOAA Diving Program.

Kipson S., Novosel M., Radić I., Kružić P., Požardomac A. (2009). The biodiversity of macrobenthos within the coralligenous community dominated by the red gorgonian *Paramuricea clavata* in the central part of the eastern Adriatic Sea (Croatia): Preliminary results. In: Pergent-Martini C., Brichet M. (Eds.), Proceedings of the first symposium on the coralligenous and other calcareous bio-concretions of the Mediterranean Sea Tabarka, Tunis, 15-16 January 2009 (pp. 211-213). UNEP/MAP-RAC/SPA: Tunis, Tunisia.

Kipson S., Fourt M., Teixidó N., Cebrian E., Casas E., Ballesteros E., Zabala M., Garrabou J. (2011). Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: a case study of Mediterranean coralligenous outcrops. PLoS ONE 6, e27103.

Kipson S., Kaleb S., Kružić P., Rajković Ž., Žuljević A., Jaklin A., Krstinic P., Zupan D., Garrabou J. (2014). Croatian coralligenous monitoring protocol: The basic methodological approach. In: Bouafif C., Langar H., Ouerghi A. (Eds.), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29-30 October 2014 (pp. 95-99). UNEP/MAP-RAC/SPA: Tunis, Tunisia.

Klein J., Verlaque M. (2009). Macrophyte assemblage associated with an invasive species exhibiting temporal variability in its development pattern. Hydrobiologia 636, 369-378.

La Mesa G., Paglialonga A., Tunesi L. (ed.), 2019. Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE e Direttiva 09/147/CE) in Italia: ambiente marino. ISPRA, serie Manuali e Linee Guida, 190/2019.

Laborel J. (1987). Marine biogenic constructions in the Mediterranean. Scientific Reports of Port-Cros National Park 13, 97-126.

Linares C., Zabala M., Garrabou J., Coma R., Díaz D., Hereu B., Dantart L. (2010). Assessing the impact of diving in coralligenous communities: the usefulness of demographic studies of red gorgonian populations. Scientific Reports of the Port-Cros National Park 24, 161-184.

Lombardi C., Rodolfo-Metalpa R., Cocito S., Gambi M.C., Taylor P.D. (2011). Structural and geochemical alterations in the Mg calcite bryozoan Myriapora truncata under elevated seawater pCO_2 simulating ocean acidification. Marine Ecology 32 (2), 211-221.

Martin S., Gattuso J. P. (2009). Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Global Change Biology 15, 2089-2100.

Martinez-Crego B., Alcoverro T., Romero J. (2010). Biotic indices for assessing the status of coastal waters: A review of strengths and weaknesses. Journal of Environmental Monitoring 12, 1013–1028.

MATTM-ISPRA (2020) Programmi di Monitoraggio per la Strategia Marina. Art.11, D.lgs. 190/2010. Schede Metodologiche Modulo 7 - Habitat coralligeno. Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Istituto Superiore per la Protezione dell'Ambiente.

Meinesz A., Hesse B. (1991). Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Méditerrané nord-occidentale. Oceanologica Acta 14, 415-426.

Mojetta A., Ghisotti A. (1994). Flora e fauna del Mediterraneo. A. Mondadori (Eds.)

Montefalcone M., Parravicini V., Bianchi C.N. (2011). Quantification of coastal ecosystem resilience. In: Wolanski E., McLusky D.S. (Eds.), Treatise on Estuarine and Coastal Science. Waltham, Academic Press 10 (3), pp. 49-70.

Montefalcone M., Morri C., Bianchi C.N., Bavestrello G., Piazzi L. (2017). The two facets of species sensitivity: stress and disturbance on coralligenous assemblages in space and time. Marine Pollution Bulletin 117, 229-238.

Mordecai G., Tyler P.A., Masson D.G., Huvenne V.A.I. (2011) Litterin submarine canyons off the west coast of Portugal. Deep-SeaResearch Part II: Topical Studies in Oceanography 58, 2489-249

Notari F., Fossati P. (2015). Meraviglie del Mediterraneo. Guida al riconoscimento degli organismi marini. Magenes (Eds.).

