ECOLOGICAL STATUS AND TRAJECTORIES OF CHANGE IN TEMPERATE AND TROPICAL REEFS

PhD Thesis: Annalisa Azzola

Academic advisor: Monica Montefalcone

University of Genoa
Department of Earth, Environment and Life Sciences

Abstract

Amongst marine temperate and tropical ecosystems, Mediterranean rocky reefs and Maldivian coral reefs are the most productive and diverse, providing essential ecosystem services. Unfortunately, due to several co-occurring local and global pressures, they are ranked as the most threatened marine habitat. The present work attempts to analyse their ecological status and change over time in a global warming scenario. With this purpose, different methodological approaches have been adopted. Many studies have been conducted in Marine Protected Areas (MPAs), highlighting that the constant updating of species inventory and surveillance is still necessary, also after their establishment. Monitoring of target species is a valuable tool in assessing ecological status of the environment they live in. Tackling the case of a species in need of protection confirmed how detailed information on species distribution is mandatory to develop effective environmental management plans. Non-destructive techniques are to be preferred when studying and monitoring target species and valuable habitats. By comparing data collected from different observers, visual methods have proven reliable and effective in the characterization of habitat and evaluation of change over time. By applying standardized protocols and monitoring water temperature, mass mortality event and target species this work unveiled effects of climate change in all study areas, regardless of different latitude and level of protection. Due to the climate change marine ecosystems are increasingly exposed to the cumulative effects of different pressures. Analysis of short-term change in a benthic community showed that the cumulative effect of multiple stressors is stronger than that of any individual stressor, no matter how intense it is. As a result of local and global human pressures, marine ecosystems have undergone important changes that can only be detected by analysing long-term data series or revisiting sites already surveyed. By assessing change over time at three different level of complexity (i.e., population, community, and ecosystems), this work addresses the issue of decreasing abundance of native species in correspondence with increasing number of invasive species. The findings confirmed that in a given reef, a turnover in species composition determine a change in community structure. Biodiversity provides a buffer to maintain ecosystem functioning in the face of environmental changes, and decrease in functional redundancy, due to the community change, demonstrated reduced ecosystem resilience. Global warming, and the consequent changes in marine ecosystems, are occurring at an unprecedented rate and on a worldwide scale. In this context, a cooperative effort between research and international environmental policy is urgently needed.

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GENERAL INTRODUCTION

Human behaviours are impacting the planet as never before in the history of Earth to the point that the current geological era is referred to as the Anthropocene (Crutzen, 2006). To provide the goods and services needed each year, humanity is using resources equivalent to those of 1.7 planets. This trend is no longer sustainable and environmental protection policies have become urgent (Mega, 2019).

Most of the debate on conservation has historically focused on terrestrial environments, while marine ecosystems have received less attention (Ormond, 1996). Humans have a very biased view of planet Earth, whose proper name should be Oceanus or Water (Roff et al., 2011). However, there is a growing awareness of the critical role that marine conservation has for human life as the oceans constitute the centre of water and nutrient cycles and global atmospheric and thermal regulation (Mega, 2019). Moreover, the ocean economy is a vital part of the global economy, valued at $1.5 trillion in 2010 (Sumaila et al., 2021).

Amongst marine temperate and tropical ecosystems, rocky and coral reefs are the most productive and diverse, providing essential ecosystem services (Birkeland, 1997; Duffy et al., 2013). Due to their proximity to anthropised areas, they are increasingly exposed to several co-occurring local human pressures (such as pollution, overfishing, coastal works) and are ranked as the most threatened marine ecosystems (Halpern et al., 2007). Under the synergic effects of such pressures and global warming, rocky and coral reefs are changing at an alarming rate (He and Silliman, 2019).

In the current scenario, the challenges of marine conservation and sustainability seem insurmountable, and all Countries urgently need to address them. However, hope must be the main trigger for science-based research and actions (Pinheiro et al., 2019). There is a need to target global efforts towards the study of human interaction with the natural system under a socio-ecological perspective, identifying and promoting action strategies and policies benefitting both the environment and society (Ortega-Rubio et al., 2021).

It is commonly recognised that knowledge is the first step for the development of effective conservation plans: one cannot conserve something which is unknown (Possingham et al., 2007). Research on the ecological status of marine ecosystems and their changes over time is a contribution towards marine conservation (Borja, 2014). Assessing the ecological status of rocky and coral reefs is a valuable tool to estimate the overall health of marine ecosystems (Aburto-Oropeza et al., 2015; Pisapia et al., 2017). For this purpose, different methodological approaches can be adopted, depending also on data availability.

Conservation is not a well-defined practice and there is no univocal strategy for the protection of marine environments (Beumer and Martens, 2013). Marine Protected Areas (MPAs) can be seen as valuable field laboratories, as they represent an effort to reduce local human impacts on ecosystems by restricting various activities such as fishing, boating, anchoring, etc. Nonetheless, MPAs cannot halt the impacts of global warming (Bazairi et al., 2013; Bruno et al., 2018). Continuous monitoring of species and habitats in MPAs is recommended and should include both inventory and periodic surveillance activities. In the case of inventories, all species and habitats should ideally be surveyed, while periodic surveillance actions should prioritize the most important species and priority habitats (Bianchi et al., 2022).

Indicator and target species are living organisms that can be easily monitored and whose health status reflects the condition of the environment (Siddig et al., 2016). For example, detailed knowledge about the distribution of protected species is a tool used in MPAs management (Sundblad et al., 2011). Analysing the spread of non-indigenous species may be useful for understanding global warming effects on local marine biodiversity (Katsanevakis et al., 2020). On the other hand, monitoring species particularly vulnerable to increasing water temperature provides information on the effects of climate change on marine ecosystems (Garrabou et al., 2019, 2022).

The use of non-destructive techniques, which do not require the collection and sacrifice of specimens, are to be preferred and highly recommended for the study and the monitoring of target species and valuable habitats (Bianchi et al., 2022). Underwater visual surveys well respond to this need, as they are based on observations and measurements made directly underwater with the advantage of obtaining data immediately, without further time-consuming analyses in the laboratory (Bianchi et al., 2004; Gatti et al., 2015). However, visual surveys may be limited by the "observer effect" when conducted by different diving scientists with dissimilar expertise (e.g., specialization) and experience (e.g., number of scuba surveys done or length of the scientific career). The design of visual monitoring activities should always take into full account the observer effect to ensure the reliability of the information provided (Azzola et al., 2022b).

An approach increasingly applied to marine ecosystems management is based on the environmental diagnostics, which includes the characterization (to identify types), and the evaluation (to define status and values) (Bianchi et al., 2012). Characterization of marine ecosystems involve, for example, analysis of community structure, while evaluation may be performed by applying diversity indices (e.g., Simpson and Shannon indices) and monitoring of target species. Evaluation should also consider that marine ecosystems are increasingly exposed to several disturbances and stresses and, hence, understanding the cumulative effects of such stressors is of fundamental importance for conservation policies (Schiel, 2009).

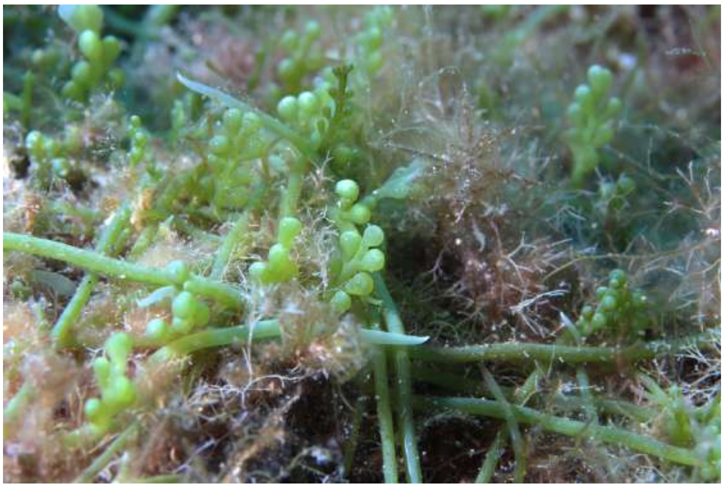
Under the effects of local and global human pressures marine ecosystems are changing increasingly faster (Hoegh-Guldberg and Bruno, 2010). The belief in equilibrium ecology, according to which marine communities tend to be stable and in perfect equilibrium with the average environmental conditions should be left aside (Coulson, 2021). Today, the concept of stability in ecology is challenged (Van Meerbeek et al., 2021) by the idea that marine communities are unpredictable and do not tend to or fluctuate around equilibrium (Coulson, 2021): they simply change (Bianchi et al., 2019a, b). This emerging school of thought should probably be defined "ecology of change" (Bianchi et al., 2022).

Identifying shifts in the diversity and composition of marine ecosystems is relevant to understanding the natural and anthropogenic drivers of ecosystem change. Long-term data series, especially those that extend beyond the typical career of individual scientists, are critical in this regard (Elhai et al., 2013), but unfortunately are rarely available (Proença et al., 2017). In absence of long-term series, change over time in marine ecosystems health may be suitably assessed by revisiting sites investigated years before (Bianchi et al., 2014).

Different levels of complexity can be considered in the study of change over time. For example, at the population level, monitoring native and non-indigenous species proves useful in assessing the effects of climate change (Galil et al., 2021). Over a larger scale, the loss of some species due to mass mortality events and the establishment of warm-water species of various origins, lead to a turnover in community composition (Bianchi et al., 2019a). In this context, it is crucial to assess whether change at the community level leads to change in ecosystem functionality. Can biodiversity provide a biological buffer to maintain ecosystem functioning in the face of environmental changes (Hong et al., 2021)?

The present work aims at analysing the ecological status and change over time of rocky reef benthic communities in the North-Western Mediterranean Sea and of coral reef benthic communities in the Maldives (Indian Ocean). Ecological status is defined by applying different methodological approaches, such as analysis of benthic community structure, diversity indices, and monitoring of mass mortality events and target species. In addition, the cumulative effect of different types of stress and disturbance (i.e., increasing sea surface temperatures, mucilaginous events, invasion of alien species, and extreme events) on rocky reef benthic communities is evaluated. With the aim of assessing change over time in Mediterranean and Maldivian reefs, historical data available in the literature are compared with current data to determine trajectories of change at different levels of complexity (population, community, and ecosystem) and as a function of local and global anthropogenic pressures.

Part one: ECOLOGICAL STATUS



ECOLOGICAL STATUS

Conservation of marine biodiversity requires knowledge and the definition of the ecological status of marine ecosystems (Cochrane et al., 2016). Ecological status, being influenced by water quality and habitat degradation, is a proxy used to assess the overall status of water bodies (Water Framework Directive 2000/60/EC).

Rocky reefs are environments of great scientific and economic value (Bianchi et al., 2004), due to the high level of biodiversity they host (Duffy et al., 2013) and the ecosystem services they offer (Paoli et al., 2017). In this framework, the assessment of their ecological status can be considered a valuable contribution to defining a marine ecosystems state of health.

METHODOLOGICAL APPROACHES

To assess the ecological status of rocky reef benthic communities, different methodological approaches could be applied according to the data availability. To this aim, the paper **1.1** presents an overview on biodiversity monitoring techniques; the paper **1.2** offers an example of how studies on target species can be useful for supporting the development of conservation strategies; the paper **1.3** investigates the observer effect in rapid visual assessment, which can be a useful tool for habitats assessment.

ENVIRONMENTAL DIAGNOSTICS: CHARACTERIZATION AND EVALUATION

The procedure to assess the ecological status through environmental diagnostics involves two levels: i) the characterization, based on the analysis of environmental abiotic and biotic parameters to identify ‘types’; and ii) the evaluation, based on the analysis of these ‘types’ to define their status and values (Bianchi et al., 2012). In this framework the paper **2.1** aims at characterizing benthic communities of different areas and evaluating the effects of climate change by monitoring water temperature and indicator species, while the paper **2.2** aims at assessing the effects of multiple stressors on a temperate rocky reef.

1.1

Biodiversity Monitoring in Mediterranean Marine Protected Areas: Scientific and Methodological Challenges

Extracted content - Reference:

Bianchi C.N., **Azzola A.**, Cocito S., Morri C., Oprandi A., Peirano A., Sgorbini S., Montefalcone M. (2022) *Diversity*, 14(1): 43

Abstract

Biodiversity is a portmanteau word to indicate the variety of life at all levels from genes to ecosystems, but it is often simplistically equated to species richness; the word ecodiversity has thus been coined to address habitat variety. Biodiversity represents the core of the natural capital, and as such needs to be quantified and followed over time. Marine Protected Areas (MPAs) are a major tool for biodiversity conservation at sea. Monitoring of both species and habitat diversity in MPAs is therefore mandatory and must include both inventory and periodic surveillance activities. In the case of inventories, the ideal would be to census all species and all habitats, but while the latter goal can be within reach, the former seems unattainable. Species inventory should be commeasured to investigation effort, while habitat inventory should be based on mapping. Both inventories may profit from suitability spatial modelling. Periodic surveillance actions should privilege conspicuous species and priority habitats. Efficient descriptor taxa and ecological indices are recommended to evaluate environmental status. While it seems obvious that surveillance activities should be carried out with regular recurrence, diachronic inventories and mapping are rarely carried out. Time series are of prime importance to detect marine ecosystem change even in the absence of direct human impacts.

INTRODUCTION

Biodiversity is a portmanteau word that expresses the value of biological variety at different levels of integration, from genes to the entire ecosystem (De Long, 1996; Boudouresque, 2014). For many authors, the meaning of biodiversity is above all linked to the concept of species richness, that is, the number of species found in a specific site, region or ecosystem. Although widespread, this acceptation is too simplistic, as it neglects the other levels of biological variety (Swingland, 2001). Perhaps as a reaction, the term ”ecodiversity” has been coined to focus on the variety of habitats, rather than species (Boudouresque and Verlaque, 2005). […]

The significance of biodiversity as an indicator of the health of the environment and the functioning of ecosystems is fully recognized, not only by scientists, but also by mass media, administrators and public opinion (Loreau et al., 2001). Conciliating human development with biodiversity conservation is at the root of the emerging discipline of socioecology (Ortega-Rubio et al., 2021). However, most of the attention and debate on biodiversity has concerned predominantly the terrestrial environment, while marine biodiversity has historically received less attention (Ormond, 1996). From this point of view, the situation in the Mediterranean Sea is no exception (Sperandii et al., 2021), despite the great cultural and economic importance that the sea has always had for the Mediterranean countries (Bianchi et al., 2012). […]

Marine Protected Areas (MPAs), although mostly designed to manage fisheries (Weigel et al., 2014), have proved capable to enhance the status of species and habitats (Guidetti and Sala, 2007; Fraschetti et al., 2013), and are thus viewed, notwithstanding some controversy (García-Charton et al., 2008), as the primary strategy for the conservation of marine biodiversity (Scianna et al., 2018, 2019). […]

If it is true - as it is true - that the natural capital due to biodiversity also epitomizes an economic capital (Costanza and Daly, 1992), then there is no doubt that its amount must be adequately quantified and then followed over time. Monitoring activities on biodiversity in MPAs must therefore include both the inventory and the surveillance (Hutto and Belote, 2013). The present viewpoint paper will discuss these aspects mostly based on experiences in Italian MPAs, expanding and updating previous contributions (Bianchi and Morri, 2000; Bianchi, 2002).

MONITORING AS AN INVENTORY

Commonly, the establishment of a MPA is preceded by a series of investigations that typically include inventories of species and habitats (Foley et al., 2010; Lee et al., 2015). This may have generated, in the minds of those who were subsequently called to manage that MPA, the idea that the local biodiversity was “already known” and that no further information was needed.

This way of thinking, much more widespread than one might imagine, undoubtedly derives from the belief that marine communities tend to be stable and in perfect balance with the average conditions of the environment; most changes, seasonal cycles apart, are thus attributable to human impacts (Bianchi, 1997). From this belief, which identifies the so-called “equilibrium ecology” and has its roots in the paradigms of the balance of nature dating back at least to the Greek philosopher Herodotus (V century BCE), can derive the falsely reassuring tranquillity that, where human impact is excluded (as in protected areas), it is no longer necessary to invest in the inventory of biodiversity. Equilibrium ecology has been challenged by non-equilibrium ecology – or “new ecology” - which denies that biologically accommodated communities are better structured (i.e., with a greater number of niches realized and therefore higher diversity) than physically controlled communities. Disturbance has assumed a key role as regulator of the composition and structure of communities (Pickett et al., 1989): communities will be more structured at intermediate disturbance levels, also through physical heterogeneity and space-time mosaics generated by the so-called “patch dynamics” (Pickett and White, 1986). In altered disturbance regime, or when disturbance intensity exceeds a certain threshold, unpredictable change may occur, leading to abrupt phase shifts (Montefalcone et al., 2011; Bianchi et al., 2019; Oprandi et al., 2020).

The new ecology led to a revolution in the way we see biological communities: equilibrium ecology describes them as deterministic and predictable; non-equilibrium ecology considers them non-deterministic and unpredictable, due to stochastic disturbance events (Bianchi et al., 1998). The idea has also emerged that biological communities are chaotic, i.e., deterministic but unpredictable due to the great sensitivity to small variations of the initial conditions (Pool, 1989). In the last decades of the 20th century, ecology went through a phase of crisis, which has been the melting pot where a new conceptual vision has been forged (Peters, 1991). Such a critical phase saw as essential to focus on change rather than on equilibrium (Margalef, 1997). Today, the concept of stability in ecology is questioned (Van Meerbeek et al., 2021), and it has been fully realized that marine communities neither tend to, nor fluctuate around, equilibrium (Coulson, 2021): they simply change, and such changes occur at time scales shorter than one might have thought (Bianchi and Morri, 2004). The emerging ecology should probably be understood as “the ecology of change” (Bianchi et al., 1998). It is from this awareness that the need arises for a diachronic (i.e., repeated over time) inventory of species and habitats within the MPAs (Ros and Gili, 2015; Montefalcone et al., 2007).

For the inventory of species, specimen sampling and/or observation (including photo and video) are a current approach. However, three innovative techniques are emerging that do not require the collection of organisms: environmental DNA, passive acoustic methods, and baited remote underwater video systems. […]

For the inventory of habitats, the main tool is mapping (Bianchi et al., 2004). […] Non-destructive mapping procedures, based on remote acoustic and optical techniques (Rowan and Kalacska, 2021) should be preferred, minimizing the collection of physical samples. Common adopted acoustic methods include single-beam echosounder, multibeam echosounder and side-scan sonar (Pandian et al., 2009). […] The combination of acoustic and optical data has been shown to be the most reliable approach to obtain high-resolution benthic habitat maps (Rende et al., 2020). Algorithms do exist for the automated interpretation of acoustic and optical outputs with an unsupervised approach; however, a supervised approach, with inspections in situ (“sea truth”) using towed video cameras, ROVs (Remotely Operated Vehicles), AUVs (Autonomous Underwater Vehicles) or diving, are typically necessary for disambiguation (Vassallo et al., 2018; Mata et al., 2021). Combining acoustic methods and ROVs, for instance, allows efficient surveying also at great depths (Fanelli et al., 2017). The characterization and definition of marine habitats may benefit of a seascape approach, which merges mesological, geological and biological data (Pittman et al., 2011; Cocito et al., 1991; Musand et al., 2014; Rovere et al., 2011) and stands at the basis of the ecotypological classification of marine ecosystems (Bianchi et al., 1984). Geological features may often contribute to the environmental value of marine habitats (Rovere et al., 2010a, b, 2011).

The scale at which the cartography of the habitats of a MPA must be produced is of fundamental importance, as it dictates the resolution and hence the number of habitats found. Large scales (>1:5,000) are suitable for illustrating in detail small areas where rocky bottoms dominate, which is often the case with MPAs (Gili et al., 1984; Parravicini et al., 2007). Intermediate scales (between 1:10,000 and 1:25,000) are useful for acquiring a synoptic view of the entire MPA when the MPA is large or to embrace the whole physiographic unit where it is located (Morri et al., 1986; Navone et al., 1992; Bianchi et al., 2002; Diviacco et al., 2004). Small scales (1:50,000 or less) can highlight the ecological situation of the MPA in the context of the natural region to which it belongs (Bianchi and Morri, 1989; Bianchi et al., 1993). To reconcile relatively large scales and regional coverage, atlases have been proposed whose pages contain contiguous or slightly overlapping maps (Parenzan, 1983; Bianchi and Peirano, 1995; Ardizzone et al., 2018; Coppo et al., 2020).

It must be emphasized that the choice of the scale does not only involve, as is obvious, a greater or lesser detail: it affects the very meaning of the phenomena represented (Burel et al., 1992). There is no “natural” scale of ecological systems (Levin, 1992), but rather a hierarchical series of spatial and temporal scales: changing the scale of study and representation of phenomena means describing intrinsically different typologies, levels of organization, and functions (Peterson and Parker, 1998). While at limited spatial scales it is not possible to predict the structure of communities - which fluctuates between different alternative states equally probable (Sutherland, 1974) - at seascape scale the probability increases, as the proportion of habitats in each state tends to remain relatively constant (Rahel, 1990). This is of considerable scientific and practical importance, since the assessment of human impacts requires, as a reference framework, the expected frequency of different states. It may therefore be informative that bionomical maps highlight not only the extent and distribution of the various habitats present, but also their state and dynamic trend, based on the presence of indicator species and other biological and ecological indicators (Navone et al., 1992; Bianchi et al., 1996; De Biasi, 1999). The same indicators may also help elaborating sets of evaluation maps, of immediate use for management (Bianchi et al., 2012; Rovere et al., 2013). Combined with maps of human pressures, habitat state maps are of prime importance for marine spatial planning, an important decision-support tool for finding efficient management solutions (Parravicini et al., 2012).

It is obviously impossible to establish conservation plans for something that has not been found yet (Azzola et al., 2021). To maximise the probability of finding what is searched for, inventories of species and habitats may benefit from suitability modelling, which allows predicting their occurrence on the basis of known environmental variables, such as depth, distance from the coast, bottom type, etc. (Vassallo et al., 2018; Montefalcone and Azzola, 2020): in this way, maps of expected occurrence for either species (Fig. 3) or habitats can be produced, verifying their actual presence with a selected number of sea truth checks. […]

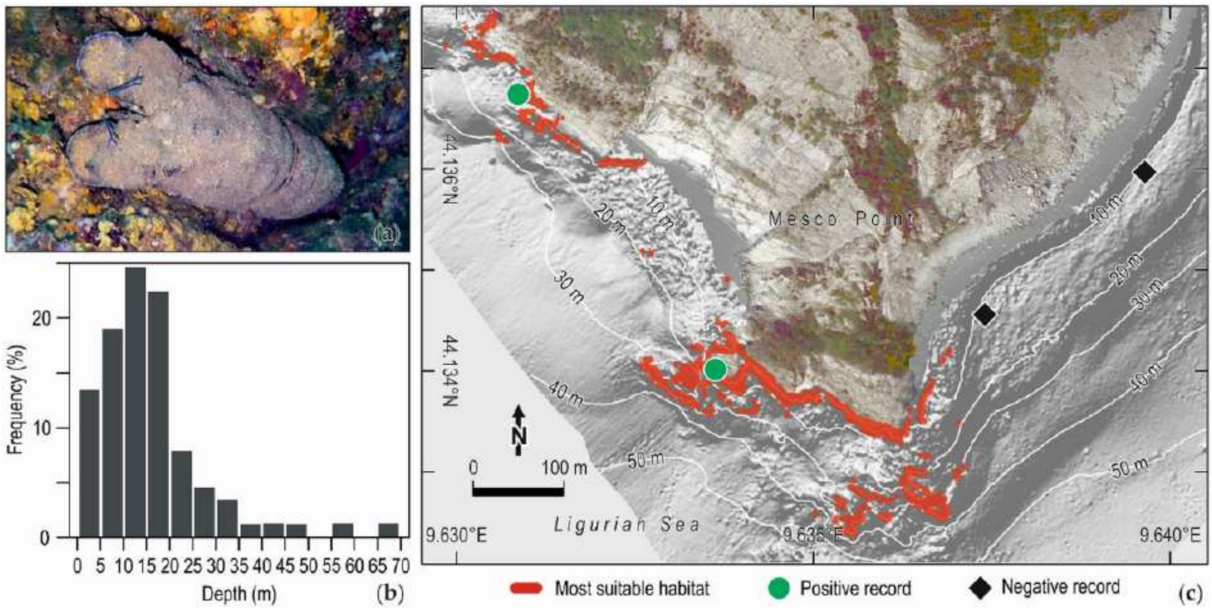


Fig. 3 Suitability modelling to locate a species of conservation interest in a marine protected area: **(a)** the slipper lobster Scyllarides latus; **(b)** its preferred depth according to literature information; **(c)** habitat suitability model superimposed to the seafloor map (obtained by merging aerial photography and multibeam survey) of Mesco Point, in the Cinque Terre MPA (NW Italy, Ligurian Sea), together with sea truth results by scuba diving (based on information in Montefalcone and Azzola, 2020).

MONITORING AS SURVEILLANCE

Carrying out exhaustive inventories with great frequency is out of the question for reasons of cost and operation. Drawing up the list of the species present in a MPA can cost thousands of euros per km of coastline, even if only considering the “most important” taxa, whatever is meant by this expression (see below); the cost per species may range from a few euros (in the case of common species) up to hundreds of euros (in the case of rare species) (Bianchi, 2002).

It is therefore essential to implement continuous surveillance plans that take into consideration a selected number of species and habitats. In this case, however, monitoring does not address the verification of the presence, but rather the detection of some quantitative parameters. In the case of species, for example, the size and demographic structure of the populations are among the most recommendable parameters (Cocito et al., 1998; Cocito and Chiantore, 2004; Pressey, 2004; Azzola et al., 2022a). Contrary to the logic of inventories, this type of monitoring does not invest most of the sampling effort in finding what is rare, but in defining as strictly as possible the quantity and conservation status of some selected species. It should be emphasized that this is the approach that usually provides the most immediate and most noticeable measure of the reserve effect to the general public (Grorud-Colvert et al., 2010): the presence of rare species is generally felt less interesting than the increase in the number and size of groupers or other popular fish (Bianchi, 2002).

As for the type of sampling to be adopted for the surveillance of biodiversity in MPAs, also in this case there is growing consensus on the use of non-destructive techniques, which do not require the collection and sacrifice of specimens. Photographic sampling responds to this need and also has the advantage of obtaining nonetheless a physical “sample” (the photographic image) that can be analysed and archived (Bianchi et al., 2001, 2004). Similarly, video imagery has acquired great relevance in the last decades (Mallet and Pelletier, 2014), especially for the monitoring of mesophotic habitats (Cánovas-Molina et al., 2016; Enrichetti et al., 2019). Another technique that has undergone great development is visual census, which can be used both for species belonging to the sessile or sedentary epibenthos (Parravicini et al., 2009) and for fish (La Mesa and Vacchi, 2004). […] Counts are carried out in permanent plots along paths (transects) or within well-defined surfaces (quadrats) suitably located in space (Fig. 5), in order to ensure the possibility of a rigorous statistical treatment of the data (Bianchi et al., 2004; Bianchi et al., 2018). The adoption of precise standardized survey protocols and training of the investigators minimizes the subjectivity and error inherent in visual censuses (Azzola et al., 2022b).

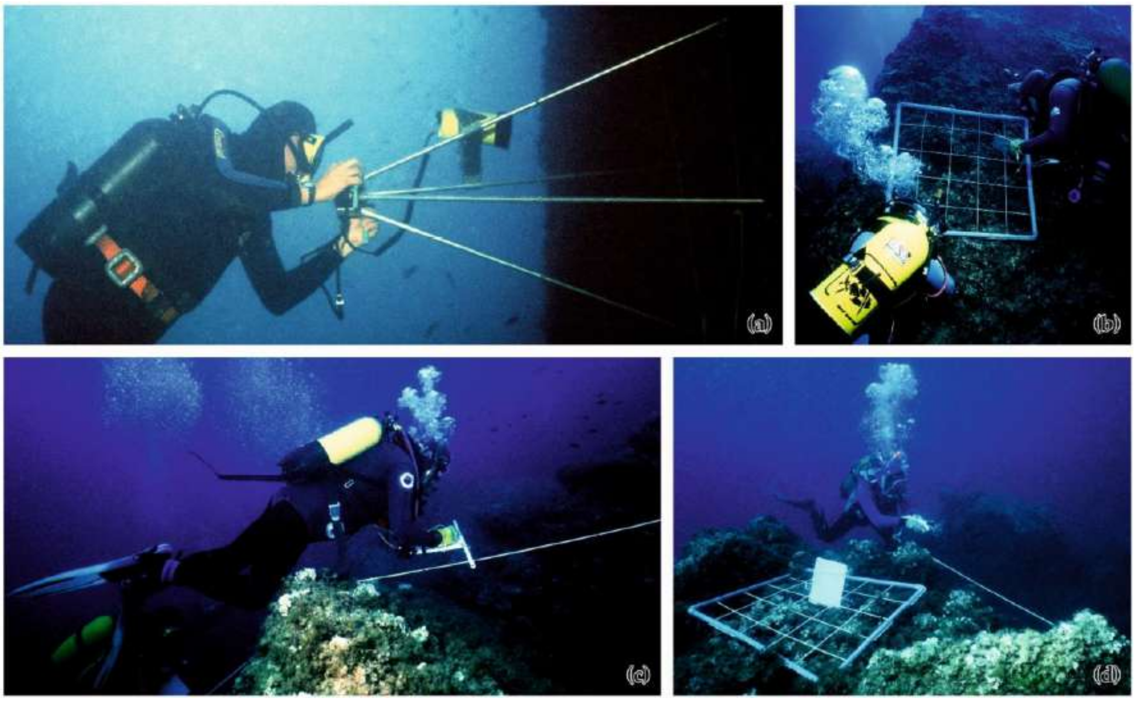


Fig. 5 Examples of non-destructive techniques for biodiversity monitoring: **(a)** underwater photography; **(b)** quadrat; **(c)** transect; **(d)** quadrats and transects can be complementary, rather than alternative, techniques.

WHAT TO MONITOR?

In the case of inventories, the ideal would be to be able to census all species (from microorganisms to vertebrates) and all habitats. While the latter goal should be within reach, the former seems unattainable (Mora et al., 2011). However, the inventory of the entire planet's biodiversity might even be an attainable goal (Wheeler et al., 2012), as was the creation of the map of the human genome when there had been a strong will to invest in the necessary resources (International HapMap Consortium, 2005). The MPAs could represent a concrete opportunity to try to become closer to the goal of taking a census of the biodiversity of our seas.

However, it should be emphasized that when asked what to monitor, different answers are possible depending on the specific purpose. Starting from the case of checking the size and state of populations of certain species, it is likely that these species belong to a category at risk. In conservation biology it is customary to distinguish three situations, according to the criteria of the IUCN (International Union for Conservation of Nature): “endangered species” are close to extinction; “threatened species” are those that risk disappearing in the foreseeable future; “endemic species” are those with a narrow distributional range (Mace, 2008).

The Mediterranean has numerous endemic species, many are currently threatened and some are probably in danger (Boudouresque et al, 1990; Coll et al., 2010). The mass media, public opinion and administrators are realizing this problem, but their interest mostly concerns the so-called “flagship species” (Clucas, 2018), i.e., charismatic species (Albert et al., 2018) mostly belonging to mammals, turtles, some fish and a small number of popular invertebrates and plants (Boudouresque et al., 1991; La Posta et al., 1992).

This attitude reflects how humans value other living beings (Small, 2011, 2012). It is relatively easy to obtain public consensus for the protection of seals and dolphins, since humans have a kind of sympathy for large mammals (Wilson, 1986; Kellert, 1996); but who cares about the extinction of a marine alga? […] The charismatic megafauna typically include wide-ranging species that may act as “umbrella”, i.e., focal or surrogate species whose protection implies the protection of many other species living within their range (Barua, 2011; Ducarme et al., 2013). The rising awareness among common people about the actual meaning of biodiversity and the importance of its monitoring has recently led to the launch of citizen science projects, in which volunteers (e.g., students, fishermen, divers) are involved in gathering data that would otherwise be impossible to collect because of limitations of time and resources (Mannino and Balistreri, 2019; Monk et al., 2008). Proper training and validation and verification by taxonomic experts can provide quality-filtered data that improve taxonomic representation and the geographic breadth of species monitoring (Kelly et al., 2020; Kasten et al., 2021).

There are therefore both scientific and opportunity reasons to suggest that surveillance activities preferentially target populations of species attributable to the aforementioned categories and familiar to the general public (fish and other marine vertebrates, large molluscs, decapod crustaceans, etc.). Frequently, these species are found in top positions in food chains and therefore exert a top-down control over the functioning of ecosystems (“keystone species”, Piraino et al., 2002). However, priority must also be given to those species which, by giving shape to the submerged landscape (erect sponges, gorgonians, seagrass, large phaeophyceans) or even by building bioherms (coralline algae and many bioconstructional invertebrates, Ingrosso et al., 2018), exert a bottom-up control of ecosystems (“structural species”, Piraino et al., 2002). Both keystone species and structural species have the capacity of modifying physically the environment in which they thrive, and have been thus defined ecosystem engineers (Braeckman et al., 2014), allogenic the former (they modify the environment with their behaviour), autogenic the latter (they modify the environment through their own mass). […]

As regards the monitoring of habitats, first of all it should be emphasized that the criteria diverge from those adopted for species. Public opinion has a different attitude in the two cases. For habitats, the criterion is essentially that of utility. It is considered useful to have healthy marine ecosystems mainly because they are more appreciated for bathing, tourism or simply for aesthetic enjoyment. For species, the main evaluation criterion is “sympathy”. Animals (especially mammals) should be protected mainly because they appear cute and friendly (Batavia et al., 2021): this has been called the “Walt Disney effect” (Bianchi and Morri, 2000). Eating dolphin meat is a crime, eating lobster meat is just a gourmet choice.

It is not always clear that protecting species and protecting habitats are the two sides of the same coin. […] Only recently a gradual shift from a species-centred to an ecosystem-based management has been emerging in biodiversity conservation (Boudouresque et al., 2020).

Most of the main marine habitats of the Mediterranean are currently in danger but national laws or European Community directives in practice mostly identify seagrass beds and, less so, rocky reefs, coralligenous reefs, and sea caves as deserving protection (Bellan-Santini et al., 1994). It seems therefore logical to consider that the surveillance activities, on a seasonal or annual basis, should primarily target these habitats, monitoring their extent and state of health.

The assessment of the health state of marine habitats typically requires numerical targets and specific reference values, to allow comparison in space and/or time (Cochrane et al., 2006). In the last decades, a vast array of biotic indices has been developed to this purpose, mostly as a consequence of European Directives (Borja et al., 2009). Indices have been devised based on indicators at different ecological complexity levels (Bavestrello et al., 2016; Oprandi et al., 2021): indices working at individual level take into account the phenology of a key species (e.g., a seagrass); indices at population level use abundance, spatial extent, density or size distribution frequency of a key species; indices at community level consider taxonomic composition, diversity, and the sensibility of different species towards environmental quality; indices at ecosystem level are based on the identification of functional compartments (e.g., in the food web) and on the evaluation of the status of each of them; indices at seascape level typify and quantify different elements and their spatial configuration, paying special attention to the morpho-structural traits of the species. […]

FINAL REMARKS

Change has always been the rule in marine biodiversity [54]. The Mediterranean marine biota has changed dramatically over the past, and is continually changing under the influence of both local and global pressures (Bianchi, 2007; Bianchi et al., 2012).

Biodiversity is probably the tool that ecosystems adopt to deal with environmental fluctuations (insurance hypothesis): through the distribution of resources and the alternation in the contribution to biomass, a flexible composition in species can allow ecosystems to maintain their functioning (Loreau et al., 2021). A high biodiversity buffers the effects of environmental variation since tolerant species can more easily be present. It seems a paradox, but in ecology change promotes stability (Lewin, 1986).

Distinguishing environmental stress due to climatic changes from that due to local anthropogenic pressure is often difficult (Morri et al., 2001; Montefalcone et al., 2017; Gissi et al., 2021). For instance, the loss of canopy forming macroalgae of the order Fucales, observed in many regions of the Mediterranean Sea, has been considered by different authors as due to either climate or human impact (Bianchi et al., 2019b, and references therein]. A network of MPAs can represent the reference system for understanding the influence of humans and for developing guidelines for an integrated management of the coastal zone for the purpose of sustainable development. MPAs must be seen as elective sites where natural changes can play their role independently from the interaction with local anthropogenic impacts, which should not represent a major driver of change there (Bianchi and Morri, 2000; Azzola et al., 2022b). Assessing change over time requires long term data series, which – notwithstanding their importance for both scientific understanding and conservation planning (Heip et al., 1997; García-Barón et al., 2021) – are rarely available (Bianchi and Morri, 2004; Proença et al., 2017). In absence of such data series, the revisitation of sites already surveyed in the past has proved successful (Pearson et al., 1985; Barry et al., 1995; Hiscock, 2005; Parravicini et al., 2013; Bianchi et al., 2014; Gatti et al., 2015, 2017; Montefalcone et al., 2018, Bianchi et al., 2019a; Azzola et al., 2022a, b).

A new marine ecological research policy is needed to address biodiversity problems, as they are becoming increasingly urgent. The MPAs can play a primary role in this field (Giakomi et al., 2018; Grorud-Colvert et al., 2021).

In the first place, a better understanding of the meaning and expressions of biodiversity requires continuous research in those scientific areas now considered out of date by the funding bodies, such as systematics, biogeography and natural history. Specialists in these disciplines who retire are not being replaced by young scholars: therefore, as biodiversity problems are growing, biodiversity skills are being lost (Boero, 2010).

Secondly, to assess the natural variability of marine ecosystems, and to understand the effects of change on their biodiversity, it is essential to start monitoring biodiversity at the scale of the entire Mediterranean. Neither species nor ecosystems recognize borders between nations, and neither climate nor human impacts do. An internationally coordinated network of MPAs in the Mediterranean would be indispensable for long-term marine biodiversity monitoring projects. These projects must be of such a duration as to include at least the life span of the dominant organisms and the time scale of the most important influencing factors, but funding and other constraints force ecologists into projects lasting 2–3 years at the most: few natural patterns have such a short duration, major changes in the biota occurring with cycles lasting 10 years or even longer (Southward, 1995). Small-scale, short-term approaches undermine the possibility to assess change in marine ecosystems, whether natural or human-induced. Only continued monitoring will help understanding the consequences of the ongoing transformations driven by sea water warming and other components of global change (Bianchi et al., 2019a). Long-term monitoring efforts are a multi-generational deal and require commitments by institutions that persist beyond the working lives of individuals (McDonald, 2019). Marine Protected Areas are the best placed institutions to accomplish this task in the years to come.

Author Contributions

Conceptualization, C.N.B. and C.M.; methodology, **A.A.**, C.N.B., S.C., M.M., C.M., A.O., A.P. and S.S.; software, **A.A.** and A.O.; validation, M.M.; formal analysis, C.N.B.; investigation, **A.A.**, C.N.B., S.C., M.M., C.M., A.O., A.P. and S.S.; resources, **A.A.**, C.N.B., S.C., M.M., C.M., A.O., A.P. and S.S.; data curation, **A.A.**, M.M. and A.O.; writing—original draft preparation, C.N.B., M.M., C.M. and A.O.; writing—review and editing, **A.A.**, C.N.B., S.C., M.M., C.M., A.O., A.P. and S.S.; visualization, C.N.B. and C.M.; supervision, M.M.; project administration, C.M.; funding acquisition, C.N.B. and C.M. All authors have read and agreed to the published version of the manuscript.

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1.2

Cannot conserve a species that has not been found: the case of the marine sponge *Axinella polypoides* in Liguria, Italy

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Azzola A., Bavestrello G., Bertolino M., Bianchi C.N., Bo M., Enrichetti F., Morri C., Oprandi A., Toma M., Montefalcone M. (2021) *Aquatic Conservation: Marine and Freshwater Ecosystems,* 31(4): 737-747

Abstract

Detailed knowledge about the distribution of species in need of protection is required for the management of Marine Protected Areas, a major tool to reduce marine biodiversity loss. Such knowledge is deficient for most marine invertebrates. *Axinella polypoides* is a marine sponge included in the list of protected species by the Barcelona Convention (1976) and the Bern Convention (1987). This large and erect species has an important ecological role in habitat forming and benthic-pelagic coupling. Bathymetrical, geographical, and ecological data over the last 60 years were collated from publications and reports, together with new surveys to assess the distribution and protection status in Liguria of *A. polypoides*. It resulted more widespread than previously thought, which points at a general need of purposely dedicated investigation on the occurrence of species that require protection. Bathymetrical distribution was trimodal, with peaks corresponding to different geomorphological settings: coastal cliff toes (around 38 m depth), inner shelf shoals (52 m), and rocks amidst coarse sediment in the outer shelf (79 m). Density was significantly greater at the shallowest depths. The species was mostly found in the coralligenous biocoenosis, in association with other characteristic species or forming monospecific facies. On (sub)vertical cliffs, *A. polypoides* often exhibited an unusual cane shape, rather than the typical bushy morphology, thus causing confusion with the congeneric *A. cannabina*, a more southern species. Records of the latter in the

Ligurian Sea need therefore confirmation. Only a minority (22.6%) of *A. polypoides* records were in MPAs, the remainder being located in areas with no current environmental protection plans in place. While the occurrence of this species in MPAs remained stable over the decades, the only quantitative historical data available indicated that populations in non-protected areas were declining due to anthropogenic impacts (fishing and anchoring).

INTRODUCTION

Human activities are negatively impacting the biodiversity of the world's ocean, causing species extinction at an unprecedented rate, with largely unknown consequences (Worm et al., 2006). Marine habitat health is currently threatened by a number of pressures, including pollution, exploitation, habitat destruction, overfishing and global warming (Dailianis et al., 2018; Montefalcone, Morri, Bianchi, Bavestrello, & Piazzi, 2017; Montefalcone, Parravicini, & Bianchi, 2011; Trush et al., 2009). All these pressures are affecting ecosystem functioning through changes in species diversity and other impacts (Lubchenco, Palumbi, Gaines, & Andelman, 2003).

Conservation is not a defined practice and there is no univocal plan for how to halt the loss of biological diversity (Beumer & Martens, 2013). Identification of priority areas, such as 'hotspots' or high-biodiversity sites, is an essential tool for conservation planning (Brooks et al., 2006; Vassallo et al., 2020). In order to develop effective policies and make decisions regarding biodiversity conservation, knowledge, at a bare minimum, about the distribution of species that require protection is mandatory (Jetz, McPherson, & Guralnick, 2012). Of course, no species conservation plan can be launched is such a species has not been found yet (Possingham, Grantham, & Rondinini, 2007), and assembling all the available information represents but a first step in trying to assess species occurrence. Knowledge of species' ecological traits – including habitat type and recurrent association with better known species – may be of help to identify sites where to concentrate surveys (Costello et al., 2015).

Spatially explicit areas of high biodiversity associated with either high or low human impact have already been selected in several regions for some vertebrate groups (Possingham et al., 2007), proving their utility in terrestrial species conservation. However, for marine species, and in particular for the invertebrates, this has not been fully accomplished yet (Selig et al., 2014). The marine environment has long been considered invulnerable and its resources inexhaustible, due to its vastness and the reproductive and dispersion capacity of the various species (Roberts & Hawkins, 1999). While the protection by law of terrestrial species started early in the 20th century, for a long time the protection of species in the marine environment was not taken into account (Boudouresque & Bianchi, 2013). At present, many regional, national and international actions have been implemented to improve the conservation of marine biodiversity, but their attention mostly concerns mammals, sea turtles, fishes and only a few invertebrates, knowledge of which, however, is far from adequate (Bianchi et al., 2012; Stuart, Wilson, McNeely, Mittermeier, & Rodriguez, 2010). The conservation status of sponges, in particular, has been rarely assessed, notwithstanding their relevant role in benthic communities (Bell et al., 2015; Gerovasileiou et al., 2018).

For the Mediterranean Sea, the Protocol of the Barcelona Convention (1976) provides a list of Endangered or threatened species in Annex II; similarly, the Bern Convention (1987) provides a list of Strictly protected fauna species in Appendix II. The aim of both protocols is to ensure the conservation of wild fauna and flora, with particular attention to vulnerable species. Contracting Parties of the former protocol, in particular, must take all the appropriate measures to protecting those Mediterranean Sea areas which are important for the safeguard of the marine vulnerable species.

The list of 49 Mediterranean marine invertebrates in need of protection according to the Barcelona Convention (Relini & Tunesi, 2009) includes the sponge *Axinella polypoides* Schmidt, 1862 (Pansini & Pronzato, 2009), which is defined vulnerable by Sitja and Maldonado (2014). It is classified as endangered in Italy (www.iucn.it/scheda.php?id=712544432), France (Boudouresque et al., 1996), Montenegro (Mačić, Petović, & Backović, 2015), and Algeria (Hussein & Talet, 2019), but considered of ‘Least Concern’ (LC) in Greece (Gerovasileiou et al., 2018), which suggests that further information is needed to improve knowledge about the conservation status of this sponge in the Mediterranean Sea.

A. polypoides is a large and long-lived marine sponge that acts as a marine ecosystem engineer: it plays a significant role in benthic-pelagic coupling (Coppari et al., 2016) and provides suitable habitat for a variety of other marine species due to its erect and bushy, three-dimensional structure (Bell, 2008; Koukouras, Russo, Voultsiadou-Koukoura, Arvanitidis, & Stefanidou, 1996).

The geographical distribution of *A. polypoides* ranges from the Mediterranean Sea to Mauritania and Senegal, including the oceanic islands of the Azores, Madeira and Canaries (www.marinespecies.org/porifera/), thus suggesting a subtropical affinity. In the Ligurian Sea, one of the coldest sectors of the Mediterranean (Bianchi et al., 2012), this species was first recorded in 1957 by Tortonese (1958) at Portofino, where a so-called 'thermophilic oasis' was said to occur (Sarà, 1985). Subsequent research reported several other records of this species both at Portofino (Gatti et al., 2017) and in other Ligurian Sea localities (Bianchi & Morri, 1994; Parravicini et al., 2015, and references therein). Knowledge of its distribution in the Ligurian Sea, however, remained fragmentary until recently, and little is known in particular about its conservation status.

The aim of this work was to collate all the existing data about the occurrence of *A. polypoides* along the coast of Liguria, an administrative region in NW Italy, which embraces the central and largest tract of the Ligurian Sea, in order to analyse the conservation status of this marine protected sponge. Information on the ecology and bathymetric distribution of this sponge was extracted from the bibliography (including grey literature) and new field observations. Protection status was assessed on the basis of its occurrence inside or outside of marine protected areas and considering the threats to which the sponge is exposed.

MATERIALS AND METHODS

Study area

This study considered the whole Ligurian coast, collating available data from 1957 (date of the first record of *Axinella polypoides*) to 2019. The Ligurian coastline spans for about 350 km: the eastern side, that includes almost two thirds of the whole coastline, is mostly composed of rocky cliffs and promontories, whereas the west side also contains extensive sandy beaches (Cánovas Molina et al., 2016; Cattaneo-Vietti et al., 2010).

Since the 1990s, three national Marine Protected Areas (nMPAs: Bergeggi, Cinque Terre and Portofino), two regional Marine Protected Areas (rMPAs: Capo Mortola and Portovenere), and 27 marine Special Areas of Conservation (SACs: www.ambienteinliguria.it) have been established in Liguria to manage and conserve marine biodiversity. However, while both nMPAs and rMPAs are more or less fully enforced, all SACs still require management plans that regulate human access and activities.