Paoli C., Morten A., Bianchi C. N., Morri C., Fabiano M., Vassallo P. (2016). Capturing ecological complexity: OCI, a novel combination of ecological indices as applied to benthic marine habitats. Ecological Indicators 66, 86-102.

Parravicini V., Micheli F., Montefalcone M., Villa E., Morri C., Bianchi C.N. (2010). Rapid assessment of benthic communities: a comparison between two visual sampling techniques. Journal of Experimental Marine Biology and Ecology 395, 21-29.

Patzner R. 1998. The invasion of *Lophocladia* (Rhodomelaceae, Lophotaliae) at the northern coast of Ibiza (Western Mediterranean Sea). Bolletí de la Societat d 'Historia Natural de les Balears 41, 75-80.

Penna M., Gennaro P., Bacci T., Trabucco B., Cecchi E., Mancusi C., Piazzi L., Rende F.S., Serena F., Cicero A.M. (2017). Multiple environmental descriptors to assess ecological status of sensitive habitats in the area affected by the Costa Concordia shipwreck (Giglio Island, Italy). Journal of Marine Biology Association of United Kingdom 98, 51-59

Pérès J. M., Picard J. (1951). Nouvelle carte des fonds du Golfe de Marseille. Vie et Milieu, 7 p. avec carte.

Pérès J., Picard J.M. (1964). Nouveau manuel de bionomie benthique de la mer Méditerrané. Recueil des Travaux de la Station Marine d'Endoume 31(47), 1-131.

Pérez T., Garrabou J., Sartoretto S., Harmelin J.G., Francour P., Vacelet J. (2000). Mass mortality of marine invertebrates: an unprecedented event in the Northwestern Mediterranean. Comptes Rendus dé l'Académie des Scinces-Serie III Sciences de la Vie 323, 853-865.

Piazzi L., Balata D. (2008). The spread of *Caulerpa racemosa* var. *cylindracea* in the Mediterranean Sea: An example of how biological invasions can influence beta diversity. Marine Environmental Research 65, 50-61.

Piazzi L., Balata D. (2009). Invasion of alien macroalgae in different Mediterranean habitats. Biological Invasion 11, 193-204.

Piazzi L., Balata D. (2011). Coralligenous habitat: patterns of vertical distribution of 22 macroalgal assemblages. Scientia Marina 75, 399-406.

Piazzi L., Ceccherelli G. (2006). Persistence of biological invasion effects: recovery of macroalgal assemblages after removal of *Caulerpa racemosa* var. *cylindracea*. Estuarine Coastal and Shelf Science 68, 455-461.

Piazzi L., Balata D., Pertusati M., Cinelli F. (2004). Spatial and temporal variability of Mediterranean macroalgal coralligenous assemblages in relation to habitat and substrate inclination. Botanica Marina 47, 105-115.

Piazzi L., Balata D., Cinelli F. (2007a). Invasions of alien macroalgae in Mediterranean coralligenous assemblages. Cryptogamie Algologie 28, 289-301.

Piazzi L., Balata D., Foresi L., Cristaudo C., Cinelli F. (2007b). Sediment as a constituent of Mediterranean benthic communities dominated by *Caulerpa racemosa* var *cylindracea*. Scientia Marina 71, 129-135.

Piazzi L., Gennaro P., Balata D. (2011). Effects of nutrient enrichment on macroalgal coralligenous assemblages. Marine Pollution Bulletin 62, 1830-1835.

Piazzi L., Gennaro P., Balata D. (2012). Threats to macroalgal coralligenous assemblages in the Mediterranean Sea. Marine Pollution Bulletin 64, 2623-2629

Piazzi L., Balata D., Cecchi E., Gennaro P., Serena F. (2014a). Effectiveness of different investigation procedures in detecting anthropogenic impacts on coralligenous assemblages. Scientia Marina 78(3), 319-328.

Piazzi L., Cecchi E., Serena F., Guala I., Cánovas Molina A., Gatti G., Morri C., Bianchi C.N., Montefalcone M. (2014b). Visual vs photographic methods to estimate the quality of coralligenous reefs under different human pressures. In: Bouafif C., Langar H., Ouerghi A. (Editors), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29-30 October 2014 (pp135-140). UNEP/MAP-RAC/SPA: Tunis, Tunisia.

Piazzi L., Gennaro P., Cecchi E., Serena F. (2015). Improvement of the ESCA index for the evaluation of ecological quality of coralligenous habitat under the European Framework Directives. Mediterranean Marine Science 16, 419-426.

Piazzi L., La Manna G., Cecchi E., Serena F., Ceccherelli G. (2016). Protection changes the relevancy of scales of variability in coralligenous assemblages. Estuarine Coastal and Shelf Sciences 175, 62-69.

Piazzi L., Bianchi C.N., Cecchi E., Gatti G., Guala I., Morri C., Stéfane Sartoretto, Fabrizio Serena, Montefalcone M. (2017a). What's in an index? Comparing the ecological information provided by two indices to assess the status of coralligenous reefs in the NW Mediterranean Sea. Aquatic Conservation: Marine and Freshwater Ecosystems 27, 1091-1100.

Piazzi L., Gennaro P., Cecchi E., Serena F., Bianchi C.N., Morri C., Montefalcone M. (2017b). Integration of ESCA index through the use of sessile invertebrates. Scientia Marina 81, 283-290

Piazzi L., Atzori F., Cadoni N., Cinti M.F., Frau F., Ceccherelli G. (2018a). Benthic mucilage blooms threaten coralligenous reefs. Marine Environmental Research 140, 145-151.

Piazzi L., Bianchi C.N., Cecchi E., Gennaro P., Marino G., Montefalcone M., Morri C., Serena F. (2018b). Il coralligeno toscano: distribuzione, struttura dei popolamenti e monitoraggio mediante utilizzo di diversi indici di qualità ecologica. Proceedings and Reports of the Seventh International Symposium "Monitoring of Mediterranean coastal areas: problems and and measurements techniques", Livorno, Italy, June 19-21 2018 (Eds.). Fabrizio Benincasa, Firenze University Press, pp. 311-316.

Piazzi L., Cecchi E., Cinti M.F., Stipcich P., Ceccherelli G. (2019a). Impact assessment of fish cages on coralligenous reefs: an opportunity to use the STAR sampling procedure. Mediterranean Marine Science 20(3), 627-635.

Piazzi L., Gennaro P., Montefalcone M., Bianchi C.N., Cecchi E., Morri C., Serena F. (2019b). STAR: an integrated and standardized procedure to evaluate the ecological status of coralligenous reefs. Aquatic Conservation: Marine and Freshwater Ecosystems 29, 189-201.

Piazzi L., Gennaro P., Cecchi E., Bianchi C.N., Cinti M.F., Gatti G., Guala I., Morri C., Sartoretto S., Serena F., Monteflacone M. (2021). Ecological Status of Coralligenous Assemblages: ten years of application of the ESCA index from local to wide scale validation. Ecological Indicators 121, 107077.

Ponti M., Falace A., Rindi F., Fava F., Kaleb S., Abbiati M. (2014). Beta diversity patterns in northern Adriatic coralligenous outcrops. In: C. Bouafif, H. Langar, Ouerghi A. (Eds.), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29-30 October 2014 (pp. 147-152). UNEP/MAP-RAC/SPA: Tunis, Tunisia.

Rodolfo-Metalpa R., Ferrier-Pagés C. (2009). Physiological responses of Mediterranean corals to temperature and pH perturbations. Comparative Biochemistry and Physiology, Part A, 2 (153), S168.

Rodriguez-Prieto C., Ballesteros E., Bosset F., Alfonso-Carrillo J. (2015). Alghe e fanerogame del Mediterraneo. Il Castello (Eds.)