Data collection

A database was organized to report year of record, locality, site, coordinates, depth, level of protection, and data source for the *A. polypoides* occurrence in Liguria. Throughout the paper, the term record refers to the species occurrence (even repeated in different years) at a specific depth in an individual site. One record may include several specimens. Locality is a geographical place that may include several sites. Thanks to the wealth of studies since the 1950s on the Ligurian Sea fauna (Bianchi, Morri, Peirano, Romeo, & Tunesi, 1987; Bianchi et al., 2019), hundreds of scientific papers and technical reports are available. Similarly to data extracted from the bibliography, novel information was obtained from scuba diving surveys carried out mostly along depth transects between 2011 and 2019 by the authors. In total (bibliography and new surveys), 388 scuba dives from 50 m to the surface, and 103 ROV (Remotely Operated Vehicle) dives from 40 to 200 m depth (Enrichetti et al., 2019a, 2020) have been taken into account. Negative records (i.e. where the species was not found) of *A. polypoides* were not included in the database, as this information is rarely available, especially in the case of literature data.

Species identification and survey methodology

In field surveys, identification of *A. polypoides* was carried out visually based on the erect shape, with a stalk and dichotomous and occasionally coalescent branches circular or oval in cross-section, yellow colour shifting to orange, smooth surface, and stellate oscula (Pansini & Pronzato, 2009; Uriz, 1982). When visual identification was not conclusive, a limited number of small voucher pieces were detached from a terminal branch for laboratory analyses of the spicules (Rützler, 1978).

When available from either the literature or recent field surveys, abundance data were used to evaluate population size. Scuba surveys employed quadrats of 4 m² (Bianchi et al., 2004) or belt transects of variable length and 1 m total width (Bianchi et al., 2018). Belt transects, of variable length and 0.5 m total width, were also employed by ROV surveys (Enrichetti et al., 2019b).

Data management and analysis

The trend of the knowledge of *A. polypoides* occurrence in Liguria from 1957 to 2019 was analysed considering the cumulative number of localities per year.

To evaluate the proportion of occurrences of *A. polypoides* within the MPAs and SACs of Liguria, the geographical coordinates of each record were loaded in Quantum GIS (QGIS) version 3.6. Information was available for 41 unprotected localities and for all MPAs (five localities), while only ten SACs out of 27 were taken into account, as the remaining 17 only include Posidonia oceanica meadows (Relini, Diviacco, & Tunesi, 2000), where *A. polypoides* is not expected to occur (Gerovasileiou et al., 2018). Potential threats were estimated through bibliographic information and field observations.

Bathymetric distribution has been investigated through frequency analysis. Observations made on the same site and depth over several years were considered as one record only. The number of bins for histogram plotting has been chosen according to the zero-stage rule of Wand (1997). To assess multimodality in data distribution, Kernel Density Estimation (Botev, Grotowski, & Kroese, 2010) has been used as a smooth estimator of the histogram according to the rule of Silverman (1986).

Most analyses were based on presence-only data, as absence can rarely be definitively assessed; presence-only data can convey sufficient information to estimate species distribution (Ward, Hastie, Barry, Elith, & Leathwick, 2009, and references therein). In the cases where abundance data were available, they were converted to density (specimens·m^{-2}).

All statistical analyses were performed with the open-access software PaSt (Hammer, Harper, & Ryan, 2001).

RESULTS

Axinella polypoides was recorded in 57 sites (out of 71 investigated) from 26 Ligurian localities (out of 35) (Fig. 1a; see also the Supporting Information). Of the 230 records, 210 came from bibliographic information, and 20 from hitherto unpublished observations. The latter were obtained between 2011 and 2019 by scuba diving at depths comprised from 25 m to 53 m. These new data came from 6 localities ranging from Capo Mortola, to the west, to Punta Manara, to the east: one locality (Portofino) is included in an nMPA, one (Capo Mortola) in an rMPA, two (Arenzano and Punta Manara) in SACs, while the remaining two localities (Albissola and Varazze) are deprived of any form of protection (Supporting Information). In all, 130 records came from scuba diving and 100 from ROV transects. Some localities (e.g. Arenzano and Porto Maurizio) and sites (e.g. Isuelina and Punta Sciusciau) have been surveyed through both scuba and ROV dives, reporting consistent information about *A. polypoides* occurrence.

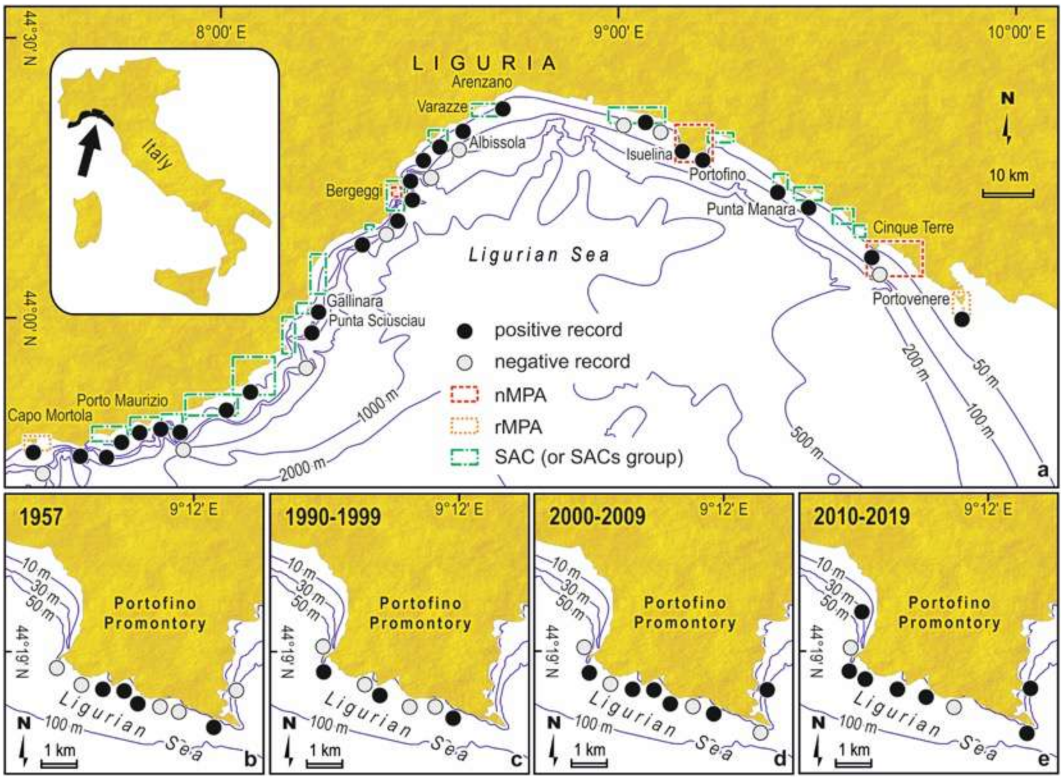

Fig. 1 Distribution of *Axinella polypoides* in Liguria: **a**) localities with positive or negative records along the whole coast; **b**, **c**, **d**, **e**) sites of occurrence at Portofino, the locality of its first historical record, in different time periods: 1957 (Tortonese, 1958); 1990-1997 (Gatti et al., 2017); 2000-2009 (Gatti et al., 2017); 2010-2019 (Enrichetti et al., 2019, 2020; Gatti et al., 2017; and unpublished data by C.N. Bianchi).

Bibliographic analysis indicated that records of *A. polypoides* in Liguria were scarce until the 1990s, and then increased rapidly after 2010 (Fig. 2). Several localities (e.g. Portofino, Gallinara, Punta Manara) have been revisited in different years and the occurrence of *A. polypoides* was always confirmed. At Portofino, in particular, *A. polypoides* was first reported in 1957 and repeatedly found again from 1990 to 2019 (Fig. 1b,c,d).

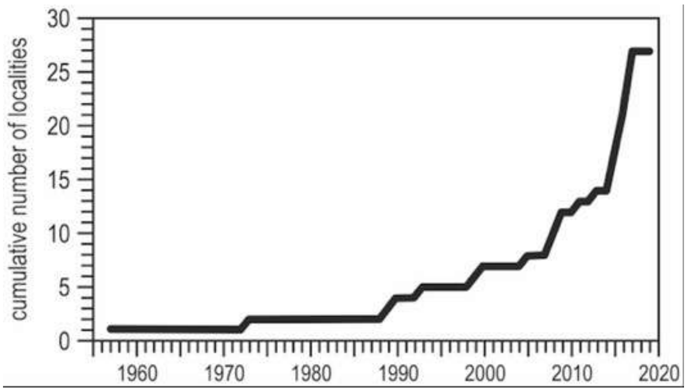

Fig. 2 Increased knowledge with time about the occurrence of *Axinella polypoides* in Liguria.

Bathymetric distribution ranged from a minimum of 15 m to a maximum of 81 m depth. Overall, the distribution was trimodal, with peaks around 38 m, 52 m and 79 m depth (Fig. 3a).

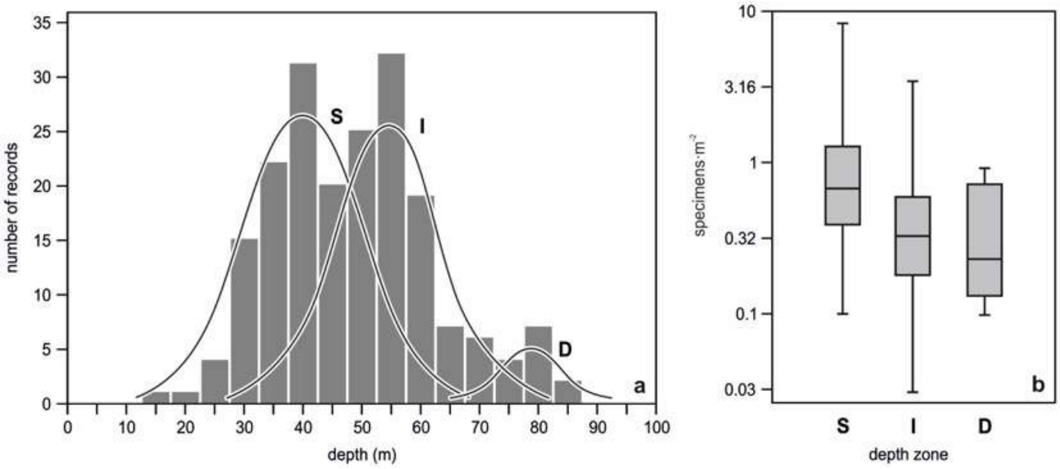

Fig. 3 Depth range of *Axinella polypoides* in Liguria: a) frequency of records by depth, with Kernel Density Estimation identifying three depth zones: S = shallow; I = intermediate; D = deep; b) relative abundance of specimens in the three depth zones (note logarithmic scale on y axis). Boxes depict the 25-75 percent quartiles, the horizontal line inside the box indicating the median; the whiskers represent minimal and maximal values.

Density data were available for 83 records (i.e. 36.1% of the total number of records). Density was greatest at shallow depths, where it could approach 10 specimens·m-2 (Fig. 3b). Although *A. polypoides* was occasionally abundant also below 50 m (Fig. 4d), there was a significant difference between the shallow and intermediate or deep zones (1-way ANOVA, p = 0.0372). However, density data for the deep zone were overall scarce.

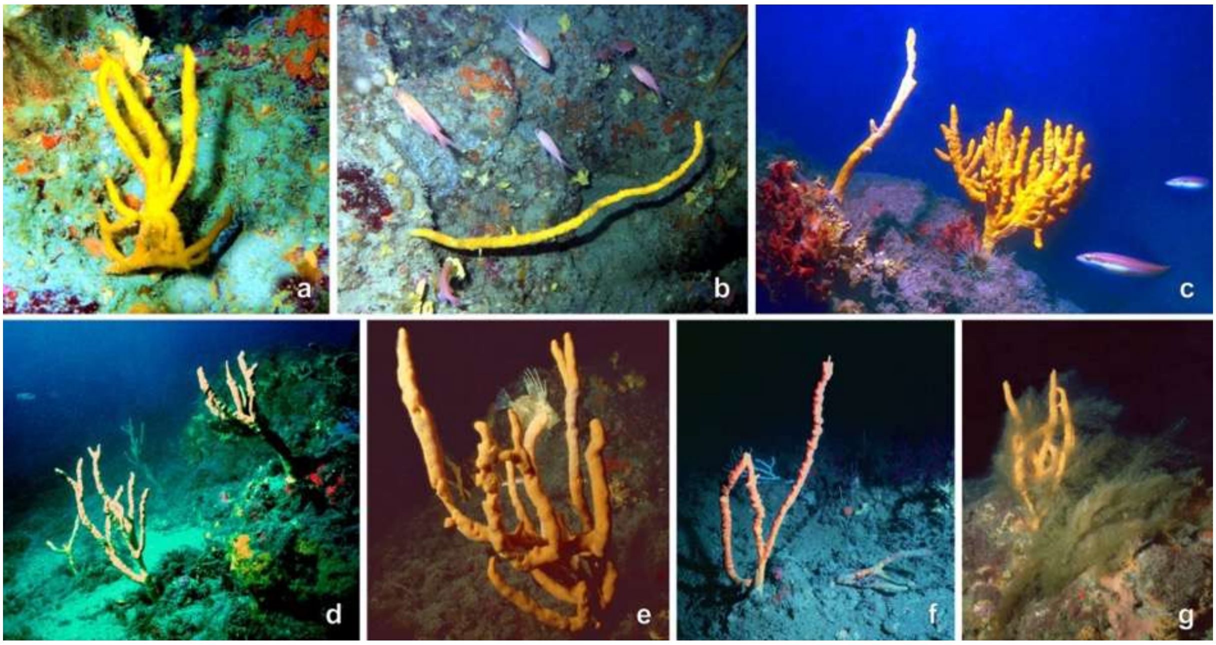

Fig. 4 Aspects of *Axinella polypoides* in Liguria. a) Bush-like growth form (Portofino, Isuelina, 47 m depth). b) Cane-like growth form (Portofino, Isuelina, 49 m depth). c) The two morphologies coexisting in close vicinity (Portofino, Altare, 52 m depth): note the different slopes of the substrate where the two specimens stay. d) A dense population at the toe of the coastal rock (Portofino, Torretta, 54 m depth). e) A juvenile of the fish Zeus faber finding refuge amidst the branches of a bush-like specimen (Gallinara, Punta Sciusciau, 36 m depth); f) Signs of mechanical damage on specimens growing on a detritic bottom (Portofino, Torretta, 56 m depth): note the regenerated tip of the largest specimen and a felled specimen on the right (a felled colony of the gorgonian *Eunicella verrucosa* is also visible in the background, and a derelict fishing line in the middle). g) A specimen entangled with mucilage filaments (Gallinara, Punta Sciusciau, 33 m depth).

In most cases, *A. polypoides* was found in the coralligenous biocoenosis, in association with the brown alga Cystoseira zosteroides (Turner) C. Agardh, 1821, the purple gorgonian Paramuricea clavata (Risso, 1826), or other large sponges, but might also form a monospecific facies (Fig. 5). It was also found on biodetritic bottoms with sparse rocks, together with the white gorgonian Eunicella verrucosa (Pallas, 1766). Both scuba and ROV observations evidenced that A. polypoides, thanks to its morphology and size, acted as a habitat former, providing spatial three-dimensionality and refuge for motile species and juvenile fish (Fig. 4e).

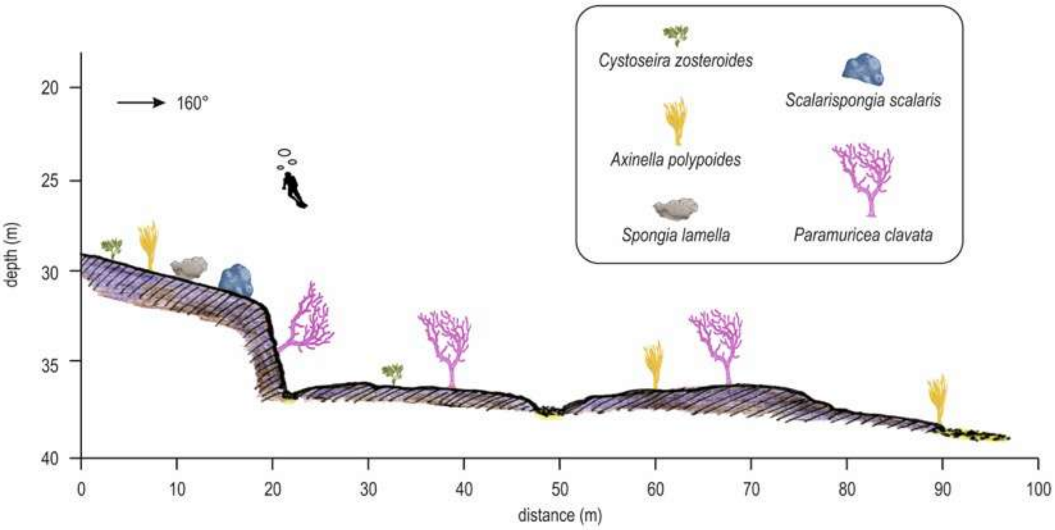

Fig. 5 Example of a scuba transect at Punta Manara shoals, with *Axinella polypoides* participating in different coralligenous facies. Species icons not to scale.

Especially in the shallowest depths, *A. polypoides* exhibited two different morphologies: a ‘bush-like’ growth form (Fig. 4a) and a ’cane-like’ growth form (Fig. 4b). However, morphological and spicule analyses of voucher samples confirmed that both growth forms were A. polypoides. The two growth forms often coexisted (Fig. 4c).

A. polypoides was recorded in nine out of the ten SACs considered, in both rMPAs, and only in two (Cinque Terre and Portofino) of the three nMPAs of Liguria (Fig. 6). At Bergeggi nMPA, it was observed just outside the boundary of the marine protected area but not inside (Fig. 7). In all, 22.6% of the records were in MPAs, 32.7% were in SACs (for which there are still no management plans) and the remaining 44.7% were in sites without any form of protection.

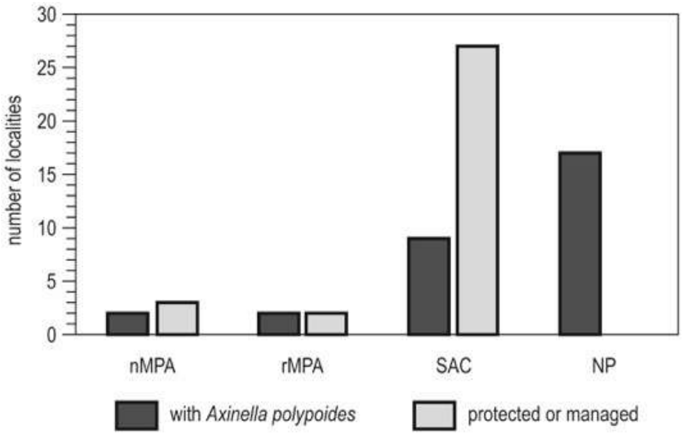

Fig. 6 Protection status of *Axinella polypoides* in Liguria. nMPA = national Marine Protected Area; rMPA = regional Marine Protected Area; SAC = Special Areas of Conservation; NP = no protection.

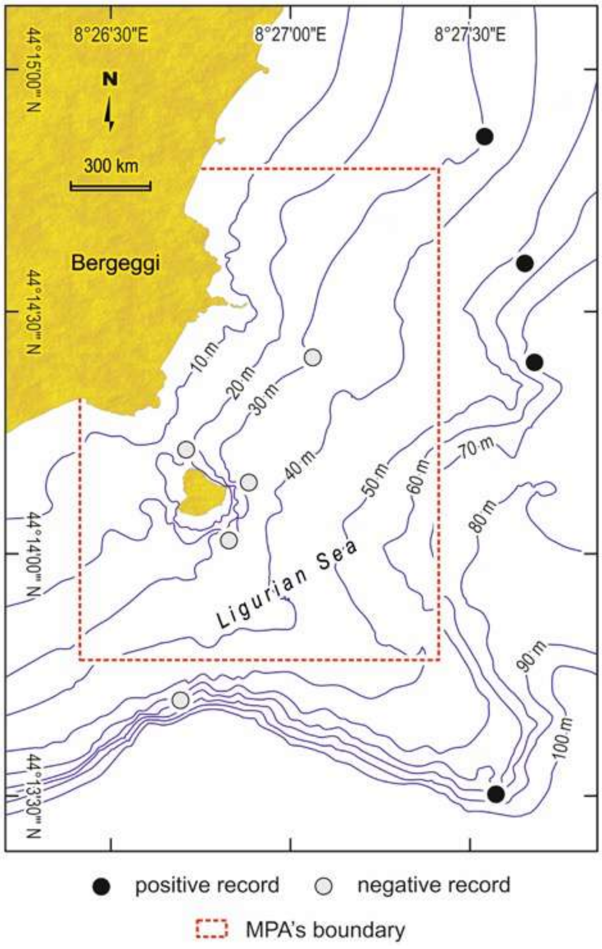

Fig. 7 Positive and negative records of *Axinella polypoides* inside and outside the boundaries of the Bergeggi nMPA (based on data in Bianchi et al., 2007, 2009; Enrichetti et al., 2019a; Parravicini et al., 2007; Rovere et al., 2008).

Independently of existing protection institutes, *A. polypoides* was found suffering by mechanical damage, either by anchoring or derelict fishing gears (Fig. 4f), and entanglement by mucilage filaments (Fig. 4g). No quantitative data are available about degree and intensity of damage.

DISCUSSION

Invertebrates inhabiting marine and freshwater ecosystems greatly contribute to global biodiversity and play a relevant role in the ecosystems functioning. However, their conservation status is often neglected in targeted aquatic conservation efforts. More than 30% of invertebrate species are considered ‘Data Deficient’, highlighting the lack of information for making such assessment (Collier, Probert, & Jeffries, 2016).

This is the case for *Axinella polypoides* in Liguria, where the species turned out widespread contrary to what was previously reported by Bianchi and Morri (1994). Despite the wealth of studies carried out since the 1950s on the marine biodiversity of Liguria (Bianchi et al., 1987), prior to the 1990s this species had been recorded only twice (Pansini & Pronzato, 1973; Tortonese, 1958), possibly due to lack of targeted searches. However, in a revision of the genus *Axinella*, based on material coming mostly from Liguria, Pansini (1984) did not mention it. *A. polypodes* was considered as a warm-water species scarce in the cold Ligurian Sea with respect to warmer Mediterranean sectors (Bianchi & Morri, 1994). Surprisingly, the abundance reported in this paper was comparable to what observed in the south-eastern Mediterranean Sea (Idan et al., 2018). Thus, and notwithstanding the species was assigned endangered status for Italy, newly collected data from Liguria confirm the ‘Least Concern’ status defined for Greece (Gerovasileiou et al., 2018). Under the hypothesis that *A. polypoides* was a thermophilic species, the increased number of records since the early 1990s, such as those coming from Portofino (i.e. the locality where it has been known for the longest time), was taken as a clue to recent spreading out in response to sea water warming (Parravicini et al., 2015); more probably it was simply the outcome of the increased popularity of scuba diving among scientists (Bianchi & Morri, 2000) and therefore of the increased number of observations. The recent development of ROV technology since the 2010s has exponentially increased our knowledge of *A. polypoides* occurrence in areas of

Liguria deeper than scuba depths (Enrichetti et al., 2019a, 2019b, 2020). Revisiting the same sites after many years evidenced that the occurrence of *A. polypoides* has remained stable for decades in protected areas (e.g. Portofino), pointing to the capacity of persistence – if undisturbed – of this long-lived species (Coppari et al., 2016); however, available quantitative data indicated that its population declined in localities deprived of any form of protection (e.g. Gallinara: Bianchi et al., 2018).

As observed in other Mediterranean regions, the species is a component of the coralligenous community (Bertolino et al., 2013; Coppari et al., 2016; Kefalas, Tsirtsis, & Castritsi-Catharios, 2003; Longo et al., 2018), where it occurs in different facies (Cánovas-Molinas et al., 2016; Enrichetti et al., 2019a, 2020). *A. polypoides* has been also reported from other habitats, such as rocks, rhodolith beds, soft substrata, caves and overhangs (Gerovasileiou et al., 2018), and mesophotic reefs (Idan et al., 2018).

Apparently, the bathymetric distribution of *A. polypoides* differs depending on the geographical region: it is reported from about 25 m to 190 m in Provence, France (Vacelet, 1976), from 66 m to 112 m in the Baleares, Spain (Santín et al., 2018), and from 0 m to 280 in the Aegean Sea (Gerovasileiou et al., 2018); Pansini and Pronzato (2009) stated it occurs from shallow waters to more than 300 m, but is commonly recorded around 30-40 m depth. As a whole, the shallowest occurrences were reported from southern Mediterranean regions (Bedulli, Bianchi, Zurlini, & Morri, 1986; Uriz, 1982; Voultsiadou, 2005a,b). For this reason, and considering the average depth (ca. 45 m) at which *A. polypoides* was observed in Liguria, Parravicini et al. (2015) argued that this sponge may be a stenothermal species which prefers the cooler but more stable deep waters to the warmer but seasonally variable shallow waters of the Ligurian Sea (Morri et al., 2017). Ligurian records confirm that *A. polypoides* avoids near-surface waters, with the shallowest record being at 15 m and the first peak of abundance around 38 m depth.

The bathymetrical distribution of *A. polypoides* in Liguria exhibited three peaks that mostly correspond to different geomorphological settings: the shallowest records were typically located at the coastal cliff toes, those at intermediate depths were on shoals in the inner shelf, and the deepest ones were on coarse sediment with sparse rocks in the outer shelf (Cattaneo Vietti et al., 2010).

Besides depth and geomorphology, substratum slope exerts a major influence on sponge distribution (Pansini, Morri, & Bianchi, 2000). In Liguria, *A. polypoides* specimens showed a bush shape (i.e. the one usually described in the literature) when settling on flat or gently sloping rock. A minority of specimens at comparatively shallow depths and on (sub)vertical cliffs, however, exhibited an unusual cane shape, which is reminiscent of the typical aspect of *Axinella cannabina* (Esper, 1794), with which it can be confused (Bertolino, Bo, Canese, Bavestrello, & Pansini, 2015; Uriz, 1982). Both *A. polypoides* and A. cannabina are examples of the so-called 'Facies with large and erect sponges' (SPA/RAC–UNEP/MAP, 2019), whose importance as habitat-providers makes the two of them important for biodiversity conservation (Koukouras, et al., 1996). Whilst the two species are well distinguished taxonomically, there is still a dearth of knowledge about their distribution and ecological traits (such as depth and substratum preference), which are of prime importance for species management and conservation (Costello et al., 2015). In the light of the present results, most records of A. cannabina in the Ligurian Sea (Cadeddu, Melis, Pronzato, & Manconi, 2013; Pansini & Longo, 2008; Tunesi, Agnesi, Di Nora, Molinari, & Mo, 2008) need confirmation: to date, the only validated record of this species is in the Eastern Ligurian Sea, within the rMPA of Portovenere (Cocito, Morganti, & Pansini, 2002).

Its erect (up to 80 cm high) and showy growth form makes *A. polypoides* susceptible to damage caused by human activities such as anchoring (Balduzzi et al., 1994), fishing (Otero et al., 2019), and collection by divers as a curio (Boudouresque et al., 1996). At Gallinara, the density of *A. polypoides* decreased tenfold between 1991 and 2016 due to the impact of anchoring and derelict fishing gears (Bianchi et al., 2018). In Ligurian nMPAs and rMPAs, mooring buoys have been deployed to avoid anchoring impacts, as done in other areas (Giglio, Ternes, Mendes, Cordeiro, & Ferreira, 2017, and references therein). In Liguria, however, most records of *A. polypoides* were outside MPAs; one third of the records were in SACs, which unfortunately do not have a management plan yet. Ligurian MPAs and SACs typically do not extend deeper than 50 m, so that a large proportion of the Ligurian *A. polypoides* populations, living deeper, remain exposed to the impact of fishing (Bo et al., 2014). In addition to direct human impacts, the species is suffering from entanglement by the filaments produced by mucilage blooms, probably a climate-related event (Bianchi et al., 2019).

Should the zoning of the existing MPAs and SACs be revised in the future, enlarging their boundaries must be recommended to include deeper areas, where *Axinella polypoides* is often represented, as especially illustrated in the example of Bergeggi nMPA. In the procedure of selecting, managing and monitoring MPAs, the successful protection of marine benthic invertebrates is not adequately considered. Less than 10% of the potential habitats of some major invertebrate phyla (Porifera, Cnidaria, Mollusca, Arthropoda and Echinodermata), is protected within MPAs. This mismatch could sometimes relate to a lack of knowledge (Greathead et al., 2020, and reference therein). Detailed information on the distribution of the species in need of protection and on habitat mapping are mandatory to develop effective environmental management plans, for either conservation or restoration (Gerovasileiou et al., 2019; Jetz et al., 2012). Studies for this purpose are particularly rare in the case of invertebrates, sponges in particular. Nevertheless, recent studies demonstrate that as far as a species has been considered rare, the increase in targeted studies may unveil a wider distribution (Jiménez-Alvarado, Meyers, Caro, Sealey, & Barker, 2020; Sloan, 2004). This is exactly what happened with *A. polypoides* in Liguria, which turned widespread in the present study. An accurate quantification of the negative records could hardly be done: in this study, however, the species was found in 26 out of 35 (i.e. 74%) of the Ligurian localities investigated; further surveys might therefore be justified in the nine localities where the species was not found. The increased knowledge about the species distribution may be useful to develop recommendations on how local and national governments can better protect the species (Jiménez-Alvarado et al., 2020). Sloan (2004) demonstrated that even an invertebrate species can be used as a flagship species to increase public awareness about marine conservation and to develop a positive attitude towards marine protected areas.

The habitat classifications produced by EUNIS (European Union Nature Information System, www.eea.europa.eu/data-and-maps/data/eunis-habitat-classification), a comprehensive system to harmonise habitat identification across Europe (Evans et al., 2016), and RAC/SPA (Regional Activity Centre for Specially Protected Areas), which provides the reference list of habitat types in the Mediterranean (SPA/RAC–UNEP/MAP, 2019), may help to establish those habitats that contain the species in need of protection. However, only RAC/SPA system describes a well-defined circalittoral 'Facies with *Axinella polypoides*' within the coralligenous habitat.

Integrated coastal management is a learning process in which decisions should be continuously reconsidered and revised so that decision making is not hampered by uncertainty (Angulo-Valdes & Hatcher, 2010). The example of *Axinella polypoides* in Liguria provides suggestive evidence that both the existing Marine Protected Areas and habitat classification schemes should be revised to accommodate new knowledge and consequently greater protection.

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SUPPORTING INFORMATION

Table S1 - Records of *Axinella polypoides* in Liguria from 1957 (year of the first record) to 2019, collated from scientific publications, technical reports and unpublished information. For each record, year, locality, site, coordinates, depth range (m), protection status, field survey and source are reported.

Year	Locality	Site	Lat. N	Long. E	Depth range (m)	Protection	Field survey	Source
1957	Portofino	Chiesa di San Giorgio	44°18'02.06"	9°12'40.00"	35	nMPA	scuba	Tortonese, 1958
1957	Portofino	L'Indiano	44°18'45.81"	9°10'10.16"	35	nMPA	scuba	Tortonese, 1958
1957	Portofino	Punta Carega	44°18'31.64"	9°10'37.89"	40	nMPA	scuba	Tortonese, 1958
1957	Portofino	Torretta	44°18'45.10"	9°10'03.40"	40	nMPA	scuba	Tortonese, 1958
1973	Bogliasco	Grotto	44°22'26.94"	9°04'06.67"	15	SCI	scuba	Pansini and Pronzato, 1973
1989	Portovenere	Tino Punta Bianca	44°01'44.87"	9°50'50.95"	27	rMPA	scuba	Bianchi et al., 2002
1990	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	25-31	SCI	scuba	Balduzzi et al., 1992
1990	Portofino	Paraggi Castello	44°18'38.40"	9°12'47.83"	20	nMPA	scuba	Gatti et al., 2017
1991	Gallinara	Punta Falconara	44°01'24.75"	8°13'33.96"	35	NP	scuba	Balduzzi et al., 1992
1991	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	24-40	SCI	scuba	Balduzzi et al., 1992
1992	Gallinara	Punta Falconara	44°01'24.75"	8°13'33.96"	35	NP	scuba	Balduzzi et al., 1992
1992	Gallinara	Punta Falconara S	44°01'24.75"	8°13'33.96"	38	NP	scuba	Balduzzi et al., 1992
1992	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	35-42	SCI	scuba	Balduzzi et al., 1992
1993	Portofino	Altare	44°18'19.74"	9°11'48.31"	47-49	nMPA	scuba	Gatti et al., 2017

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Year	Locality	Site	Lat. N	Long. E	Depth range (m)	Protection	Field survey	Source
1993	Punta Manara	Secche	44°14'57.78"	9°24'22.26"	30-33	SCI	scuba	Bianchi and Morri, 1994
1997	Portofino	Torretta	44°18'45.10"	9°10'03.40"	38	nMPA	scuba	Gatti et al., 2017
1998	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	40	NP	scuba	Bianchi et al., 2009
1999	Arenzano	Scogli della Torretta	44°23'16.30"	8°41'30.91"	38-42	SCI	scuba	Relini et al., 1999
2000	Cinque Terre	Capo Montenero	44°05'28.90"	9°44'23.28"	26	nMPA	scuba	Bianchi et al., 2009
2000	Portofino	Secca Gonzatti	44°18'31.64"	9°10'37.89"	50	nMPA	scuba	Gatti et al., 2017
2002	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	48	nMPA	scuba	Gatti et al., 2017
2002	Portovenere	Secca di Dante	44°01'59.73"	9°50'49.55"	25	rMPA	scuba	Bianchi et al., 2002
2002	Portovenere	Tinetto	44°01'23.07"	9°51'05.70"	20	rMPA	scuba	Bianchi et al., 2002
2002	Portovenere	Tino, Cala del Bunker	44°01'44.07"	9°50'58.92"	30	rMPA	scuba	Bianchi et al., 2002
2002	Portovenere	Tino, Punta Bianca	44°01'44.87"	9°50'50.95"	27	rMPA	scuba	Bianchi et al., 2002
2003	Portofino	Altare	44°18'19.74"	9°11'48.31"	50	nMPA	scuba	Bianchi et al., 2009
2003	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	47-50	nMPA	scuba	Gatti et al., 2017
2003	Portofino	Torretta	44°18'45.10"	9°10'03.40"	41	nMPA	scuba	Gatti et al., 2017
2005	Bergeggi	Scoglietti	44°14'53.00"	8°27'32.00"	30	NP	scuba	Parravicini et al., 2007; Rovere et al., 2008
2005	Bergeggi	Secca del Banano	44°14'36.00"	8°27'11.99"	45	NP	scuba	Rovere et al., 2008; Bianchi et al., 2009
2005	Bergeggi	Secca della Travacca	44°14'51.00"	8°27'42.00"	55	NP	scuba	Bianchi et al., 2007; Rovere et al., 2008
2005	Punta Manara	Secche	44°14'57.78"	9°24'22.26"	45	SCI	scuba	Rovere et al., 2006
2008	Capo Mortola	Canyon Pertuso	43°46'35.22"	7°32'21.18"	26	rMPA	scuba	Bianchi et al., 2009, 2010

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Year	Locality	Site	Lat. N	Long. E	Depth range (m)	Protection	Field survey	Source
2008	Portofino	Altare	44°18'19.74"	9°11'48.31"	48	nMPA	scuba	Gatti et al., 2017
2008	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	55	nMPA	scuba	Gatti et al., 2017
2008	Santo Stefano	Canyon Terra	43°48'46.44"	7°54'18.72"	35	SCI	scuba	Previati et al., 2009
2009	Capo Berta	Le Stelle	44°12'03.60"	8°25'55.20"	34-38	SCI	scuba	Previati et al., 2009, 2011
2009	Capo Berta	Scoglio dell'Ariete	43°52'46.96"	8°04'53.51"	50-56	SCI	scuba	Previati et al., 2009, 2011
2009	Gallinara	Punta Falconara S	44°01'24.75"	8°13'33.96"	32	NP	scuba	Bianchi et al., 2009
2009	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	32-33	NP	scuba	Bianchi et al., 2009
2009	Gallinara	Scoglio Giare	44°01'27.66"	8°13'23.09"	29	SCI	scuba	Bianchi et al., 2009
2009	Porto Maurizio	Le Gorgonie	43°51'06.19"	8°01'31.40"	34-40	SCI	scuba	Previati et al., 2009, 2011
2009	Porto Maurizio	Le Maldive	43°50'36.42"	8°01'33.38"	50-56	NP	scuba	Previati et al., 2009, 2011
2009	Portofino	L'Indiano	44°18'45.81"	9°10'10.16"	30	nMPA	scuba	Gatti et al., 2017
2010	Portofino	Paraggi Castello	44°18'38.40"	9°12'47.83"	16	nMPA	scuba	Gatti et al., 2017
2011	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	45-50	nMPA	scuba	Gatti et al., 2017
2011	Portofino	Paraggi Castello	44°18'38.40"	9°12'47.83"	20	nMPA	scuba	Gatti et al., 2017
2011	Varazze	Scogliera dei Forsé	44°20'38.76"	8°34'12.84"	33	NP	scuba	Present paper
2012	Bergeggi	Corallone	44°13'30.00"	8°27'32.40"	69-72	SCI	ROV	Enrichetti et al., 2019
2012	Portofino	Faro	44°17'13.20"	9°13'15.60"	77-80	SCI	ROV	Enrichetti et al., 2019,2020
2012	Punta Manara	Secche	44°14'57.78"	9°24'22.26"	40	SCI	scuba	Bertolino et al., 2013
2013	Albissola	Secca dei Forzieri	43°19'24.00"	8°31'53.00"	32	NP	scuba	Present paper

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Year	Locality	Site	Lat. N	Long. E	Depth range (m)	Protection	Field survey	Source
2013	Arenzano	Scoglietti di Lerone	44°23'11.72"	8°40'57.14"	32	SCI	scuba	Present paper
2013	Arenzano	Secca Due Balconi	44°23'11.72"	8°40'57.14"	43	SCI	scuba	Present paper
2013	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	54-55	nMPA	scuba	Gatti et al., 2017
2013	Portofino	Paraggi Castello	44°18'38.40"	9°12'47.83"	20	nMPA	scuba	Gatti et al., 2017
2014	Punta Manara	Secche	44°14'57.78"	9°24'22.26"	43	SCI	scuba	Present paper
2015	Arenzano	Scogli della Torretta	44°23'13.20"	8°41'42.00"	39-42	NP	ROV	Enrichetti et al., 2019, 2020
2015	Bogliasco	Zina	44°21'28.80"	9°03'57.60"	54-55	NP	ROV	Enrichetti et al., 2019
2015	Capo Mortola	Scogli	43°46'35.10"	7°32'19.80"	32	rMPA	scuba	Present paper
2015	Diano Marina	Scogli sparsi	43°52'55.20"	8°05'09.60"	50-52	SCI	ROV	Enrichetti et al., 2019, 2020
2015	Noli	Canyon	44°12'03.60"	8°25'55.20"	75	NP	ROV	Enrichetti et al., 2020
2015	Porto Maurizio	Scogli di Punta Chiappe	43°50'52.80"	8°00'36.00"	33-52	NP	ROV	Enrichetti et al., 2019
2015	Punta Baffe	Secca Rebuccian	44°13'44.40"	9°26'09.60"	54-70	NP	ROV	Enrichetti et al., 2019, 2020
2015	Punta Manara	Secche	44°14'45.60"	9°24'00.00"	42-61	SCI	ROV	Enrichetti et al., 2019
2015	Santo Stefano	Secca	43°47'45.60"	7°55'12.00"	60	NP	ROV	Enrichetti et al., 2019, 2020
2015	Vado Ligure	Scogli del Magazzino	44°15'41.04"	8°27'54.72"	67	NP	ROV	Enrichetti et al., 2019, 2020
2015	Varazze	Scogliera dei Forsé	44°20'24.00"	8°34'22.80"	39-44	NP	ROV	Enrichetti et al., 2019, 2020
2016	Bordighera	Scogliera dei Gronchi	43°46'12.00"	7°40'30.00"	71-81	NP	ROV	Enrichetti et al., 2019
2016	Bordighera	Capo di Sant'Ampelio	43°46'12.00"	7°40'30.00"	60	NP	ROV	Enrichetti et al., 2019
2016	Capo Mortola	Scogli	43°44'34.80"	7°33'07.20"	46	rMPA	ROV	Enrichetti et al., 2019

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Year	Locality	Site	Lat. N	Long. E	Depth range (m)	Protection	Field survey	Source
2016	Cinque Terre	Punta Mesco	44°07'58.80"	9°37'51.60"	52-58	nMPA	ROV	Enrichetti et al., 2019
2016	Gallinara	Punta Falconara S	44°01'24.75"	8°13'33.96"	30	SCI	scuba	Bianchi et al., 2018
2016	Gallinara	Punta Sciusciau	44°01'30.43"	8°13'47.71"	23	SCI	scuba	Bianchi et al., 2018
2016	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	39-60	nMPA	ROV	Enrichetti et al., 2019, 2020
2016	Portofino	San Rocco	44°20'13.20"	9°08'52.80"	34	NP	ROV	Enrichetti et al., 2019, 2020
2016	Punta Manara	Secche	44°14'45.60"	9°24'00.00"	60	SCI	ROV	Enrichetti et al., 2020
2016	Savona	Scogliera della Fortezza	44°16'49.80"	8°31'26.76"	46-56	NP	ROV	Enrichetti et al., 2019, 2020
2017	Albenga	Scogliera	44°01'34.50"	8°14'50.53"	59-60	NP	ROV	Enrichetti et al., 2019
2017	Arma di Taggia	Scogliera	43°48'13.82"	7°50'28.10"	47	NP	ROV	Enrichetti et al., 2019
2017	Bussana	Scogliera	43°48'11.88"	7°48'49.93"	45-59	NP	ROV	Enrichetti et al., 2019
2017	Finale Ligure	Scoglio del Campanile	44°09'22.58"	8°21'51.20"	78-81	NP	ROV	Enrichetti et al., 2019
2017	Gallinara	Punta Sciusciau	44°00'32.40"	8°13'33.60"	37-39	NP	ROV	Enrichetti et al., 2019
2017	Ospedaletti	Rosa di Scogli	43°46'03.22"	7°43'49.48"	57-66	NP	ROV	Enrichetti et al., 2019, 2020
2017	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	42-54	nMPA	scuba	Present paper
2017	Sanremo	Capo Nero	43°46'51.53"	7°45'34.06"	46-77	NP	ROV	Enrichetti et al., 2019, 2020
2017	Sanremo	Capo Verde	43°47'34.19"	7°47'35.27"	59-69	NP	ROV	Enrichetti et al., 2019, 2020
2019	Portofino	Grotta Gamberi	44°19'18.22"	9°08'44.73"	41	nMPA	scuba	Present paper
2019	Portofino	Isuelina	44°19'12.81"	9°08'40.12"	50-53	nMPA	scuba	Present paper
2019	Portofino	Secca Gonzatti	44°18'31.64"	9°10'37.89"	50	nMPA	scuba	Present paper

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1.3

Variability between observers in rapid visual assessment of coralligenous assemblages in the Marine Protected Area of Portofino (Ligurian Sea)

***Reference*:**

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Abstract

Coralligenous reefs are a habitat of high conservation value, which needs continuous monitoring for its management. Among the vast array of existing methods, visual criteria are to be preferred as they are not destructive and make immediately available the data collected. However, a problem often neglected is the variability between observers, which might blur the efficiency of habitat characterization and evaluation. Observers might differ for both their expertise (e.g., specialization) and experience (e.g., number of scuba surveys done or length of the scientific career). In this paper, we compare the output of vertical transects carried out by an expert diving scientist and a trainee on five coralligenous reefs in the Marine Protected Area of Portofino (Ligurian Sea). Both observers recorded on a diving slate the occurrence and semi-quantitative abundance of conspicuous epibenthic sessile species every 5 m of depth between 25 m and 50 m. Three main assemblages were recognized, discriminated mainly by depth: i) Association with algae such as *Zanardinia typus*, *Dictyota dichotoma* and *Dictyopteris polypodioides* at around 25-30 m depth; ii) Association with *Cystoseira zosteroides* at around 30 m depth; and iii) Facies with *Paramuricea clavata* at around 40 m depth. Differences between observers were always significantly lower than environmental variability, the latter being essentially due to species patchiness.

INTRODUCTION

Coralligenous reefs represent, in terms of extent, biodiversity and production, one of the most important coastal marine habitats in the Mediterranean Sea, capable of creating an extremely complex and diverse seascape (Ballesteros, 2006; Montefalcone et al., 2017), whose value is comparable to that of tropical coral reefs (Bianchi, 2001). Coralligenous reefs are a vulnerable ecosystem, due to its susceptibility to mechanical damage (caused for example by fishing gear) and to the slow growth rates of its component species (Ferrigno et al., 2017).

Despite its vulnerability and high conservation value, coralligenous reefs are not formally protected, being not included in the list of Sites of Community Importance (SCI) defined by the Habitats Directive (92/43/EEC). Only recently, in the framework of the Marine Strategy Framework Directive, they have been defined as a "special habitat": the assessment of its health status is considered a valuable contribution in defining the Environmental Status of Mediterranean marine regions or sub-regions (E.C., 2008). Coralligenous reefs have also been included in the European Red List of marine habitats, where they are classified as "data deficient", highlighting the need of surveys and accurate monitoring plans.

As for the type of surveying method to be adopted, the use of non-destructive techniques, which do not require the collection and sacrifice of specimens, are to be preferred and highly recommended for the study and the monitoring of valuable habitats such as coralligenous reefs (Bianchi et al., 2022). Rapid Visual Assessment (RVA) responds to this need, based on observations and measurements made directly underwater. This method allows for adequate taxonomic detail and for a comprehensive data collection, as it integrates topographical and ecological information (Gatti et al., 2015).

Although visual surveys are the most appropriate techniques in the study of coralligenous, they undoubtedly may have an inherent limit in the "observer effect": information collected by different diving scientists may be biased by their dissimilar expertise (e.g., specialization) and experience (e.g., number of scuba surveys done or length of the scientific career) possibly influencing sampling quality and data analysis (Azzola et al., 2022 and reference therein).

The aim of the present work is to evaluate observer effect in the characterization and evaluation of the coralligenous assemblages of Portofino Marine Protected Area (MPA).

MATERIALS AND METHODS

Portofino (MPA) covers 345 ha of sea in the Ligurian Sea (NW Mediterranean). The sea bottoms of Portofino MPA are mainly characterized by vertical and sub-vertical rocky cliffs that, below 20-25 m depth, host extensive coralligenous formations (Bavestrello et al., 2022).

Underwater surveys were carried out in five sites by two diving scientists, with different expertise and level of experience (one expert and one trainee). In each site, they carried out vertical transects of 4 m total width, every 5 m depth from 50 m to 25 m, also recording substratum slope. Along the vertical transects, the percentage cover of conspicuous species (i.e., species of a size that would allow their recognition and identification underwater) was visually estimated (Gatti et al., 2015, 2017) and directly recorded on a diving slate. The percentage cover of all the conspicuous species was organized in a data matrix (depth × slope × observer) × species to perform statistical analyses.

Different assemblages of the Portofino MPA coralligenous were recognised through Cluster Analysis and then assimilated and named according to the SPA/RAC habitat classification (SPA/RAC–UNEP/MAP, 2019; Montefalcone et al., 2021).

To highlight observer effect in the characterization of each assemblage, variability between observers was compared with environmental variability. The percent dissimilarity between the two diving scientists was measured by the following formula:

$$\text{Dissimilarity } \% = (1 - BC) \times 100$$

where BC is the Bray Curtis Index; environmental variability was estimated by subtracting the variability between observers from the overall variability within the assemblage. Differences between observer variability and environmental variability were evaluated by Student's t-test.

All the statistical analyses were performed by the free software PaSt (Hammer et al., 2001).