Ros J., Romero J., Ballesteros E., Gili J.M. (1985). Diving in blue water: the benthos. In: Margalef R. (Eds.), Western Mediterranean. Oxford, Pergamon, pp. 233-295.

Ruitton S., Personnic S., Ballesteros E., Bellan-Santini D., Boudouresque C. F., Chevaldonné P., Bianchi C.N., David R., Feral J.P., Guidetti P., Harmelin J.G., Montefalcone M., Morri C., Pergent G., Pergent-Martini C., Sartoretto S., Tanoue H., Thibaut T., Vacelet J.,Verlaque M. (2014). An ecosystem-based approach to evaluate the status of the Mediterranean coralligenous habitat. In: Bouafif C., Langar H., Ouerghi A. (Eds.), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29–30 October 2014 (pp. 153-158). UNEP/MAP–RAC/SPA: Tunis, Tunisia.

Sala E., Garrabou J., Zabala M. (1996). Effects of diver frequentation on marine sub-littoral population of the bryozoan *Pentapora fascialis*. Marine Biology 126, 451-459.

Sartoretto S. (1996). Vitesse de croissance et bioérosion des concrétionnements "coralligènes" de Méditerranée nord-occidentale. Rapport avec les variations Holocènes du niveau marin. Thèse Doctorat d'Ecologie, Universitè dAix-Marseille, II.

Sartoretto S., Francour P. (1997). Quantification of bioerosion by *Sphaerechinus granularis* on "coralligène" concretions of the western Mediterranean. Journal of the Marine Biological Association of the United Kingdom 77, 565-568.

Sartoretto S., David R., Aurelle D., Chenuil A., Guillemain D., Thierry De Ville D'Avray L., Féral J.P., Cinar M.E., Kipson S., Arvanitidis C., Schohn T., Daniel B., Sakher S., Garrabou J., Gatti G., Ballesteros E. (2014). An integrated approach to evaluate and monitor the conservation state of coralligenous bottoms: The INDEX-COR method. In: Bouafif C., Langar H., Ouerghi A. (Eds.), Proceedings of the second Mediterranean symposium on the conservation of coralligenous and other calcareous bio-concretions, Portorož, Slovenia, 29-30 October 2014 (pp. 159-165). UNEP/MAP-RAC/SPA: Tunis, Tunisia.

Sartoretto S., Schohn T., Bianchi C. N., Morri C., Garrabou J., Ballesteros E., … Gatti G. (2017). An integrated method to evaluate and monitor the conservation state of coralligenous habitats: the INDEX-COR approach. Marine Pollution Bulletin, 120, 222-231.

Schiaparelli S., Castellano M., Povero P., Sartoni G., Cattaneo-Vietti R. (2007). A benthic mucilage event in North-Western Mediterranean Sea and its possible relationships with the summer 2003 European heatwave: short term effects on littoral rocky assemblages. Marine Ecology 28, 1–13.

Simkiss K. (1964). Phosphates as crystal poisons of calcification. In: Kangwe J.W. (1999). Effects of Land Based pollution on Reef Building Calcareous algae in the Reefs near Zanzibar town. Master of Science Thesis, University of Dar Es Salaam, Tanzania, pp. 97.

SPA/RAC–UN Environment/MAP, 2019. Updated Classification of Benthic Marine Habitat Types for the Mediterranean Region. UNEP/MAP-SPA/RAC publ., Tunis, 23 pp.

Teixidó N., Casas E., Cebrian E., Linares C., Garrabou J. (2013). Impact on coralligenous outcrop biodiversity of a dramatic coastal storm. PLoS ONE 8, e53742.

Trainito E. (2004). Atlante di flora & fauna del Mediterraneo. Il Castello (Eds.).

Trainito E., Baldaconi R. (2013). Spugne del Mediterraneo. Il Castello (Eds.).

Trainito E., Baldaconi R. (2016). Coralli del Mediterraneo. Il Castello (Eds.).

Trygonis V., Sini, M. (2012). photoQuad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. Journal of Experimental Marine Biology and Ecology 424/425, 99-108.