RESULTS

Three assemblages were identified (Fig. 1): i) one dominated by the brown algae *Zanardinia typus*, *Dictyota dichotoma*; and *Dictyopteris polypodioides*; ii) one dominated by the brown alga *Cystoseira zosteroides*; and iii) one dominated by the sea fan *Paramuricea clavata*. They closely match the SPA/RAC habitats MC1.513a (Association with algae, except Fucales, Laminariales, Corallinales and Caulerpales), MC1.512a (Association with Fucales or Laminariales), MC1.514b (Facies with Alcyonacea). All the habitats identified showed some typical coralligenous traits: MC1.513a, despite being dominated by algae, is also characterized by the presence of the sea fans *Paramuricea clavata* and *Eunicella cavolini*; while MC1.512a and MC1.514b, despite being dominated by two different species, are both characterized by a considerable abundance of encrusting corallinales (*Lithophyllum/Mesophyllum* complex).

The distribution of the three assemblages recognised in the coralligenous of Portofino MPA was mainly discriminated by depth, and secondarily by substratum slope: MC1.513a was distributed between 25 m and 40 m depth, and especially at around 30 m; MC1.512a occurred between 30 m and 40 m depth; and MC1.514b was widely distributed between 25 m (on sub-vertical substrates) and 50 m (on gently sloping substrates) peaking at 40 m depth. MC1.513a was located in the depth zone most prone to the colonization by the invasive alga *Caulerpa cylindracea* and the development of mucilaginous aggregates (*Acinetospora crinita*) (Fig. 1).

Within each assemblage, dissimilarity between the two observers was always significantly lower than environmental variability (Fig. 2). This may be due to species patchiness: for MC1.513a none of the species was present in all the observations made in that assemblage in different sites; for MC1.512a and MC1.514b only the dominant species (i.e., *C. zosteroides* and *P. clavata*, respectively) was recorded in all the relevant observations.

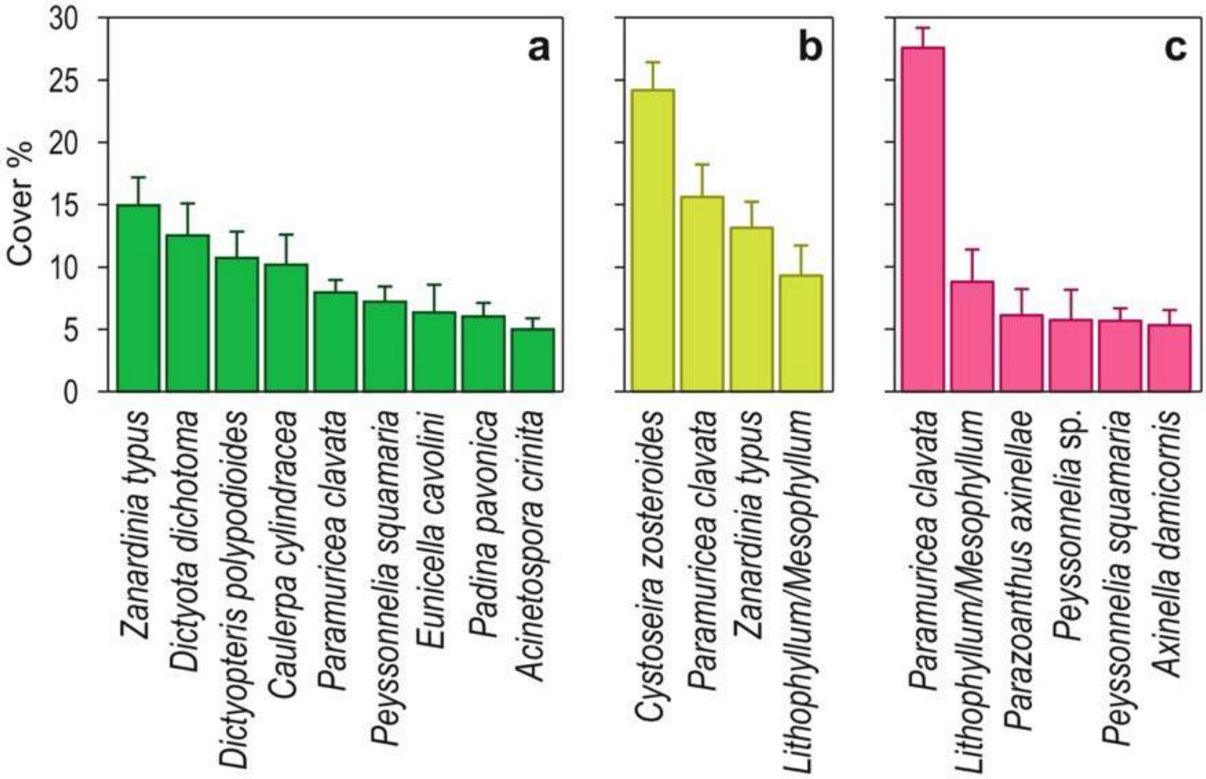

Fig. 1 Cover (%) of the most important species (i.e., species with a mean cover > 5%) of the three coralligenous assemblages identified with Cluster Analysis: **a)** MC1.513a Association with algae, except Fucales, Laminariales, Corallinales and Caulerpales; **b)** MC1.512a Association with Fucales or Laminariales; **c)** MC1.514b Facies with Alcyonacea.

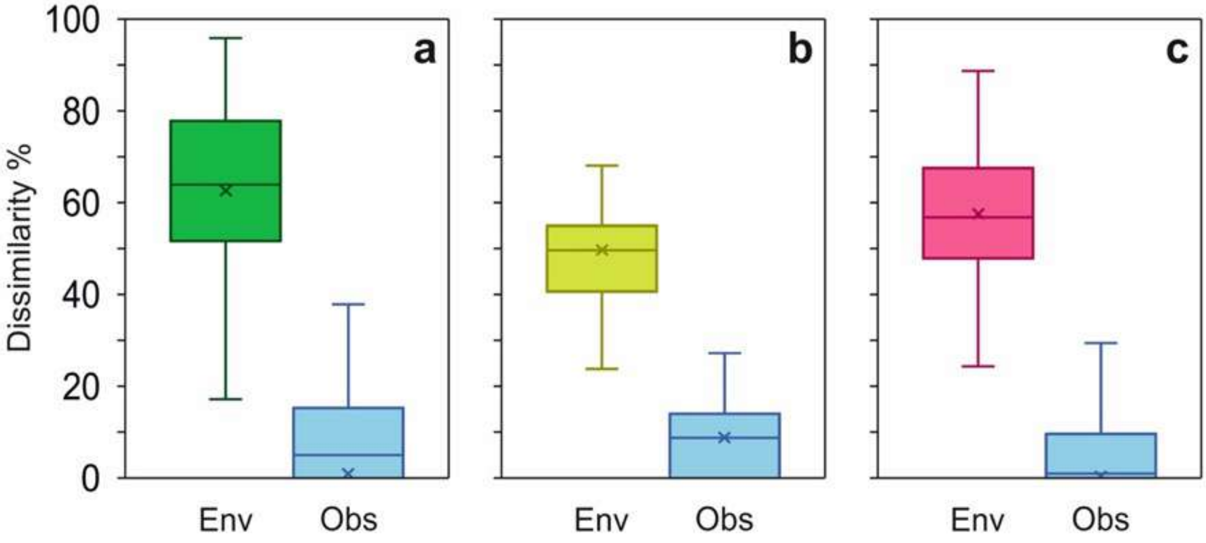

Fig. 2 Dissimilarity (%) due to the environmental variability (Env) was always significantly higher compared to the differences between observers (Obs): a) MC1.513a (p = 5E-24); b) MC1.512a (p = 7E-17); c) MC1.514b (p = 3E-88).

DICUSSION AND CONCLUSIONS

The rocky reefs of Portofino MPA have been the stage of one of the first experiences of study based on visual observations by diving (Tortonese, 1958, 1961; Bianchi and Morri, 2000), thanks to the unprecedented collaboration between the sport diver Duilio Marcante (1914-1985) and the natural scientist Enrico Tortonese (1911-1987). Nearly 70 years after, our study - however preliminary and partial - indirectly corroborates the results of that pioneer investigation. Gatti et al. (2017) were indeed capable of comparing the data of Marcante and Tortonese with later visual data collected in the 1980s through the 2000s to detect change over time and estimate the magnitude of such change, mostly linked to the climate shift of the 1980s-90s (Bianchi et al., 2019).

Although the present work involved surveys at only five sites by only two observers, the significance of the results suggested that the difference between observers may be negligible in the characterization and evaluation of coralligenous assemblages. Gatti et al. (2015) showed that the assessment of coralligenous state of health was not affected by the experience of different diving scientists who collected visual data. Field observations resulted objective and reliable, with the advantage of obtaining data immediately, without further time-consuming analyses of photos or videos in the laboratory (Bianchi et al., 2004). Similarly, in a study on the rocky reefs of Capo Carbonara MPA, conducted at two different times by four observers through visual surveys, it was shown that the observer effect did not hamper detecting change over time in the benthic communities under the effect of global and local pressures (Azzola et al., 2022).

Our result confirm that visual methods may be considered robust to observer bias, and should become part of the fundamental training of diving scientists. On the other hand, visual surveys protocols should always take into account the assessment of observer variability in the characterization and evaluation of coralligenous assemblages.

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Cold-water species mortality and warm-water species occurrence in a warmer Mediterranean Sea

In preparation:

Azzola A., Bianchi C.N., Morri C., Oprandi A., Mancini M., Montefalcone M.

Abstract

Due to the climate change, marine heatwaves, mass mortality events and the spread of invasive species are increasingly frequent, resulting in dramatic changes in marine communities structure and ecosystem functioning. The semi-enclosed nature of the Mediterranean Sea makes this basin particularly exposed to the increase of temperature. This paper presents the results of a three-year (2019 - 2021) monitoring of the effects of global warming on benthic rocky reef communities in four study areas located along the Italian coast. Following the protocol provided by the T-MEDNet monitoring network, temperature data along the water column were collected by placing data loggers on rocky reefs. Ecological status of benthic communities was evaluated by applying diversity indices. Moreover, data on indicator species of climate change (i.e., species vulnerable to water warming and thermophilic species) were collected and compared amongst the four different areas. Abnormally high temperature and the effects of global warming were observed in all the study areas. In addition, where historical data were available, a significant change in benthic communities structure was observed. These findings are consistent with many other studies conducted in the Mediterranean Sea, highlighting the urgent need for global actions to mitigate the effects of climate change.

INTRODUCTION

The first evidence of global warming linked to anthropogenic activities dates back to the 1970s, and today the increasing trend of ocean temperatures is widely documented (von Schuckmann et al. 2019). Despite sea surface temperature is the most used parameter to analyse this phenomenon (Pisano et al., 2020), climate change also affects deep water temperature, and in situ measurements by placing data loggers on the sea bottom are needed (Ceccherelli et al., 2020; Garrabou et al., 2022).

The semi-enclosed nature of the Mediterranean Sea makes it highly vulnerable to rising water temperature, to the point that its region has been identified as a ‘‘hotspot’’ for climate change (Group T.M.M. et al. 2011). A direct consequence of climate change is an increase in the frequency of prolonged periods of elevated temperatures, called “heat waves” (Frölicher et al., 2018).

In the Mediterranean Sea, since the 1990s, the heat waves have been associated with a rapid increase in mass mortality events of benthic species (Garrabou et al., 2009). Depending on the biological characteristics of species (e.g., longevity, size, reproductive rates), they may exhibit different levels of sensitivity to environmental stress (Santangelo et al., 2015; Montefalcone et al., 2017). However, if stress persists for long time, it generally leads to mortality of entire populations. In the Mediterranean Sea, during the 2015-2019, between 40% and 75% of gorgonians, sponges, molluscs and bryozoans populations were subject to mortality (Garrabou et al., 2022).

The rise in yearly mean temperatures and the mitigation of the winter temperatures have greatly favoured the distribution and dispersal of invasive species across the Mediterranean basin (Osland et al., 2021). Thermophilic native species, which usually inhabit southern areas, have become stable in colder northernmost sectors of the Mediterranean; and tropical species are to date abundant (Bianchi et al., 2019). These species are generally more adaptable to different environmental conditions and stronger in competition with native species, which have often been replaced.

The loss of native species and the expansion of thermophilic ones can result in drastic changes in community structure and ecosystem functioning (Doney et al., 2012). Rocky reefs in a good ecological status have been found to be more resilient to the impacts of climate change. Stable communities are better able to recover after species mortality events and are better able to tackle invasion by invasive species (Strauss et al., 2006).

This paper presents the results of a three-year (2019 - 2021) monitoring of the effects of global warming on the rocky reef benthic communities in four study areas located along the Italian coast (Portofino MPA, Elba Island, Capo Carbonara MPA, and Plemmirio MPA). Data on water column temperature, ecological status of rocky benthic communities, and indicator species of climate change (i.e., species vulnerable to water warming and thermophilic species) are compared between the different study areas. In addition, where historical data were available, change over time is assessed.

MATERIALS AND METHODS

Study area

Four areas of the North-Western Mediterranean Sea located at different latitudes, along the Italian coast, and subjected to different levels of protection were monitored: Portofino Marine Protected Area (MPA) in northern Italy (44°18’44’’ N, 9°10’23’’ E), Elba Island in central Italy (42°49’30’’ N, 10°09’09’’ E), Capo Carbonara MPA (39°05’32’’ N, 9°31’32’’ E) and Plemmirio MPA (37°00’06’’ N, 15°20’09’’ E) in southern Italy (Fig. 1).

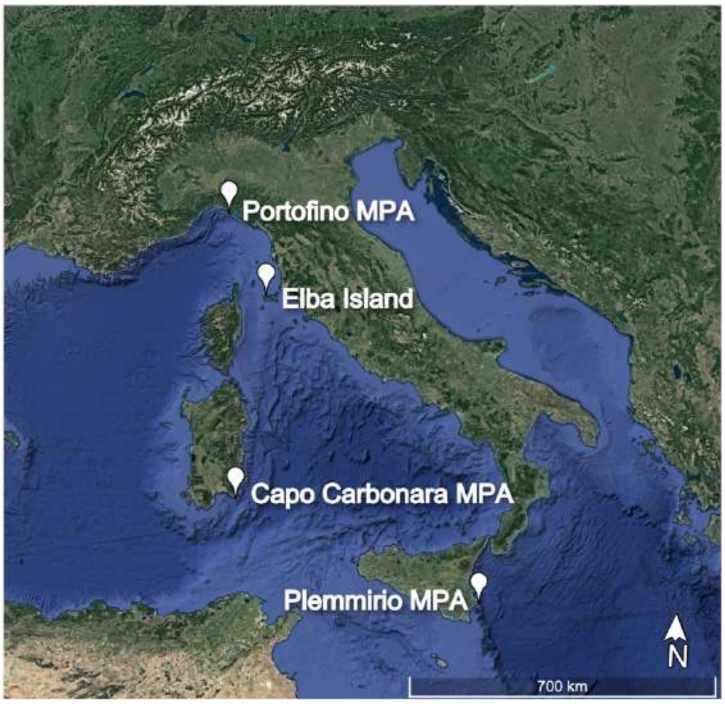

Fig. 1 Map of Italy with the location of the study areas

Temperatures data management

Seawater temperatures monitoring was conducted following the protocol provided by the T-MEDNet network (www.t-mednet.org), which is a long-term collaborative initiative dedicated to building a pan-Mediterranean observation network on the effects of climate change in marine coastal ecosystems (www.t-mednet.org). The T-MEDNet protocol consists of high-frequency (hourly) data collection obtained using HOBO data loggers (accuracy ±0.21°C) placed at standard depths along rocky reefs, every 5 m from the surface to 40 m depth (Garrabou et al., 2009, 2019, 2022). Data collected were processed by the software Ocean Data View (www.odv.awi.de), which provides a graphical output by which temperature trends over time in relation to different depths can be visualized through a colour scale. Temperature data were available: from 17 September 2019 to 9 September 2022 for the Portofino MPA, from 26 November 2019 to 22 October 2022 for Elba Island, from 8 September 2020 to 19 October 2022 for Capo Carbonara MPA, and from 10 September 2020 to 10 November 2022 for the Plemmirio MPA.

Field activities and data collection

In each study area, data were collected during the summers of 2020 and 2021 in three different sites by underwater visual surveys. In each site vertical transects of variable length (depending on the maximum depth of individual sites) and 4 m total width were conducted from the bottom to the surface to characterize the benthic communities and to assess their ecological status. The semi-quantitative abundance (based on three scores: 1 = scarce; 2 = abundant; 3 = very abundant; with absence being 0) of conspicuous species (i.e., species of a size that would allow their recognition and identification underwater) was recorded, with reference to the depth where they had been observed (Gatti et al., 2017).

Along the vertical transects, presence and abundance data on indicator species of climate change (i.e., thermophilic species) were also recorded. Thermophilic species were classified into three categories: alien species, cryptogenic species (i.e., species for which their origin cannot be defined with certainty), and native species. The list of indicator species has been adapted according to different study areas.

Mortality events were monitored following the protocol provided by the T-MEDNet. The total number of individuals/colonies of some target species (e.g., *Cladocora caespitosa*, *Eunicella cavolini*, *Eunicella singularis*, *Paramuricea clavata*) and the percentage cover of encrusting corallines, was recorded. Of the total number, the percentage of individuals/colonies (or cover for encrusting corallines) displaying signs of recent mortalities (such as denuded skeletons in gorgonians, signs of bleaching for calcareous organisms and empty valves in bivalves) was calculated. According to the T-MEDNet protocol, an individual/colony is considered affected by mortality when it showed recent tissue necrosis over 10% of its surface (Garrabou et al., 2009, 2019, 2022).

Community analysis

Conspicuous species recorded and their relative abundances collected along vertical transects were organized into (site × depth) × species matrix to characterize the composition of rocky reef benthic communities of study areas.

Ecological status of each community was evaluated applying two diversity indices: Shannon Index, to measure the diversity, and Simpson Index, to measure the dominance among the species. All the analyses were performed by the software PaSt (Hammer et al., 2001).

Change over time data management

The analysis of change over time in benthic communities structure was conducted by comparing historical data available for Portofino MPA (Gatti et al., 2017), Elba Island (unpublished data), and Capo Carbonara MPA (Azzola et al., 2022). For each study area, data were organised in a (time × depth) × species matrix. Based on the availability of historical data, four time periods were identified for Portofino MPA (i.e., 1950s, 1990s, 2010s, and 2020s) and Elba Island (i.e., 1990s, 2000s, 2010s, and 2020s) and two time periods (2000s and 2020s) for Capo Carbonara MPA. To evaluate the change as a function of depth gradient, four depths were considered: 10 m, 20 m, 30 m, and 40 m. A non-metric multidimensional scaling (nMDS) based on Euclidean Distance was applied using the software PaSt (Hammer et al. 2001). Centroids of each period at the four depths and trajectories of change were graphically illustrated on the nMDS plot.

RESULTS

Water column temperature

Temperature monitoring of the Portofino MPA and Elba Island highlighted abnormally high temperatures in June 2020 affecting the water column down to about 20-25 m with temperatures of 20°C (Fig. 2, 3). A second particularly hot period was detected in all the areas in July and August 2022 affecting the water column down to about 15 m with temperature of 26-27°C (Fig. 2, 3, 4, 5).

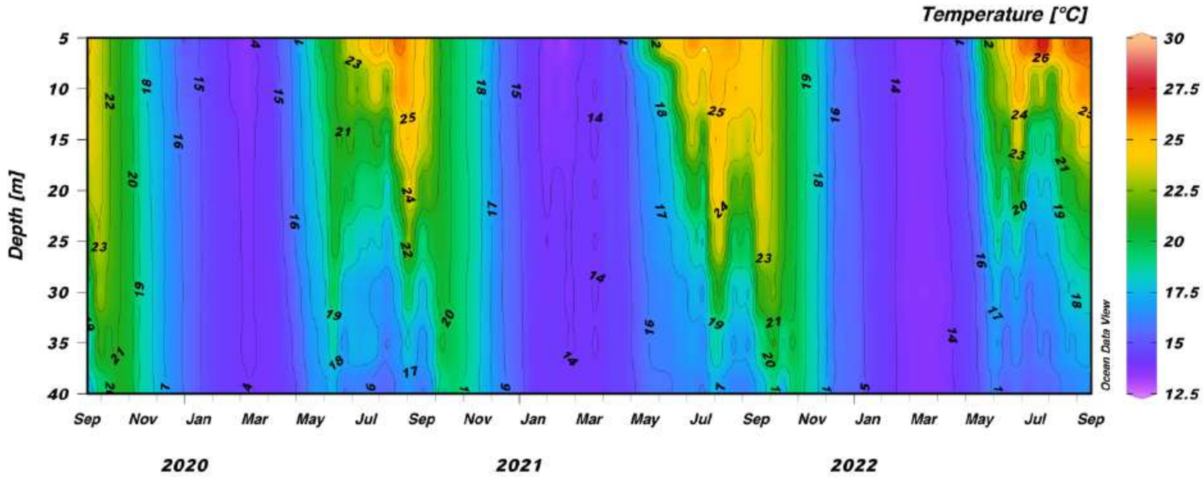

Fig. 2 Temperature (°C) trend along the water column of the Portofino MPA. Graphical output processed by Ocean Data View software

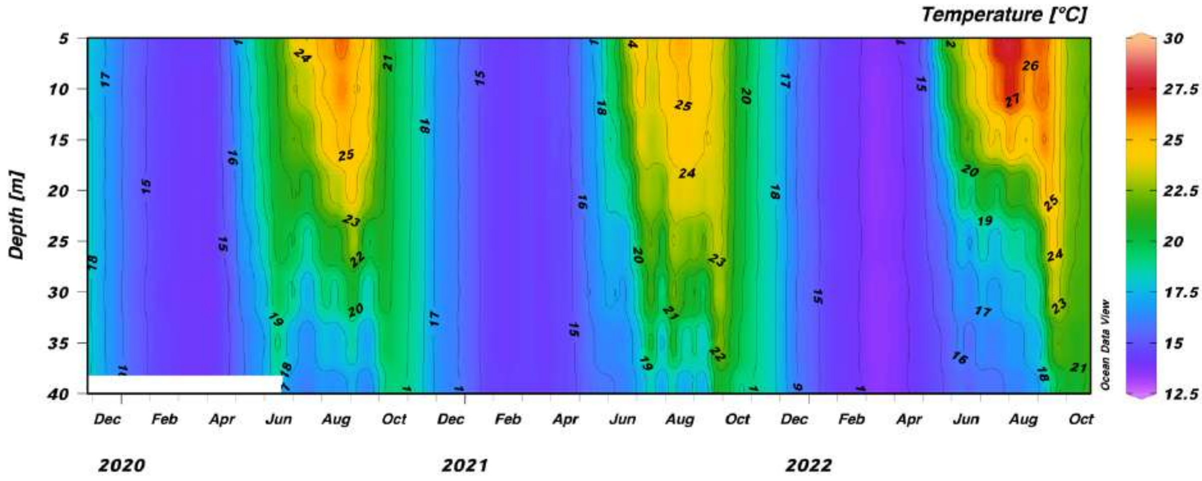

Fig. 3 Temperature (°C) trend along the water column of the Elba Island. Graphical output processed by Ocean Data View software

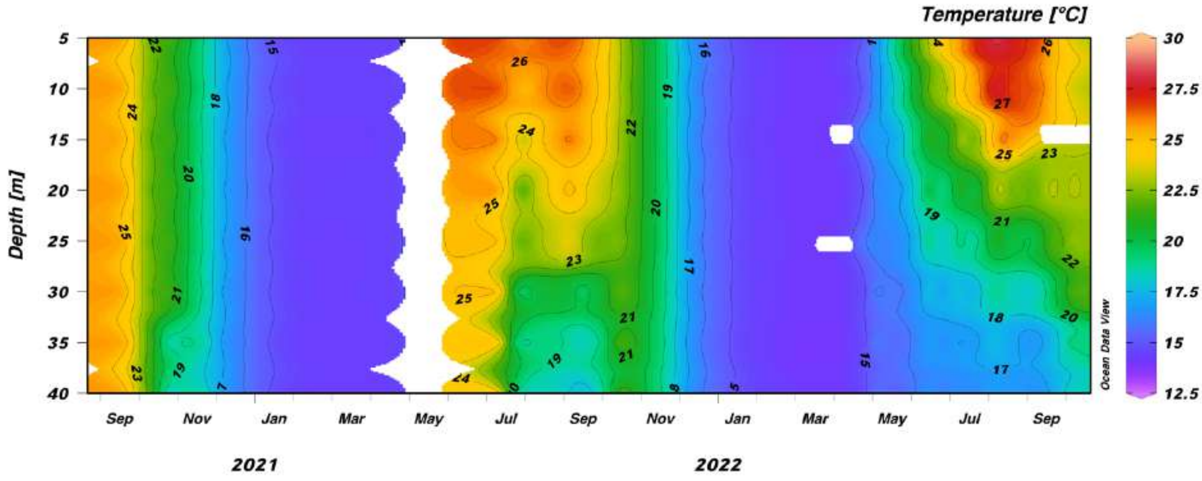

Fig. 4 Temperature (°C) trend along the water column of the Capo Carbonara MPA. Graphical output processed by Ocean Data View software. White areas represent missing data

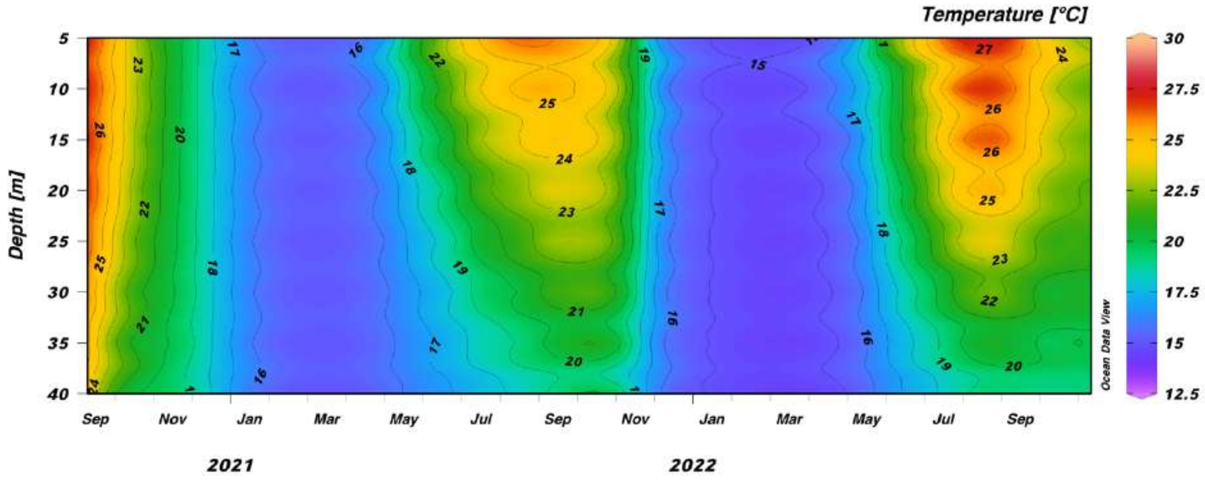

Fig. 5 Temperature (°C) trend along the water column of the Plemmirio MPA. Graphical output processed by Ocean Data View software

Benthic community composition and ecological status

Capo Carbonara MPA was the area with the highest values of biodiversity in terms of number of species (N=82), followed by Plemmirio MPA (N=71) and Elba Island (N=70), while Portofino MPA showed the lowest value (N=38). For all the benthic communities, the most represented groups in terms of number of species are algae, porifera, cnidarians, and bryozoans (Fig. 6).

Shannon Index values indicated a high ecological status for the Capo Carbonara MPA, and a good ecological status for Elba Island and Plemmirio MPA. A moderate ecological status, on the other hand, was obtained for the Portofino MPA (Fig. 7a). Simpson Index values indicated that the communities, although rich, are strongly dominated by few species and, consequently, may be less resilient (Fig. 7b).

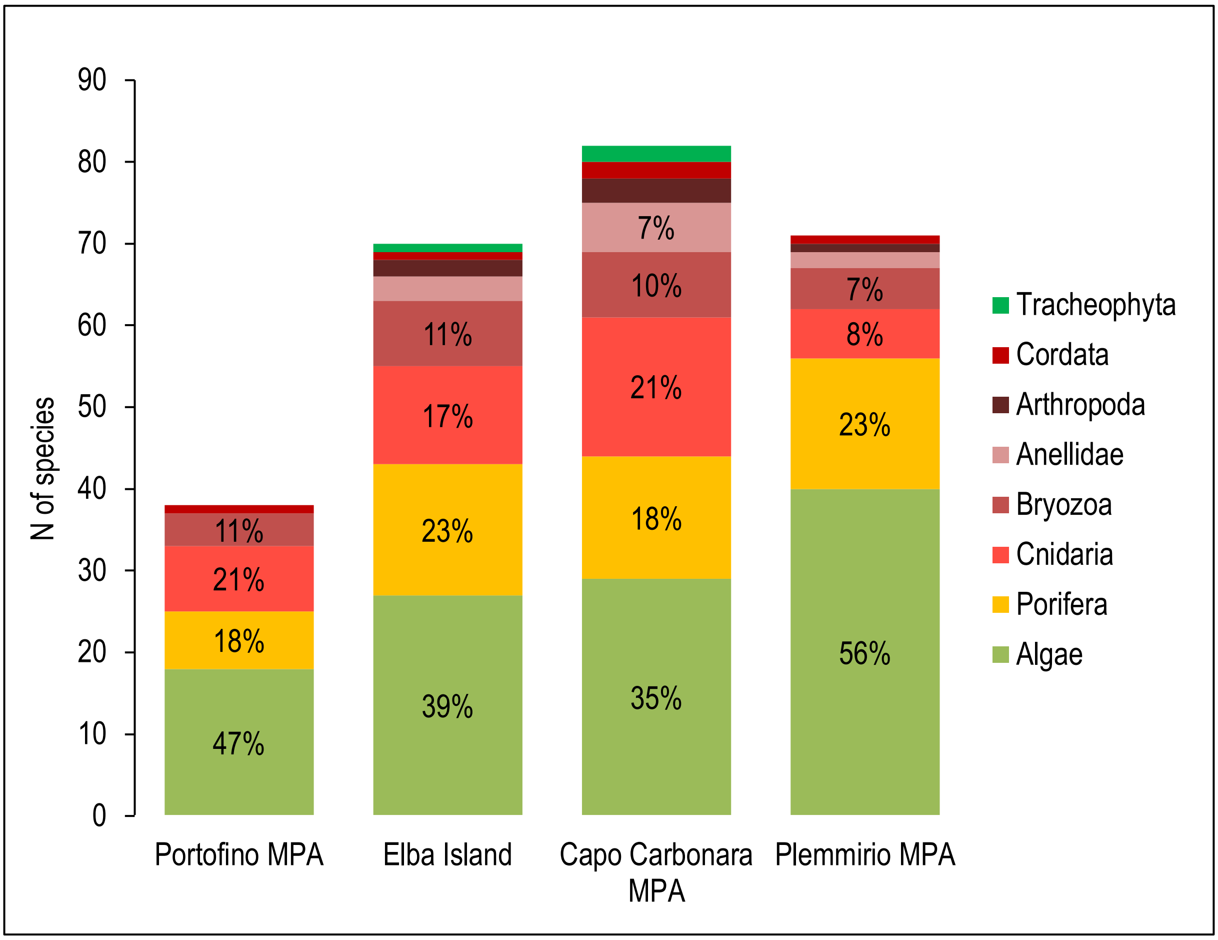

Fig. 6 Number of species for each phylum that compose benthic communities of the study areas

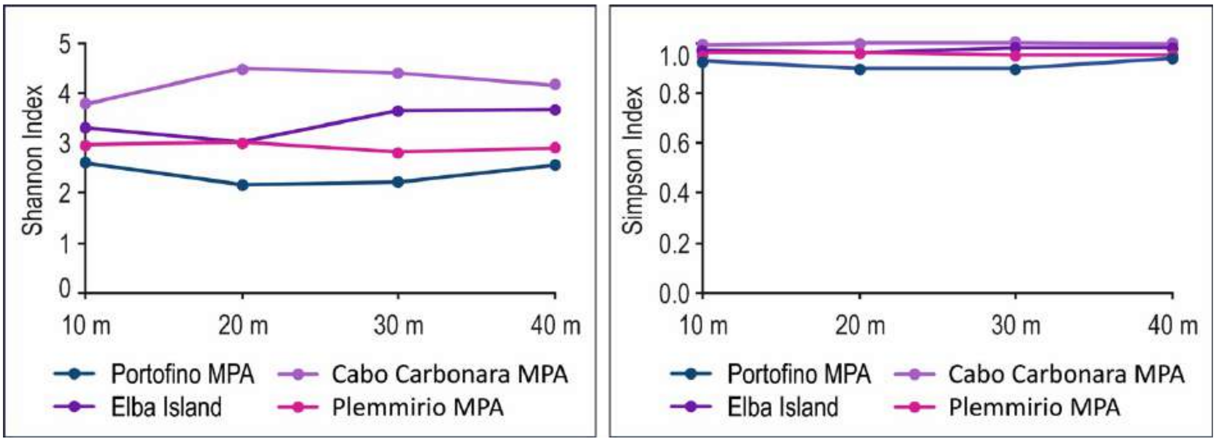

Fig. 7 Shannon (**a**) and Simpson (**b**) values at the four depths monitored in the four study areas

Mortality assessment

Portofino MPA resulted the area where the lowest impact values were recorded for both cnidarian species and calcareous algae. In Capo Carbonara MPA and Elba Island the greatest impacts on gorgonians were recorded: 20-30% of the monitored colonies of *Eunicella cavolini* and *Eunicella singularis* showed signs of necrosis. Moreover, in Capo Carbonara MPA the 50% percent of colonies of *Paramuricea clavata* were affected. *Cladocora caespitosa* resulted the most impacted species with mortality values between 60-100% recorded in three of the four monitored areas (Fig. 8). For calcareous algae, the greatest signs of impact were observed in Capo Carbonara MPA, where 65% of the monitored area of encrusting algae were bleached.

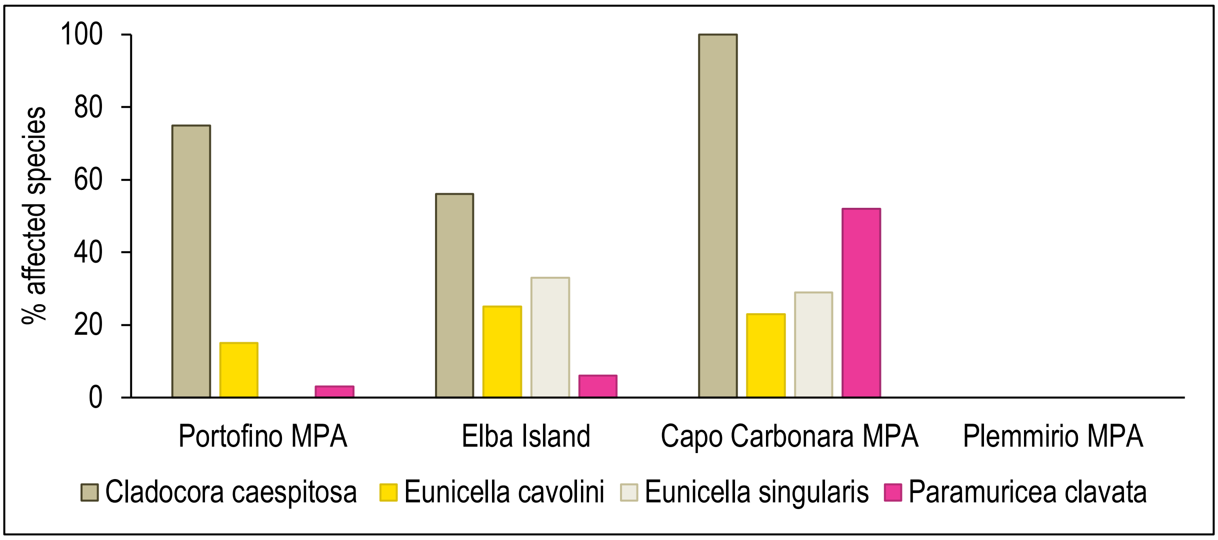

Fig. 8 Percentage (%) of Cnidarian colonies reporting signs of necrosis or bleaching in the four study areas

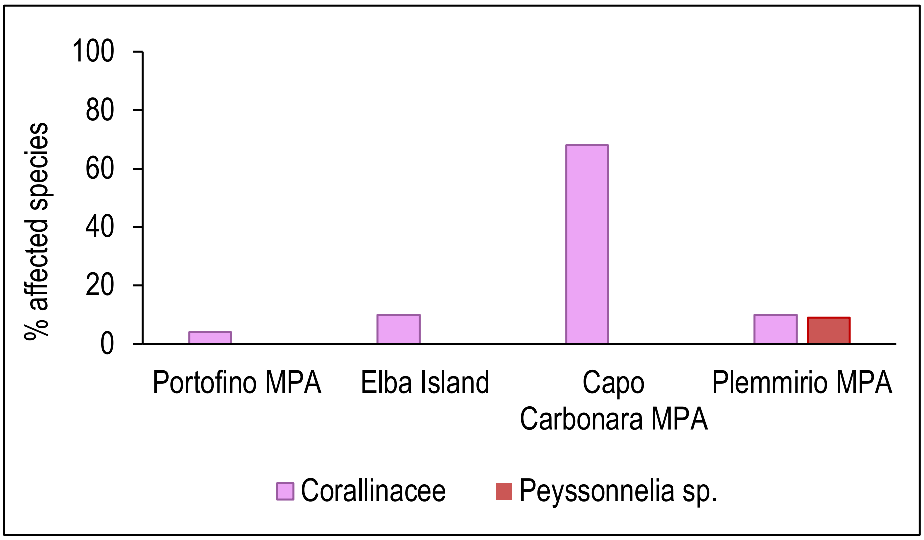

Fig. 9 Percentage (%) of bleached calcareous algae in the four study areas

Termophilic species

Data on thermophilic species showed a general latitudinal gradient with greater abundances from north to south. A greater presence of thermophilic species was observed in the Capo Carbonara and Plemmirio MPAs, where native thermophilic species naturally abound; but several alien species (from the Red Sea and the Atlantic Ocean) were also recorded. Elba Island and Portofino MPA were the areas with the lowest number of thermophilic species, according to the increasing latitudinal gradient (Fig. 10).

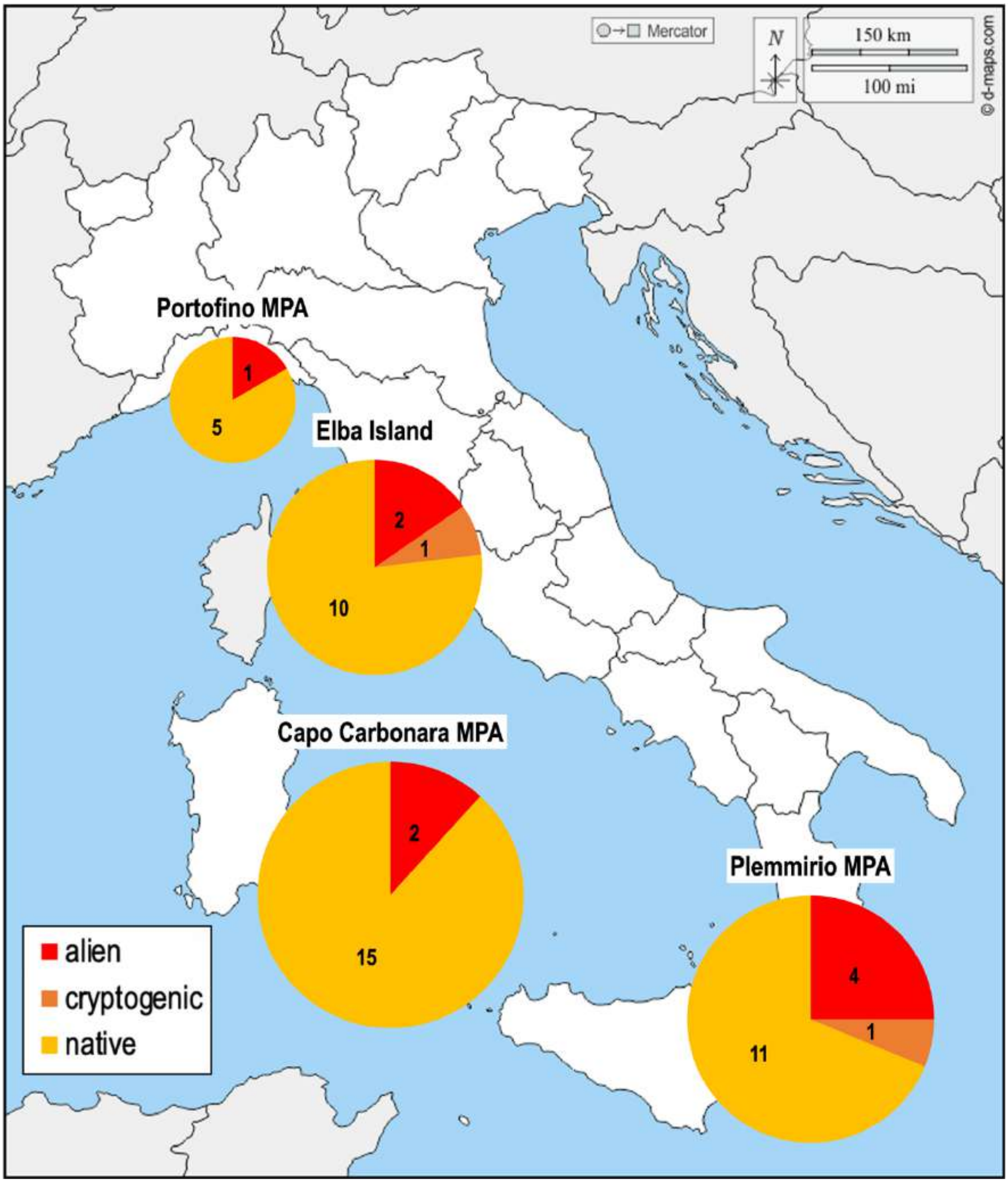

Fig. 10 Number of alien, cryptogenic and native species recorded in each study area.

Change over time

As already observed by Gatti et al. (2017), since the 1950s the structure and composition of the Portofino MPA benthic communities have significantly changed at all depths monitored (Fig. 11a). A strong change was observed between the 1950s and the 1990s, and a further marked change occurred between the 2010s and the 2020s (Fig. 11a).

Multivariate analysis (nMDS) applied to the data matrix of the benthic community of Elba Island showed an irreversible change for the stations at 10 m and 20 m and a clear signal of return to the initial condition for the station at 40 m. At 30 m depth the community seemed to be returning to the initial condition in the 2010s, but the 2020s data show a new change in direction showing again a probably irreversible change, in the benthic community (Fig. 11b).

Multivariate nMDS analysis applied to the data matrix of the benthic community of Capo Carbonara MPA showed that the structure of rocky reef communities has changed at all depths investigated in the last 20 years (Fig. 11c).

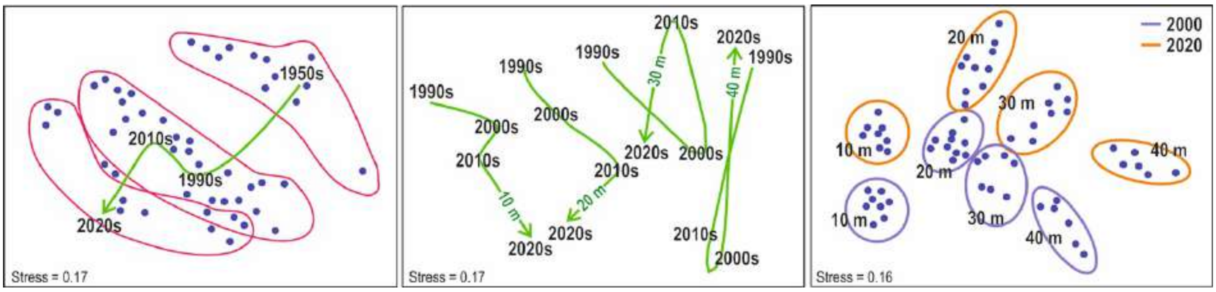

Fig. 11 nMDS plot of (**a**) Portofino MPA, (**b**) Elba Island, and (**c**) Capo Carbonara MPA

DISCUSSION AND CONCLUSION

Multidecadal time series of water temperature data are essential to detect climate change signals (such as thermal anomalies) and impacts on marine ecosystems (Ducklow et al., 2009). Time and spatial surveys can be used to evaluate temporal trends and empirical relationships between biological variables and environmental forcing (Doney et al., 2012). Our water temperatures data, from only three years of monitoring, are obviously not representative for the Mediterranean setting and do not allow for the detection of thermal anomalies in absence of a multidecadal data series. However, the high temperature values detected during the summers of 2020 and 2022 provide further insights in the detection of thermal anomalies of the Mediterranean Sea (www.t-mednet.org).

Among the four areas surveyed, Capo Carbonara MPA showed the highest biodiversity value, followed by Plemmirio MPA, Elba Island and Portofino MPA, where the lowest number of species was recorded. This is obviously consistent with the results of Shannon Index, which highlighted a high ecological status for Capo Carbonara MPA, a good ecological status for Plemmirio MPA and Elba Island, and a low ecological status for Portofino MPA. However, results of Simpson Index showed a high dominance value for all the study areas, highlighting a low resilience to disturbance of their benthic communities. Low value of equitability makes a community more prone to loss of biodiversity (in the case of disappearance of the least abundant species) and ecosystem functioning (in the case of disappearance of keystone species) (Doney et al. 2012). The low biodiversity value obtained for Portofino MPA and the high dominance values of all the study areas confirmed that local scale conservation measures (such as the establishment of MPAs) are not sufficient to maintain a good ecological status of marine ecosystems under a climate change scenario.

No mass mortality events comparable to those occurred in the 1990 and 2003 were observed during the three years monitoring (Cerrano et al., 2000; Garrabou et al., 2009). However, bleaching and necrosis signs attributable to the effect of water warming were observed in all the study areas for target species. Analyses conducted on thermophilic species revealed a latitudinal gradient consistent with temperature data. In the southernmost Capo Carbonara and Plemmirio MPAs the greatest number of thermophilic species were recorded. Native thermophilic species are naturally abundant in these areas; however, their potential increase and the spread of alien species could lead to an impoverishment of native communities (Occhipinti-Ambrogi, 2007). Several thermophilic species were also unexpectedly observed at Elba Island and in the Portofino MPA.

Historical data series on benthic communities is a valuable tool to understand the extent of the impact of global warming on marine ecosystems (Gatti et al. 2015). Comparison with historical data available from the 1950s for the Portofino MPA, from the 1990s for the Elba Island area, and from the 2000s for the Capo Carbonara MPA allowed to assess the change over time in the structure and composition of benthic reef communities due to the increasing temperature occurred in recent decades.

Our finding unveiled effects of climate change in all study areas, regardless of different latitude and level of protection. Proper management of local human pressures should be accompanied by synergic global actions that have become urgent to mitigate the effects of climate change on marine ecosystems.

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2.2

Troubles never come alone: outcome of multiple pressures on a temperate rocky reef

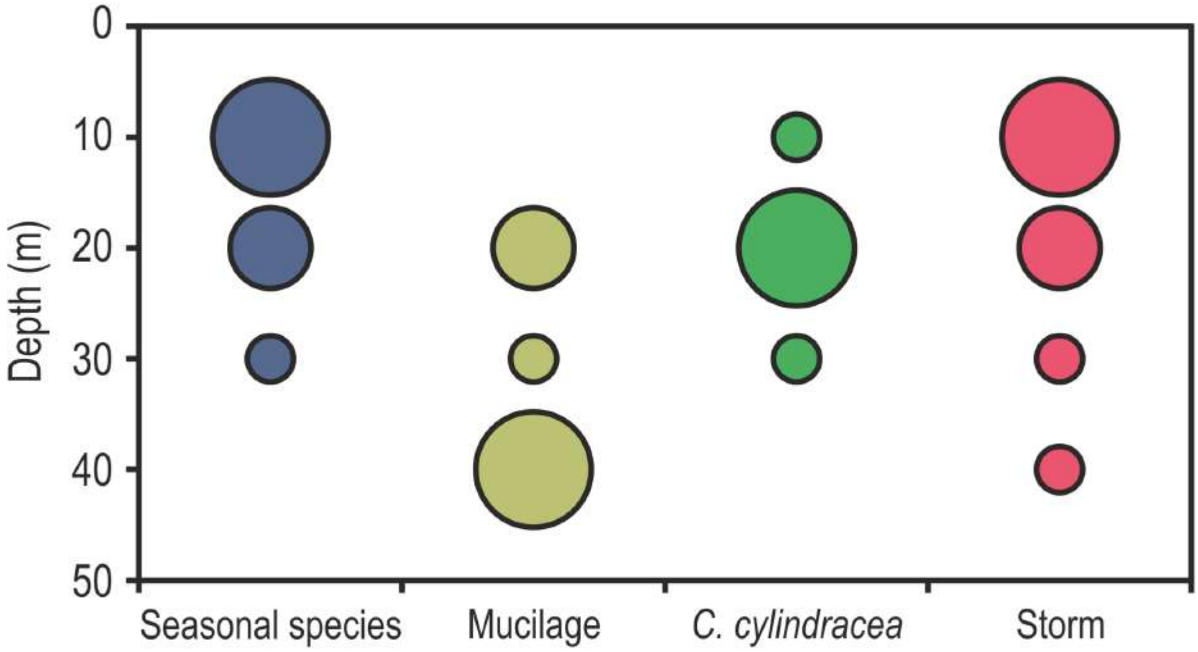
Under review:

Azzola A., Picchio V., Asnaghi V., Bianchi C.N., Morri C., Oprandi A., Montefalcone M., *Frontiers in Marine Science,* under review

Abstract

Climate change is affecting rocky reef ecosystems in a multitude of ways at global scale. Among the direct and indirect effects of climate change, the major threats to ecosystems structure and functioning are represented by extreme events, thermal anomalies, expansion of alien species and mucilaginous events. During summer 2018, the rocky reef communities of Portofino Marine Protected Area (MPA) in the Ligurian Sea (NW Mediterranean) were affected by thermal anomalies, a mucilaginous event, and the seasonal expansion of the alien species *Caulerpa cylindracea*. Moreover, a severe storm occurred on 29 October. The effects of these different affectors on the rocky reef communities were analysed through photographic sampling at different depths (10 m, 20 m, 30 m, and 40 m) and at three times (June, October, December) to evaluate the change at short temporal scale. Portofino MPA rocky reef communities significantly changed under the cumulative effects of the multiple affectors occurred in 2018. Seasonality was taken into account to reduce the confounding effect due to natural variability and, as expected, its greatest influence was observed at 10 m and 20 m depth. Mucilage affected first communities in shallow water (10 m and 20 m depth) and later communities in deep water (30 m and 40 m depth), where mucilaginous aggregates typically fall in late summer. The greatest impact due to the expansion of *Caulerpa cylindracea* was detected at 20 m depth, where negative consequences on diversity and spatial heterogeneity were already demonstrated for the Portofino MPA. The severe storm directly impacted communities in shallow and intermediate waters by uprooting algal species, while it had indirect effects at greater depths through sediment redistribution. Disentangling the effects of multiple affectors on coastal ecosystems is one of the most pressing goals in marine ecology and

biodiversity conservation. This study represents an attempt in this direction as applied to the short-term dynamics of rocky reef communities under a climate change scenario.



INTRODUCTION

Rocky reef ecosystems are globally exposed to several co-occurring impacts (Korpinen et al., 2021) due to many different global and local pressures (e.g., pollution, overfishing, coastal works, spread of alien species, ocean acidification, and global climate change), which threat their state of health (Halpern et al., 2007). Any abiotic or biotic agent capable of altering ecosystem state has been defined as an affector (Montefalcone et al., 2011, 2017), which may be either a stress (i.e., the condition induced by variations in the levels of environmental factors that reduce the productivity within the ecosystem because of the energetic cost of adaptation) or a disturbance (i.e., an unpredictable, episodic event, due to an external agent, which disrupts the state of the ecosystem causing abrupt mortality, and, hence, subtraction of biomass).

When subjected to a single affector, ecosystems are generally able to recover, but under the pressure of multiple affectors they may undergo irreversible change (Jaiswal et al., 2021). The sequential and interactive consequences of multiple affectors alter the mechanisms that regulate biotic community composition, leading to ecosystem phase shifts (Davis et al., 2010). To date, most ecological studies have investigated the response of ecosystems to one single affector; however, the dynamics and structure of an ecosystem are rarely influenced by a single environmental factor (Polazzo and Rico, 2021). The need to better understand the cumulative effects of multiple affectors is one of the most pressing goals in marine ecology and conservation (Ellis et al., 2015).

Climate change is perhaps the most serious threat to marine ecosystems, triggering unpredictable consequences on their structure and functioning (Henson et al., 2017). Both on land and in the ocean, the first direct effect of climate change is due to positive thermal anomalies such as the so-called heat waves, abnormally high temperature for a short period in a particular marine region (Hobday et al., 2016; Frölicher et al., 2018). In recent decades, abrupt increase in sea water temperature has caused mass mortality events of marine species (Garrabou et al., 2009, 2019, 2022) and changes in community structure (Bianchi et al., 2019a). In addition, high temperatures have promoted the development of mucilage, with negative effects on benthic ecosystems (Schiaparelli et al., 2007; Bianchi et al., 2019b).

Another effect of the increasing temperature is the range expansion of alien species (Galil, 2007). Much research has been conducted on the impacts of their invasion on marine biodiversity, but the results are still contradictory. Some studies report negligible or no negative effects of biological invasions on native biodiversity (Pusceddu et al., 2016, and references therein), while others state that alien species represent a worrisome threat to the integrity of ecosystems, to economy and even to human health (Streftaris and Zenetos, 2006, and references therein). A commonly recognised conclusion is that the impacts of alien species can be context dependent, varying at different spatial and temporal scales (Rizzo et al., 2020, and references therein). In the Mediterranean Sea, one of the most concerning invasive species is the green alga *Caulerpa cylindracea* (Montefalcone et al., 2015; Piazzi et al., 2016; Tsirintanis et al., 2022). In the Marine Protected Area (MPA) of Portofino (Ligurian Sea, NW Mediterranean), its strong invasion caused negative effects on diversity and spatial heterogeneity, leading to benthic habitat homogenization (Morri et al., 2019).

As a consequence of climate change, the intensity and frequency of extreme events are increasing (Stott, 2016). Hurricanes and storms have caused important impacts on marine ecosystems (Renaud et al., 1996; Baird et al., 2018). At the end of October

2018, an unprecedented storm hit the region of Portofino, severely damaging coastal benthic communities (Betti et al., 2020; Oprandi et al., 2020).

As many other coastal habitats, rocky reefs are subjected to both local and global pressures. While many studies have considered intertidal communities (Thompson et al., 2002; Micheli et al., 2016; Kunze et al., 2021), the effects of multiple affectors on subtidal communities have rarely been investigated (Rogers-Bennett and Catton, 2019; Moore and Smale, 2020). Rocky reefs represent environments of great scientific and economic value (Bianchi et al., 2004), due to the high biodiversity they host (Duffy et al., 2013) and the ecosystem services they offer (Paoli et al., 2017). Understanding the effects of multiple affectors on rocky reefs is of fundamental importance for conservation policies (Schiel, 2009).

This paper aims at evaluating the short-term change (June to December) underwent by the rocky reef communities of the Portofino MPA during 2018, when different stressors (i.e., thermal anomalies, a mucilaginous event, and the seasonal expansion of *Caulerpa cylindracea*) came in action contemporaneously (mostly in full summer) and a major disturbance (the severe storm) occurred on 29 October. The study has been carried out at four different depths (10 m, 20 m, 30 m, and 40 m), under the hypothesis that the expected consequences are different along a vertical gradient.

The starting assumptions on which this study was based on are: i) the main driver of change in a temperate rocky reef should be seasonality (Garrabou et al., 2002), which mainly affects shallow water communities (at 10 m and 20 m depth); ii) mucilage should affect first the shallow water communities and later the deep water ones (at 30 m and 40 m depth), where mucilaginous aggregates typically fall in late summer covering erect organisms, such as gorgonian corals (Bianchi et al., 2019b); iii) the greatest impacts due to the expansion of *C. cylindracea* are expected at 20 m depth, as already reported by Morri et al. (2019); and iv) the severe storm is expected to have mainly caused impacts on shallow water communities.

MATERIALS AND METHODS

Study area

The Portofino Marine Protected Area (MPA), established in 1999, covers an area of 345 ha in the Ligurian Sea (NW Mediterranean) and encircles a rocky promontory, which spans out towards the sea for about 5 km. The southern front of the promontory is characterized by high vertical or sub-vertical cliffs, which continue underwater to about 50 m depth, while the eastern and western sides are comparatively shallower (Bavestrello et al., 2022).

Increasing urbanisation of the Ligurian coast has amplified large-scale and chronic impacts, affecting marine ecosystems. Together with climate change, local human pressures have in particular led to a phase shift in the rocky reef communities of Portofino MPA in the 1990s (Gatti et al., 2017; Bianchi et al., 2019b).

Sea Surface Temperatures data management

Trend of the yearly mean Sea Surface Temperatures (SST) from 1948 till 2019 were examined to evaluate the potential role of temperature as a driver of change in the rocky reef communities. As 2018 had been the warmest year on record (Cheng et al., 2019), we compared SST monthly values of 2018 with the climatological mean (monthly mean values) of the previous 70 years (1948 to 2017). For each month of 2018, thermal anomalies (aSST) were detected applying the formula:

$$a_{\mathrm{SST}} = \frac{\mathrm{SST}_{2018} - \mathrm{SST}_{\mathrm{mean}}}{\sigma}$$

where SST2018 is the mean for 2018, SSTmean is the climatological mean, and σ is the standard deviation of the climatological mean. The differences between 2018 and the climatological mean were evaluated through a Mann-Kendall test (Jiménez-Muñoz et al., 2015).

SST data were derived from NOAA (US National Oceanic and Atmospheric Administration) satellite records (www.esrl.noaa.gov/psd/cgi-bin/data/timeseries/timeseries1.pl).

Field activities and data collection

Data on rocky reef communities of the Portofino MPA were collected by scuba diving using photoquadrats in three sites (Figure 1): Altare (44°18'20'' N, 9°11'48'' E), Colombara (44°18'35'' N, 9°10'38'' E), and Torretta (44°18'45'' N, 9°10'03'' E). The three sites have similar environmental and morphological characteristics and are equally exposed to the considered affectors. To assess the differences in community structure before and after the onset of seasonal and recurrent stressors (i.e., thermal anomalies, mucilaginous events, expansion of *Caulerpa cylindracea*), data were collected from 19 to 21 June and from 18 to 23 October 2018. Following the unpredictable storm that hit the Ligurian coast on 29 October, a further data collection was carried out between 12 and 13 December 2018 to quantify the impact of this disturbance. To analyse the effects of all the affectors in relation to depth, data collection was carried out at 10 m, 20 m, 30 m, and 40 m depth. In each combination of site and depths, 32 random photoquadrats (each covering an area of 50×50 cm²) were taken (Figure 2).

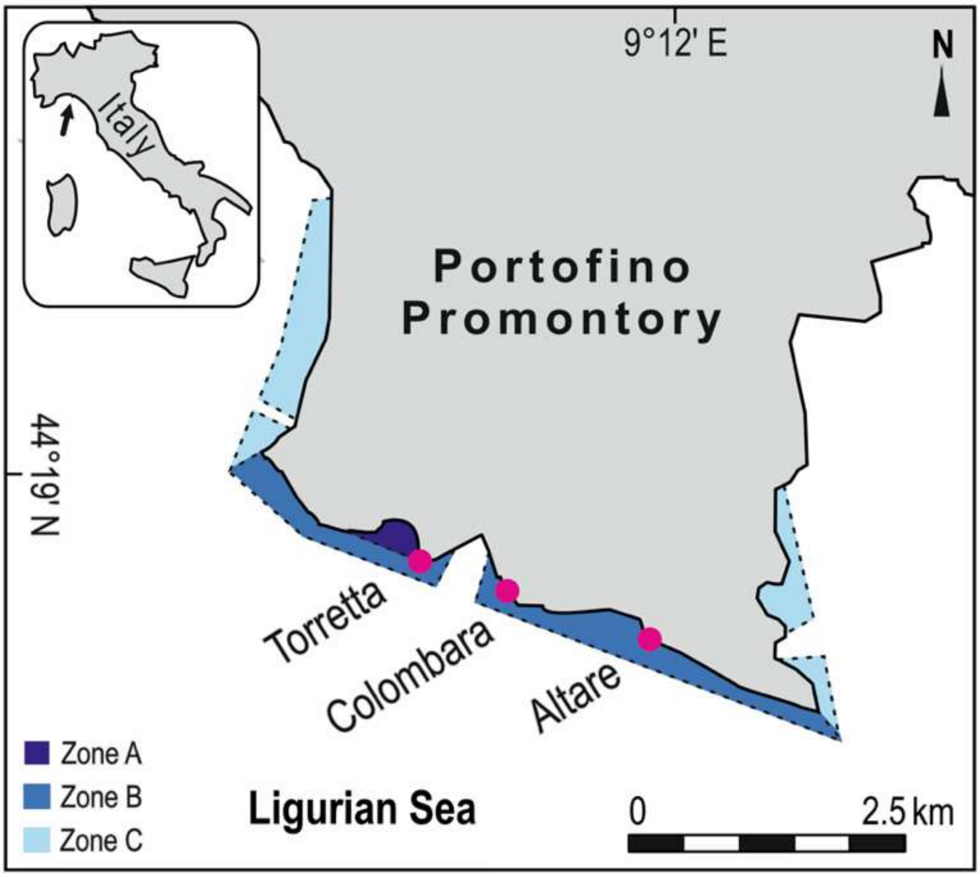


Fig. 1 Map of the Portofino Marine Protected Area (Ligurian Sea, NW Mediterranean) with the three sites surveyed (Altare, Colombara, and Torretta) represented by hot pink circles. Geographic coordinates in WGS84

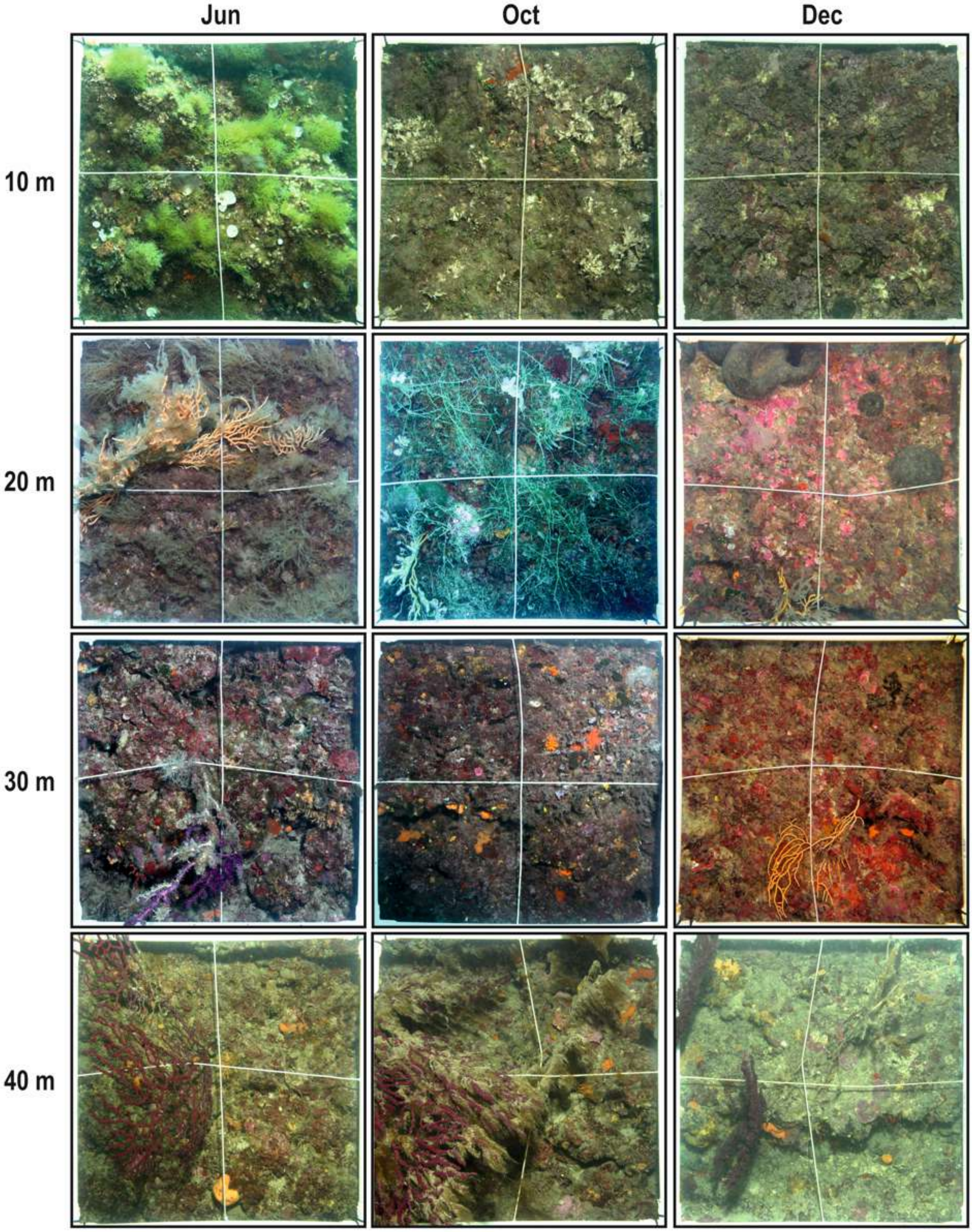


Fig. 2 Examples of photoquadrats (each covering an area of 50×50 cm²) carried out in three times (June, October and December) at four depths (10 m, 20 m, 30 m and 40 m)

Photographic analysis

Each photoquadrat was analysed using the software Image J (Ferreira and Rasband, 2012) to calculate the percent cover of any organism presents at the lowest possible taxonomic level (usually species). When species identification was not possible, lumped categories based on similar morphological and structural characteristics (i.e., OTUs = Operational Taxonomic Units) were adopted. The abiotic component (sediment and bare rock) was also recorded.

Community data management

Cover data of species and OTUs were organized in a matrix (time × site × depth) × species/OTUs and transformed applying arcsine$\sqrt{(x/100)}$. A non-metric multidimensional scaling (nMDS) based on Bray-Curtis index was applied to quantify change over time in rocky reef communities.

A two-way permutational multivariate analysis of variance (PERMANOVA), followed by a PAIRWISE test for significant terms, was performed with the factor ‘time’ (3 levels: June, October, and December) and the factor ‘depth’ (4 levels: 10 m, 20 m, 30 m, 40 m) fixed and orthogonal to test the differences in percent cover over time. The factor ‘site’ was not considered since the differences among sites were not under investigation.

Two time intervals were considered to highlight when the major changes occurred in the Portofino MPA rocky reef communities: June to October and October to December. The Pythagoras theorem was applied to the first two nMDS axes scores to measure the time trajectories between the two time intervals at the four depths (De Cáceres et al., 2019). For example, the time trajectory between June and October at 10 m depth was calculated as the geometric distance between the 10 m centroids of June and October as follow:

$$Tt_{10} = \sqrt{\left(x_{\mathrm{O}} - x_{\mathrm{J}}\right)^2 + \left(y_{\mathrm{O}} - y_{\mathrm{J}}\right)^2}$$

where Tt10 is the length of the time trajectory at 10 m, xJ and yJ are the axes scores of the 10 m centroid of June, and xO and yO are the axes scores of the 10 m centroid of October. Analogous formulas were applied to measure the time trajectories between June and October and between October and December at all the depths surveyed.

Differences between time trajectories at each depth have been evaluated by Student's t-test.

A SIMPER analysis based on Bray-Curtis index was applied to identify the descriptors (species or OTUs) that mostly contributed to differences in community structure among the three times at each depth.

All analyses were performed using the free software PaSt (Hammer et al., 2001), Primer 6 + PERMANOVA (Clarke and Gorley, 2005) and RStudio (R Core Team, 2021).

Role of seasonality in community change

Cover data of seasonal species (i.e., the sum of all the seasonal species, the alien *Caulerpa cylindracea* excluded) were analysed to highlight their trend in the period investigated. Differences among the three times at each depth were evaluated by one-way ANOVA and Tukey's post-hoc comparison.

A new simulated data matrix (time × depth) × species/OTUs, excluding seasonal algae, was generated to distinguish the community changes due to the seasonality from those referable to the other affectors. We used the term 'simulated' to make clear that we did not perform a real, physical removal experiment in the field. This procedure has already been applied for studies involving a so called 'inclusion versus exclusion' approach, in which the descriptor of interest is an integral part of the response (Morri et al., 2019 and reference therein). An nMDS analysis based on the Bray-Curtis index was applied to the simulated data matrix, and the time trajectories between June and October and between October and December have been measured using the axes scores, as done for the original data matrix. Differences between time trajectories of the original data matrix and the simulated one have been evaluated by Student's t-test.

Data management for mucilage, *Caulerpa cylindracea* and the severe storm

Cover data of mucilage and *C. cylindracea* were analysed to highlight their trends in the period investigated. Differences among the three times at each depth were evaluated by one-way ANOVA and Tukey's post-hoc comparison.

As done for seasonal species, two new simulated data matrixes (time × depth) × species/OTUs, each excluding one affector (mucilage or *C. cylindracea*), were generated to test the effect of such affector on the rocky reef community structure. An nMDS analysis based on the Bray-Curtis index was applied to each simulated data matrix, and the time trajectories between June and October and between October and December have been measured using the axes scores, as done for the original data matrix. Differences between time trajectories of the original data matrix and the simulated ones have been evaluated by Student's t-test.

Differences in cover of abiotic components and perennial species, which are not expected to show seasonal variation, between October and December at each depth were analysed by Student's t-test to evaluate the effect of the storm. The species or OTUs that mostly contributed to differences were identified through SIMPER analysis. Seasonal species, mucilage and *C. cylindracea* were not considered in the latter analysis.

RESULTS

Sea Surface Temperature and thermal anomalies

Analysis of the climatological mean SST showed a positive trend for the last 70 years (Figure 3a). In 2018, the SST yearly mean value was 17.4±1.42 °C, 1.2 °C higher than the yearly mean for 1948-2017. The yearly mean SST of the 2018 was slightly higher than to that of the year 2003 (17.2±1.4 °C), when a severe mass mortality event occurred in the North-Western Mediterranean rocky reef communities (Garrabou et al., 2009). Comparing the SST monthly values of 2018 with the SST monthly mean values of the previous 70 years unveiled thermal anomalies from August till November uninterruptedly (Figure 3b, Table 1).

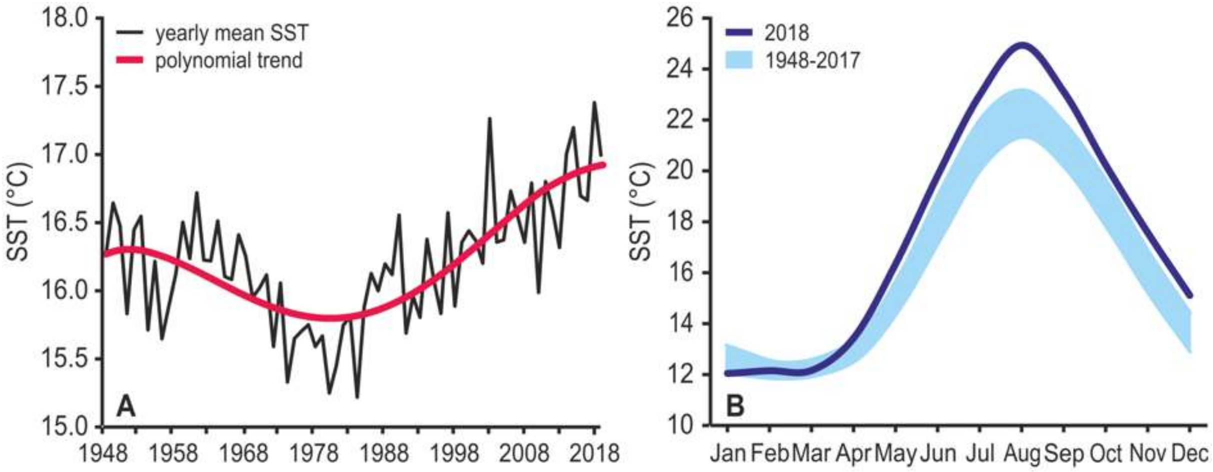


Fig. 3 (A) Fourth degree polynomial trend (r = 0.75) of yearly mean sea surface temperature (SST) in the Portofino MPA from 1948 to 2019; and **(B)** 2018 SST monthly values compared to the SST overall monthly mean values of the previous 70 years. SST values were derived from NOAA satellite data (www.esrl.noaa.gov/psd/ cgi-bin/data/timeseries/timeseries1.pl)

Table 1 The 2018 thermal anomalies (in bold) detected applying the method proposed by Jiménez-Muñoz et al. (2015)

	Jen	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Thermal anomalies (°C)	-0.7	0.1	-0.4	0.6	1.4	1.4	1.8	**3.0**	**2.6**	**1.9**	**2.0**	2.0
Mann-Kendall test (p)	0.470	0.061	0.203	0.216	0.743	0.116	0.060	**0.001**	**0.009**	**0.001**	**0.003**	0.237

Changes in community structure

A total of 91 species and 7 OTUs were recorded (Supplementary material). The nMDS applied to cover data ordered photoquadrat points according to two gradients: a spatial one along the 1st (horizontal) axis, and a temporal one along the 2nd (vertical) axis. The spatial gradient was an expression of increasing depth from the left (10 m) to the right (40 m) of the 1st axis. The temporal gradient was expressed from bottom to top by seasonality, with the points of June, October and December well separated from each other (Figure 4a, b). The nMDS did not show any ordination of photoquadrat points according to the factor site (Figure 4a).

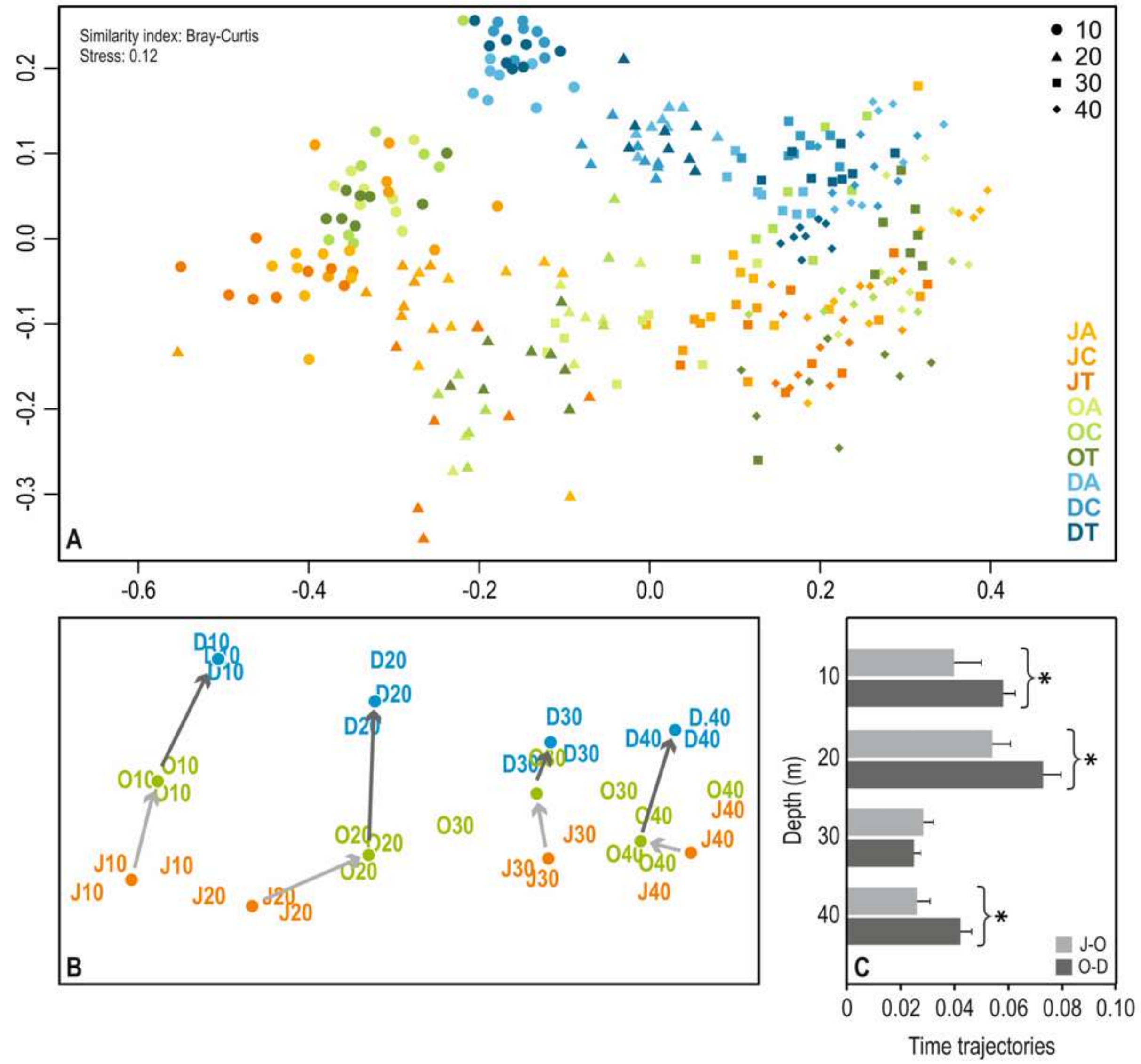


Fig. 4 (A) Multivariate analysis (nMDS) plot of Portofino MPA species/OTUs. Individual observation points are represented by different colours and shapes according to time (J = June in orange, O = October in green, and D = December in blue) and depth. The colour gradations represent different sites in the three times (A = Altare, C = Colombara, T = Torretta). **(B)** nMDS plot showing only the centroids and with time trajectories represented by arrows (light grey for trajectories between June and October; and dark grey for trajectories between October and December). **(C)** Differences between time trajectories values (derived from axis scores and analysed with Student's t-test) of June to October and October to December at each depth (10 m, 20 m, 30 m, and 40 m)

Results of PERMANOVA evidenced significant differences for the interaction between the factors time and depth (Table 2). The PAIRWISE results showed significant differences for each combination of the two factors, indicating a pervasive change with time along the whole depth gradient in the rocky reef communities of Portofino MPA.

Table 2 Results of PERMANOVA applied on rocky reef communities of Portofino MPA

	PERMANOVA				
Source	*SS*	*df*	*MS*	*F*	*p*
Time	6.489	2	3.244	52.694	**0.0001**
Depth	17.394	3	5.798	94.163	**0.0001**
Interaction	5.529	6	0.921	14.965	**0.0001**
Residual	16.994	276	0.062		
Total	46.405	287			

Comparing the time trajectories between June and October and between October and December at each depth by Student's *t*-test, significant differences were observed at 10 m (p = 0.004), 20 m (p = 0.009) and 40 m (p = 0.200). The magnitude of change between October and December was always greater than that between June and October, highlighting that the major changes in rocky reef communities occurred from October to December (Figure 4c).

The SIMPER analysis identified the descriptors that mostly contributed to community change at each depth in the two time intervals considered (Table 3): i) mucilage at 40 m in both time intervals; ii) *Caulerpa cylindracea* especially at 20 m, and secondarily at 10 m and 30 m, in both time intervals; iii) *Ellisolandia elongata* at 10 m in both time intervals; iv) encrusting corallines at 10 m and secondarily at 20 m between October and December; v) *Halopteris scoparia* at 10 m and 20 m in both time intervals; and vi) sediment at all depths investigated in both time intervals.

Table 3 Descriptors that mostly contributed (> 5%) to the similarity between June-October and between October-December identified by SIMPER analysis (based on Bray-Curtis index)

SIMPER Analysis

	10 m		20 m		30 m		40 m	
	J-O	O-D	J-O	O-D	J-O	O-D	J-O	O-D
Affectors								
Mucilage			5.99				13.66	12.50
Caulerpa cylindracea	4.76	7.69	14.08	17.42	6.28	6.44		
Others								
Ellisolandia elongata	5.37	7.13						
Encrusting corallines		7.57		5.29				
Halopteris scoparia	10.64	8.15	5.36	3.87				
Sediment	5.62	5.25	6.59	7.90	7.92	4.08	4.63	10.43

Seasonal variations

One-way ANOVA and Tukey's post-hoc comparison applied to cover data of seasonal species in the three times evidenced significant differences at 10 m, 20 m, and 30 m depth (Figure 5a). The greatest changes were observed at 10 m, where the cover of seasonal species decreased significantly ($p = 3.78 \cdot 10^{-33}$) from June (67.7±1.9) to October (37.1±2.1) and again in December (5.3±0.5). Significant changes also occurred at 20 m, where the cover increased significantly ($p = 3.42 \cdot 10^{-14}$) from June (33.1±4.2) to October (41.5±3.5) and decreased from October to December (1.4±0.2). The algal species that exhibited the greatest variation were *Jania* spp. and *Padina pavonica*, more abundant in October.

Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix confirmed significant differences in rocky reef communities between October and December at 10 m ($p = 0.02$) and 20 m ($p = 0.02$) depth (Table 4).

Proliferation of mucilaginous aggregates

One-way ANOVA and Tukey's post-hoc comparison applied to mucilage cover data in the three times evidenced major differences at 20 m and 40 m (Figure 5b). Mucilage decreased significantly (p = 0.003) from June (10.2±4.1) to October (0.2±0.1) at 20 m; on the contrary, it increased significantly ($p = 1.26 \cdot 10^{-16}$) from June (0.1±0.1) to October (17.7±2.2) at 40 m. At both depths, mucilage disappeared completely in December.

Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix showed significant differences at 40 m depth (Table 4), both between June and October (p = 0.005) and between October and December ($p = 7.89 \cdot 10^{-5}$).

Caulerpa cylindracea expansion

One-way ANOVA and Tukey's post-hoc comparison applied to the cover of *C. cylindracea* in the three times evidenced major changes at 20 m depth, where *C. cylindracea* increased significantly ($p = 2.34 \cdot 10^{-22}$) from June (1.8±0.3) to October (35.8±3.4), to disappear in December (Figure 5c).

Student's *t*-test applied to the time trajectories of the original data matrix and the simulated data matrix confirmed significant differences between October and December at 20 m (p = 0.002) depth (Table 4).

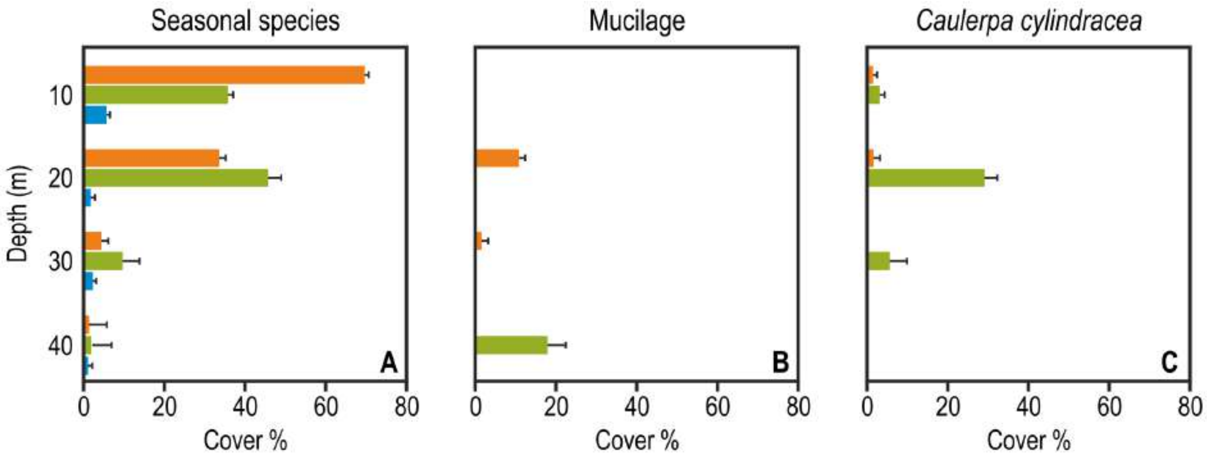


Fig. 5 Percent cover (+ se) of **(A)** seasonal species, **(B)** mucilage and **(C)** *Caulerpa cylindracea* in the three times (June in orange, October in green, December in blue) at the four surveyed depths (10 m, 20 m, 30 m, and 40 m)

Table 4 p values of the Student's t-test, applied to analyse the differences between time trajectories (J-O: June to October; O-D: October to December) of the original data matrix and the simulated ones at the four depth investigated. The significant differences are in bold

	p values ANOVA					
	Seasonal species		**Mucilage**		***Caulerpa cylindracea***	
	J-O	**O-D**	**J-O**	**O-D**	**J-O**	**O-D**
10 m	0.265	**0.021**	0.102	0.956	0.375	0.181
20 m	0.547	**0.022**	0.665	0.710	0.391	**0.002**
30 m	0.531	0.547	**0.037**	0.183	0.109	0.342
40 m	0.256	0.094	**0.005**	**0.000**	0.115	**0.003**

Effects of the storm

SIMPER analysis highlighted the following perennial species and abiotic components as responsible for the main significant differences within communities between October and December: *Ellisolandia elongata,* encrusting corallines, *Halopteris scoparia*, and sediment (Table 3). All exhibited significant variation in cover between the two times (Figure 6). *E. elongata* increased significantly from 7.7±1.2 to 23.6±1.9 ($p = 1.69 \cdot 10^{-6}$) at 10 m depth. Encrusting corallines increased significantly from 5.3 ± 0.6 to 22.7±1.4 ($p = 2.71 \cdot 10^{-10}$) at 10 m depth and from 5.1±0.4 to 15.8±1.3 ($p = 3.18 \cdot 10^{-7}$) at 20 m. *H. scoparia* decreased significantly from 32.5±2.5 to 8.8±0.5 ($p = 7.19 \cdot 10^{-9}$) at 10 m and from 4.3±1.8 to zero ($p = 2.11 \cdot 10^{-2}$) at 20 m. Sediment increased significantly from 5.4±0.7 to 8.8±0.6 ($p = 1.18 \cdot 10^{-3}$) at 10 m depth and from 7.6±1.4 to 11.5±1.4 ($p = 5.42 \cdot 10^{-2}$) at 20 m depth, while it decreased significantly from 20.8±1.3 to 16.2±0.7 ($p = 9.93 \cdot 10^{-3}$) at 30 m depth and from 25.9±2.1 to 19.1±3.3 ($p = 9.36 \cdot 10^{-2}$) at 40 m depth.

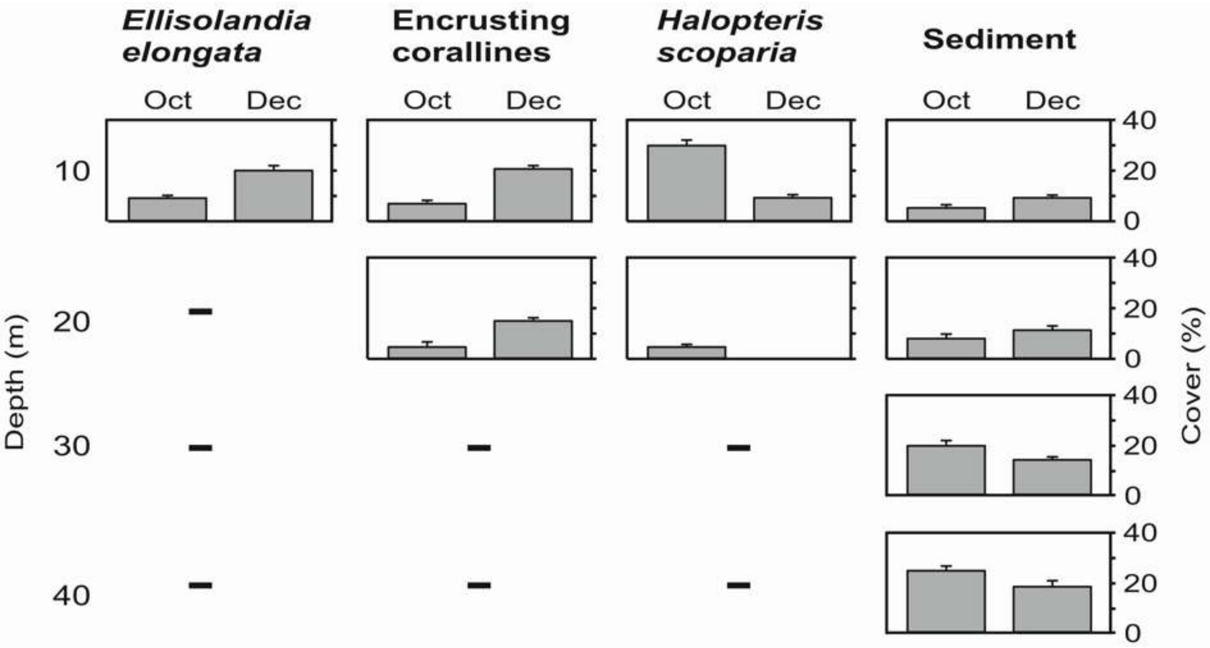


Fig. 6 Percent cover (+ se) of the species or abiotic component indicating the effects of the storm and identified by the SIMPER analysis (see Table 3), before (Oct = October) and after (Dec = December) the disturbance at four different depths (10 m, 20 m, 30 m, and 40 m)

DISCUSSION

In 2018, the rocky reef communities of Portofino MPA underwent three major stresses (high temperature, mucilage, and the invasive alga species *Caulerpa cylindracea*) and were subjected to a severe disturbance (the storm of 29 October). Sea Surface Temperature (SST) was the highest of the last 70 years, slightly surpassing that of the hot 2003 (Garrabou et al., 2009); similarly, the mucilaginous event equalled the one of 2003 (Schiaparelli et al., 2007; Bianchi et al., 2019b), and a peak in the cover of *C. cylindracea* occurred (Mancini et al., 2022). The October storm was the most severe that ever hit the Ligurian coast (Oprandi et al., 2020). The cumulative action of all these affectors triggered significant changes on the rocky reef communities from June to December.

When multiple affectors act simultaneously, as happened at Portofino MPA, each one may produce similar or distinct effects (Bianchi et al., 2012; Azzola et al., 2022). Distinguishing the outcome of every single affector, even excluding natural variability, is difficult (Bianchi and Morri, 2004; Gissi et al., 2021). Taking into account four depths, under the hypothesis that every single affector exhibited different impacts along the vertical gradient, this study explored the possibility to tackle this difficulty.

Mediterranean coastal benthic ecosystems are strongly subjected to the seasonal variation of environmental factors (Coma et al., 2000), but the effects of seasonality are damped with depth (Garrabou et al., 2002). As expected, at Portofino MPA the major variation in the cover of seasonal species was recorded at 10 m depth. However, the observed decrease in perennial species, such as *Halopteris scoparia*, and the increase in *Ellisolandia elongata* and encrusting corallines cannot be explained by the effects of seasonality. A possible explanation is that the October storm cleaned off the soft-bodied species *H. scoparia* from of the upper layer, making more visible the hard calcified, and therefore more resistant, species (*E. elongata* and the encrusting corallines) of the basal layer.

Overall, the time trajectories illustrated that the greatest change in the rocky reef community between June and December occurred at 20 m depth, in response not only to seasonality but also to other affectors. Mucilaginous aggregates formed mostly at this depth, just below the summer thermocline (Bianchi et al., 2019b). The invasive alga *C. cylindracea* showed the highest cover there, conforming to previous years (Morri et al., 2019). As observed at 10 m depth, the decrease of *H. scoparia* and the increase of calcified corallines in December point at the cleaning effect of the storm.

At 30 m depth, only small differences in community structure were observed from June to December; this result is consistent with the trend of all affectors that did not show noteworthy change at this depth. A slight quantity of mucilage was observed only in October, and a negligible cover of *C. cylindracea* was recorded in June. Although significant, the variation in the cover of seasonal species was overall modest.

The significant change at 40 m depth is attributable to the fall of mucilage in late summer: not present in June, the mucilaginous aggregates covered a wide extent of the seafloor in October (Bianchi et al., 2019b), to completely disappear in December. Seasonal species and *C. cylindracea* were little or no represented at this depth.

The only affector that impacted all the four depths investigated was the severe storm that occurred at the end of October. Besides the cleaning effect on the species of the upper layer, the storm redistributed sediment along the depth gradient. Also in this case, the removal of the upper layer, which occurred mostly in the algal-dominated shallow-water communities, may have made the sediment more visible on the rocky substrate.

The initial hypothesis that exploring different depths could have disentangled the effects of the four affectors has been corroborated by the results of the present study, which adopted three different methodological approaches: i) a multivariate analysis that allowed measuring the time trajectories of change; ii) the variation in cover of selected descriptors (species/OTUs) used as proxies for natural variability and the three affectors considered; and iii) the exploration of simulated matrices where three of those proxies (i.e., seasonality, mucilage, and *C. cylindracea*) have been excluded in turn.

The effect of seasonality was more evident at 10 m depth, and so was – but later in time – that of the storm. Mucilage, after a first bloom at 20 m, mostly impacted the community at 40 m depth. *C. cylindracea* peaked at 20 m. The cumulative effect of the four affectors peaked at 20 m depth, where the greatest changes in the rocky reef community were documented. As a general result, this study suggests that the cumulative effect of multiple affectors is stronger than that of any individual affector, no matter how intense it is.

Notwithstanding the consistent results, our approach has undoubtedly several inherent limits: i) despite the analyses conducted on the seasonal species, it is difficult to completely exclude natural variability when assessing change over time due to the other affectors considered, two of which (mucilage and *C. cylindracea*) also exhibit seasonal trends; ii) the lack of comparable information in the years before and after 2018 limits the possibility to infer cause-effect relationships between affectors and community change; and iii) considering only one sampling time before/after each affector may not be enough to explore thoroughly the sources of variation.

Interactions among multiple affectors, which can trigger abrupt changes in marine communities, are still poorly understood and their consequences pose important challenges to researchers and conservationists (Turner et al., 2020). Historical data series and the revisitation of sites already surveyed in the past are essential to illustrate ecological change (Gatti et al., 2015; Azzola et al., 2022), but short-term studies, which allows considering all the multiple affectors acting almost simultaneously, may help disentangling their effects. The importance of analysing ecological change at different scales has been underlined on several occasions (Bianchi et al., 2022 and reference therein). This study represents an attempt in this direction as applied to the short-term dynamics of rocky reef communities under a climate change scenario.