True M.A. 1970. Étude quantitative de quatre peuplements sciaphiles sur substrat rocheux dans la région marsellaise. Bulletin de l'Institut Océanographique (Monaco) 69 (1401), 1-48.

UNEP/MAP-RAC/SPA (2008). Action Plan for the Conservation of the Coralligenous and Other Calcareous Bio-concretions in the Mediterranean Sea. RAC/SPA publ., Tunis, 21 p.

UNEP/MAP-RAC/SPA (2014). Monitoring Protocol for Reefs - Coralligenous Community. By Garrabou J., Kipson S., Kaleb S., Kruzic P., Jaklin A., Zuljevic A., Rajkovic Z., Rodic P., Jelic K., Zupan D. (Eds.) RAC/SPA - MedMPAnet Project, Tunis. 35 pages + annexes.

UNEP/MAP-RAC/SPA (2017). Action Plan for the Conservation of the Coralligenous and Other Calcareous Bio-concretions in the Mediterranean Sea. Athens, Greece: UNEP/MAP. http://www.rac-spa. org/sites/default/files/action_plans/pa_coral_en.pdf

UNEP/MED (2019). Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species, which includes the Guidelines for monitoring marine benthic habitats in the Mediterranean (WG.467/16). UNEP/MAP Athens, 301 pp.

Virgilio M., Airoldi L., Abbiati M. (2006). Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. Coral Reefs 25, 265-272.

Watters D.L., Yoklavich M.M., Love M.S., Schroeder D.M. (2010). Assessing marine debris in deep seafloor habitats off California. Marine Pollution Bulletin 60, 131-138

Zabala M. (1986). Fauna dels briozous dels Països Catalans. Arxius Secció Ciències 84, 1-833.

ANNEX A

Tables for calculation of the ecological quality indices

Table 1 – ESCA index: Sensitivity Level (SL) values of the main morphological taxa/groups [(1)]

TAXA/GROUPS	SL
Algal turf (e.g. *Lophosiphonia* spp., *Sphacelaria* spp.)	1
Hydrozoans (e.g. *Sertularella* spp., *Eudendrium* spp.)	2
Siphonal/siphonocladal Chlorophyta with separates filaments (e.g. *Pseudochlorodesmis* spp.; *Cladophora* spp.)	2
Perforating sponges (e.g. *Cliona* spp.)	2
Dictyotales (e.g. *Dictyota* spp., *Dictyopteris* spp.)	3
Encrusting sponges (e.g. *Phorbas* spp., *Spirastrella cunctatrix*)	3
Encrusting bryozoans (e.g. *Schizoporella* spp., *Schizomavella* spp.)	3
Encrusting ascidians (also epibionts) (e.g. *Diplosoma* spp., *Botryllus* spp.)	3
Encrusting calcified Rhodophyta (e.g. *Mesophyllum* spp., *Neogoniolithon* spp.)	4
Articulated calcified Rhodophyta (e.g. *Amphiroa* spp., *Tricleocarpa fragilis*)	4
Peyssonnelia spp.	4
Siphonal Chlorophyta with vesicle thallus (*Valonia* spp., *Codium* spp.)	4
Prostrate/hemispherical sponges (e.g. *Chondrosia reniformis*; *Agelas oroides*)	5
Large serpulids (e.g. *Protula intestinum*, *Serpula vermicularis*)	5
Parazoanthus axinellae	5
Leptogorgia sarmentosa	5
Flabellia petiolata	6
Erect cylindrical Ochrophyta (e.g. *Halopteris* spp., *Sporochnus* spp.)	6
Encrusting Ochrophyta (e.g. *Aglaozonia* spp., *Zanardinia typus*)	6
Azooxantellate scleractinians (e.g. *Leptopsammia pruvoti*, *Phyllangia americana mouchezii*)	6
Ramified bryozoans (e.g. *Cellaria fistulosa*, *Caberea boryi*)	6
Palmophyllum crassum	7
Arborescent/massive sponges (e.g. *Axinella polypoides*; *Sarcotragus* spp.)	7
Salmacina-Filograna complex	7
Myriapora truncata	7
Turbicellepora avicularis	7
Erect cylindrical Rodophyta (e.g. *Botryocladia* spp., *Osmundea pelagosae*)	8
Bushy sponges (e.g. *Aplysina* spp., *Axinella damicornis*)	8
Eunicella verrucosa	8
Alcyonium acaule	8
Erect ascidians (e.g. *Halocynthia papillosa*)	8
Corallium rubrum	9
Paramuricea clavata	9
Alcyonium coralloides	9
Zooxantellate scleractinians (e.g. *Cladocora caespitosa*, *Balanophyllia europaea)*	9
Pentapora fascialis	9
Reteporella grimaldii	9
Erect flattened Rhodophyta (e.g. *Kallymenia* spp., *Phyllophora* spp.)	10
Halimeda tuna	10
Erect flattened Ochrophyta (e.g. *Laminaria* spp., *Phyllariopsis* spp.)	10
Fucales (e.g. *Cystoseira* spp., *Sargassum* spp.)	10
Eunicella cavolini	10
Eunicella singularis	10
Savalia savaglia	10
Adeonella calveti, *Smittina cervicornis*	10