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Supplementary Material

Supplementary Table 1. List of the 91 species and 7 OTUs recorded in the Portofino MPA, ordered alphabetically. For each species the presence (1) and absence (0) in the three months surveyed at the four different depths are reported (see Results)

Species	June				October				December			
	10 m	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**
Acanthella acuta	0	0	1	1	0	0	1	1	0	0	1	1
Acetabularia acetabulum	1	0	0	0	0	0	0	0	0	1	0	0
Acrosymphyton purpuriferum	1	1	0	0	0	0	0	0	0	0	0	0
Agelas oroides	1	1	1	1	1	1	1	1	1	1	1	1
Amphiroa rigida	1	1	0	0	1	1	1	0	1	1	1	1
Aplysina cavernicola	0	0	0	0	0	0	1	1	0	0	1	1
Ascandra contorta	0	0	1	0	0	0	1	1	0	1	1	1
Axinella spp.	1	1	1	1	0	1	1	1	1	1	1	1
Balanophyllia europaea	0	0	1	1	1	0	0	1	1	0	0	0
Cacospongia mollior	1	0	0	0	1	0	1	1	1	1	1	1
Caryophyllia inornata	0	0	1	1	1	0	0	1	1	0	0	1
Caulerpa cylindracea	1	1	1	1	1	1	1	1	1	1	1	0
Cellaria fistulosa	0	0	0	0	0	0	1	1	0	0	1	0
Cerianthus membranaceus	0	0	0	1	0	0	1	0	0	0	0	0
Chondrilla nucula	0	0	0	0	0	0	0	0	1	1	0	0
Chondrosia reniformis	1	1	1	1	1	1	1	1	1	1	1	1
Chrysymenia ventricosa	0	1	0	0	0	0	0	0	0	1	0	0
Cladocora caespitosa	0	1	0	0	1	1	0	0	1	1	1	0

Species	**June**				**October**				**December**			
	10 m	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**
Clathrina clathrus	0	0	0	0	0	0	1	1	0	0	1	1
Cliona spp.	0	0	0	0	0	0	0	0	1	1	1	1
Codium bursa	1	1	1	1	1	1	1	1	1	1	1	0
Corallium rubrum	0	0	1	1	0	1	1	1	0	0	1	1
Crambe crambe	1	1	0	0	1	1	1	0	1	1	0	0
Crella elegans	0	0	1	1	0	0	1	1	1	1	1	1
Crella pulvinar	0	0	1	1	0	0	1	1	0	0	1	1
Cyanobacteria	1	0	0	0	0	0	0	0	0	0	0	0
Cystoseira spp.	0	0	1	1	1	0	1	0	1	1	1	0
Dictyopteris polypodioides	0	1	1	0	1	1	1	1	0	1	0	0
Dictyota dichotoma	1	1	1	1	1	1	1	1	1	1	1	1
Dudresnaya verticillata	1	0	0	0	1	0	0	0	1	1	0	0
Dysidea avara	0	0	0	1	0	0	0	0	1	1	0	1
Dysidea fragilis	0	0	1	1	1	0	1	1	1	0	1	1
Ellisolandia elongata	1	0	0	0	1	1	1	1	1	1	0	0
Encrusting corallines	1	1	1	1	1	1	1	1	1	1	1	1
Encrusting dark sponge	0	0	1	0	0	0	1	1	0	1	0	1
Encrusting white sponge	0	0	1	1	0	1	1	1	1	1	1	1
Eunicella cavolini	1	1	1	1	0	1	1	1	0	1	1	1
Eunicella singularis	0	1	0	0	0	0	0	0	0	0	0	0
Eunicella verrucosa	0	0	1	0	0	0	0	0	0	0	0	0
Flabellia petiolata	1	1	1	1	1	1	1	1	1	1	1	1
Frondipora verrucosa	0	0	0	0	0	0	0	1	0	0	0	1
Haliclona fulva	0	0	1	1	0	0	1	1	0	0	1	1
Haliclona mucosa	0	0	0	0	0	0	0	1	0	0	0	1
Halimeda tuna	1	1	1	1	1	1	1	1	1	1	1	1

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Species	June				October				December			
	10 m	20 m	30 m	40 m	10 m	20 m	30 m	40 m	10 m	20 m	30 m	40 m
Halocynthia papillosa	1	0	1	1	0	1	1	1	0	1	1	1
Halopteris scoparia	1	1	0	0	1	1	0	0	1	0	0	0
Hemimycale columella	0	0	0	0	1	1	1	1	1	1	0	0
Hydroids	0	0	1	1	0	1	1	1	1	1	1	1
Ircinia oros	1	1	1	0	1	1	1	1	1	1	1	1
Ircinia variabilis	0	1	1	1	1	0	1	1	1	0	1	1
Jania spp.	1	1	1	0	1	1	1	1	1	1	1	0
Laurencia spp.	1	0	0	1	1	0	1	0	1	0	0	0
Leptogorgia sarmentosa	0	0	0	0	0	0	1	1	0	0	0	0
Leptopsammia pruvoti	1	1	1	1	1	1	1	1	0	1	1	1
Microcosmus sabatieri	1	0	0	0	0	0	0	1	1	0	0	0
Mucilage	1	1	1	1	1	1	1	1	0	0	0	0
Myriapora truncata	0	1	1	1	0	1	1	1	0	1	1	1
Oscarella lobularis	0	0	1	1	1	0	1	1	1	0	1	1
Padina pavonica	1	1	1	1	1	1	1	1	1	0	1	0
Palmophyllum crassum	1	1	0	0	1	1	1	1	1	1	1	0
Paramuricea clavata	0	0	1	1	0	1	1	1	0	1	1	1
Parazoanthus axinellae	1	1	1	1	1	1	1	1	0	1	1	1
Pentapora fascialis	0	1	1	1	0	0	1	1	0	0	1	1
Petrosia ficiformis	0	0	1	1	0	1	1	1	1	1	1	1
Peyssonnelia spp.	1	1	1	1	1	1	1	1	1	1	1	1
Phorbas tenacior	1	1	1	1	1	1	1	1	1	1	1	1
Phyllangia americana mouchezii	0	0	0	0	0	0	0	1	0	0	0	1
Phyllophora crispa	0	0	0	0	0	0	0	1	0	1	0	0
Pinna nobilis	1	0	0	0	0	0	0	0	0	0	0	0
Pleraplysilla spinifera	0	0	1	1	0	0	1	1	0	0	1	1

Species	June				October				December			
	10 m	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**
Plocamium cartilagineum	0	0	0	0	0	0	1	0	1	0	0	0
Posidonia oceanica	0	1	0	0	0	0	0	0	0	1	0	0
Protula tubularia	1	1	0	0	1	1	1	1	1	1	1	1
Pseudochlorodesmis furcellata	0	1	1	1	1	1	1	0	1	0	0	0
Reteporella grimaldii	0	1	1	1	0	1	1	1	1	0	1	1
Rocellaria dubia	1	1	1	0	1	0	0	1	1	1	1	1
Bare rock	1	1	1	1	1	1	1	1	1	1	1	1
Sabella spallanzanii	0	1	1	1	0	1	1	0	0	0	0	0
Salmacina-Filograna complex	0	0	0	1	0	1	1	1	0	0	1	1
Sarcotragus spp.	1	1	1	1	1	1	1	1	1	0	1	0
Savalia savaglia	0	0	1	0	0	0	1	0	0	0	0	0
Schizomavella mamillata	1	1	1	1	0	1	1	1	1	1	1	1
Schizoporella dunkerii	0	0	1	1	0	0	1	1	1	1	0	1
Scrupocellaria spp.	0	0	1	1	0	0	0	1	1	0	1	1
Sediment	1	1	1	1	1	1	1	1	1	1	1	1
Serpula vermicularis	1	0	1	1	0	1	0	1	1	1	1	1
Smittina cervicornis	0	1	1	1	0	0	1	1	0	0	1	1
Sphaerococcus coronopifolius	1	0	0	0	0	0	1	0	0	0	0	0
Spirastrella cuncatrix	0	1	1	1	0	0	1	1	0	1	1	1
Spongia officinalis	0	1	0	0	1	0	0	0	1	1	0	1
Sporochnus pedunculatus	0	0	0	0	0	0	0	1	0	1	1	0
Terpios gelatinosus	0	0	0	0	0	0	0	0	1	1	1	0
Tricleocarpa fragilis	1	1	0	0	1	1	1	0	0	0	0	0
Turbicellepora avicularis	0	0	1	1	0	0	1	1	0	0	1	1
Turf	1	1	1	1	1	1	1	1	1	1	1	1
Valonia sp.	0	0	0	1	0	0	0	0	0	0	0	1

Species	**June**				**October**				**December**			
	10 m	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**
Womersleyella setacea	1	0	0	0	1	0	1	1	1	1	0	1
Zanardinia typus	0	1	1	1	1	1	1	1	1	1	1	1

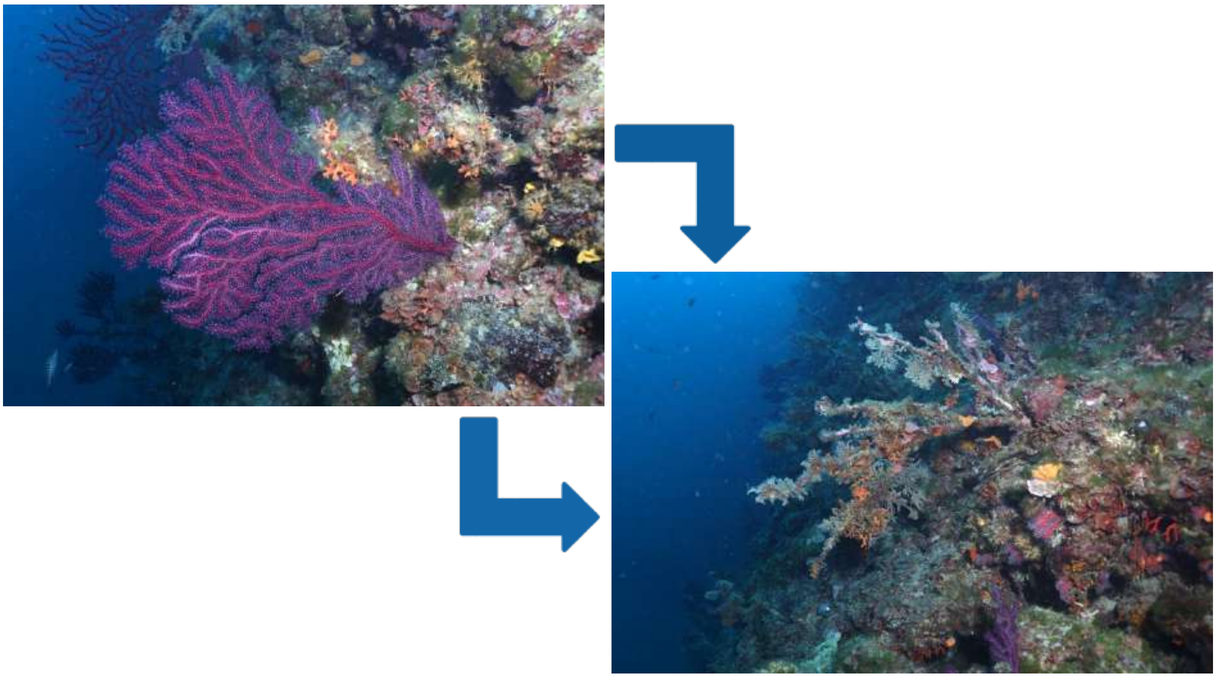
Supplementary Table 2. List of the conspicuous species and OTUs (cover > 1%) recorded in the Portofino MPA, ordered alphabetically. For each species the mean cover (± se) in the three months surveyed at the four different depths are reported (see Results).

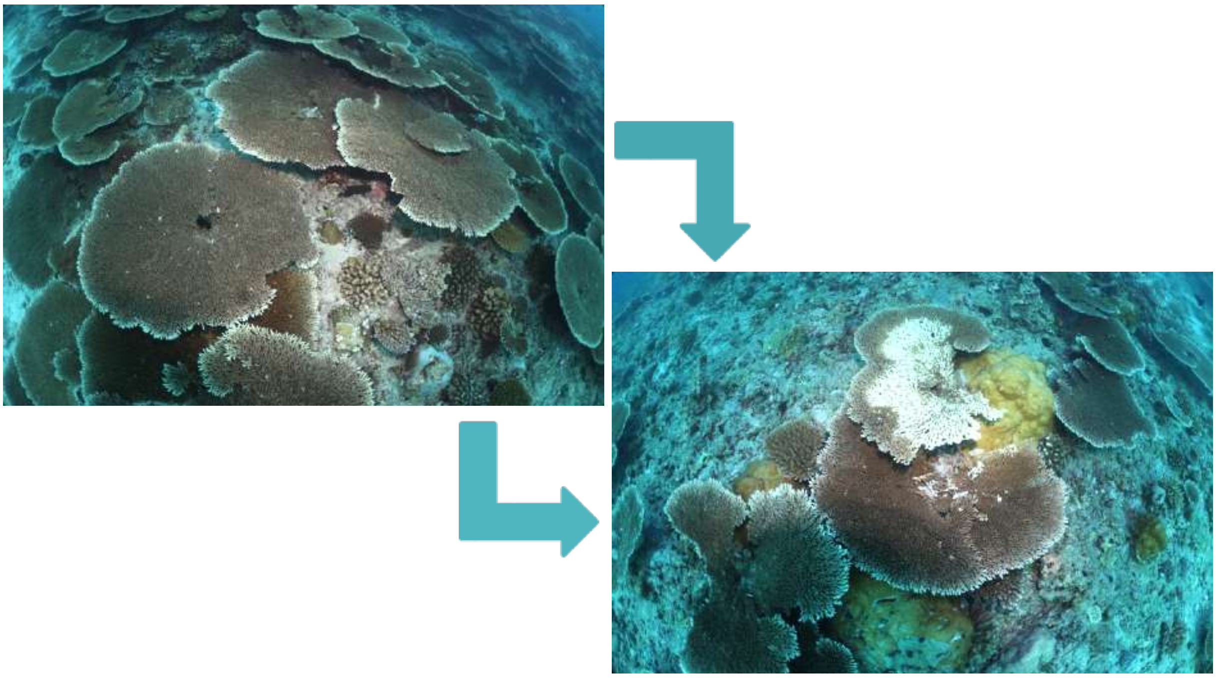
Species	June				October				December			
	10 m	20 m	30 m	40 m	10 m	20 m	30 m	40 m	10 m	20 m	30 m	40 m
Acrosymphyton purpuriferum	1.4 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Axinella spp.	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	1.2 ± 0.1
Cacospongia mollior	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0
Caulerpa cylindracea	2 ± 0.3	1.8 ± 0.3	0 ± 0	0 ± 0	7.1 ± 0.7	35.8 ± 3.4	9 ± 2.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Codium bursa	0.5 ± 0.2	2.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	1.2 ± 0.3	0 ± 0	0 ± 0	0 ± 0	2.8 ± 0.7	0 ± 0	0 ± 0
Corallium rubrum	0 ± 0	0 ± 0	2.6 ± 0.8	1.8 ± 0.7	0 ± 0	0 ± 0	2.8 ± 0.5	1.5 ± 0.5	0 ± 0	0 ± 0	1.7 ± 0.6	1.6 ± 0.5
Crambe crambe	1.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	1.9 ± 0.5	1.2 ± 0.4	0 ± 0	0 ± 0	2.9 ± 1	1.9 ± 0.4	0 ± 0	0 ± 0
Dictyota dichotoma	36.2 ± 3.2	23.7 ± 3.4	1.7 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ellisolandia elongata	2.9 ± 0.9	0 ± 0	0 ± 0	0 ± 0	7.7 ± 1.2	0 ± 0	0 ± 0	0 ± 0	23.6 ± 1.9	1.1 ± 0.2	0 ± 0	0 ± 0
Encrusting corallines	2.6 ± 0.5	4.7 ± 0.7	4.1 ± 0.6	8 ± 0.8	5.3 ± 0.6	5.1 ± 0.4	7.1 ± 0.8	7.1 ± 0.7	22.7 ± 1.4	15.8 ± 1.3	10.6 ± 0.9	7.7 ± 0.8
Encrusting orange sponges	0 ± 0	0 ± 0	0 ± 0	1.4 ± 0.5	0 ± 0	0 ± 0	1.5 ± 0.3	1.8 ± 0.5	0 ± 0	0 ± 0	1.5 ± 0.7	1.4 ± 0.2
Eunicella cavolini	0 ± 0	1.2 ± 0.6	0 ± 0	0 ± 0	0 ± 0	1.6 ± 0.3	0 ± 0	0 ± 0	0 ± 0	1.8 ± 0.4	1.1 ± 0.3	0 ± 0
Flabellia petiolata	1.9 ± 0.5	1.9 ± 0.7	2 ± 0.4	0 ± 0	3.4 ± 0.8	1.3 ± 0.4	0 ± 0	0 ± 0	3.4 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Haliclona fulva	0 ± 0	0 ± 0	0 ± 0	1 ± 0.3	0 ± 0	0 ± 0	1.1 ± 0.3	1.1 ± 0.3	0 ± 0	0 ± 0	1 ± 0.2	0 ± 0
Halopteris scoparia	7.6 ± 1.3	5.9 ± 1.2	0 ± 0	0 ± 0	32.5 ± 2.5	4.3 ± 1.8	0 ± 0	0 ± 0	8.8 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Hydroids	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2.7 ± 0.3	1 ± 0.2	1.7 ± 0.2
Jania spp.	15.5 ± 2	3.7 ± 0.9	0 ± 0	0 ± 0	18.5 ± 2.1	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.1	0 ± 0	0 ± 0	0 ± 0
Leptopsammia pruvoti	0 ± 0	0 ± 0	1.2 ± 0.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.5	0 ± 0
Mucilage	0 ± 0	10.2 ± 4.1	1.3 ± 0.4	0.1 ± 0.1	0 ± 0	0.2 ± 0.1	0 ± 0	17.7 ± 2.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Padina pavonica	4.1 ± 0.4	1.7 ± 0.2	0 ± 0	0 ± 0	4.7 ± 0.6	8.6 ± 1.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Paramuricea clavata	0 ± 0	0 ± 0	7.5 ± 2.2	7.1 ± 1.2	0 ± 0	0 ± 0	6.4 ± 2.1	6.8 ± 1.2	0 ± 0	0 ± 0	6.1 ± 2	5.6 ± 1.1
Parazoanthus axinellae	0 ± 0	0 ± 0	1.2 ± 0.2	1.6 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.2 ± 0.2

Species	June				October				December			
	10 m	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**	**10 m**	**20 m**	**30 m**	**40 m**
Peyssonnelia spp.	4.7 ± 0.9	8.3 ± 1.4	20.1 ± 2.1	28 ± 2.8	4.4 ± 0.8	18.1 ± 1.3	25.6 ± 1.9	18.4 ± 1.9	8.4 ± 0.8	25.2 ± 2.3	28.3 ± 1.9	13.6 ± 1.6
Bare rock	0 ± 0	1.1 ± 0.4	1.9 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Schizomavella mamillata	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	1.8 ± 0.3	0 ± 0
Sediment	1.7 ± 0.4	6.5 ± 1.6	15.9 ± 2.1	28 ± 2.5	5.4 ± 0.7	7.6 ± 1.4	20.8 ± 1.3	25.9 ± 2.1	8.8 ± 0.6	11.5 ± 1.4	16.2 ± 0.7	19.1 ± 3.3
Spirastrella cuncatrix	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.2	0 ± 0
Turf	9.7 ± 1.5	20.9 ± 3.1	32.7 ± 2.2	10.7 ± 1.6	3.4 ± 0.7	11 ± 1.9	11.7 ± 1.4	7.9 ± 1.3	10.9 ± 1.2	29.6 ± 1.9	17.2 ± 1.5	35.7 ± 1.8
Womersleyella setacea	5.9 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Zanardinia typus	0 ± 0	0 ± 0	1.2 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.9 ± 0.2	2.1 ± 0.2	2.8 ± 0.4	2.4 ± 0.3

Azzola A. et al., 2022. Troubles never come alone: outcome of multiple pressures on a temperate rocky reef. Frontiers in Marine Science

Part two: CHANGE OVER TIME





CHANGE OVER TIME

The concept of change plays a central role in studying natural ecosystems, which are constantly affected by local and global human pressures. However, this concept is more recent than one might think. In the 1960s, the idea that marine communities tend to be stable and in perfect equilibrium with the average environmental conditions was widespread, and communities were therefore described as deterministic and predictable (Bianchi, 1997). The so-called “equilibrium ecology” was challenged by the “new ecology” in the 1980s. The latter considered marine communities as non-deterministic and unpredictable, due to disturbance that plays a key role as regulator of community composition and structure (Pool, 1989; Bianchi et al., 1998). Today, it is generally understood that marine communities do not tend to or fluctuate around equilibrium (Coulson, 2021): they simply change, and such changes occur faster than previously thought (Bianchi and Morri, 2004; Bianchi et al., 2019a). The emergent ecology could probably be considered as "ecology of change" (Bianchi et al., 2022). This awareness has led to monitoring change over time in marine ecosystems (Ros and Gili, 2015). Despite the availability of historical data series is unfortunately rare (Hampton et al., 2019), revisiting previously studied sites has yielded valuable information on the degree of change in marine ecosystems (Bianchi et al., 2014, 2019a; Montefalcone et al., 2018a).

POPULATION, COMMUNITY, and ECOSYSTEM

The analysis of change over time can be done at different levels of complexity, such as at population, community, and ecosystem level. At population level, paper **3.1** investigates the change over time in a Mediterranean coral population under the effect of increasing water temperature, while paper **3.2** examines the expansion rate of an alien species population in the Mediterranean Sea under a climate change scenario. At community level, paper **4.1** investigates the change over time in the community structure of a temperate reef in an Italian Marine Protected Area, while article **4.2** assesses the change over time in a coral reef of a remote atoll in the Maldives. At ecosystem level, papers **5.1** and **5.2** assess the change in the functionality of a temperate and a tropical ecosystem, respectively.

3.1

Population structure change in a temperate reef coral after a quarter of century

Reference:

Azzola A., Bianchi C.N., Morri C., Oprandi A., Peirano A., Montefalcone M. (2022) *Estuarine, Coastal and Shelf Science*, 270: 107851

Abstract

Seawater warming is affecting corals worldwide. Mass mortality events are increasingly frequent, and the bioconstruction potential of coral reefs is jeopardized. Revisiting sites surveyed in the past may represent a valuable tool to better understand the health state and ongoing trend of coral reefs. In the Mediterranean Sea the only reef-building coral is the endemic species *Cladocora caespitosa*. Several mortality events of this coral, due to thermal anomalies, have been reported in the last decades. An impressive change occurred in a population of *C. caespitosa* revisited 25 years after: smaller colonies became the prevalent size class and larger colonies disappeared, highlighting a loss in the bioconstruction potential. Data collected on *C. caespitosa* colonies in the Eastern Ligurian Sea (NW Mediterranean) revealed that mortality rate was correlated with colony size, while reproductive potential apparently increased, suggesting hormesis (i.e., enhanced reproductive output under pre-lethal conditions).

INTRODUCTION

Mass mortality events are affecting corals worldwide (Eakin et al., 2019) with negative consequences on the bioconstruction potential of coral reefs (Montefalcone et al., 2018; but see Carlot et al., 2021). A proper evaluation of the present coral health status requires long-term data series which are available for a few regions only, such as Australia (Mellin et al., 2020), Caribbean (Gross and Edmunds, 2015), and Maldives (Morri et al., 2015). In absence of long-term series, change over time in coral health may be suitably assessed revisiting sites investigated years before (Bianchi et al., 2014).

Reef corals have been extensively studied in the tropics, whereas less is known about shallow-water temperate corals (Kružić et al., 2012). In the Mediterranean, the only zooxanthellate reef-building coral is the endemic species *Cladocora caespitosa* (L.). The calcification rates of this species may exceed 1.7 kg·CaCO3·m^{2}·a^{-1}, which is comparable to the values of many tropical reef corals (Peirano et al., 2001). When abundant, *C. caespitosa* typically forms 'beds', made of small (10-30 cm in diameter) sub-spherical colonies in dense populations; largest colonies in beds may coalesce to create reef-like structures called 'banks', reaching some decimetres in height and covering several square metres in surface area (Peirano et al., 1998; Morri et al., 2000).

During the last decades, climate anomalies have caused mortality events in *C. caespitosa*, reported for several Mediterranean areas mainly in the years 1997, 1999, 2003, 2012 and 2015 (Cerrano et al., 2000; Perez et al., 2000; Rodolfo Metalpa et al., 2005; Garrabou et al., 2009; Kersting et al., 2013, 2017; Jiménez et al., 2016; Rubio-Portillo et al., 2016). Due to its basin-wide decline and poor capacity to recover after disturbances (Peirano et al., 1998; Kersting and Linares, 2012), *C. caespitosa* has been recently included in the IUCN Red List of endangered species (Kersting et al., 2017).

With the aim to assess change over time in the population structure of *C. caespitosa* under the effect of seawater warming, we revisited a bed at Bocca di Magra (Ligurian Sea, W Mediterranean) 25 years after the first studies (Peirano et al., 1998). Additionally, data on mortality rates in relation with colony size were collected across the Eastern Ligurian Sea, where *C. caespitosa* is abundant (Morri et al., 1994).

MATERIALS AND METHODS

The *Cladocora* bed of Bocca di Magra develops near the mouth of the Magra River (44°02'31.51'' N, 9°59'03.89'' E) at 3-5 m depth (Fig. 1), and was firstly mapped in 1994 by Peirano et al. (1998). To analyse the population structure, density (mean ±

se) and size (average diameter in cm) of the colonies were recorded in 4 replicate 5×5 m quadrats, for a total surveyed area of 100 m^2. In 2019, the site was resurveyed with the same technique to measure change in *C. caespitosa* population structure 25 years later. In both years, colonies were divided into six classes according to their size (i.e., <5, 5-10, 10-15, 15-20, 20-25, 25-30 cm) to calculate their percent frequency distributions.

To assess the possible correlation between mortality and colony size, 172 field observations were collected in 17 different sites of the Eastern Ligurian Sea between 2017 and 2021. For each observation, colony size and mortality rate (percentage of necrosis or epibiosis, mostly by filamentous algae) were recorded.

Maximum sea surface temperature (SST) anomalies were examined to investigate the potential effect of warming on *C. caespitosa* over the last 25 years. Yearly maxima of SST from 1993 till 2020 were derived from NOAA (US National Oceanic and Atmospheric Administration) satellite records (freely available at www.esrl.noaa.gov/psd/cgi-bin/data/timeseries/timeseries1.pl). Anomalies were computed with respect to the underlining trend of the period.

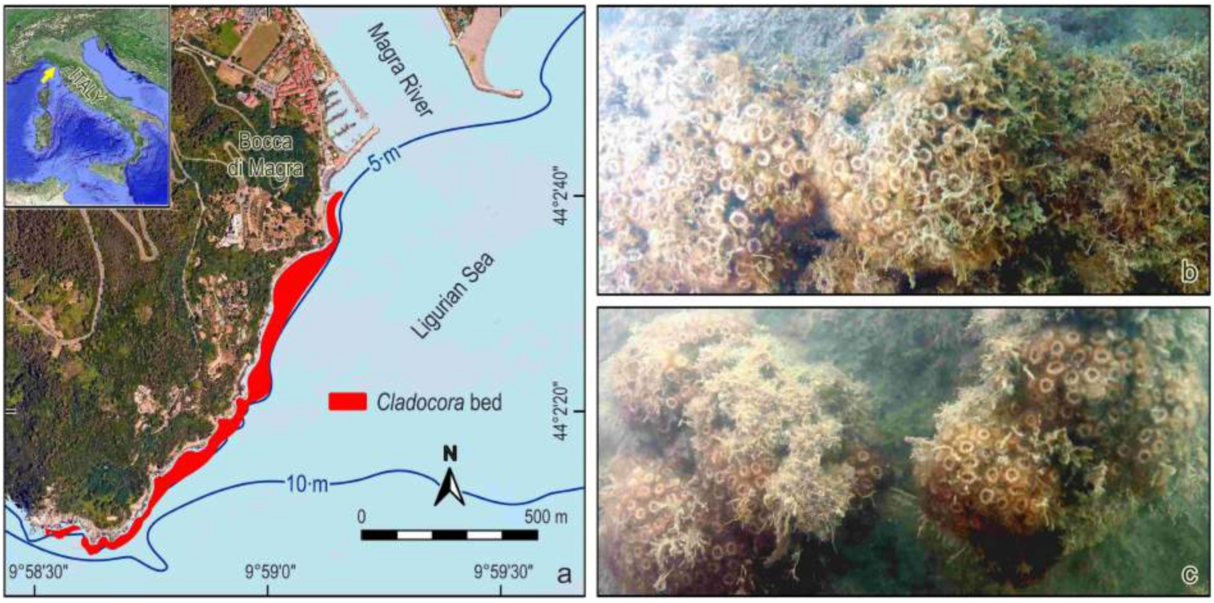


Fig. 1 Geographical situation of Bocca di Magra in Italy (arrow in the inset) and location of the *Cladocora* bed (**a**). Colonies of the bed showing some cover of filamentous algae (**b**, **c**).

RESULTS AND DISCUSSION

Revisiting the *Cladocora* bed at Bocca di Magra, an increase of 33% in colony density was observed: the number of colonies per 100 m^2 passed from 27±10.8 in 1994 to 36±7.2 in 2019. Population structure changed over time, showing a marked increase in the frequency of small colonies and a severe decrease in the frequency of the bigger ones (Fig. 2a). In 1994 the mean value was 12±1.2 cm, while in 2019 was 5±0.5 cm. *C. caespitosa* population in 1994 was dominated by medium-sized colonies (Fig. 2a): the most represented size class was 10-15 cm (30.9%), with few colonies belonging to the <5 cm (7.7%) and to the 25-30 cm (5.6%) classes. In 2019, the population showed a prevalence of small colonies belonging to the <5 cm (44.0%) and 5-10 cm (39.2%) classes, followed by intermediate colonies of 10-15 cm (14.9%). No colonies of the 20-25 cm and 25-30 cm classes were observed (Fig. 2a).

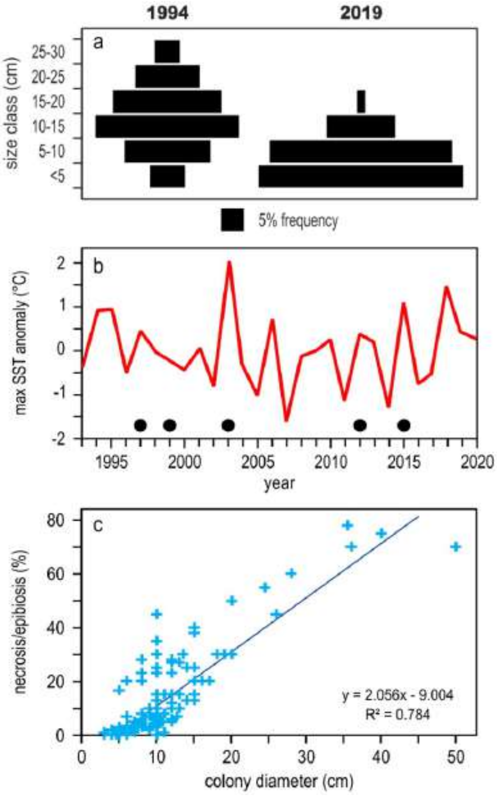


Fig. 2 Change in population structure (size frequency distribution) of *Cladocora caespitosa* in the bed of Bocca di Magra between 1994 and 2019 (a). Trend of maximum sea surface temperature (SST) in the Eastern Ligurian Sea between 1993 and 2020. Black circles indicate years with Mediterranean-wide mortality events of *Cladocora caespitosa* (see text) (b). Relationship between colony diameter and necrosis/epibiosis rate in *Cladocora caespitosa* of the Eastern Ligurian Sea (c).

Five major mortality events of *C. caespitosa* at basin-wide scale (i.e., in at least two distant sites) occurred in the last decades (Cerrano et al., 2000; Perez et al., 2000; Rodolfo Metalpa et al., 2005; Garrabou et al., 2009; Kersting et al., 2013, 2017; Jiménez et al., 2016; Rubio-Portillo et al., 2016), most of which coinciding with positive anomalies in SST maxima (Fig. 2b). Recent field observations in the Eastern Ligurian Sea indicated an increased abundance of juveniles and showed that mortality affected predominantly largest colonies (Fig. 3). Percent occurrence of necrosis and epibiosis was significantly correlated with colony size ($r = 0.89$, $p < 0.01$), small colonies being always healthier than big ones (Fig. 2c), as observed in tropical reef corals (Bak and Meesters, 1998). In tropical corals, the proliferation of juveniles associated to mortality of large colonies has been interpreted as an example of hormesis, whereby an increase in reproductive rate occurs under pre-lethal conditions (Bianchi et al., 2003, and references therein). However, high mortality rates of *C. caespitosa* juveniles have been observed elsewhere (Kersting et al., 2014), indicating that recruitment will hardly balance the recurrent climate-related mortalities.

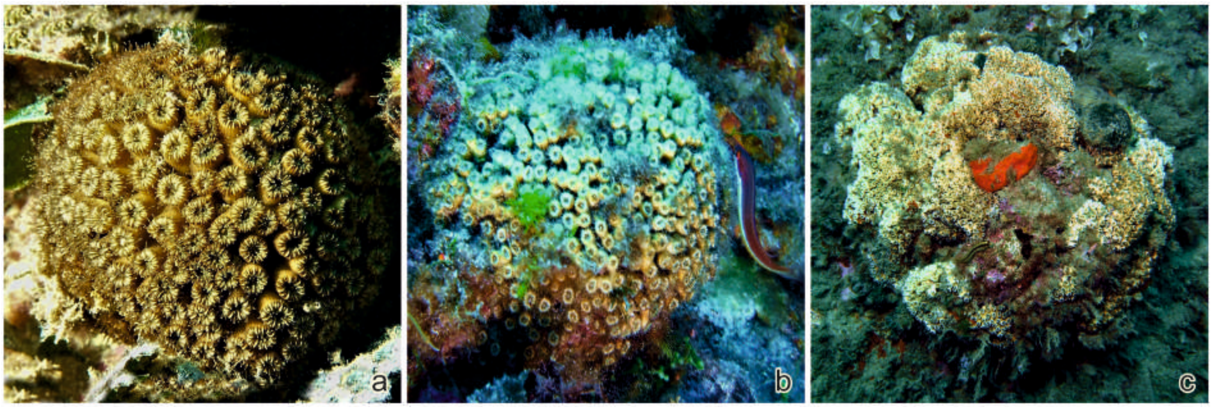


Fig. 3 Aspect of *Cladocora caespitosa* in the Ligurian Sea. A small (5 cm in diameter) healthy colony (a). An intermediate sized (ca. 12 cm) colony with a few necrotic areas covered by algae (b). A comparatively large (ca. 35 cm) colony with many necrotic areas covered by algae and encrusting sponges (c).

A similar population structure, with scarce big colonies, was observed in other Western Mediterranean localities (Kersting and Linares, 2012; Kersting et al., 2017), but lack of historical data there prevents a comparable evaluation of population change over time. In the Adriatic Sea, the *Cladocora* bed first described by Schiller

(1993) was revisited 30 years after by Zunino et al. (2018), who did not find major changes in population structure. This idiosyncratic pattern among different Mediterranean areas has equivalents in the tropics: a change in size-frequency distribution of coral populations caused by positive thermal anomalies similar to that of the Bocca di Magra bed was observed in the Maldives (Pisapia et al., 2019), while a relative stability in the abundance of large coral colonies and a systematic decline in smaller colonies were observed on the Great Barrier Reef (Dietzel et al., 2020).

Tropicalization of the Mediterranean Sea has been supposed to favour the growth of *C. caespitosa* reefs (Bianchi, 2007), as happened in warmer phases of the Quaternary (Peirano et al., 2004, 2009). The highest mortality of big colonies in the Eastern Ligurian Sea and their present dearth at Bocca di Magra contradict the above hypothesis and cast doubts about the future constructional capacity of *C. caespitosa*, banks possibly going to get even rarer than they already are (Kersting and Linares, 2012). In its evolutionary history, *C. caespitosa* has adapted to the temperate Mediterranean climate, but the speed of present warming rate might not allow it to acclimatize. However, the paucity of consistent information blurs any prediction about future trends. More studies in different sites and sustained monitoring activities are needed, as ongoing change in coral population structure may help delineating the future of Mediterranean coral reefs.

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3.2

Sea water warming favours the northward range expansion of Lessepsian species in the Mediterranean Sea: the cephalaspidean *Lamprohaminoea ovalis*

***Reference*:**

Azzola A., Furfaro G., Trainito E., Doneddu M., Montefalcone M. (2022) *Journal of the Marine Biological Association of the United Kingdom*, 102: 167-173

Abstract

Monitoring the spread of non-indigenous species is fundamental to study global warming effects on marine biodiversity. A valuable tool is represented by the continuous updating of the geographic range expansion of non-indigenous species. *Lamprohaminoea ovalis* is a Lessepsian mollusc firstly recorded in the Mediterranean Sea in the 2001. This paper reports its range expansion over the last 20 years related to water warming. The first record of *Lamprohaminoea ovalis* from the Ligurian Sea (NW Mediterranean) is also reported. Two individuals (about 10 mm length) were observed on a sub-vertical rocky bottom at 26 m depth in the potential trailing behaviour. This recent finding extends the currently known distributional range of *Lamprohaminoea ovalis*, representing the northernmost occurrence of this non-indigenous species in the Western Mediterranean Sea.

MARINE RECORD

As a result of global warming, the spread of non-indigenous species is one of the major drivers of change in marine ecosystems, with drastic consequences on their biodiversity (Bonanno and Orlando-Bonaca, 2019). Considering the number of non-indigenous species and the rate of introduction, the Mediterranean is one of the most affected seas by biological invasions (Zenetos et al., 2010). Spatio-temporal information on non-indigenous species and monitoring their geographic range expansion represent valuable tools for conservation of marine ecosystems and biodiversity (Katsanevakis et al., 2020).

Following a taxonomic revision of the Order Cephalaspidea, *Haminoea cyanomarginata* Heller and Thompson 1983 was described from individuals of the Red Sea (Heller and Thompson 1983; Dekker and Orlin, 2000) and initially considered junior synonym of *Lamprohaminoea cyanomarginata* (Oskars and Malaquias, 2019). Further taxonomic studies demonstrated that both *H. cyanomarginata* and *L. cyanomarginata* are junior synonyms of *Lamprohaminoea ovalis* (Pease, 1868; Oskars and Malaquias, 2020). This heterobranch, characterized by highly variable body colour pattern, was firstly described from Tahiti (French Polynesia, France). Nowadays it is widespread from the Red Sea to the Indo-West Pacific. Anyway, its fairly recent records from Greece, Turkey, Malta, Italy, Cyprus, Croatia, and Spain, more than 20 years after its first finding in the Red Sea, suggest that it is an invasive Lessepsian species that entered and established into the Mediterranean Sea via the Suez Canal (Rizgalla et al., 2018; Oskars and Malaquias, 2020). Nowadays, *L. ovalis* has been reported from the whole Mediterranean basin (Zenetos et al., 2010; Mifsud, 2007; Crocetta and Vazzana, 2009; Fernández-Vilert et al., 2018 and reference therein; Ragkousis et al., 2020), except from the Gulf of Lion and the Ligurian Sea.

With the aim of investigating *L. ovalis* range expansion in the Mediterranean Sea in the last 20 years, all available records have been collected. *L. ovalis* expansion rate has been evaluated by measuring the geographical distances (kilometres) between the location of its first record in 2001 and the locations of the following new records from other Mediterranean areas. The expansion rate of the species has been compared with change in time of the sea surface temperature (SST), to evaluate if the spread of this non-indigenous species is a direct consequence of sea water warming. The first record of *L. ovalis* from the Ligurian Sea (NW Mediterranean) has also been reported.

The first occurrence of *L. ovalis* in the Mediterranean Sea was reported at Porto Germano (Greece) in the year 2001 (Zenetos et al., 2003) (Fig. 1a; Table 1). In the following years this species remained confined to the eastern basin of the

Mediterranean, where Lessepsian species more typically settle (Gambi et al., 2009). Since the second half of the 2010s the species crossed the 20° meridian East reaching the westernmost portions of the Mediterranean and since 2014 it crossed the 40° parallel North starting to approach the northernmost and coldest sectors of the basin (Fig. 1a; Table 1). During the last 20 years the yearly mean SST continuously increased, and the isotherms shifted northwards (Pisano et al., 2020) (Fig. 1b, c), favouring the north-western expansion of *L. ovalis* (Bianchi, 2007).

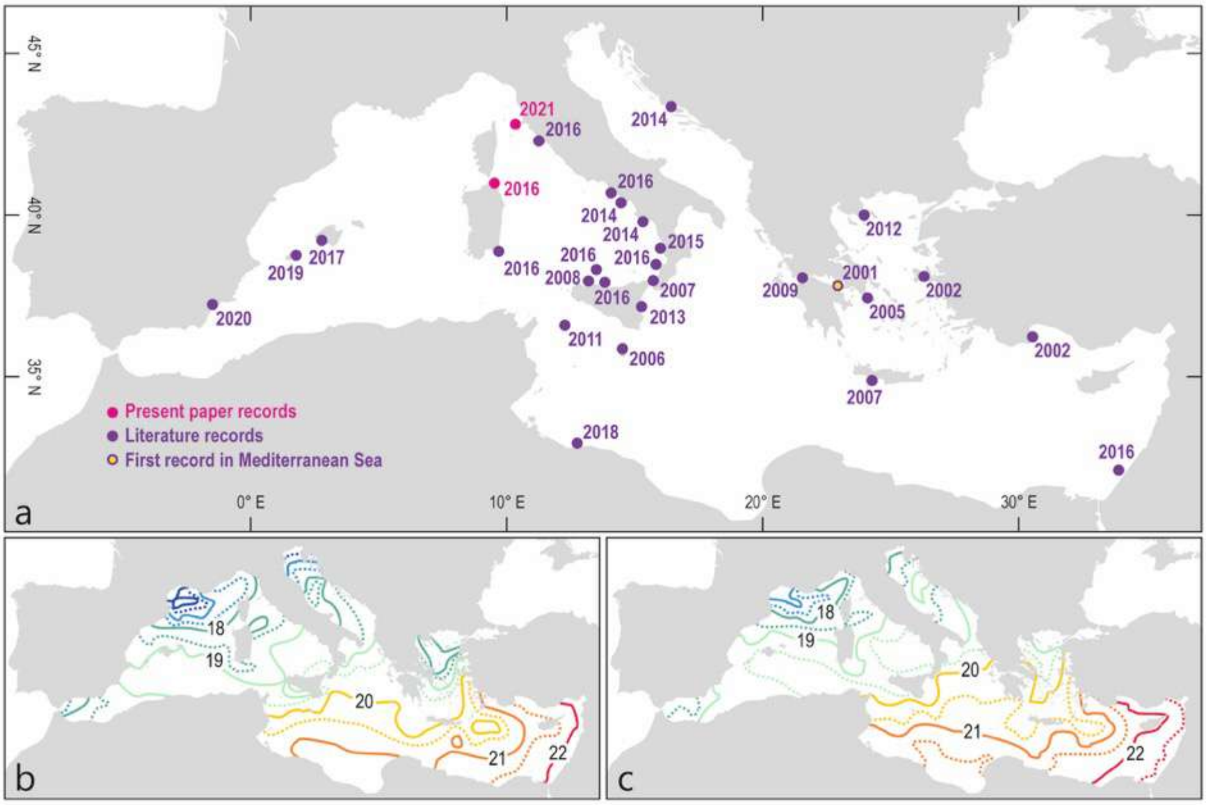

Fig. 1 Map of the available records of *Lamprohaminoea ovalis* in the Mediterranean Sea (**a**). Yearly mean SST from 1994 to 2005 (**b**) and from 2006 to 2018 (**c**) (modified from Pisano et al. 2020).

Since 2016 the geographic range of *L. ovalis* expanded northward in the Tyrrhenian Sea (NW Mediterranean) and an individual has been recorded in 2016 at Porto Ercole, Tuscany (Fig. 1a, Table 1). In the same year the species has been observed multiple times by the authors in the Tavolara-Punta Coda Cavallo Marine Protected Area (MPA), which represents its first record in the North Sardinia (Tyrrhenian Sea) (Fig. 1a, Table 1). Four individuals have been observed at Reulino (Isola Rossa) at 13 m depth in a mixed habitat with rocks, detritus and algae (Fig. 2a). In the following years two other individuals have been observed in the MPA, one at 30 m depth on a coralligenous outcrop at Punta Papa (Tavolara Island), and one at Porto San Paolo at 3 m depth (Fig. 2b). In the subsequent years *L. ovalis* became common in the MPA along the south-eastern coast of Tavolara Island.

Fig. 2 Individuals of *Lamprohaminoea ovalis* observed at: (**a**) Isola Rossa in 2016 (Sardinia, Tyrrhenian Sea), (**b**) Porto San Paolo in 2017 (Sardinia, Tyrrhenian Sea), (**c**, **d**) Elba Island (Tuscany, Ligurian Sea). Photo credit: Egidio Trainito (a, b), Lorenzo Moscia (c), Monica Montefalcone (d).

On the 21st October 2021, during a scientific expedition conducted by University of Genova in collaboration with Greenpeace to monitor non-indigenous species, two *L. ovalis* individuals of about 10 mm in length were observed and photographed at Portoferraio, Elba Island (Ligurian Sea) (Fig. 1a; Fig. 2c, d; Table 1). The individuals were found at 26 m depth on a sub-vertical rocky bottom, covered by photophilic algae and *Posidonia oceanica*. They were observed in the potential trailing behaviour. In Heterobranchia trailing action is aimed at reproduction: an individual follows the mucous trail of a conspecific and keeps contact with it by touching the tail.

In this way, rows of up to 4 individuals can be formed (Betti et al., 2011; Rizgalla et al., 2018). The unambiguous determination of the species from the photographs taken in Sardinia and at Elba Island has been possible thanks to the distinctive unornamented bubble shell and body colour pattern. The mantle bordered with purple perfectly matches the '*L. cyanomarginata* purple morph' (Fig. 3) reported in the recent systematic revision of the genus *Lamprohaminoea* Habe, 1952 and ascribed to *L. ovalis* species (Oskars and Malaquias, 2020).

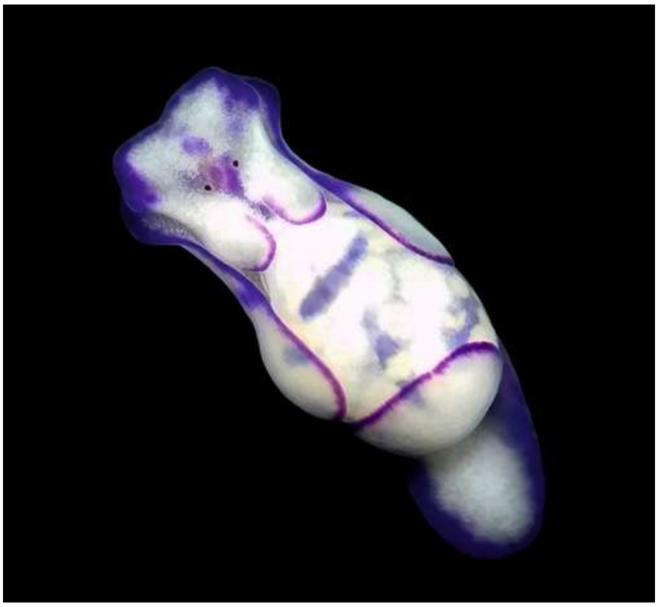

Fig. 3 An individual of *Lamprohaminoea ovalis* collected at 10 m depth at Cala Grande, Argentario (Tuscany, Tyrrhenian Sea) in October 2017 and photographed in laboratory. Photo credit: Giulia Furfaro.

The expansion rate of *L. ovalis* showed a significant increasing trend since 2001 (Fig. 4a), and two distinct phases can be identified: i) 2001-2013, when the species spread slowly westward; and ii) 2014-2021, when its spread was faster and northward (Fig. 1a; Fig. 4a). The mean distance 'travelled' by *L. ovalis* in the second period (2014-2021) was significantly higher than in the first one (Fig. 4b). This species increased its range expansion in correspondence of a warming phase of the SST started in the 2014 (Bianchi et al., 2019). As it is known that high summer temperatures allow the spread of alien species and mild winter temperatures allow their establishment (Osland et al., 2021), both minimum and maximum mean SST in the two considered periods showed the same significant increase as *L. ovalis* expansion rate (Fig. 4c, d).

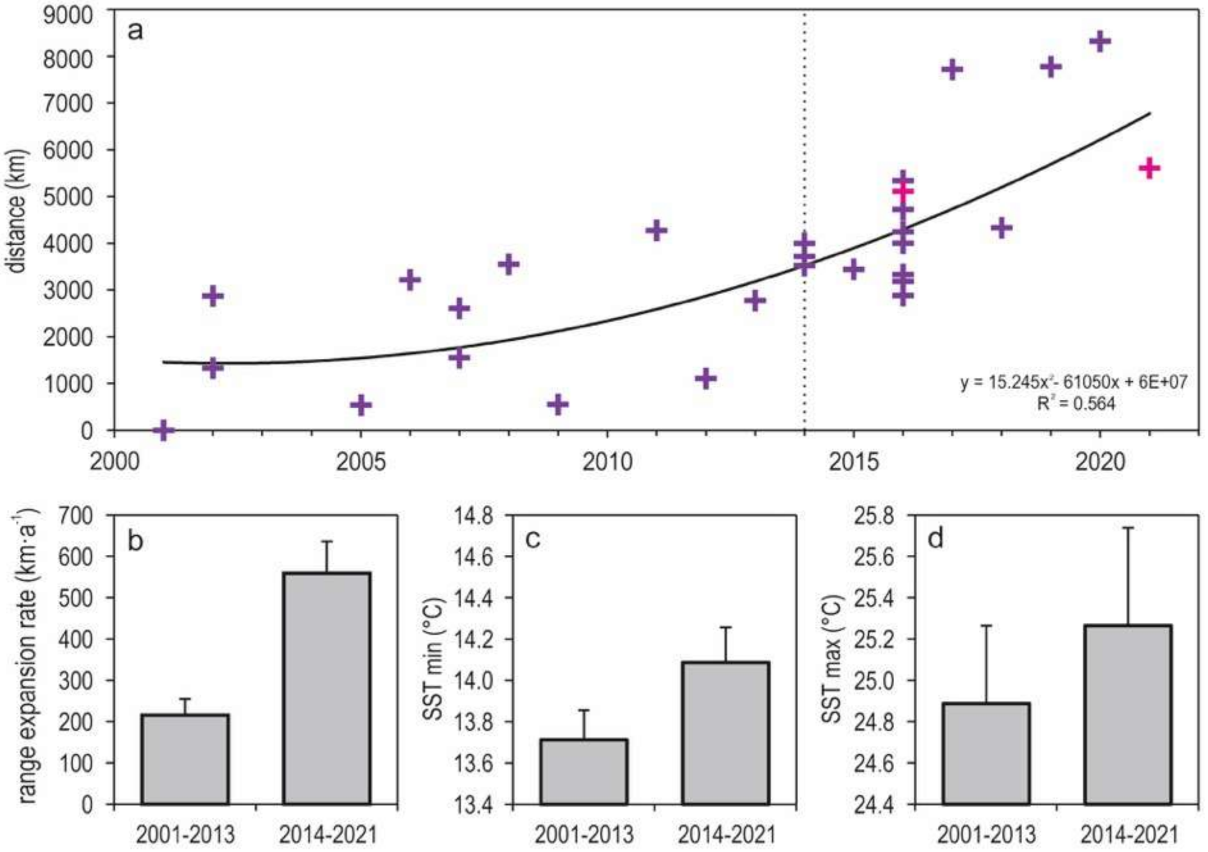

Fig. 4 Expansion rate of *Lamprohaminoea ovalis* in the Mediterranean Sea since its first record in 2001 (**a**). Mean range expansion rate in the two investigated time periods: 2001-2013 and 2014-2021 (**b**). SST minimum (**c**) and maximum (**d**) yearly mean in the two investigated time periods 2001-2013 and 2014-2021.

The expansion of non-indigenous species is an increasing and recurrent phenomenon, and the Mediterranean Sea is going towards a phase of tropicalization (Bianchi and Morri, 2003). In the Ligurian Sea, one of the northernmost portions of the Mediterranean Sea, reports of thermophilic southern species are becoming frequent (Parravicini et al., 2015; Bianchi et al., 2018). The continuous observations of *L. ovalis* in the Mediterranean, particularly after the year 2014, demonstrate that its spread is mainly favoured by sea water warming. In the coldest Mediterranean sectors, where the yearly mean SST is still around 18°C or lower, this species is not yet widespread. Nevertheless, the first observation of *L. ovalis* in the Ligurian Sea reported in this paper represents the northernmost record of occurrence of this non-indigenous species in the Western Mediterranean Sea, providing further important evidences of the ongoing global water warming also in the coldest sectors of the Mediterranean.

Table 1 List of records of *Lamprohaminoea ovalis* in the Mediterranean Sea (see also Fig. 1).

First record	Locality	Lat. (N)	Long. (E)	Reference	Author of the record
2001	Porto Germano, Greece	38.155064	23.222459	Zenetos et al. 2003; CIESM	Ernesto Mollo
2002	Üç Adalar, Antalya, Turkey	36.459593	30.549790	Sea Slug Forum - http://www.seaslugforum.net	Adnan Buyuk
2002	Çeşme, Izmir, Turkey	38.326670	26.294391	Sea Slug Forum - http://www.seaslugforum.net	Namzi Kural
2003	Üç Adalar, Antalya, Turkey	36.459593	30.549790	Sea Slug Forum - http://www.seaslugforum.net	Baki Yokes
2004	Üç Adalar, Antalya, Turkey	36.459593	30.549790	Sea Slug Forum - http://www.seaslugforum.net	
2005	Çeşme, Izmir, Turkey	38.326670	26.294391	Sea Slug Forum - http://www.seaslugforum.net	Aziz Saltik
2005	Saronikos Kolpos, Greece	37.768312	23.895814	Sea Slug Forum - http://www.seaslugforum.net	Panaiotis Ovalis
2006	Rdum id-Delli, Malta	35.951865	14.338688	Mifsud 2007	
2006	Fomm ir-Rih Bay, Malta	35.907289	14.339596	Mifsud 2007	
2006	Ġnejna Bay, Malta	35.920942	14.342461	Mifsud 2007	
2007	Cirkewwa, Malta	35.983213	14.332776	Sea Slug Forum - http://www.seaslugforum.net	Patricia Peels
2007	Schinaria, Plakias, Creta, Greece	35.166232	24.421272	Sea Slug Forum - http://www.seaslugforum.net	Jim Anderson
2007	Saline Joniche, Italy	37.937922	15.707796	Crocetta and Vazzana 2009	
2008	Castellammare del Golfo, Italy	38.036258	12.876490	Stasolla et al. 2014	Santo Tirnetta and Anna Macaluso
2009	Lazzaro, Italy	37.971763	15.662174	Crocetta et al. 2009	
2009	Salamis, Attiki, Greece	37.934791	23.466596	NMR_Mollusca - http://www.marinespecies.org	Joop Trausel and Frans Slieker
2009	Golfo di Patrasso, Greece	38.317006	21.642224	Medslugs - http://www.medslugs.de	George Tryfonopoulos
2011	Scauri, Pantelleria, Italy	36.765097	11.969795	Stasolla et al. 2014	Maria Ghelia and Francesco De Marchi
2012	Kalogrias Beach, Chalkidiki, Greece	40.177022	23.714886	Social media - Facebook	Nicholas Samaras
2013	Saline Joniche, Italy	37.937922	15.707796	Stasolla et al. 2014	Domenico Tripodi
2013	Acicastello, Italy	37.554525	15.150620	Stasolla et al. 2014	Alessandro Pagano
2013	Scilla, Italy	38.258340	15.715148	Stasolla et al. 2014	Domenico Tripodi
2014	Mgarr, Gozo, Malta	36.027588	14.308428	Medslugs - http://www.medslugs.de	Silke Baron
2014	Baia di Puolo, Massa Lubrense, Italy	40.626660	14.341940	Katsanevakis et al. 2014	Fabio Russo
2014	Acicastello, Italy	37.554525	15.150620	Social media - Facebook	Alessandro Pagano
2014	Casteldaccia, Palermo, Italy	38.057863	13.542987	Social media - Facebook	Dario Messina
2014	Palinuro, Italy	40.029065	15.266873	Tiberti et al. 2015	Fabio Barbieri
2014	Uvala Travna, Šolta, Croatia	43.368852	16.267800	Social media - Facebook	Jakov Prkic
2015	Ammouliani island, Greece	40.313241	23.944508	Social media - Facebook	Yiannis Iliopoulos
2015	Praiano, Italy	38.930623	16.209802	Social media - Facebook	Raffaele Livornese
2016	Porto Ercole, Grotta Azzurra, Italy	42.370636	11.188660	Social media - Facebook	Gabriele Ziino
2016	Lago Faro, Italy	38.269288	15.637137	Vitale et al. 2016	
2016	Munxar, Gozo, Malta	36.021566	14.218147	Social media - Facebook (Nudibranch lovers)	Vera Wittenberg
2016	Agios Minas, Greece	40.587246	22.881911	Underwater Photography in Greece - http://www.greeceme.com	Tasos Drosopoulos
2016	Costa Rei, Muravera, Sardegna, Italy	39.249898	9.579118	Social media - Facebook	Giorgio Zara e Silvia Crippa
2016	Nemesis wreck, Protraras, Cyprus	32.049633	34.0366	Yokes et al. 2018	Fabio Crocetta and Vasilis Andreou
2016	Ammouliani island, Greece	40.313241	23.944508	Underwater Photography in Greece - http://www.greeceme.com	Yiannis Iliopoulos

2016	Greben Štit, southern side, Croatia	42.77166	17.33250	Chartosia et al. 2018	Branko Dragicevic, Marija Despalatovic and Jakov Dulcic
2016	Capo Vaticano, Italy	38.618792	15.825806	Social media - Facebook	Francesco Pacienza
2016	Ustica, Italy	38.705300	13.196120	Social media - Facebook	Jonathan Cecchinel
2016	Ischia Island	40.737956	13.853602	Personal communication	Paolo Mariottini and Giulia Furfaro
2016	Secca della Formica, S. Flavia, Italy	38.083431	13.550167	Social media - Facebook	Giovan Ombrello
2016	Isola Reulino, AMP Tavolara, Italy	40.877310	9.673453	Present paper	Egidio Trainito
2016	Kašuni, Split, Croatia	43.510980	16.400696	Social media - Facebook	Jakov Prkic
2016	Praiano, Italy	38.930623	16.209802	Social media - Facebook	Francesco De Rosa
2017	Reggio Calabria, Italy	38.104215	15.626211	Social media - Facebook	Domenico Tripodi
2017	Porto Ercole, Grotta Azzurra, Italy	42.370636	11.188660	Social media - Facebook	Gabriele Ziino
2017	Argentario Promontory, Italy	42.400000	11.091666	Personal communication	Giulia Furfaro
2017	Capo Figari, Golfo Aranci, Italy	40.991111	9.6577778	Personal communication	Renato Romor
2017	Porto Santo Stefano, Italy	42.444388	11.114297	Social media - Facebook	Flavio Mattucci
2017	Punta Papa, AMP Tavolara, Italy	40.911873	9.743434	Present paper	Egidio Trainito
2017	Porto San Paolo, Italy	40.881635	9.637065	Present paper	Egidio Trainito
2017	Illa del Toro, Mallorca, Spain	39.462182	2.471748	Fernandez-Vilert et al. 2018	Rubén Castrillo
2018	Tripoli, Lybia	32.854028	13.054639	Rizgalla et al. 2018	
2019	Ibiza, Spain	39899546	1.429359	Ragkousis et al. 2020	Miquel Pontes, Fabio Crocetta and Ro Ba
2020	Nova Tabarca Island, Alicante, Spain	38.172250	-0.483639	Ragkousis et al. 2020	Miquel Pontes and Fabio Crocetta
2020	Gozo, Malta			Social media - Facebook (nudibase)	Flow Curlebee
2020	AMP Tavolara, Italy	40.896533	9.707200	Present paper	Egidio Trainito
2021	Porto Ferraio, Elba Island, Italy	42.821707	10.328733	Present paper	Annalisa Azzola and Monica Montefalcone

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4.1

Variability between observers does not hamper detecting change over time in a temperate reef

Reference:

Azzola A., Atzori F., Bianchi C.N., Cadoni N., Frau F., Mora F., Morri C., Oprandi A., Orrù P.E., Montefalcone M. (2022) *Marine Environmental Research*, 177: 105617

Abstract

Marine ecosystems are subject to global and local impacts, both contributing to dramatic changes in coastal communities. Assessing such changes requires time series or the revisitation of sites first surveyed in the past. In both cases, data are not necessarily collected by the same observers, which could lead to a bias in the results. In the Marine Protected Area (MPA) of Capo Carbonara (Sardinia, Italy), established in 1998, rocky reef communities were first assessed in 2000 by two diving scientists. Twenty years later, the same rocky reefs were resurveyed using the same method by two other diving scientists. In both surveys, semi-quantitative data on conspicuous species were collected at five sites in four depth zones, providing the possibility of assessing change over time. To explore the influence of climate and local pressures, existing data on sea surface temperature, resident population, tourism and diving activities were analysed. The reef communities of the Capo Carbonara MPA have distinctly changed over time, mostly under the effect of seawater warming, as highlighted by the occurrence of thermophilic species and by other climate-related indicators. On the other side, species vulnerable to local human pressures have increased over time, demonstrating the effectiveness of the protection measures undertaken by the MPA. Comparing data collected by four different observers in the two periods demonstrated that change over time was significantly greater than variability between the observers.

INTRODUCTION

In the era defined as the Anthropocene, coastal marine ecosystems have undergone important changes worldwide as a consequence of human-induced impacts (de Bakker et al., 2017). The health of rocky reef habitats, in particular, is threatened by the synergistic effects of several affectors acting at both local and global scales (Halpern et al., 2007; Montefalcone et al., 2017). Among the local anthropogenic impacts, the most significant are overfishing, pollution, eutrophication and coastal development (Thrush et al., 2009). The species affected by these impacts are those subjected to commercial or recreational harvesting, species sensitive to contaminants, and benthic species whose habitat is modified by coastal works (Thibaut et al., 2005; O'Hara et al., 2021). Further localised habitat degradation may be due to recreational divers activities, especially for fragile species (e.g., bryozoans) that are subjected to damaging contact from careless diving behaviour (Barker et al., 2004; Terrón-Sigler et al., 2016; Casoli et al., 2017; Betti et al., 2019). On a global scale, one of the most serious threats is seawater warming, which has caused mass mortality events over the past decades (Cerrano et al., 2000; Garrabou et al., 2009, 2021). The increase in seawater temperature also favours the spread and settlement of alien and thermophilic species (Bianchi et al., 2018, 2019a; Dimitriadis et al., 2021).

Due to the speed with which climate and local anthropogenic impacts are occurring, species may not be able to adapt, leading to dramatic consequences on marine communities (Ceccherelli et al., 2020). Change in environmental factors that persists over time, namely 'regime shift', can lead to a drastic modification in community structure and composition, namely 'phase shift' (Montefalcone et al., 2011). The individuation of phase shifts in benthic communities represents a valuable tool to understand the effects of global change on coastal habitats (Gatti et al., 2015), particularly in a closed basin such as the Mediterranean Sea (Bianchi et al., 2012; Gravina et al., 2020).

Assessing change over time requires long term data series, which are rarely available (Bianchi and Morri, 2004; Proença et al., 2017). In absence of such data series, the revisitation of sites already surveyed in the past has proved successful in detecting change over time of coastal communities (Pearson et al., 1985; Barry et al., 1995; Hiscock, 2005; Bianchi et al., 2014, 2019b; Montefalcone et al., 2018a). Over long time periods (decades), data may rarely be collected by the same researcher

(Bianchi et al., 2014). Level of experience, ability and knowledge of the different observers may influence sampling quality, possibly producing a bias in data analysis. In a study aiming at measuring change in seagrass meadows, Montefalcone et al. (2013) concluded that the variability between observers induced differences greater than those due to change over time. Similarly, Sales and Ballesteros (2010) stated that the differences obtained from a long-term comparison of algal assemblages could be due to misidentifications or different abilities of the observers. Unfortunately, the available comparisons of long term data rarely quantify the incidence of the observer effect.

With the aim of assessing change over time due to seawater warming, marine protected areas (MPAs) may provide valid field laboratories, since they represent a cornerstone of the efforts to reduce local human impacts on ecosystems by restricting different economic activities, such as fishing, shipping, anchoring, mining, oil and gas extraction, etc. (García-Barón et al., 2020), but cannot halt the impacts of global warming (Bruno et al., 2018).

The rocky reef communities of Capo Carbonara MPA were initially surveyed in 2000, short after its establishment (Bianchi and Morri, 2001), while subsequent investigations evaluated water quality and selected marine habitats (Ferrigno and Guala, 2015; Andromède, 2017; Piazzi et al., 2018). In 2020 the survey of 2000 has been repeated with the same method and in the same sites by different observers (Montefalcone and Azzola, 2020). This paper uses the Capo Carbonara MPA as example of an area where local anthropogenic impacts should not represent a major driver of change. Such a situation would be of help to reduce confounding effects in assessing change over time due to global warming. As data have been collected 20 years apart by different observers, comparing the outcomes of the two surveys will also allow assessing whether the variability between observers may blur the detection of change over time.

MATERIAL AND METHODS

Study area

The Capo Carbonara MPA (39°07'04" N; 09°30'35" E), established by the Italian Ministry of the Environment in 1998, is located near the town of Villasimius (SE

Sardinia, Italy). The MPA covers an area of 14,360 ha and includes Cavoli Island, Serpentara Island, and numerous minor islets and shoals (Fig. 1).

According to the regulations, the Capo Carbonara MPA is divided into four zones subject to different levels of protection: i) zone A (integral reserve), where only rescue, surveillance activities, scientific research, and recreational scuba diving are allowed under the authorization of the MPA; ii) zone B (general reserve), where the activities allowed in the A zone are also allowed, together with swimming, regulated navigation, authorized sport fishing by Villasimius residents, and anchoring on sandy bottoms deprived of Posidonia oceanica meadows; iii) zone C (partial reserve), where navigation, sport fishing and anchoring are also allowed with the same restrictions of the zone B; iv) zone D (experimental reserve), where the same activities for the zone C are allowed, together with access for tourist recreational vessels.

Fig. 1 Map of the study area with the five surveyed sites and the indication of protection zones. Dashed lines represent depth contours redrawn from the nautical chart IT300045 of the Italian Navy Hydrographical Institute.

Data collection and field activities

The first survey was conducted in 2000 by the expert diving scientists Carlo Nike Bianchi (1200 previous scientific dives) and Carla Morri (700 previous scientific dives) with the aim of characterizing the reef communities of Capo Carbonara MPA and defining a fruition plan for divers (Bianchi and Morri, 2001; Bianchi et al., 2002). They surveyed five different sites subjected to different levels of protection (Fig. 1, Fig. 2, Table 1): i) Variglioni (zone A, a rock followed by large boulders ending on coarse sand at about 40 m); ii) Caterina (zone A, a rocky shoal ending on coarse sand at about 40 m); iii) Serpentara (zone B, a rock with patches of Posidonia oceanica ending on sand at about 45 m); iv) Madonnina (zone B, rocks and meadows of Cymodocea nodosa or Posidonia oceanica on sandy bottom at about 15 m); v) Berni (zone B, a shallow rocky shoal ending on sand with Posidonia oceanica meadow at about 25 m). In each site they carried out vertical transects of variable length (depending on the maximum depth of individual sites) and 4 m total width from the bottom to the surface. The semi-quantitative abundance (based on three scores: 1 = scarce; 2 = abundant; 3 = very abundant; with absence being 0) of conspicuous species (i.e., species of a size that would allow their recognition and identification underwater) was recorded, with reference to the depth where they had been observed (Bianchi et al., 2004; Gatti et al., 2017). Twenty years later, the same sites were revisited by two different diving scientists, the expert Monica Montefalcone (600 previous scientific dives) and the trainee Annalisa Azzola (50 previous scientific dives), using the same survey methodology (Montefalcone and Azzola, 2020). Both surveys were carried out in summer. The coordinates of the surface bench mark of each transect were set by GPS in both years (Table 1).

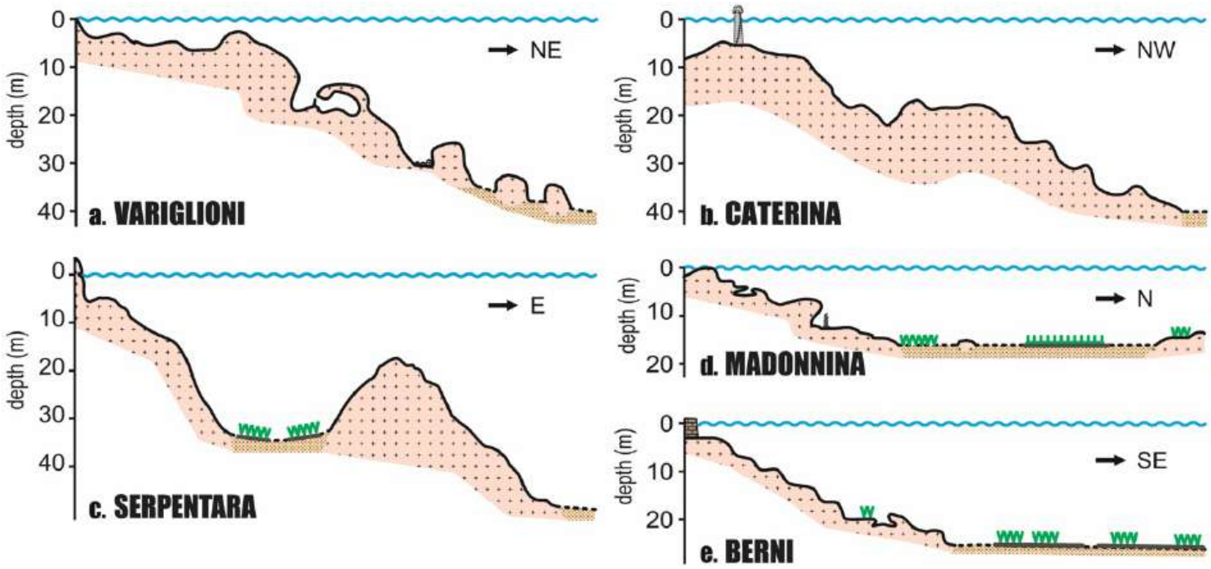

Fig. 2 Bottom profiles of the five surveyed sites: Variglioni, a rocky bottom ending on coarse sand at about 40 m (**a**); Caterina, a rocky shoal ending on a coarse sand at about 40 m (**b**); Serpentara, a rocky bottom with patches of *Posidonia oceanica* ending on a sand at 45 m (**c**); Madonnina, a sandy bottom at 20 m with rocks and *Cymodocea nodosa* and *Posidonia oceanica* meadows (**d**); Berni, a shallow rocky shoal ending on sand at 25 m with a *Posidonia oceanica* meadow (e). Pink colour with crosses is granite rocks, beige colour with dots is sand, green ⊥ symbols are *Cymodocea nodosa*, and green ᵛ symbols are *Posidonia oceanica*.

Table 1 Methodological details about field activities. Coordinates, MPA zone and transect direction are reported for each site. Date, maximum and minimum depth, and dive time are also showed for each sampling year.

Site	**Variglioni**		**Caterina**		**Serpentara**		**Madonnina**		**Berni**	
	(zone A)		(zone A)		(zone B)		(zone B)		(zone B)	
Coordinates at surface	39°4.941' N; 9°32.520' E		39°5.019' N; 9°30.892' E		39°8.565' N; 9°36.554' E		39°5.077' N; 9°32.329' E		39°7.422' N; 9°33.129' E	
Transect direction	NE		NW		E		N		SE	
Year	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020
Max depth (m)	40.7	39.0	38.9	40.5	49.2	44.5	16.6	15.5	26.3	24.8
Min depth (m)	0.5	0.5	5	6	0.5	0.5	0.5	0.5	0.5	1
Time (min)	54	57	36	47	42	49	35	38	29	31

Data on climate

Sea surface temperature (SST) was examined to evaluate the potential role of global warming as a driver of change in the rocky reef communities. As no time series of water temperature data exist for the area of Capo Carbonara MPA, SST data were derived from NOAA (US National Oceanic and Atmospheric Administration) satellite records, freely available at www.esrl.noaa.gov/psd/cgi-bin/data/timeseries/timeseries1.pl. Yearly maximum, mean and minimum sea water temperature anomalies were analysed to illustrate their trends in the last two decades (i.e., between 2000 and 2020). Temperature anomalies were calculated as deviations from overall annual mean (actual value of each year minus the average for the period 2000-2020).

Data on human pressures

To evaluate local human pressures, data about resident population, tourism and diving activities were collated from official sources. Data on resident population were provided by ISTAT (the Italian National Institute of Statistics), updated to December 31 each year (https://www.istat.it). Data on tourism were obtained by the ‘Assessorato del Turismo, Artigianato e Commercio’ of the ‘Regione Autonoma della Sardegna’ (http://osservatorio.sardegnaturismo.it); tourism data, available for the period 2013 to 2019 only, were divided in ‘visitors’ (i.e., number of customers hosted in receptive exercises) and ‘stays’ (i.e., number of nights spent by the customers in the receptive exercises). Finally, data on diving activities carried out in 2001, 2007, 2015, 2016, 2018, 2019 and 2020 in the Capo Carbonara MPA (in term of number of dives per site) were provided by the MPA. Unfortunately, the number of dives for the site Madonnina was not available.

Data management and analysis

Semi-quantitative abundances of conspicuous species were organized in a data matrix (time × site × depth zone × observer) × species. Two time periods were defined: 0, for the data sampled in the 2000; 2, for the data sampled in the 2020. All the five sites were considered: V = Variglioni; C = Caterina; S = Serpentara; M = Madonnina; B = Berni. Four depth zones were defined following Riedl (1964): 1 = 0.3-3 m, water’s edge; 2 = 3-18 m, zone of oscillatory water movement; 3 = 18-30 m, zone of unidimensional flow; 4 = >30 m, zone of laminar flow (Morri et al. 2004).

Finally, the three expert observers and the trainee were coded as follows: A = Annalisa Azzola; C = Carla Morri; M = Monica Montefalcone; N = Carlo Nike Bianchi.

The data matrix was submitted to non-metric multidimensional scaling (nMDS), based on Euclidean Distances. Inspection of the nMDS plot suggested that the first axis was mostly an expression of depth, and the second axis of time (see Results). Thus, axis scores were grouped according to these two factors, and differences inside both groups were tested by permutational multivariate analysis of variance (PERMANOVA) based on a Euclidean Distance matrix (Anderson 2001).

To explore the possibility of a link with climate or human pressures, the species found were divided into categories according to their sensitivity to different threats. Five groups of species indicated the effects of global warming: i) aliens (tropical or subtropical species recently introduced in the Mediterranean Sea); ii) southerners (thermophilic native species previously confined to the southernmost areas of the Mediterranean but presently migrating northward); iii) mortality (species particularly affected by the effects of increasing temperatures and for which mass mortality have been reported due to heatwaves); iv) mucilage (aggregations of algal exudates whose development is favoured by high temperatures); v) others (species that do not suffer the effects of climate change). Likewise, five groups of species were identified based on their vulnerability to local human impacts: i) harvesting (species that are harvested or fished as food resources); ii) Fucales (an order of macroalgae known to be negatively affected by pollution); iii) protection (species included in Annex II of the Barcelona Convention); iv) diving (erect and fragile species, such as bryozoans, particularly exposed to mechanical impacts from divers); v) others (species presumably little influenced by local human impacts). To analyse change with time of these species groups between the two time periods, for each species the following index of relative dominance was calculated (Parravicini et al., 2008):

$IRD = (AS2020 – AS2000) \times (AS2020 + AS2000)-1$

where IRd is the index of relative dominance, AS2020 is the abundance score of a species in the year 2020, and AS2000 is the abundance score of the same species in 2000. IRd ranges from -1 (species found in 2000 only) to +1 (species found in 2020 only). IRd values of individual species were averaged for each group.

To assess change over time in the four depth zones and in the five sites, subjected to different levels of protection and diving pressures, a dissimilarity matrix based on

Euclidean Distances has been generated. Euclidean Distance values of each depth zone and site in the two years were averaged, and differences were evaluated by the non-parametric test Krustal-Wallis, as the unequal number of depth zones within sites did not allow for a parametric test.

To estimate the observer effect in detecting change over time, the values of Euclidean Distances between the observers of each pair that had surveyed in the same year (i.e., 2000 N vs 2000 C and 2020 M vs 2020 A) or in different years (i.e., 2000 N vs 2020 M, 2000 N vs 2020 A, 2000 C vs 2020 M, 2000 C vs 2020 A), were compared with ANOVA and Tukey's post-hoc comparison.

Statistical analyses were performed with the open-access software PaSt (Hammer et al., 2001) and with Primer 6 + PERMANOVA.

RESULTS

Climate pressures

Analysis of sea surface temperature (SST) data showed a positive trend for the maximum, mean and minimum anomalies (Fig. 3). From 2000 to 2020 the maximum SST has increased by 1.92 °C, the mean SST has increased by 0.32 °C and the minimum SST by 0.29 °C. Recurrent marine heatwaves (i.e., temperature > 1 °C than the average monthly values) were recorded in the study period with the highest peak (nearly reaching 27 °C) in the 2003 (Fig. 3).

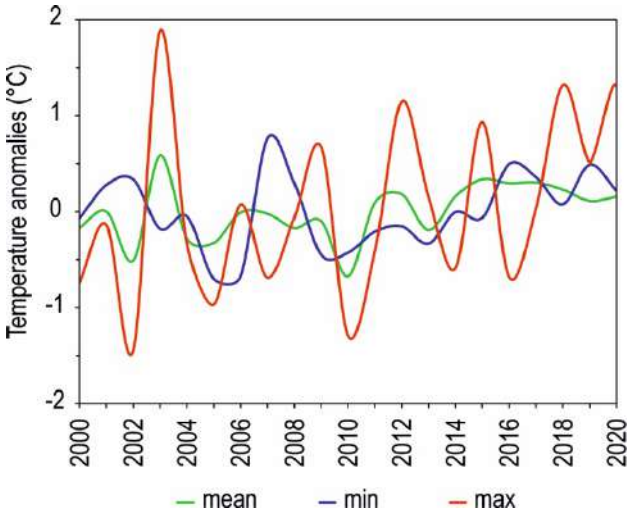

Fig. 3 Trends of yearly maximum, mean and minimum sea surface temperature (SST) anomalies in the Capo Carbonara MPA from 2000 to 2020, derived from NOAA satellite data

Human pressures

Data on the resident population of Villasimius showed a significant (r = 0.97) increasing trend in the last two decades (Fig. 4a). In the year 2020, 870 more inhabitants were registered with respect to the year 2000 (Fig. 4a). Similarly, the analysis of tourist flow showed a significant increase in both visitors (r = 0.82) and stays (r = 0.95) (Fig. 4b). Between 2013 and 2019, the number of visitors increased by about 35,000 tourists, and the number of stays increased by about 110,000 (Fig. 4b).

The yearly number of dives in the MPA showed a not-significant (r = 0.36) increase from 2001 to 2020 (Fig. 5). In particular, the number of dives in the MPA remained stable till 2015, then started to increase reaching the maximum value in 2018 (n = 9550). In 2020 the number of dives decreased (n = 6331) due to the Coronavirus pandemic, which reduced tourist flow. In only three sites, the highest number of dives exceeded one thousand per year: Variglioni (n = 3083), Serpentara (n = 1903) and Caterina (n = 1022).

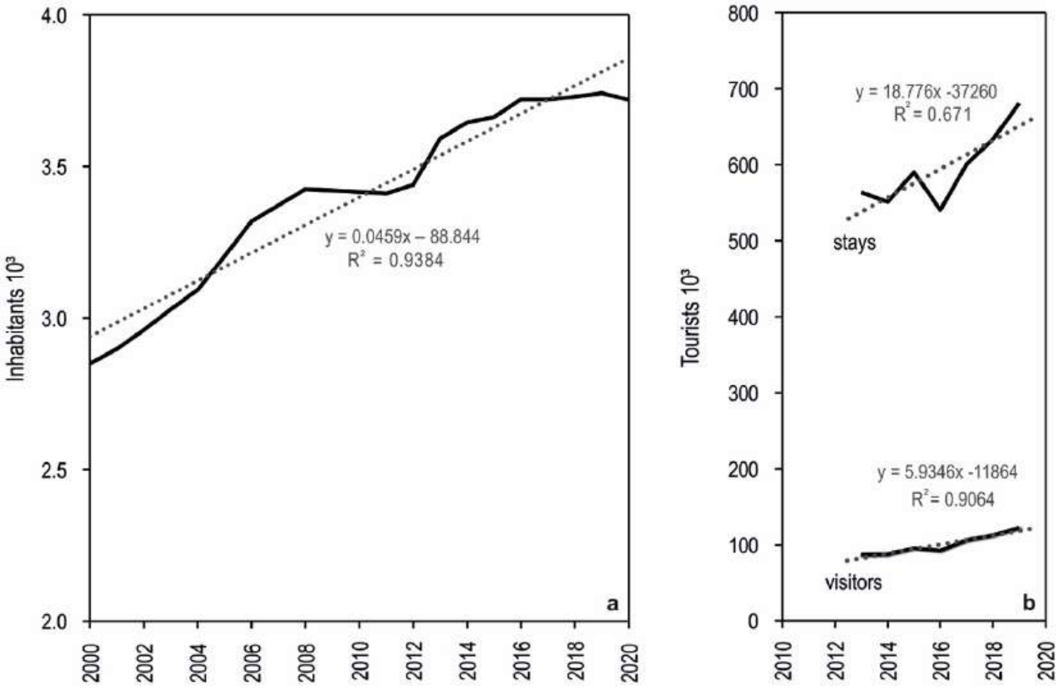

Fig. 4 Twenty year (2000–2020) trends of human pressure: total number of Villasimius inhabitants, derived from ISTAT (Italian National Institute of Statistics) (**a**); number of tourists, in term of ‘visitors’ (i.e., number of customers hosted in the receptive exercises in the considered period) and ‘stays’ (i.e., number of nights spent by the customers in the receptive exercises), from 2013 to 2019 from ‘Assessorato del Turismo, Artigianato e Commercio’ of the ‘Regione Autonoma della Sardegna’ (**b**).

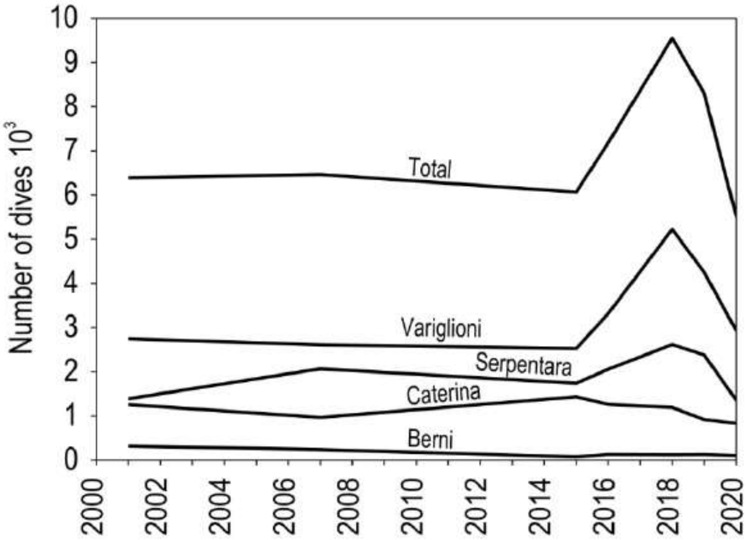

Fig. 5 Trends in the total number of dives and in the number of dives per site in the Capo Carbonara MPA from 2001 to 2020. Data provided by the MPA for the years 2001, 2007, 2015, 2016, 2018, 2019 and 2020. Information for the site Madonnina is not available

Change in reef communities

Multivariate analysis (nMDS) on semi-quantitive data ordered observation points along two gradients: a spatial one along the 1st (horizontal) axis, and a temporal one along the 2nd (vertical) axis (Fig. 6a). The spatial gradient was an expression of increasing depth from the left (zone 1, 0.3-3 m) to the right (zone 4, >30 m) of the 1st axis. The temporal gradient was expressed along the 2nd axis, with the points of the year 2000 separated from those of the year 2020. Points related to the two observers who surveyed in 2000 were close to each other, as well as the points related to the two observers who surveyed in 2020 (Fig. 6). Results of PERMANOVA evidenced significant differences for depth, time and interaction between these two factors (Table 2), indicating a pervasive change with time along the whole depth gradient in the reef communities of Capo Carbonara MPA.

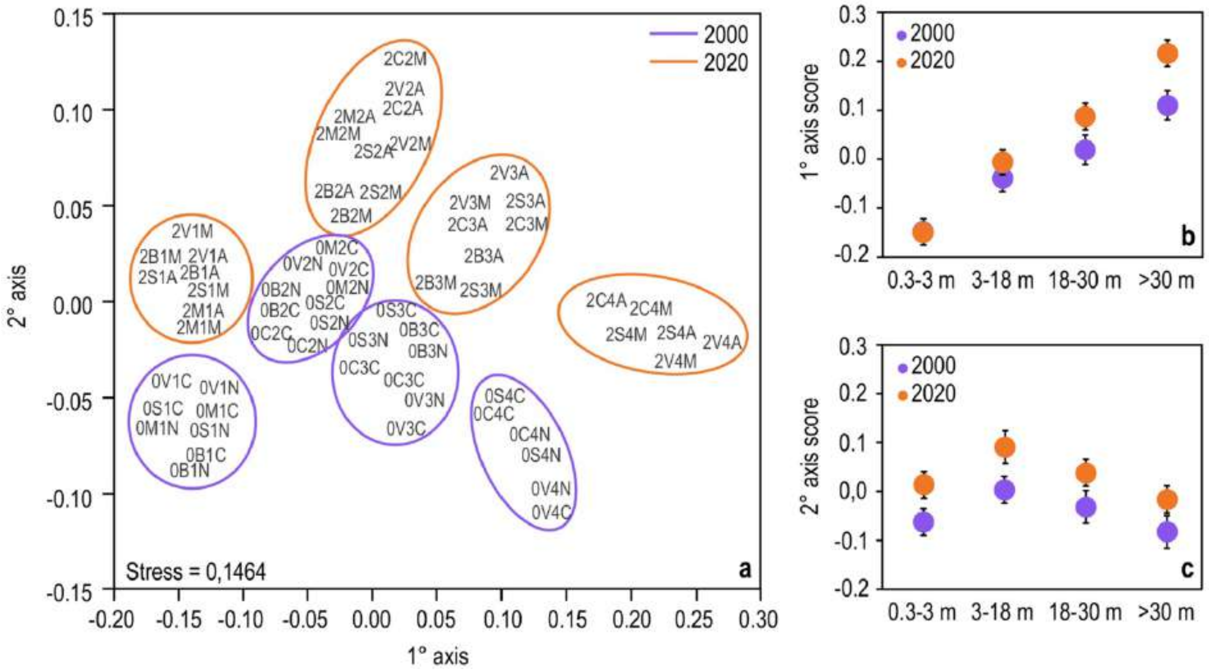

Fig. 6 Multivariate analysis (nMDS) plot of Capo Carbonara MPA survey data. Individual observation points are represented by alphanumeric codes with year (0: 2000; 2: 2020) followed by site (V = Variglioni; C = Caterina; S = Serpentara; M = Madonnina; B = Berni), depth zones (1: 0.3-3 m; 2: 3-18 m; 3: 18-30 m; 4: >30 m) and observer (A = Annalisa Azzola; C = Carla Morri; M = Monica Montefalcone; N = Carlo Nike Bianchi) (**a**). Mean (± se) value of the 1st (**b**) and 2nd (**c**) axis scores for each depth zone (0.3-3 m, 3-18 m, 18-30 m, and >30 m) in 2000 (violet) and 2020 (orange)

Table 2 Results of PERMANOVA on reef communities of Capo Carbonara MPA

	SS	df	MS	Pseudo-F	P(perm)	Unique perms
Depth zones	2992.2	3	997.41	24.50	0.001	999
Time	576.33	1	576.33	15.31	0.001	997
De × Ti	879.45	3	293.15	7.79	0.001	999
Residuals	2108	56	37.643			
Total	6591.7	63				

Indicator species

In total, 129 species were found: 108 in the year 2000, 126 in the year 2020. Three species found in 2000 were not found again in 2020, while 21 species newly appeared in 2020. The latter include mostly alien and southern species (Supplementary Information Table S1).

The relative dominance index (IRd) of the species responding to climate change showed an increase for the categories 'mucilage' (IRd = 0.35), 'aliens' (IRd = 0.33) and 'southerners' (IRd = 0.21). The category 'others' also increases (IRd = 0.16), while the category 'mortality' decreased (IRd = -0.14), indicating a reduced abundance of species vulnerable to heatwaves in 2020 (Fig. 7a; Fig. 8a-d).

As for local anthropogenic impacts, IRd showed a clear increase for the categories 'harvesting' (IRd= 0.24) and 'protection' (IRd= 0.19), indicating that the corresponding species were more abundant in 2020 than in 2000. The increase in categories 'diving' (IRd= 0.16), 'Fucales' (IRd= 0.02) and 'others' (IRd= 0.09) was less remarkable (Fig. 7b; Fig. 9a-d).

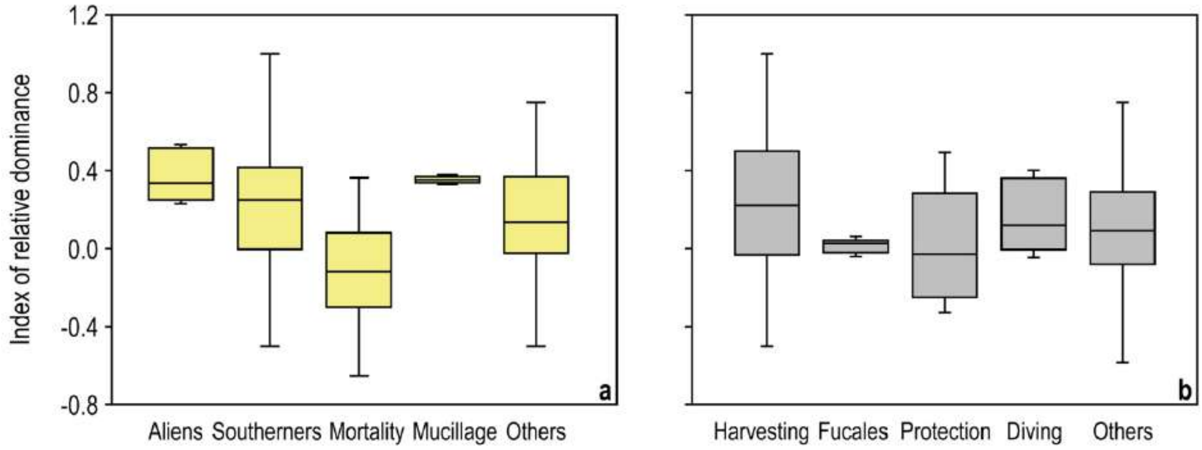

Fig. 7 Change over time of the relative dominance index of indicator species of climate impacts (**a**) or human impacts (**b**)

Fig. 8 Selected examples of indicator species of climate impacts: the alien alga *Caulerpa cylindracea* (**a**); the southern species *Hermodice carunculata* (**b**); mortality induced by heatwaves in the gorgonian *Eunicella singularis* (**c**); mucilage (*Acinetospora crinita*) entangling the gorgonian *Eunicella cavolini* (**d**)

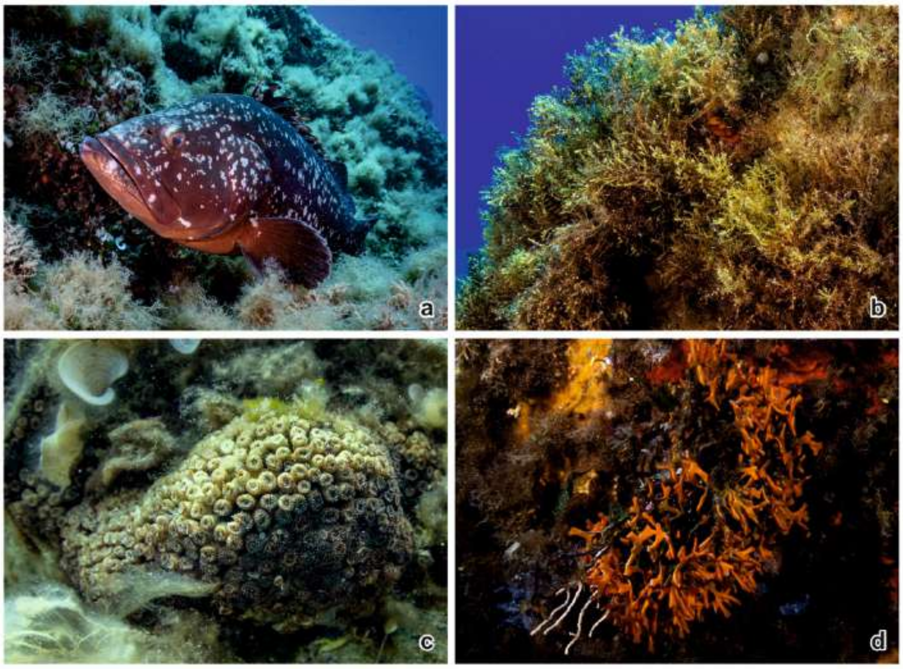

Fig. 9 Selected examples of indicator species of human impacts: the highly-priced fish *Epinephelus marginatus* (**a**); the brown alga *Ericaria amentacea* (*Fucales*) (**b**); the protected coral *Cladocora caespitosa* (**c**); the fragile and erect bryozoan *Pentapora fascialis*, vulnerable to divers' impact (**d**)

Trajectories of change

Results of Krustal-Wallis test on Euclidean Distances between the two time periods were significant for all the four depth zones (p = 4.19E-06), with the greatest changes for zone 4, followed by zone 3, zone 2, and zone 1 (Fig. 10a).

Krustal-Wallis indicated that change over time in the sites surveyed was also significant (p = 3.34E-04) with the greatest changes for Caterina, followed by Variglioni, Berni, Serpentara and Madonnina (Fig. 10b).

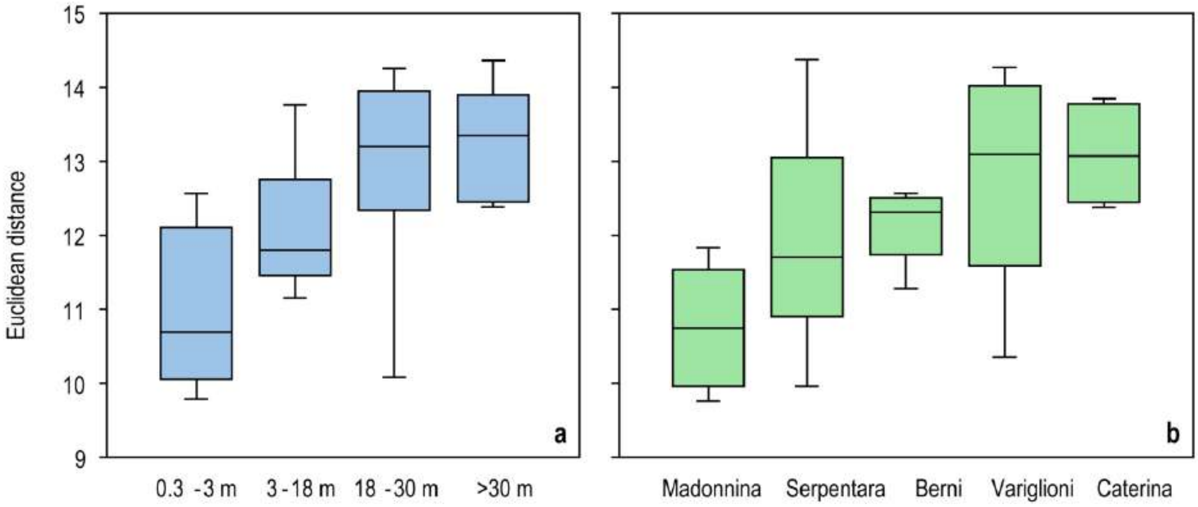

Fig. 10 Magnitude of change (evaluated by Euclidean Distances) between 2000 and to 2020 in the four depth zones (0.3-3 m; 3-18 m; 18-30 m; >30 m) (**a**) and in the five sites (Madonnina; Serpentara; Berni; Variglioni; Caterina) (**b**)

Observer effect

ANOVA applied on Euclidean Distances among all the observer pairs showed significant differences (p = 5.60E-44). The most important differences, however, were only those that implied different years of survey, with the corresponding dissimilarity values being nearly the same (Fig. 11). Tukey's comparison showed that the difference between the combinations containing the two years (2000 N vs 2020 M, 2000 N vs 2020 A, 2000 C vs 2020 M, and 2000 C vs 2020 A) and the combinations containing the pair of observers in the same year (2000 N vs 2000C and 2020 M vs 2020 A) was highly significant (p = 4.35E-10; Table 3). The difference between the two pairs that surveyed in the same year was also significant (p = 0.002; Table 3), indicating the existence of an observer effect. The differences between the two observers of 2000 (who were both similarly expert) were slightly lower than those between the two observers of 2020 (one of them being a trainee). However, the Euclidean Distances between the two observers were definitely low as compared with those involving different years (Fig. 11).