Note:
[(1)] Adapted from Piazzi et al. 2017b (modified). Only some of the examples from Sheet 2 are reported for each taxon/group

Table 2 – COARSE index: scores assignment for descriptors of the three coralligenous layers [(1)]

BASAL LAYER	
1) % cover of benthic categories (BCs) The formula *(cover × score)/100* is applied to each group and results are summed to obtain the final score	1: TURF/SED 2: NCEA, EA 3: ECR
2) Thickness/consistency of calcareus matrix (PEN) By averaging the value of the 6 penetration measures	1: null penetration 2: penetration > 1 cm 3: penetration up to 1 cm
3) Perforators frequency (PER)	1: common = n° borer marks > 2 2: occasional = n° borer marks < 2 3: absent
INTERMEDIATE LAYER	
1) Species richness (S)	1: S < 5 2: 5 ≤ S ≤ 8 3: S > 8
2) Number of erect calcified organisms (ECO) (madreporans, bryozoans, etc.)	1: ECO ≤1 2: 1 < ECO ≤ 3 3: ECO > 3
3) Sensitive bryozoans (SB)	1: *Myriapora truncata*, *Turbicellepora avicularis* 2: *Pentapora fascialis*, *Reteporella grimaldii* 3: *Adeonella calveti*, *Smittina cervicornis*
UPPER LAYER	
1) Total cover of taxa/groups (%)	1: cover < 5% 2: 5% ≤ cover ≤ 25% 3: cover > 25%
2) Maximum height (MH) LMH = maximum height reported in literature for each species	1: MH < 0.3 LMH 2: 0.3 LMH ≤ MH ≤ 0.6 LMH 3: MH > 0.6 LMH
3) Necrosis (N)	1: N > 75% 2: 10% ≤ N ≤ 75% 3: N < 10%

Note:
[(1)] Adapted from Gatti et al. 2012, 2015a (modified)

Table 3 – ISLA index: Integrated Sensitivity Level (ISL) values of the main morphological taxa/groups(1) DSL = Disturbed Sensitivity Level; SSL = Stress Sensitivity Level; nd = not determined