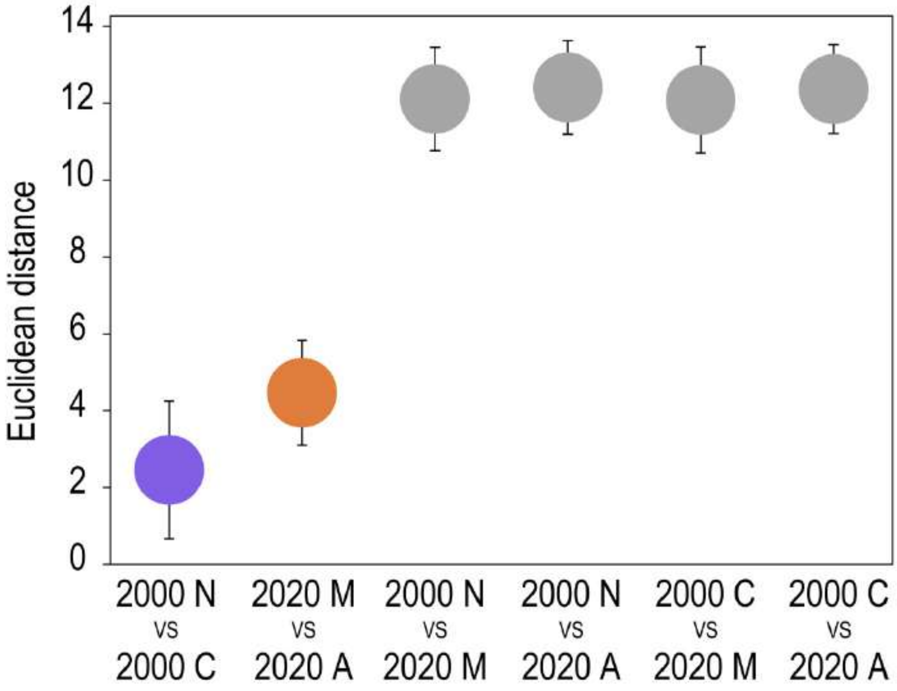

Fig. 11 Differences (Euclidean Distances) between the pairs of observers in the same year (2000 N vs 2000 C; 2020 M vs 2020 A) or in different years (2000 N vs 2020 M; 2000 N vs 2020 A; 2000 C vs 2020 M; 2000 C vs 2020 A). N: Carlo Nike Bianchi, C: Carla Morri, M: Monica Montefalcone, A: Annalisa Azzola

Table 3 Results of one-way ANOVA and Tukey's pairwise comparison on the difference between observers' pairs

	__SS	df	Ms	F	p
Between groups:	1677.09	5	335.42	167.20	**5.60E-44**
Within groups:	180.50	90	2.01		
Total:	1857.59	95	1.00E-05		

	0N-0C	2M-2A	0N-2M	0N-2A	0C-0M	0C-2A
0N-0C		**0.002**	**4.35E-10**	**4.35E-10**	**4.35E-10**	**4.35E-10**
2M-2°	5.67		**4.35E-10**	**4.35E-10**	**4.35E-10**	**4.35E-10**
0N-2M	27.24	21.57		0.99	1.00	0.99
0N-2A	28.09	22.42	0.85		0.99	1.00
0C-0M	27.19	21.52	0.05	0.90		0.99
0C-2A	27.99	22.32	0.75	0.10	0.80	

DICUSSION AND CONCLUSIONS

An often overlooked problem in long term data series is that information collected by different observers may be biased by their dissimilar expertise and personal attitudes (Peperzak, 2010; Bernard et al., 2013). Even when the observer is the same but collects data several years or even decades apart (Bianchi et al., 2014), the greater experience gained with respect to early career years must be considered. Errors due to different observers may be so great to hamper the detection of change over time (Thompson and Mapstone, 1997; Sales and Ballesteros, 2010; Montefalcone et al., 2013) and underwater visual surveys should be preferably conducted by diving scientist with the same experience level (Hinojosa et al., 2021). Nevertheless, rapid visual survey has been proven to be an efficient tool for assessing the integrity of temperate reefs, being robust to observer bias (Gatti et al., 2015). Our study, with considered not only different observers, but also experts and a trainee, provided evidence that the observer effect, although significant, was negligible with respect to change over time in a temperate reef.

Over the last 20 years, the Capo Carbonara MPA rocky reefs have been subjected to multiple affectors linked to climate change and local human pressures. Consistently with global trends (Yang et al., 2021), sea surface temperature (SST) in this area has increased by 0.32 °C from 2000 to 2020. Maximum SST values have shown the greatest increasing trend, with recurrent marine heatwaves recorded in the years for which major thermal anomalies and mortality events have been reported for north-western Mediterranean (Schiaparelli et al., 2007; Garrabou et al., 2009; Bianchi et al., 2019a). Minimum SST values have also showed an increasing trend. Higher winter temperatures and the decreases in the frequency of cold winter events (that can lead to physiological damage and mortality) are believed to favour the establishment of both alien, which are usually of tropical or subtropical origin, and native thermophilic species (Bianchi et al., 2019a; Osland et al., 2021). Both inhabitants and tourists have also shown a significant increasing trend between 2000 and 2020. On the contrary, recreational diving, which can represent a threat to reef species (Betti et al., 2019), has not shown a substantial increase.

Marine protected areas represent one of the most effective tools for conserving biodiversity and mitigating the effects of climate change (Scianna et al., 2018, 2019; Maestro et al., 2019), even if the impacts of sea water warming cannot be kept outside the boundaries of MPAs (Bruno et al., 2018). The reef communities of the Capo Carbonara MPA have significantly changed over time, similarly to what observed in other regions of the world ocean (de Young et al., 2008; de Bakker et al., 2017; Montefalcone et al., 2018b). Comparing only two points in time may not be sufficient to assess when changes in reef community have occurred (Parravicini et al., 2013; Bianchi et al., 2014). Nevertheless, it is possible to quantify the magnitude of change in relation to different global and local impacts (Ellis et al., 2015). Although the five sites surveyed were subjected to different levels of diving pressure, no relation was observed between number of dives and magnitude of change. The greatest change was recorded in the deepest zone (>30 m), where only a minority of recreational divers adventure (Demers, 1994) and where gorgonian mass mortality events due to summer heatwaves have been described (Piazzi et al., 2021).

Distinguishing the effects of climate change from those due to local human pressures is often difficult (Bianchi and Morri, 2004; Gissi et al., 2021). Multiple drivers of change act in connection and different ecological factors may produce the same or similar effects (Bianchi et al., 2012). Interactions among multiple drivers, that can trigger abrupt changes in marine communities, are still poorly understood and the consequences of changes over long time scales (hardly covered by the duration of most scientific studies) pose important challenges to researchers (Turner et al., 2020).

The comparison with outside control areas may prove that change over time in MPAs communities is to be ascribed to climate change. In the present study, only sites included within the Capo Carbonara MPA were surveyed and this may be a limitation. Nevertheless, indicator species were effective in identifying the specific drivers of change (Lindenmayer and Likens, 2011). In Capo Carbonara MPA, mucilage, aliens and southerners have increased from 2000 to 2020, while the species vulnerable to heatwaves (such as gorgonians) have decreased. Both results are likely effect of water warming. The increase in alien and southern species did not imply a decrease in the local counterparts: both the total number of species and the non-indicator species ('others') increased. This suggests that no competitive exclusion occurred under the new climatic regime: coexistence of invaders and native species may lead to complex cascading effects on the receiving communities (Dimitriadis et al., 2021).

None of the species sensitive to anthropogenic impacts has decreased. On the contrary, species subjected to harvesting and fishing, protected species, and species usually impacted by divers have increased, indicating that the protection measures enforced by the MPA in these two decades have been effective (Scianna et al., 2018, 2019). Fucales, which have disappeared from several Mediterranean areas under the effect of either coastal anthropization or seawater warming (Thibaut et al., 2005; Harley et al., 2012; Bianchi et al., 2019a), exhibited little change.

The revisitation of sites investigated years before provides a valuable tool for assessing change over time in marine ecosystems (Bianchi et al., 2014, 2019b). However, the original sampling design often shows limits due to methodological approaches that may appear obsolete or inadequate with respect to more modern standards (Guidetti et al., 2014). In most cases, the issue is complicated by the impossibility to have replicates in historical surveys. Thus, our study has undoubtedly several inherent limits: i) lack of surveys in years intermediate between 2000 and 2020 does not allow to identify when exactly changes has occurred (Spencer et al., 2012); ii) no proper replication of observers was available because in 2000 both surveyors were experts while in 2020 there were an expert and a trainee; iii) using only visual semiquantitative data may blur the comparison of data collected by different observers (Harvey et al. 2004; Edgar et al., 2020); iv) adopting transects, instead of having a confined area, increases the possibility of haphazard effects (Bianchi et al., 2014). This notwithstanding, our results proved successful in illustrating change over time in a temperate reef community.

Sustained monitoring of biodiversity in MPAs is indispensable (Bianchi et al., 2022) to detect change over time due to climate or local human pressures and to the effectiveness of the protection measures enforced (Bravo et al. 2020, and reference therein). We recommend that within the design of such monitoring activities, the variability between observers is always taken into full account in order to assure reliable information: the present case study may provide a positive example.

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SUPPLEMENTARY INFORMATION

Table S1 List of the conspicuous species surveyed in the Capo Carbonara MPA, ordered alphabetically. For each species the category based on the vulnerability to climate and human pressures is reported. I_{Rd} = index of relative dominance (see Materials and Methods)

Species	Climate pressures	Human pressures	I_{Rd}
Acetabularia acetabulum	Others	Others	0.43
Acinetospora crinita	Others	Mucillage	0.35
Aiptasia mutabilis	Others	Others	0.00
Alcyonium acaule	Others	Others	-0.08
Alcyonium coralloides	Others	Mortality	0.09
Alicia mirabilis	Others	Others	0.34
Amphiroa rigida	Others	Others	-0.06
Anadyomene stellata	Others	Southerners	-0.24
Anthias anthias	Harvesting	Others	0.27
Apogon imberbis	Harvesting	Others	0.27
Arbacia lixula	Harvesting	Others	-0.08
Asparagopsis taxiformis	Others	Aliens	0.25
Atherina hepsetus	Harvesting	Others	0.75
Axinella damicornis	Others	Others	0.19
Axinella verrucosa	Others	Others	0.38
Balanophyllia europaea	Others	Mortality	-0.29
Balanophyllia regia	Others	Southerners	0.40
Bonellia viridis	Others	Others	-0.01
Caryophyllia inornata	Others	Others	0.00
Caulerpa cylindracea	Others	Aliens	0.52
Centrolabrus melanocercus	Harvesting	Others	-0.09
Centrostephanus longispinus	Protection	Southerners	0.25
Cerianthus membranaceus	Others	Others	0.50
Chama gryphoides	Others	Others	-0.38
Chelon labrosus	Harvesting	Others	-0.12
Chondrilla nucula	Others	Others	0.75
Chondrosia reniformis	Others	Mortality	0.01
Chromis chromis	Harvesting	Others	0.30
Cladocora caespitosa	Protection	Mortality	-0.33
Cladophora prolifera	Others	Others	0.25
Clathrina clathrus	Others	Others	0.09
Clavelina lepadiformis	Others	Others	0.54

Species	**Climate pressures**	**Human pressures**	**I_{Rd}**
Codium bursa	Others	Others	0.01
Coris julis	Harvesting	Others	0.27
Crambe crambe	Others	Others	0.02
Cymodocea nodosa	Protection	Others	-0.03
Cystoseira brachycarpa	Fucales	Others	0.03
Dardanus calidus	Others	Others	0.50
Dasycladus vermicularis	Others	Southerners	0.42
Dentex dentex	Harvesting	Others	-0.02
Dictyopteris polypodioides	Others	Others	-0.04
Dictyota dichotoma	Others	Others	0.38
Dictyota fasciola	Others	Others	0.04
Diplodus puntazzo	Harvesting	Others	0.56
Diplodus sargus	Harvesting	Others	0.74
Diplodus vulgaris	Harvesting	Others	0.30
Echinaster sepositus	Others	Others	0.50
Ellisolandia elongata	Others	Others	0.26
Epinephelus costae	Harvesting	Southerners	0.50
Epinephelus marginatus	Harvesting	Others	0.22
Ericaria amentacea	Fucales	Others	0.04
Eudendrium racemosum	Others	Others	-0.15
Eunicella cavolini	Others	Mortality	-0.01
Eunicella singularis	Others	Mortality	-0.07
Exaiptasia diaphana	Others	Others	-0.50
Filograna implexa	Others	Others	0.50
Flabellia petiolata	Others	Others	0.05
Hacelia attenuata	Others	Southerners	0.05
Haliclona fulva	Others	Others	0.21
Halocynthia papillosa	Others	Others	0.56
Halopteris scoparia	Others	Others	0.07
Hemimycale columella	Others	Others	0.19
Hermodice carunculata	Others	Southerners	0.25
Hexaplex trunculus	Others	Others	0.25
Holoturia tubulosa	Others	Others	0.75
Ircinia oros	Others	Others	0.67
Ircinia variabilis	Others	Mortality	-0.51
Jania rubens	Others	Others	0.45
Labrus merula	Harvesting	Others	0.16

Species	**Climate pressures**	**Human pressures**	**I_{Rd}**
Laurencia obtusa	Others	Others	0.11
Leptopsammia pruvoti	Others	Others	0.25
Lithophyllum incrustans	Others	Mortality	-0.17
Lithophyllum stictiforme	Others	Mortality	0.16
Mullus surmuletus	Harvesting	Others	0.68
Muraena helena	Harvesting	Others	0.00
Myriapora truncata	Diving	Others	0.00
Oblada melanura	Harvesting	Others	0.19
Octopus vulgaris	Harvesting	Others	-0.29
Ophidiaster ophidianus	Protection	Southerners	0.49
Oscarella lobularis	Others	Others	-0.33
Osmundaria volubilis	Others	Others	0.24
Padina pavonica	Others	Others	0.10
Paracentrotus lividus	Harvesting	Mortality	-0.04
Paramuricea clavata	Others	Mortality	-0.21
Parazoanthus axinellae	Others	Mortality	0.36
Patella caerulea	Harvesting	Others	-0.05
Peltaster placenta	Others	Southerners	0.25
Pentapora fascialis	Diving	Others	0.40
Percnon gibbesi	Others	Aliens	0.25
Perforatus perforatus	Others	Mortality	-0.58
Petrosia ficiformis	Others	Mortality	0.13
Peyssonnelia spp.	Others	Others	0.03
Phorbas tenacior	Others	Mortality	0.08
Phyllophora crispa	Others	Others	-0.01
Pinna nobilis	Protection	Mortality	-0.25
Polycyathus muellerae	Others	Others	-0.29
Posidonia oceanica	Protection	Others	0.28
Protula tubularia	Others	Others	0.48
Reptadeonella violacea	Others	Others	-0.24
Reteporella grimaldii	Others	Others	0.05
Rhynchozoon neapolitanum	Others	Others	-0.10
Sabella spallanzani	Others	Others	0.25
Sarcotragus foetidus	Protection	Mortality	-0.17
Scalarispongia scalaris	Others	Mortality	-0.65
Schizoporella dunkeri	Others	Others	-0.15
Sciaena umbra	Harvesting	Others	-0.07

Species	**Climate pressures**	**Human pressures**	**I_{Rd}**
Seriola dumerili	Harvesting	Others	0.75
Serranus cabrilla	Harvesting	Others	0.75
Serranus scriba	Harvesting	Others	0.01
Smittina cervicornis	Diving	Others	0.24
Sparisoma cretense	Harvesting	Southerners	1.00
Sparus aurata	Harvesting	Others	0.50
Sphaerechinus granularis	Others	Others	-0.47
Sphyraena viridensis	Harvesting	Southerners	0.29
Spicara maena	Harvesting	Others	0.38
Spirastrella cunctatrix	Others	Others	0.05
Spirobranchus triqueter	Others	Others	-0.08
Spongites fruticulosa	Others	Others	0.21
Symphodus roissali	Harvesting	Others	-0.02
Symphodus tinca	Harvesting	Others	0.22
Synodus saurus	Harvesting	Southerners	-0.50
Thalassoma pavo	Harvesting	Southerners	0.00
Thylacodes arenarius	Others	Others	-0.66
Treptacantha ballesterosii	Fucales	Others	-0.02
Tricleocarpa fragilis	Others	Southerners	0.27
Turbicellepora avicularis	Diving	Others	-0.01
Vermetus triquetrus	Others	Others	-0.46
Zanardinia typus	Others	Others	0.17
Zonaria tournefortii	Others	Southerners	-0.31

4.2

Coral reefs in a changing climate regime: revisiting lagoon and ocean sites in a remote Maldivian atoll

Under review:

Zampa G.*, **Azzola A.***, Bianchi C.N., Morri C., Oprandi A., Montefalcone M. *PeerJ*

*Greta Zampa and Annalisa Azzola are first co-authors

Abstract

In the last two decades, Maldivian reefs (Indian Ocean) have been affected by two major mass coral bleaching events (1998 and 2016), which caused a remarkable coral mortality. Coral recovery after such extreme events may be negatively influenced by the combined effect of global and local pressures. Huvadhoo, a remote atoll in the south of the Maldives, was first surveyed in 2009 and revisited in 2020. Eight coral reefs, four within the lagoon and four exposed to oceanic conditions, were surveyed in both years, using the same field methods. A general dramatic decrease in tabular *Acropora* cover was observed, with possible negative consequences on the three-dimensionality of reefs. Lagoon reefs of Huvadhoo Atoll showed a better recovery capacity than ocean reefs, contrary to the trend observed in the more anthropized central atolls of the Maldives. This study provides an example of how comparative analysis between atolls subjected to different levels of local human pressures can be a valuable tool to better understand recovery capacity of coral reefs after extreme thermal events.

INTRODUCTION

Coral reefs are declining worldwide due to several global and local human pressures, such as ocean warming, water acidification, pollution, and overexploitation of marine resources (Hoegh-Guldberg et al., 2007). Climate change is perhaps the major threat for coral reefs, and the increase in intensity and frequency of extreme thermal anomalies, not only may cause mass mortality events but also affects coral recovery capacity (Hughes et al., 2017a; Hughes et al., 2017b; Mohan et al., 2021).

Coral reefs play an essential role in coastline protection from erosion, by increasing the structural habitat complexity that reduces wave energy (Sheppard et al., 2002; Ferrario et al., 2014). Hard corals, in particular, act as ecosystem engineers and their cover is the most commonly used indicator of reef health, representing the reef ability to maintain three-dimensionality and positive vertical accretion (Montefalcone et al., 2018).

The Maldives (Indian Ocean) is a nation that literally lives on coral (Morri et al., 2010) and, with a maximum elevation of 2.4 m above sea level (Graham & Nash, 2013; Stevens & Froman, 2019), is particularly exposed to the negative consequences of coral reef decline (Khan et al., 2002; Gerrard & Wannier, 2013).

In the last two decades, Maldivian reefs were affected by several thermal anomalies (El Niño events), two of which were particularly severe, leading to high coral mortality rates (Cowburn et al., 2019; Pisapia et al., 2016). After El Niño event of 1998, a general loss of 80-100% of corals was observed depending on species and reef location (Edwards et al., 2001; Bianchi et al., 2006; Lasagna et al., 2008). In 2015, an almost complete recovery was recorded with a hard coral cover of 65%. The following year, a second extreme El Niño event hit the Maldivian coral reefs, leading to a further decline in hard coral cover to around 20% (Montefalcone et al., 2020).

In the central atolls of Maldives, local human pressures are exponentially growing because of population increase, tourism intensification, and activities such as reclamation and land filling (Pancrazi et al., 2020). Such pressures make coral reefs more vulnerable to climate change effects and reduce their recovery capacity (Nepote et al., 2016). After the El Niño event of 2016, reefs in atolls with higher human pressure showed greater coral mortality than reefs in atolls with lower human pressure (Montefalcone et al., 2020).

Assessing the effects of global and local pressures on coral reefs and evaluating their present state of health requires long-term data series (Gross & Edmunds, 2015; Morri et al., 2015; Mellin et al., 2020). In absence of such historical data, revisiting sites already surveyed in the past represents a useful tool in assessing change over time of coral reef communities (McClanahan, 2017; Bianchi et al., 2022 and references therein).

In the present study, eight coral reefs of Huvadhoo Atoll (Southern Maldives), firstly surveyed in 2009, were revisited in 2020 using the same field methods. Analyzing coral reef communities of lagoon and ocean reefs separately allowed evaluating change over time depending on reef exposure. The comparison of the remote Huvadhoo Atoll with the more anthropized Malé Atoll represents an attempt to understand the influence of local human pressures on the recovery capacity of coral reefs after extreme thermal events.

MATERIAL AND METHODS

Study area

Huvadhoo, located in the southern part of the Maldives (0.533333°N; 73.283333°E), is the largest atoll of the archipelago, including 241 coral reefs with a total area of 437.9 km^2 (Naseer & Hatcher, 2004). Reaching the depth of 80 m, its lagoon is the deepest of the Maldives.

The Huvadhoo Atoll is divided into two administrative provinces: the northern one, called Gaafu Alifu, and the southern one, called Gaafu Dhaalu. In the northern province, tourism development was allowed by the Ministry of Tourism Maldives in 2006, and in 2009 the first tourist facilities were developed. Gaafu Dhaalu remained closed from tourism until 2011.

Data on climate

Sea surface temperature (SST) was examined to evaluate the potential role of climate change as a driver of change in the coral reef communities. Yearly maximum SST data from 1948 to 2020 were derived from NOAA (US National Oceanic and Atmosphere Administration) satellite records (http://coralreefwatch.noaa.gov/vs/gauges/maldives.php). These data were calibrated by linear regression with discontinuous temperature data collected during the field activities.

Data on local human pressures

Data about resident population and tourism were collected from official sources to evaluate local human pressures. The number of Huvadhoo inhabitants was obtained by the National Bureau of Statistics of the Ministry of National Planning (https://statisticsmaldives.gov.mv/population-and-households/), which carried out national censuses in 1995, 2000, 2006, and 2014. Data on tourism (in terms of number of beds occupied in tourist facilities), between 1996 and 2020, were obtained by the Ministry of Tourism (http://statisticsmaldives.gov.mv/yearbook/2020/). The same data were collected for Malé, to compare local human pressure of Huvadhoo with the most anthropized atoll in the Maldives.

Field surveys

The first surveys were conducted in 2009, to characterize coral reef communities of Huvadhoo Atoll. Data were collected in 8 different sites, whose positions were recorded using a GPS (Fig. 1). Two reef types, characterized by different topography and exposure, were considered: i) 4 lagoon reefs (i.e., reefs located on the side of the atoll facing the lagoon); and ii) 4 ocean reefs (i.e., reefs located on the side of the atoll facing the ocean).

Data were collected by scuba diving at 5 m, 10 m, and 15 m depth. At each depth, the percent cover data of 15 benthic descriptors (Morri et al. 2010; Morri et al., 2015; Table 1) was visually estimated by the plain view technique of Wilson et al. (2007). Divers hovered 1–2 m above the bottom observing an area of 20 m2, in three replicate spots (tens of meters apart) at each reef site (Montefalcone et al., 2020). In 2020, the same sites were revisited using the same field methods.

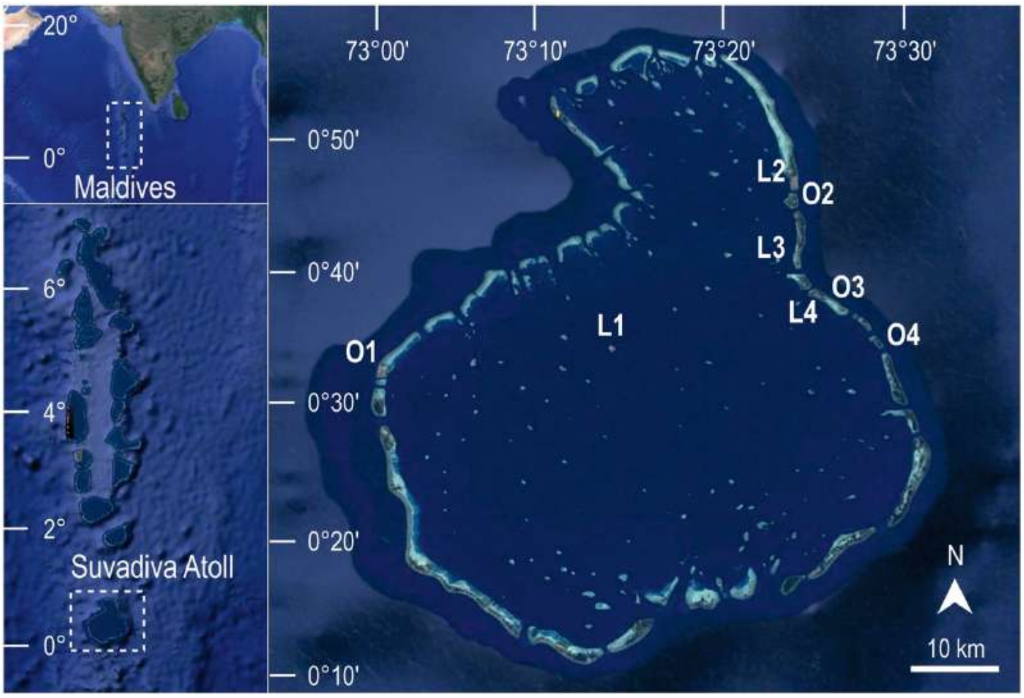

Fig. 1 Location of the 8 sites (L: lagoon reefs; O: ocean reefs) of Huvadhoo Atoll (Maldives, Indian Ocean) surveyed in 2009 and 2020

Ecological data analysis

The data collected were organized in a matrix (time × reef type × depth) × descriptors and transformed applying arcsine $\sqrt{(x/100)}$ to the percent cover data (Legendre & Legendre, 1998). Two different time periods were considered: i) 09, for the data collected in 2009; ii) 20, for the data collected in 2020. Two reef types were defined: i) L = lagoon reefs; and ii) O = ocean reefs. All the three depths surveyed were taken into account: i) 1 = 5 m; ii) 2 = 10 m; and iii) 3 = 15 m.

The data matrix was submitted to non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity index. The analysis of the nMDS plot suggested that the first axis was mostly an expression of reef type, while the second axis was an expression of time. On the contrary, a distribution of the observation points according to depth was not observed (see Results). Thus, axis scores were grouped according to the former two factors, and differences inside both groups were tested by permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis matrix.

Time-trajectories between two years in lagoon and ocean reefs have been visualized on the nMDS plot, and their length calculated as difference between axis scores.

Similarity Percentage Analysis (SIMPER) was applied to highlight the descriptors that mostly contributed to the differences in coral reef communities between the two years and between lagoon and ocean sites.

To evaluate the descriptors that mostly changed over the time, in both lagoon and ocean sites, their percent cover was compared by Student's t-test between the years 2009 and 2020. Lastly, the 15 benthic descriptors (Table 1) were lumped a posteriori into three categories: i) hard corals (HCC); ii) others (OTH); and abiotic descriptors (ABT), to better synthesize the major modification in coral reef structure. Differences in percent cover of HCC, OTH and ABT between lagoon and ocean sites and between 2009 and 2020 were evaluated by the Student's t-test.

All statistical analyses were performed using the open-source software PaSt (Hammer et al., 2001). All data presented in the text, figures and tables are expressed as mean ± SE.

Table 1. List of the benthic descriptors (together with their codes) used to characterize the coral reef communities of Huvadhoo Atoll

Descriptor	**Code**
Hard coral	**HCC**
Tabular *Acropora*	CAT
Branching *Acropora*	CAB
Digitate *Acropora*	CDA
Branching coral	BC
Columnar coral	CC
Foliose coral	CF
Encrusting coral	CE
Massive coral	CM
Fungiidae	F
Heliopora coerulea	Hcoe
Others	**OTH**
Soft bodied organisms (zooxanthellatae and azooxanthellatae soft corals, whip and wire corals, sea fans, sponges, tunicates, Actiniaria, Zoantharia)	SF
Coralline algae	CA
Fleshy algae	FA
Large clam (mostly *Tridacna*)	LC
Abiotic descriptors (Dead coral, Coral rock, Coral rubble, Sand)	**ABT**

RESULTS

Climate pressures

The analysis of sea surface temperature (SST) data from 1996 to 2020 showed four peaks of SST that exceeded the moderate regional bleaching threshold (30.9°C). The highest peaks were recorded in 1998 and 2016, coinciding with the known thermal anomalies that affected the Maldives causing mass coral bleaching events (Fig. 2).

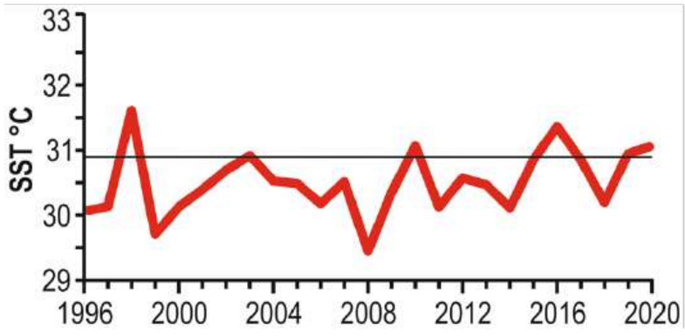

Fig. 2 Twentyfour-year trend (1996–2020) of yearly maximum Sea Surface Temperature (SST, red line) of Huvadhoo Atoll. The solid black line indicates bleaching thresholds defined for the Maldives, corresponding to 30.9°C (Montefalcone et al., 2020).

Local human pressures

Since 1996, the resident population of Huvadhoo Atoll has remained stable at around 20,000 inhabitants, compared to the resident population of Malé Atoll that doubled from ca. 62,500 inhabitants in 1996 to over 129,000 in 2014 (Fig. 3a).

The total number of beds in Huvadhoo increased from 200 in 2009, at the first opening to tourists of Gaafu Alifu (North Huvadhoo), up to 1,216 in 2019. In Gaafu Dhaali (South Huvadhoo) the total number of beds increased from 200 in 2011 to 616 in 2019, when a total of 1,832 beds in the entire atoll was recorded (Fig. 3b). Overall, the increase of tourists in Huvadhoo, although significant, was negligible in contrast to Malé, where the number of beds increased from 7,368 in 1996 to 19,078 in 2020 (Fig. 3b).

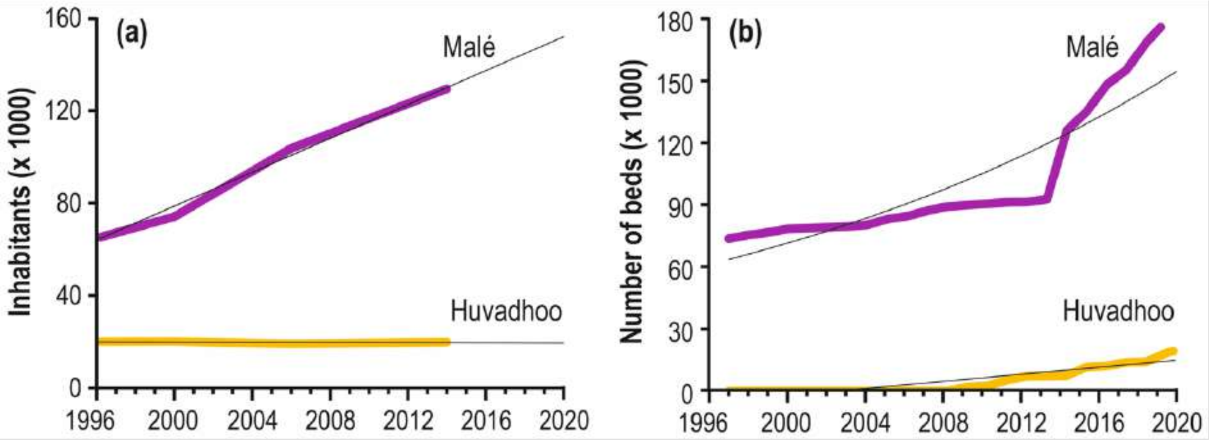

Fig. 3 (a) Twenty-four year trend (1996-2020) of the total number of inhabitants of Huvadhoo Atoll (number of inhabitants = -0.0204x + 60.764; R2 = 0.1672) compared with the total number of inhabitants of Malé Atoll (number of inhabitants = 3.6596x - 7240.5; R2 = 0.987), according to the national censuses carried out by the National Bureau of Statistics of the Ministry of National Planning (available at http://statisticsmaldives.gov.mv/yearbook/ statisticalarchive); **(b)** Twenty-four year trend (1996-2020) of the number of beds in tourist facilities of Huvadhoo Atoll (number of beds = 76.933x – 154039; R2 = 0.7908) compared with the total number of beds of Malé Atoll (number of beds = 9E-27e0.0345x; R2 = 0.7791) (from Ministry of Tourism data, compiled from annual reports and available at https://www.tourism.gov.mv/downloads/stats)

Reef communities

Non-metric multidimensional scaling (nMDS) plot provided a visualization of the differences in coral reef communities of the lagoon and ocean sites in the two time periods (Fig. 4). The analysis ordered observation points along two gradients: i) a spatial one along the 1st axis, which represents reef types with lagoon sites on the right and ocean sites on the left; and ii) a temporal one along the 2nd axis, which represents the two time periods with sites surveyed in 2009 separated from those surveyed in 2020 (Fig. 4). Coral reef communities did not show differences according to the depth gradient (5 to 15 m). In the years 2009 and 2020, a higher variability was observed for lagoon sites (whose points are scattered in the nMDS plot) in contrast with a greater spatial homogeneity observed for ocean sites (Fig. 4).

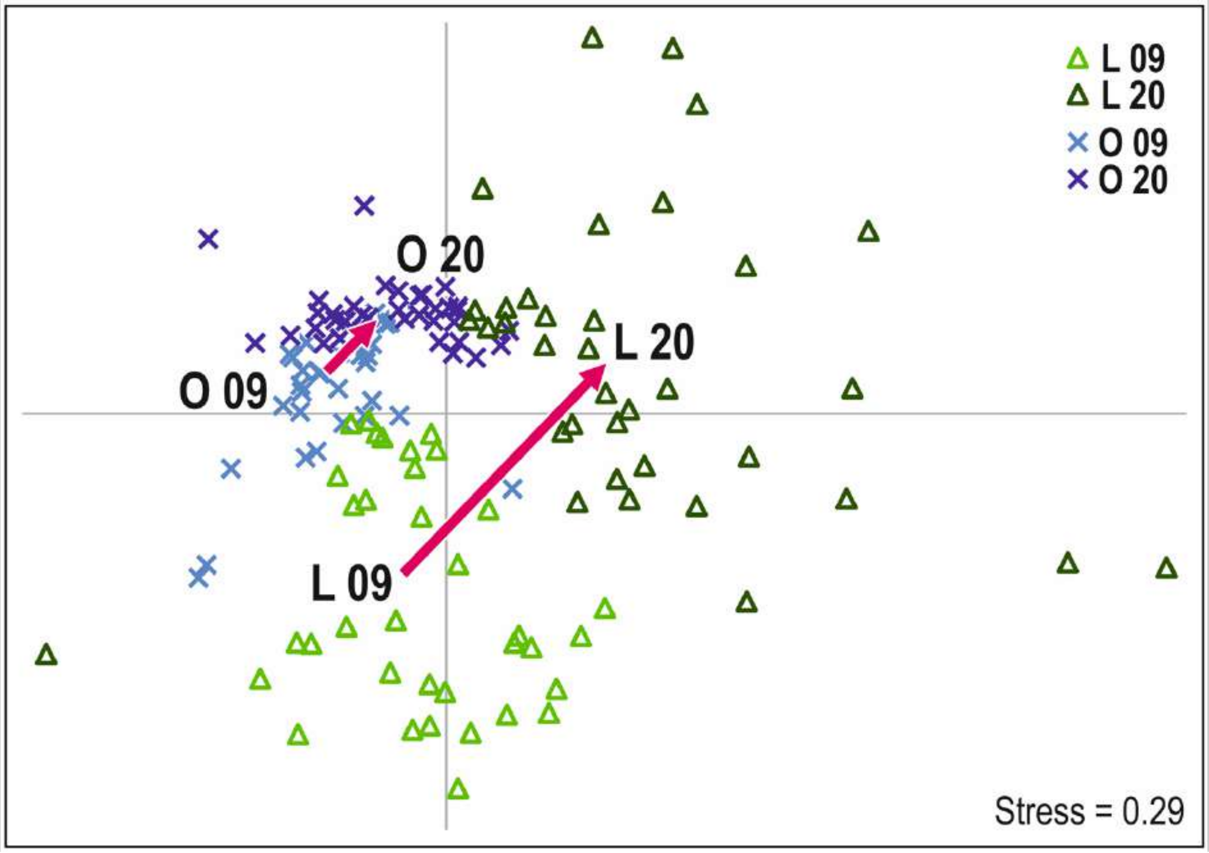

Fig. 4 Non-metric multidimensional scaling (nMDS) plot of Huvadhoo Atoll survey data. Individual observation points are represented by triangle for lagoon sites and cross for ocean sites. Light blue and light green indicate observation points of 2009; dark blue and dark green of 2020. Centroids are identified by alphanumeric codes with reef types (L: lagoon; O: ocean) followed by year (09: 2009; 20: 2020)

Results of PERMANOVA evidenced significant differences for reef type, time, and interaction between these two factors (Table 2), indicating a pervasive change with time in both lagoon and ocean coral reef communities of Huvadhoo Atoll.

Table 2. Results of PERMANOVA on the coral reef communities of Huvadhoo Atoll

	SS	df	MS	Pseudo-F	p	Unique perms
Reef type	2.2992	1	2.2992	39.87	0.0001	997
Time	2.5615	1	2.5615	44.42	0.0001	998
Interaction	0.5653	1	0.5653	9.80	0.0001	999
Residuals	8.0733	140	0.0577			
Total	13.499	143				

The time-trajectories of lagoon sites were always higher than those of ocean sites, highlighting that the major changes in coral reef communities occurred in the former ones (Fig. 5).

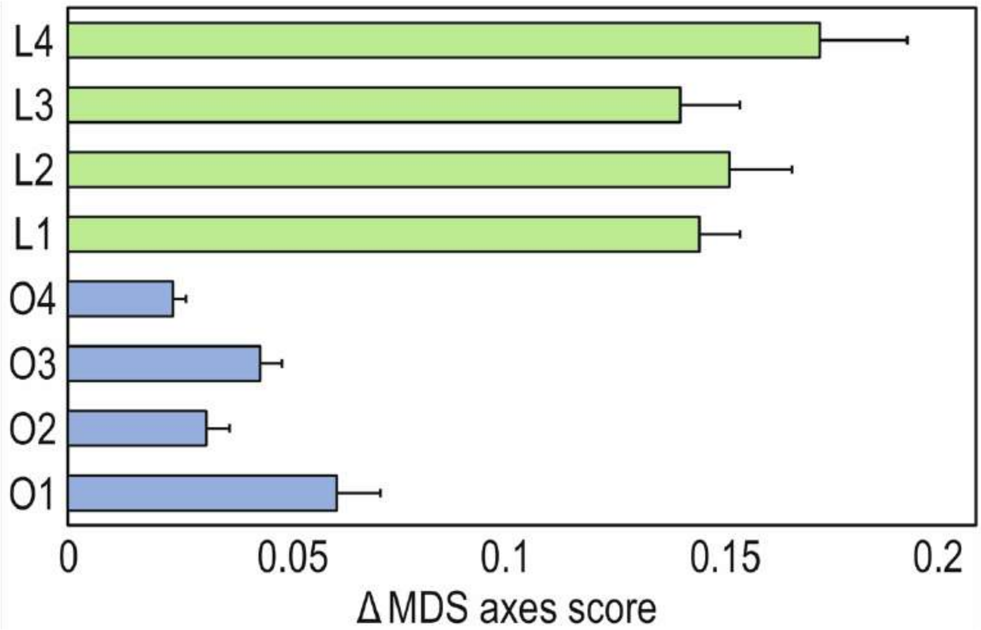

Fig. 5 Time-trajectories calculated as the mean (± SE) differences of the second nMDS axes scores between 2009 and 2020 observation points in lagoon and ocean sites

SIMPER analysis highlighted that in the lagoon sites the differences between 2009 and 2020 were mainly due (50%) to the decrease of CAT and to the increase of CAB, CDA and CE (Table S1). Student’s t-test applied on the percent cover of descriptors showed a significant decrease of CAT ($p = 8.90E-14$), SF ($p = 5E-05$), CA ($p = 0.04$), ABT ($p = 4E-04$), and a significant increase of CDA ($p = 4.34E-05$), CE ($p = 9E-04$), CM ($p = 0.02$), F ($p = 1.69E-05$) and FA ($p = 8.8E-08$) (Fig. 6a).

In the ocean sites, SIMPER analysis highlighted that the differences between 2009 and 2020 were mainly due (47%) to the decrease of CAT and CE, and to the increase of CM and ABT (Table S2). Student’s t-test applied on the percent cover of descriptors showed a significant decrease of CAT ($p = 3.91E-06$), CDA ($p = 0.03$), CE ($p = 0.03$) and a significant increase of CAB ($p = 0.01$), CM ($p = 0.01$), F ($p = 7E-04$), FA ($p = 0.002$) and ABT ($p = 0.02$) (Fig. 6b).

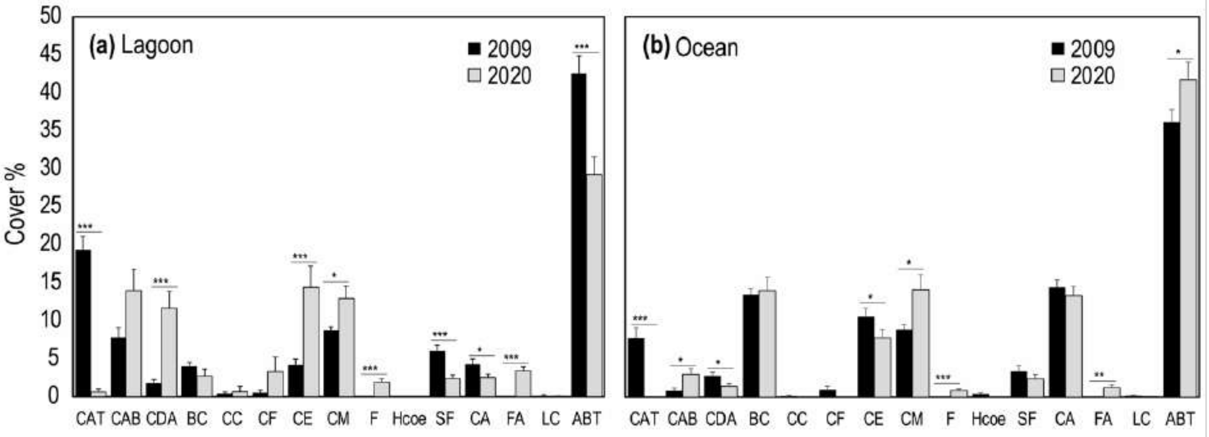

Fig. 6 Mean (+ SE) percent cover of the 15 benthic descriptors (coded as in Table 1), recorded in **(a)** lagoon and **(b)** ocean sites in 2009 and 2020 at Huvadhoo Atoll. Significant differences tested by Student’s t-test are represented as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The Student’s t-test applied on percent cover of the three lumped categories of descriptors showed a significant increase for HCC in lagoon sites ($p = 9.64E-05$) and a significant ($p = 0.04$) decrease in ocean sites, between 2009 and 2020 (Fig. 7a). OTH did not show significant differences in the two time periods, in lagoon and ocean sites (Fig 7b). ABT significantly ($p = 4E-04$) decreased in lagoon sites and significantly ($p = 0.02$) increased in ocean sites (Fig. 7c).

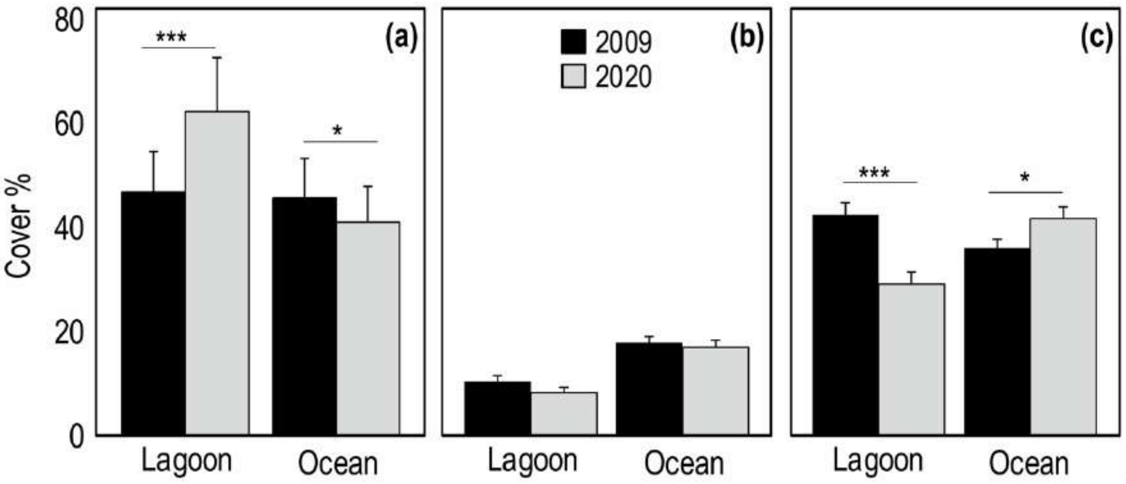

Fig. 7 Mean (+ SE) percent cover of the three categories of descriptors **(a)** hard corals, **(b)** others, and (c) abiotic descriptors, recorded in lagoon and ocean sites in 2009 and 2020 at Huvadhoo Atoll. Significant differences tested by Student's t-test are represented as follow: * p <0.05, ** p <0.01, *** p <0.001

DISCUSSION

Revisiting Huvadhoo Atoll eleven years after the first studies, illustrated significant change in coral reef communities from 2009 to 2020. In the two years of survey, the most important factor influencing coral reef communities was exposure: the lagoon sites significantly differed from the ocean ones. As for the central atolls of Maldives, differences between these two reef types were expressed not only in morphology and topography (Rovere et al., 2018), but also in the species composition of the communities (Lasagna et al., 2010a). Maldivian lagoon reefs are typically dominated by fast-growing tabular and branching Acropora, compared to ocean reefs dominated by encrusting and massive corals (Bianchi et al., 1997; Lasagna et al., 2010b).

At Huvadhoo Atoll, such differences were reflected in the changing pattern over time of the two reef types. The major changes were observed in the lagoon sites compared to the minor changes occurred in the ocean ones. From 2009 to 2020, in the lagoon reefs encrusting corals and digitate Acropora significantly increased, while soft body corals and abiotic component significantly decreased. On the contrary, in the ocean reefs an increase of abiotic descriptors was observed. Fungiidae and foliose corals showed a positive increase in all reefs surveyed. In both lagoon and ocean reefs, the major change observed was the reduction of tabular Acropora.