TAXA/GROUPS	DSL	SSL	ISL
Alien species (e.g. *Caulerpa cylindracea*, *Asparagopsis* spp.)	nd	nd	-1
Algal turf (e.g. *Lophosiphonia* spp., *Sphacelaria* spp.)	6	1	0
Small hydroids (e.g. *Sertularella* spp.)	7	1	0
Siphonal/siphonocladal Chlorophyta with separate filaments (e.g. *Pseudochlorodesmis* spp.; *Cladophora* spp.)	8	1	1
Siphonal Chlorophyta with vesicle thallus (*Valonia* spp., *Codium* spp.)	8	2	1
Encrusting sponges (e.g. *Phorbas* spp., *Spirarstrella cunctatrix*)	8	3	1
Dictyotales (e.g. *Dictyota* spp., *Dictyopteris* spp.)	8	3	2
Encrusting calcified Rhodophyta (e.g. *Mesophyllum* spp., *Neogoniolithon* spp.)	8	4	2
Articulated calcified Rhodophyta (e.g. *Amphiroa* spp., *Tricleocarpa fragilis*)	9	4	3
Encrusting Ochrophyta (e.g. *Aglaozonia* spp., *Zanardinia typus*)	6	6	2
Peyssonnelia spp.	8	4	2
Perforating sponges (e.g. *Cliona* spp.)	9	2	2
Large hydroids (e.g. *Eudendrium* spp.)	11	1	2
Encrusting bryozoans (e.g. *Schizoporella* spp., *Schizomavella* spp.)	11	2	2
Encrusting ascidians (also epibionts) (e.g. *Diplosoma* spp., *Botryllus* spp.)	10	2	2
Erect cylindrical Ochrophyta (e.g. *Halopteris* spp., *Sporochnus* spp.)	9	6	3
Flabellia petiolata	8	6	3
Palmophyllum crassum	7	8	3
Erect cylindrical Rodophyta (e.g. *Botriocladia* spp., *Osmundea pelagosae*)	9	9	4
Macroforaminifera (e.g. *Miniacina miniacea*)	11	6	4
Prostrate/hemyspherical sponges (e.g. *Chondrosia reniformis; Agelas oroides*)	12	4	4
Parazoanthus axinellae	12	4	4
Stolonifera (e.g. *Cornularia cornucopiae*)	12	6	4
Erect flattened Rhodophyta (e.g. *Kallimenia* spp., *Phyllophora* spp.)	9	10	5
Halimeda tuna	9	10	5
Erect flattened Ochrophyta (e.g. *Laminaria* spp., *Phyllariopsis* spp.)	10	10	5
Bushy sponges (e.g. *Aplysina* spp., *Axinella damicornis*)	13	7	5
Leptogorgia sarmentosa	16	4	5
Azooxantellate solitary scleractinians (e.g. *Leptopsammia pruvoti*)	15	4	5
Bivalve molluscs (e.g. *Lithophaga lithophaga*, *Arca barbata*)	15	5	5
Large serpulids (e.g. *Protula intestinum*, *Serpula vermicularis*)	14	5	5
Salmacina-Filograna complex	13	6	5
Ramified bryozoans (e.g. *Cellaria fistulosa*, *Caberea boryi*)	14	5	5
Fucales (e.g. *Cystoseira* spp., *Sargassum* spp.)	10	11	6
Arborescent/massive sponges (e.g. *Axinella polypoides*; *Sarcotragus* spp.)	16	6	6
Actinians (e.g. *Cribrinopsis crassa*)	15	7	6
Eunicella cavolini	16	7	6
Azooxantellate colonial scleractinians (e.g. *Phyllangia americana mouchezi*)	16	5	6
Vermetids (e.g. *Thylacodes arenarius*)	16	5	6
Erect ascidians (e.g. *Halocynthia papillosa*)	15	7	6
Alcyonium acaule	16	8	7
Alcyonium coralloides	16	9	7
Corallium rubrum	17	8	7
Eunicella verrucosa	16	7	7
Paramuricea clavata	16	8	7

Note:

(1) Adapted from Montefalcone et al. 2017 (modified). Only some of the examples from Sheet 2 are reported for each taxon/group

Zooxantellate solitary scleractinians (e.g. *Balanophyllia europaea*)	15	9	7
Myriapora truncata	17	6	7
Turbicellepora avicularis	17	6	7
Pentapora fascialis	17	8	7
Reteporella grimaldii	17	8	7
Savalia savaglia	16	11	8
Zooxantellate colonial scleractinians (e.g. *Cladocora caespitosa*)	17	9	8
Eunicella singularis	16	12	9
Adeonella calveti, *Smittina cervicornis*	17	12	9

MANUALI E
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