Tabular Acropora are corals with a high growth rate, but they are also among the most susceptible to thermal anomalies, reporting the greatest damage after mass coral bleaching events (Lasagna et al., 2008; Baker et al., 2008; Morri et al., 2010; Pisapia et al., 2016; Sakai et al., 2019). The dramatic decrease of such corals observed at Huvadhoo Atoll from 2009 to 2020, could be explained as a consequence of the El Niño event that occurred in 2016. After this extreme event, the same reduction of tabular Acropora was observed in the central atolls of Maldives, where a loss of reef three-dimensionality, with possible negative effects on Maldivian island coastlines, was described (Wild et al., 2011; McClanahan & Muthiga, 2014; Tkachenko 2014; Duvat & Magnan, 2019; Bessell-Browne et al., 2021).

At Huvadhoo Atoll, lagoon reefs showed a better recovery capacity than ocean reefs: the cover of hard corals significantly increased from 2009 to 2020. This trend was the opposite of that observed in the central atolls, where corals proved to be more resistant to the 2016 bleaching event in the ocean reefs than those in the lagoon (Montefalcone et al., 2020). Ocean reefs are close to deep cooler waters and have higher water movement that may ensure mixing of water (Muir et al., 2017). Compared to Huvadhoo, the central atolls of Maldives are much more anthropized and the lagoon sites are treated by the combination of global and local human pressures that can cause slow recovery of coral cover (Montefalcone et al., 2020; Pancrazi et al., 2020). Huvadhoo is a remote atoll, where local human pressures did not severely increase in the last two decades. The resident population remained almost stable from the first census carried out in 1995 to the last one in 2014. Likewise, thanks to the location of the atoll, the tourist flow did not increase as much as in the central atolls. At Huvadhoo, the slower recovery of hard corals in ocean reefs, compared to that of lagoon reefs, can be due to their exposure to waves which cause constant turnover associated with breaks, scratches, and abrasions (Madin & Connolly, 2006; Lange et al., 2021). Furthermore, the topography of the Huvadhoo lagoon, which is the deepest of the Maldives, may ensure better environmental conditions in term of water quality.

CONCLUSION

Revising sites surveyed in the past may be a useful tool to better understand change over time and future trend of coral reef communities (McClanahan 2017; Bianchi et al., 2022 and references therein). Comparing only two points in time may not be sufficient to assess when changes in coral reef communities have occurred (Azzola et al. 2022, and references therein). Nevertheless, the analysis of SST data was strongly suggestive that changes occurred in correspondence of the thermal anomaly of 2016. In all, our study proved effective in illustrating the magnitude of change of coral reef communities in relation to global and local impacts and depending on reef exposure.

Distinguishing the effects of climate change from those due to local human pressures is often difficult (Gissi et al., 2021). Comparative analysis between atolls subjected to different level of anthropization may allow to better understanding in recovery capacity of coral reefs under a changing climate regime. By showing a different trend between Huvadhoo reefs compared to the Malé reefs, this study highlighted the possibility that local human pressure may impair the resilience capacity of hard corals. Continued monitoring and further studies are needed to foresee the future of Maldivian coral reefs.

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SUPPLEMENTAL INFORMATION

Table S1. Results of Similarity Percentage Analysis (SIMPER) on lagoon coral reef communities of Huvadhoo Atoll

LAGOON REEF						
Descriptor	**Code**	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Mean 2009**	**Mean 2020**
Tabular *Acropora*	CAT	8.49	17.72	17.72	0.44	0.03
Encrusting coral	CE	5.31	11.09	28.81	0.16	0.35
Branching *Acropora*	CAB	5.25	10.96	39.77	0.22	0.30
Digitate *Acropora*	CDA	4.94	10.32	50.09	0.08	0.27
Abiotic descriptors	ABT	4.25	8.88	58.97	0.71	0.56
Soft bodied organisms	SF	3.21	6.70	65.67	0.23	0.11
Fleshy algae	FA	3.14	6.55	72.21	0.00	0.16
Branching coral	BC	3.08	6.42	78.64	0.17	0.10
Massive coral	CM	2.97	6.19	84.83	0.29	0.33
Coralline algae	CA	2.76	5.75	90.58	0.16	0.12
Fungiidae	F	2.08	4.34	94.92	0.00	0.10
Foliose coral	CF	1.59	3.31	98.23	0.02	0.06
Columnar coral	CC	0.62	1.28	99.51	0.019	0.02
Large clam	LC	0.23	0.49	100.00	0.01	0.01
Heliopora coerulea	Hcoe	0.00	0.00	100.00	0.00	0.00

Table S2. Results of Similarity Percentage Analysis (SIMPER) on ocean coral reef communities of Huvadhoo Atoll

OCEAN REEF						
Descriptor	**Code**	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Mean 2009**	**Mean 2020**
Tabular *Acropora*	CAT	4.32	14.34	14.34	0.21	0.00
Massive coral	CM	3.52	11.70	26.04	0.29	0.34
Encrusting coral	CE	3.30	10.97	37.01	0.30	0.25
Abiotic descriptors	ABT	2.10	9.97	46.98	0.64	0.70
Soft bodied organisms	SF	2.79	9.27	56.25	0.13	0.10
Branching *Acropora*	CAB	2.48	8.25	64.49	0.03	0.12
Digitate *Acropora*	CDA	2.40	7.96	72.45	0.12	0.07
Coralline algae	CA	2.29	7.60	80.06	0.39	0.36
Branching coral	BC	2.28	7.58	87.63	0.37	0.37
Fungiidae	F	1.17	3.88	91.51	0.00	0.06
Fleshy algae	FA	1.11	3.70	95.21	0.00	0.06
Foliose coral	CF	0.64	2.14	97.35	0.03	0.00
Heliopora coerulea	Hcoe	0.37	1.22	98.56	0.02	0.00
Large clam	LC	0.38	1.06	99.62	0.01	0.06
Columnar coral	CC	0.12	0.38	100.00	0.01	0.00

5.1

Maintained functional diversity but loss of redundancy in a temperate reef ecosystem

In preparation:

Azzola A., Bianchi C.N., Morri C., Oprandi A., Montefalcone M.

Abstract

The study of change in coastal marine ecosystems is of fundamental importance, considering their decline on a spatial and temporal scale. Their state of health is currently threatened by several disturbances and stresses, which in the last decades have caused the loss of many species, altering the biodiversity of marine ecosystems. As widely recognised, biodiversity represents the variety of living organisms at all levels of complexity from genes to ecosystems. Nevertheless, species richness is not the only component of biodiversity. The relative abundance of different species and their functional roles are also crucial. The analysis of functional diversity and redundancy can provide information on the ability of ecosystems to maintain their integrity. Amongst coastal ecosystems, the coralligenous reef represents one of the most important habitats in the Mediterranean Sea in terms of extent, biodiversity, and production. This paper analyse the coralligenous reef of Punta Mesco (Ligurian Sea), where quantitative data have been collected since the 1960s. Biological Traits Analysis (BTA) was applied to analyse whether the change over time in species composition has affected the functionality of this ecosystem. As the BTA is a method based on the morphological and functional characteristics of species (i.e., biological traits) three main functions were considered: trophism, three-dimensionality of populations and bioconstruction. The functional diversity did not significantly change over time, indicating that ecosystem functions have been maintained. On the other hand, redundancy significantly decreased for all the functions considered, highlighting a reduced resilience of the Punta Mesco coralligenous.

INTRODUCTION

Ecosystems worldwide are rapidly losing biological and functional diversity (Naeem et al., 2012). Species extinction is a natural process, but the rapidity with which it is globally occurring is worrisome (Chapim et al., 2000). Drawing attention to biodiversity is a need that found a first governmental response in the Convention on Biological Diversity (1992) with the aim of defining conservation strategies. In this framework, the concept of biological diversity found its first definition, and, in the following years, several others have been proposed (Long, 1996; Gaston and Spicer, 1998; Swingland, 2013). Nowadays is commonly recognised that biodiversity expresses the variety and variability of living organisms at all organisational levels from genes to ecosystems (Chapim et al., 2000).

Naeemen (1997) defined biodiversity as a form of 'biological insurance' against the loss of species. Disturbance, depending on its magnitude and duration, differently affects the species, based on their specific resilience and recovery capacity (Duarte, 1991; Santangelo et al., 2015). In a community rich in biodiversity, the ability to repopulate ecological niches left empty by disturbance is greater (Duarte, 2000). Species with a high growth rate are more able to recover (Gärdmark et al., 2003) and contribute more to ecosystem functioning (Duarte, 2000). The importance of a species is therefore not only based on its abundance, but also on its functional role: relatively rare species can strongly influence the dynamics of a community (Hooper et al., 2005).

Species richness is therefore not the only component of biodiversity: functional diversity and redundancy are also crucial (Grime, 1998; Wardle et al., 2000; Lavorel & Garnier, 2002; Van der Plas, 2019). Functional diversity comprises the set of functional characteristics of species occupying an ecosystem. High values of functional diversity imply that there is a more efficient use of resources in the community and a greater ability to maintain ecosystem functionality (Duarte, 2000). Redundancy is the number of species with similar functions present in a community and high values of redundancy imply a more probability that at least some of the species will survive drastic changes in the environment and maintain the different functions of the ecosystem (Chapim et al., 2000). A species can be considered redundant when it coexists in the ecosystem with other functionally similar species, and its disappearance does not result in a loss of functionality. In this way, an ecosystem may be initially insensitive to species loss if there are others that play similar functional roles (Duarte, 2000).

The coralligenous represents, in terms of extent, biodiversity and production, one of the most important coastal marine habitats in the Mediterranean Sea, capable of creating an extremely complex and diverse landscape (Ballesteros, 2006), whose value is comparable to that of tropical coral reefs (Bianchi, 2001). Due to its susceptibility to local and global human pressures, the coralligenous reef is defined as a vulnerable ecosystem, also considering the slow growth rate typical of its many structuring species (Ferrigno et al., 2017). The coralligenous reef has been defined by the Marine Strategy Framework Directive as a 'special habitat type' as the assessment of its quality status is a valuable contribution to defining the Environmental Status (ES) of Mediterranean marine regions or sub-regions (EC, 2008).

Quantitative data (obtained through underwater wire-frame still photography) are available for the Punta Mesco coralligenous since 1961 (Rossi, 1965), offering one of the longest, although discontinuous, Mediterranean data series on benthic communities (Peirano et al., 1992; Peirano et al., 2000; Bianchi et al., 2001; Roghi et al., 2010; Azzola et al., 2019). Analysis of cover species over time led to the conclusion that the benthic community composition and structure changed abruptly in the 1990s to assume a new configuration that in turn remained stable till 2017 (Bianchi et al., 2019). The aim of this work is to analyse whether the change in the specific composition undergone by coralligenous communities of Punta Mesco was reflected in a reduction of ecosystem functionality and redundancy.

MATERIALS AND METHODS

Study area and sampling activities

Punta Mesco is a rocky promontory located in the zone A of the Marine Protected Area of Cinque Terre (Ligurian Sea). The rocky reef studied lies immediately off the point, between ca 15 m and ca 55 m depth, and hosts a typical coralligenous community (Bianchi et al., 2019).

The sampling activities was carried out in the 2017 (Azzola et al., 2019), consisted in photographic surveys as already done in 1961 (Rossi, 1965), 1990 (Peirano et al., 1992), 1996 (Peirano et al., 2000; Bianchi et al., 2001), and 2008 (Roghi et al., 2010), always in summer. Five stations between 20 m and 40 m depth were selected (one at 20 m, one at 30 m and three at 40 m), with 3 replicates per station, for a total of 12 photoquadrats of 1 m^2 per year.

Biological Traits Analysis (BTA)

Each photoquadrat was visually analysed to calculate the percent cover of any organism present at the lowest possible taxonomic level (usually species). Based on the BTA, three functional traits most representative of the coralligenous habitat were considered to analyse change over time in functional diversity and redundancy: trophic guilds (expressing trophism), growth forms (expressing three-dimensionality) and calcification (expressing bioconstruction capacity). Depending on the source of food, the species were then divided in active ciliated filters, active suction filters, autotrophs, organic matter absorbers or passive filters. Based on the growth forms, the species were classified as arborescents, bushy, encrusters, erect mounds (h>r), flattened mounds (h<r), folioses or hemispherical mounds (h=r). Based on the calcification, the species were divided in bafflers, dwellers, bioconstructors or sediment providers. Species with different trophic strategies, growth forms and different levels of calcification were assigned to more than one category of functional traits. In this case, the ratio of the most represented strategy to the total species cover was calculated and the cover was distributed proportionally across the different categories. The total cover of each category of trophic guild, growth form and calcification were calculated by summing the cover of all species belonging to the given category. Shannon Index was then applied to evaluate change over time of functional diversity, while Simpson Index was applied to evaluate change over time in redundancy. Significant difference between years was evaluated by the analysis of variance (ANOVA).

RESULTS

By applying Shannon index, no significant changes were detected for the functional diversity of any function investigated (Fig. 1). On the other hand, a significant increase in the Simpson Index values was observed over the time for almost all the traits investigated, showing a reduction in redundancy (Fig. 2, 3, 4).

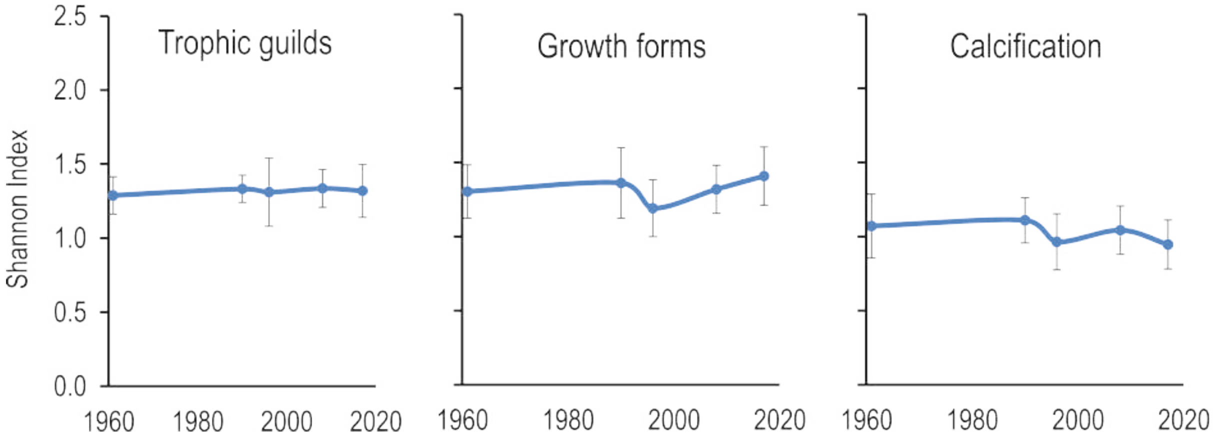

Fig. 1 Shannon Index values which express the trend of functional diversity over the time for all the traits considered

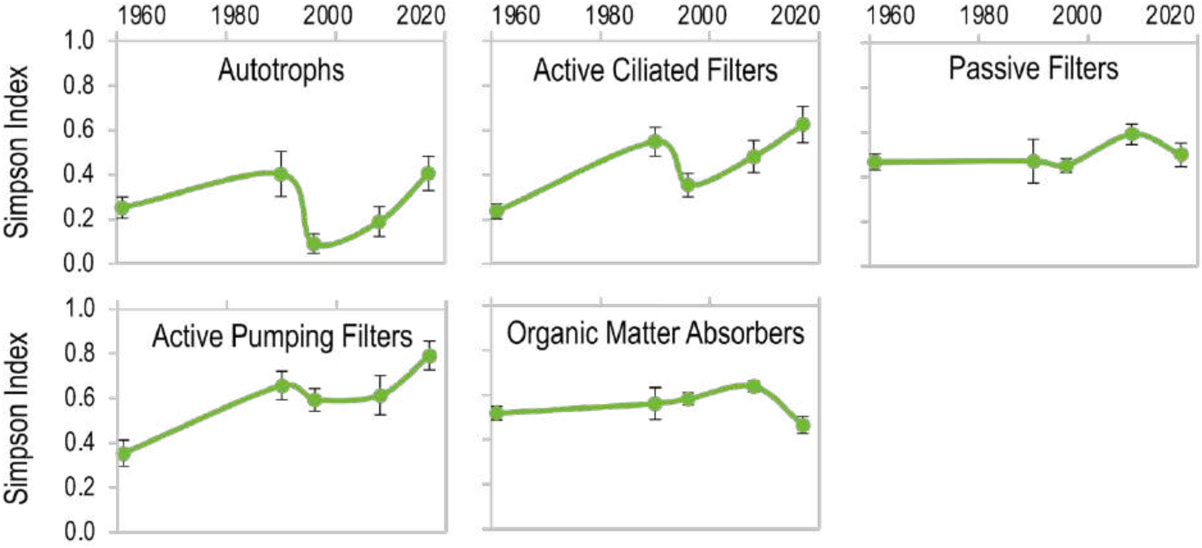

Fig. 2 Simpson Index values which express the trend of redundancy over the time for the trophic guilds

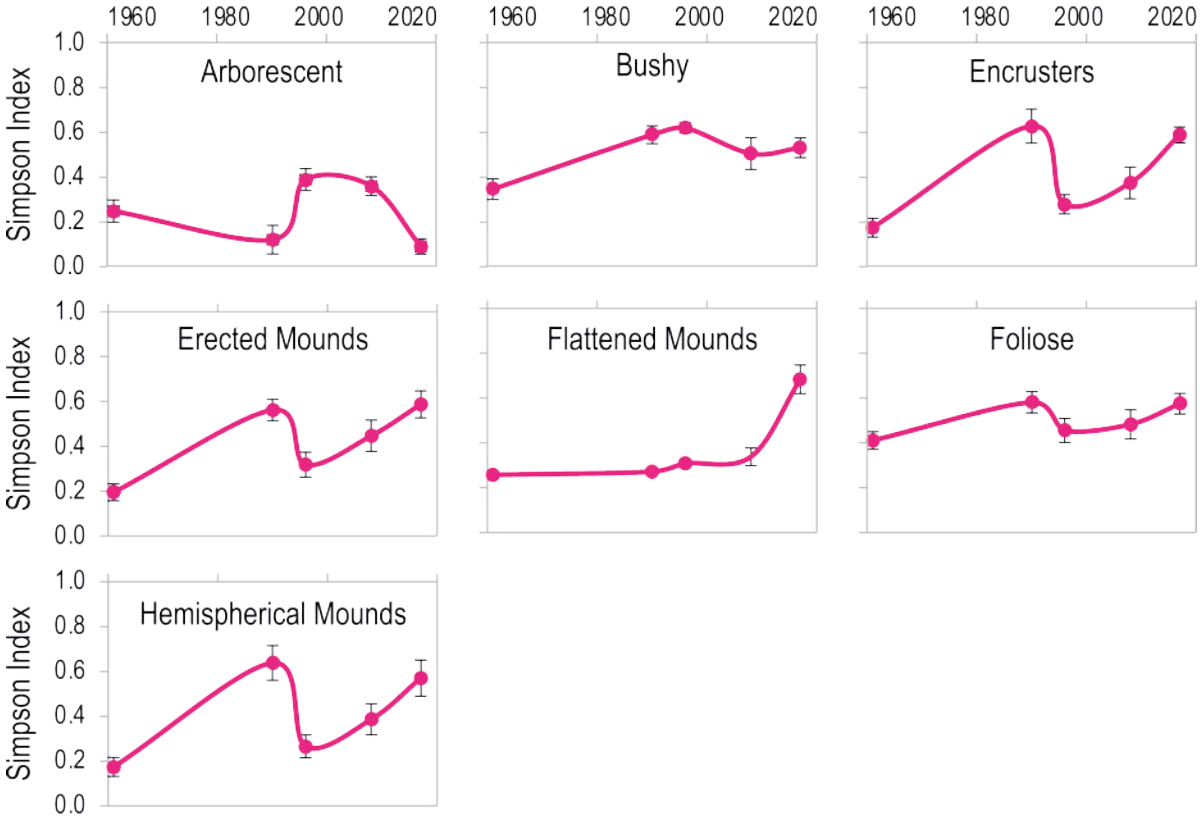

Fig. 3 Simpson Index values which express the trend of redundancy over the time for the growth forms

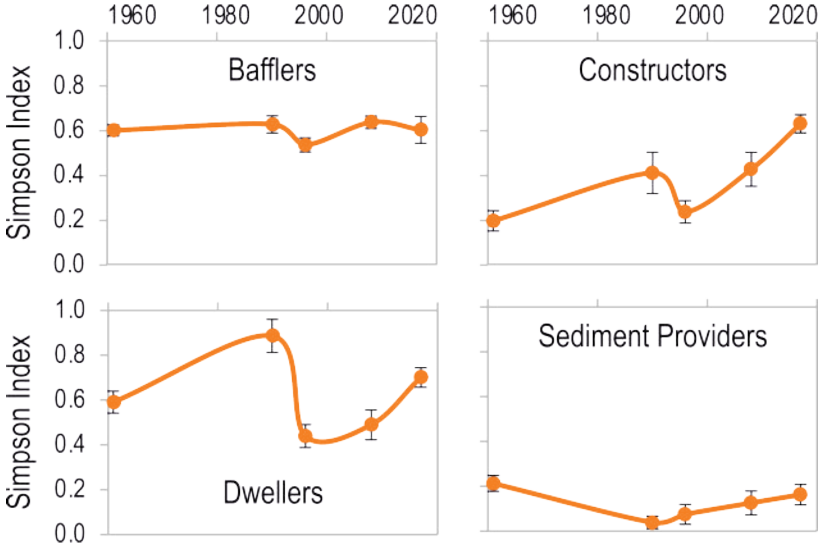

Fig. 2 Simpson Index values which express the trend of redundancy over the time for the calcification

DISCUSSION

Thanks to the historical data series of Punta Mesco, one of the longest exist for rocky reefs in the Mediterranean, it was possible to assess the evolution of coralligenous habitat over the past 60 years. Analysis of change over time showed a phase shift in benthic communities composition and structure, occurred between 1990 and 1996, with the disappearance or reduction of some of structural species (e.g., *Cellaria fistulosa*, *Paramuricea clavata*) and the arrival of alien species (e.g., *Caulerpa cylindracea*, *Womersleyella setacea*) that gradually became dominant. The community changed from a more complex, three-dimensional structure to a more homogeneous community with reduced three-dimensionality (Gatti et al., 2015; Azzola et al., 2019; Bianchi et al., 2019).

The change in community structure and composition did not result in significant changes in functional diversity which was maintained over the time for all three aspects considered (trophism, three-dimensionality and bioconstruction capacity). On the other hand, redundancy has been significantly decreased, highlighting a reduced ecosystem resilience and thus a high risk of losing ecosystem functions in the face of disturbance and/or stress.

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5.2

Little change in ecosystem functioning of a Blue Hole after 15 years

In preparation:

Azzola A., Bianchi C.N., Morri C., Montefalcone M.

Abstract

The Maldivian Blue Hole (Indian Ocean) is a large cavity of karstic origin, which opens on a lagoon floor at 30 m depth, with an entrance of 70 m diameter, and descends to 85 m depth. The water column shows a physicochemical composition according to the depth gradient: a first marine water zone occurs between 30 m and 45/50 m depth; a transition zone (chemocline) occurs at about 50 m depth, where temperature, salinity, pH and Eh decrease dramatically; a bottom zone is found below 50 m depth, where oxygen (O_2) concentration collapses, while both carbon dioxide (CO_2) and hydrogen sulphide (H_2S) significantly increase. In this paper we provide a first characterization of the Maldivian Blue Hole benthic communities trophic organization along the water column and across the transition zone that separates the shallow and deep waters. The first data collection was conducted in 2004, taking photoquadrats every 5 m depth, from 30 m to 75 m along two vertical transects. Fifteen years later, the same transects were resurveyed using the same method, allowing analysis of change over time in benthic communities of the Blue Hole. The photoquadrats were visually analysed to estimate the percentage cover of species and descriptors, which have been divided in trophic guilds. The results showed that trophic organization of the different benthic communities along the depth gradient reflected the zonation of the water column according to the physicochemical composition. The comparison between 2004 and 2019 data showed that the benthic communities did not change significantly, underlining a stable functionality of the Maldivian Blue Hole over time.

INTRODUCTION

Blue holes are subsurface voids that develop several meters underwater in carbonate banks and islands and formed by karstic processes, that is, dissolution and/or a fracture-type collapse of carbonate rocks (Mylroie et al., 1995; Yao et al., 2020 and references therein). Most of the oceanic blue holes formed during the last glacial period, when the sea level was 100-120 m below present, and due to the subsequent rise in sea level these cavities were submerged (Hatcher, 2006).

Blue holes are generally circular and have steep walls. They may contain marine, fresh, or mixed-chemistry waters, influenced by tides and/or bottom water sources. Some blue holes are open at the surface, which makes them susceptible to tides and allows water exchange at the ocean (Whitaker & Smart, 1997). Others present a limited water exchange with external environment and are characterized by stable and vertically stratified physicochemical gradients (Yao et al., 2020). The water column can be divided into three layers: the upper freshwater layer influenced by the tide, the hypoxic halocline layer typically characterized by corrosive reactions, and the bottom saltwater layer (Martin et al., 2012). Some blue holes are characterized by extreme conditions, such as anoxia and high concentrations of hydrogen sulphide (Canganella et al., 2004, 2007; He et al., 2020).

To date, the Faanu Mudugau Blue Hole in the Maldives (3°55.507'N, 72°56.559'E) is the only one known in the Indian Ocean. It opens on a lagoon floor at 30 m depth, with an entrance of 70 m diameter, and descends to 85 m depth. Along its vertical to overhanging walls, in some lateral recesses or ledges at 50 m depth, there are speleothems (i.e., stalactites and stalagmites), as proof of the karstic origin of the cave (Colantoni et al., 2003). The physicochemical composition of the Faanu Mudugau Blue Hole water shows significant variation along the depth gradient. A transition zone (chemocline) occurs at about 50 m depth, where temperature, salinity, pH and Eh decrease dramatically. Below 50 m depth, oxygen (O_2) concentration collapses, while both carbon dioxide (CO_2) and hydrogen sulphide (H_2S) significantly increase (Colantoni et al., 2003).

In this paper we provide a first characterization of the trophic organization of the Faanu Mudugau Blue Hole benthic communities along the water column and across the oxic-anoxic interface that separates a shallow layer of normal marine water from a deep sulphide-rich layer. Thanks to the availability of historical data, change over time in the functionality of Faanu Mudugau Blue Hole benthic communities is also assessed.

MATERIAL AND METHODS

Data collection and field surveys

Data on the benthic reef communities of Faanu Mudugau Blue Hole were first collected by scuba diving in 2004. Along two vertical transects three photoquadrats (each covering an area of 24×36 cm²) were taken every 5 m depth, from 30 m to 75 m to characterize the trophic organization of benthic communities. In 2019, the same transects were resurveyed using the same photographic sampling method to analyse the change over time in the ecosystem functionality (Fig. 1, 2).

Photographic analysis and trophic guilds

Each photoquadrat was visually analysed to calculate the percent cover of any organism present at the lowest possible taxonomic level (usually species). When species identification was not possible, a generic classification category, grouping different taxa based on similar morphological and structural characteristics, was adopted (i.e., OTUs = Operational Taxonomic Units).

To evaluate functional change in the benthic community of the Faanu Mudugau Blue Hole, the species and OTUs recorded were grouped in non-taxonomic descriptors, that is trophic guilds (TG). TG are used as trophic descriptors to evaluate the energy sources exploited by sessile organisms (Parravicini et al., 2010). Eight TG were defined considering the feeding mechanisms used to get food (Montefalcone et al. 2018): ciliary active suspension feeders (e.g., serpulids), chemiotroph (e.g., sulpho-bacteria), combo (e.g., zooxanthellate corals), macroautotrophs (e.g., encrusting corallines and other macro-algae), microautotrophs (e.g., Cyanobacteria), mixotroph (e.g., *Diatomeae* spp.), passive suspension feeders (e.g., azooxanthellate corals), pumping active suspension feeders (e.g., sponges and ascidians). The abiotic component (i.e., bare rock and sediment) was also considered.

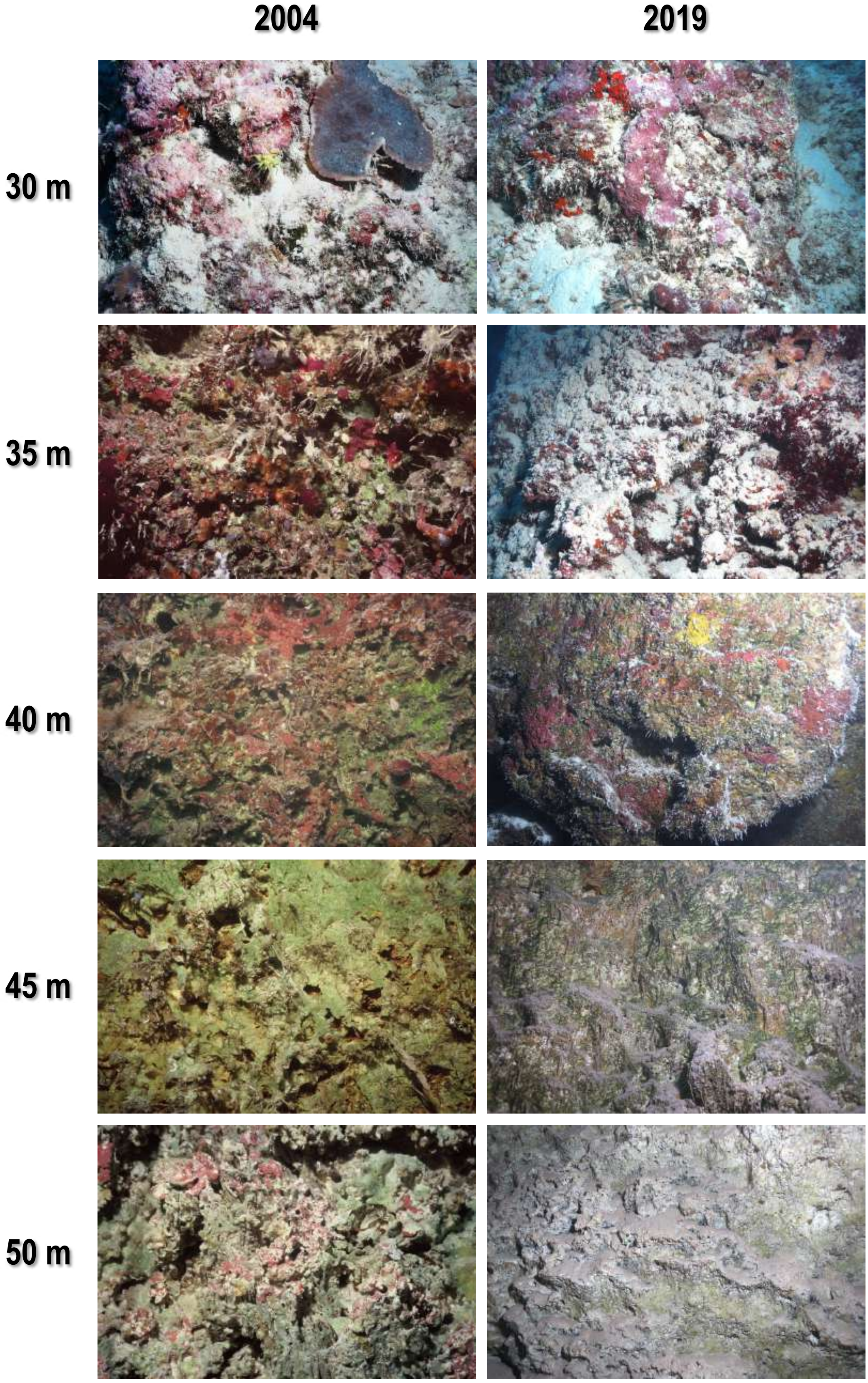

Fig. 1 Examples of photoquadrats (each covering an area of 24×36 cm²) carried out in two times (2004 and 2019) at five of the ten depths surveyed (from 30 m to 75 m every 5 m depth)

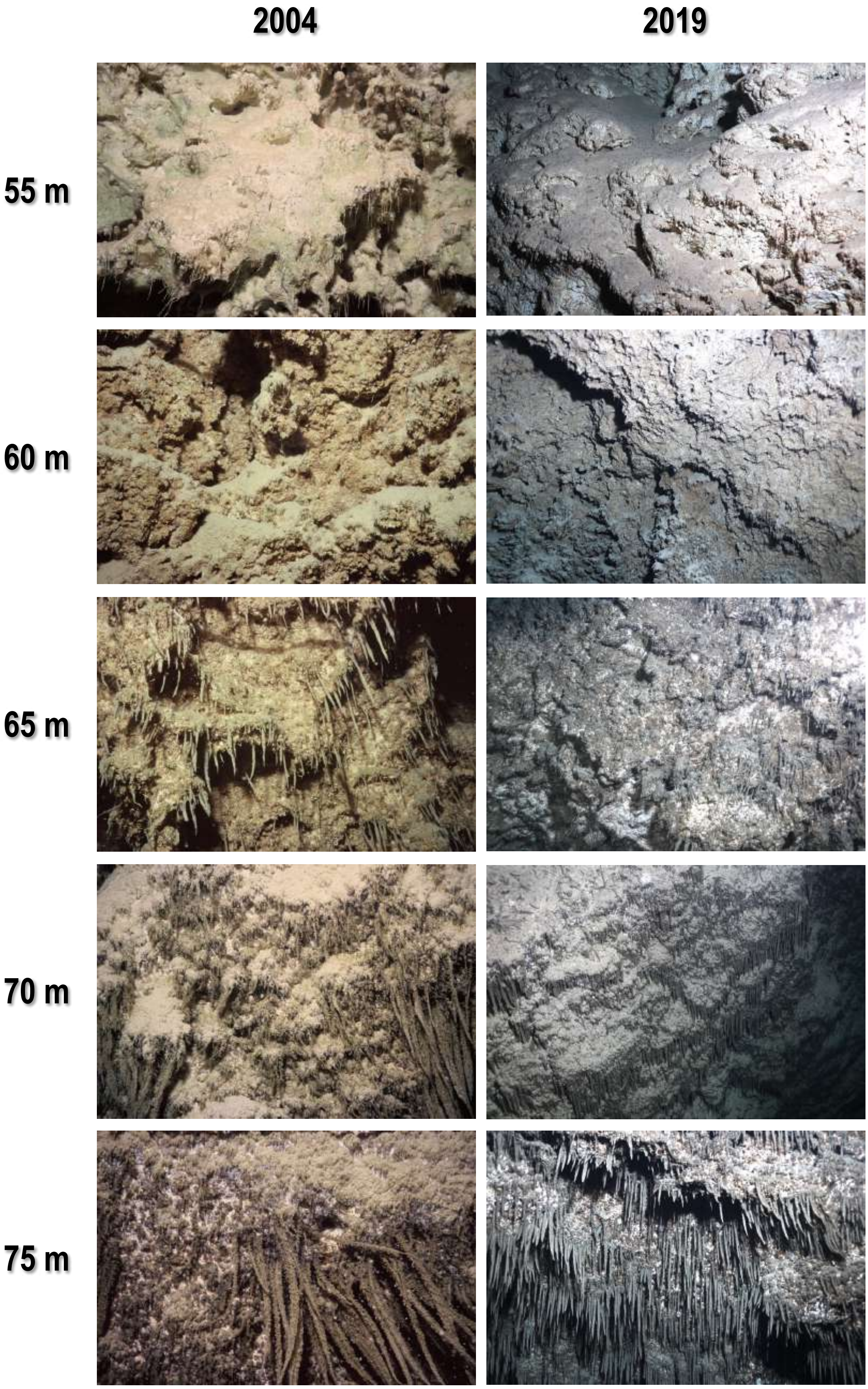

Fig. 2 Examples of photoquadrats (each covering an area of 24×36 cm²) carried out in two times (2004 and 2019) at five of the ten depths surveyed (from 30 m to 75 m every 5 m depth)

Data management and statistical analysis

Cover data of any trophic guild was organized in a matrix (time × depth) × TG and transformed applying arcsine$\sqrt{(x/100)}$. A corrispondence analysis (CA) was applied to analyse the trophic functionality of benthic communities. Three depth-related zones with different communities were identified (see Results), in each depth-related zones centroids of the 2004 (time before) and 2019 (time after) were visualised to outline time trajectories.

Cover of each trophic guild recorded in 2004 was compared to the cover of 2019 by Student's t-test to highlight which descriptor changed most.

RESULTS

A total of 36 descriptors were identified and grouped in the trophic guilds (Table 1).

Corrispondence analysis applied to cover data ordered photoquadrat points according to a depth gradient along the 1st (horizontal) axis which was an expression of increasing depth from the left (30 m) to the right (75 m) (Fig. 3). The two first axes explain 48.2% and 27.5% of the total variation (Lebart's test). Three depth-related zones with different communities can be recognized: i) between 30 m and 40 m depth the benthic community is characterized by combo, macroautotrophs, passive suspension feeders and pumping active suspension feeders; ii) between 45 m and 55 m depth the benthic community is characterized by ciliary active suspension feeders, microautotrophs, and mixotrophs; and iii) the between 55 m and 75 m depth the benthic community is only characterized by chemotroph (Fig. 3). The abiotic group does not of course fit into the benthic communities but is nevertheless much shifted toward the 50-55 m depths.

Time trajectories showed little changes in all the three depth-related zones visualized on the CA plot (Fig. 3).

Table 1. List of the Blue Hole benthic descriptors grouped in the trophic guilds

DESCRIPTORS	**TROPHIC GUILDS**
Corrosion holes Dead encrusting Corallinales Rock Skeleton of Scleractinian Sediment	ABIOTIC
Annelida, *Phyllochaetopterus* sp. Annelida, serpulids, large Annelida, serpulids, small	CILIARY ACTIVE SUSPENSION FEEDERS
Bacterial filaments, long (>10cm) Bacterial filaments, medium (3cm - 10cm) Bacterial filaments, short (<3cm)	CHEMIOTROPH
Cnidaria, hard corals, zooxanthellate, encrusting Cnidaria, hard corals, zooxanthellate, *Mycedium* sp. Cnidaria, hard corals, zooxanthellate, *Pachyseris speciosa* Cnidaria, soft corals, zooxanthellate *Sinularia brassica*	COMBO
Rhodophyta, Corallinales, encrusting Rhodophyta, *Peyssonnelia sp.*	MACROAUTOTROPH
Microbial mat, green (Cyanobacteria)	MICROAUTOTROPH
Microbial mat, golden (Diatom) Microbial mat, yellow (Crysophiceae)	MIXOTROPH
Cnidaria, hard corals, axooxanthellate, *Tubastraea faulkneri* Cnidaria, hard corals, azooxanthellate, Caryophylliidae Cnidaria, hard corals, azooxanthellate, *Dendrophyllia gracilis* Cnidaria, Hydrozoa Cnidaria, wire corals Antipatharia	PASSIVE SUSPENSION FEEDERS
Ascidians, encrusting Ascidians, zoocyanellate, *Didemnum molle* Sponges, bushy orange Sponges, bushy, *Pseudaxinella* sp. Sponges, Calcispongiae, *Clathrina* sp. Sponges, Calcispongiae, *Leuclathrina translucida* Sponges, encrusting orange Sponges, encrusting purple Sponges, massive orange Sponges, massive purple Sponges, massive white	PUMPING ACTIVE SUSPENSION FEEDERS

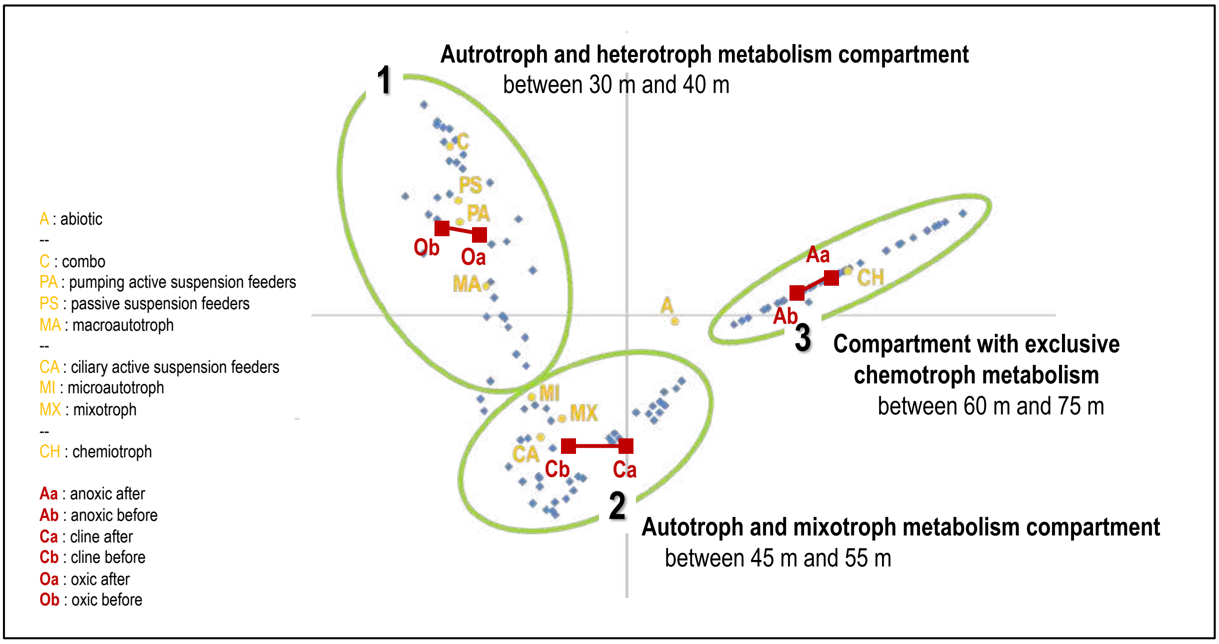

Fig. 3 Corrispondence analysis (CA) plot of Blue Hole photoquadrats (blue points) and trophic guilds (yellow codes). Centroids of each depth-related zone are represented by alphanumeric codes with zone (A: anoxic; C: cline; O: oxic) followed by time period (b: before related to 2004; a: after related to 2019). Time trajectories are visualized by red line.

T-test student applied on the trophic guilds cover of 2004 and 2019 highlighted significant ($p < 0.001$) differences only for: abiotic at 35 m, 45 m, 50 m and 55 m depth; macroautototroph at 50 m depth; microautotroph at 50 m and 55 m depth; pumping active suspension feeders at 35 m depth (Fig. 4).

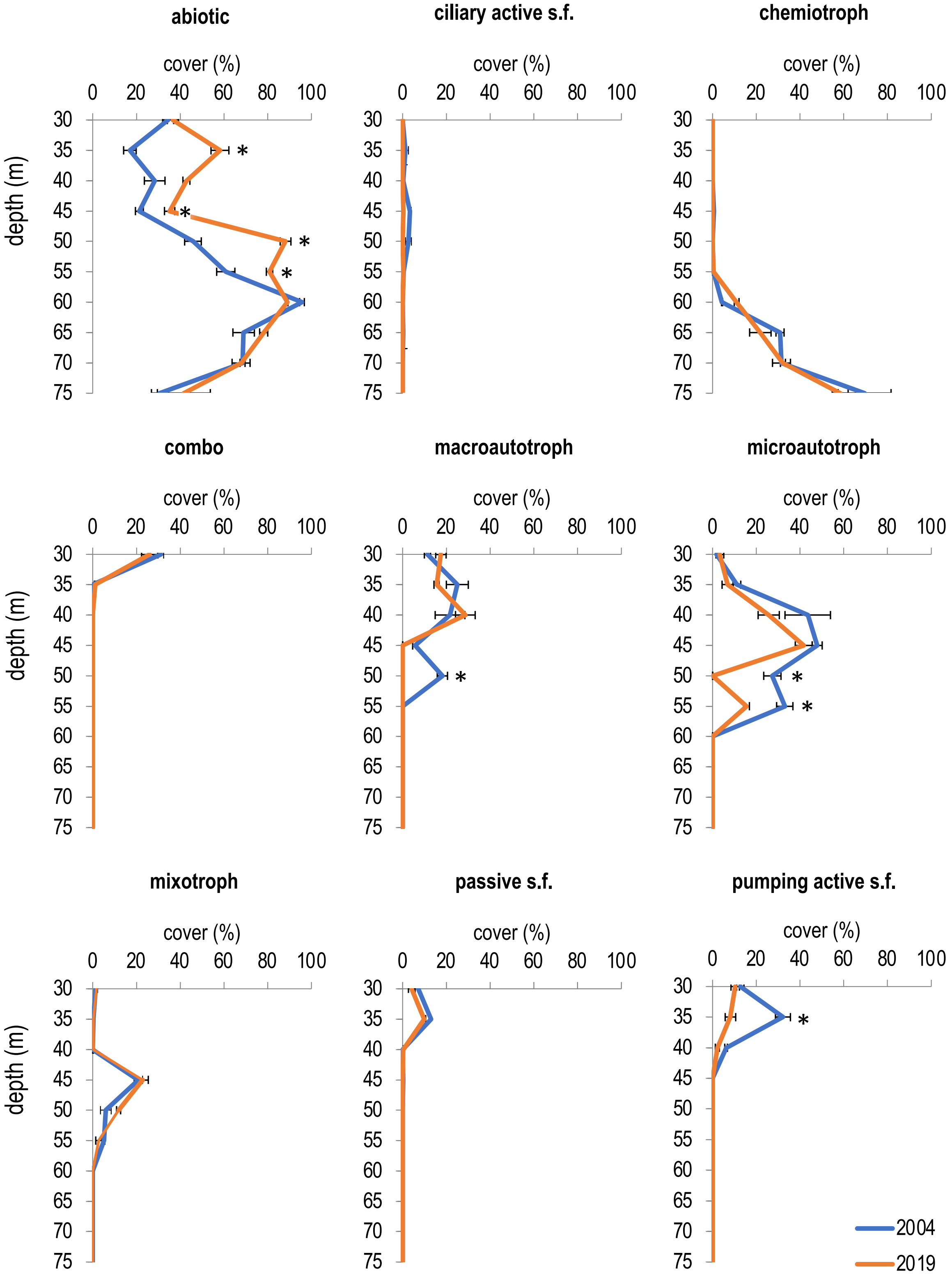

Fig. 4 Cover (%) of trophic guilds recorded in 2004 (blue line) and 2019 (orange line) at 10 depths. The asterisks highlight the significant differences between the 2004 and 2019 cover.

DISCUSSION AND CONCLUSION

The Faanu Mudugau Blue Hole is a peculiar ecosystem characterized by a water column divided into three different zones according to a physicochemical gradient: the first zone of normal marine water occurs between 30 m and 40 m depth, the second zone occurs between 45-50 m depth, where temperature, salinity, pH and Eh decrease dramatically, and the third zone occurs below 50-55 m depth characterized by deep sulphide-rich water (Colantoni et al., 2003).

The trophic organization of benthic communities reflects the stratification of the water column and is adapted to environmental parameters. Three depth-related zones with different communities have been identified: the first zone can be defined as a high-metabolism zone with a benthic community characterized by combo, macroautotrophs and suspension feeders; the second one is a low-metabolism zone as it is mainly characterized by microautotrophs and mixotrophs; and in the third zone the benthic community is characterized only by chemotroph.

Only little changes have been observed in benthic communities of the Blue Hole, mainly attributable to the abiotic component. Sediment and rock increase significantly between 35 m and 55 m depth, where a decrease in macroatotroph (such as encrusting corallines) and microautotroph was observed. However, the other descriptors did not show a significant change in their cover, underlining a stability of the Blue Hole benthic communities for over 15 years.

On this basis, the Faanu Mudugau Blue Hole can be defined as a stable system in a changing global context. Blue holes can be seen as natural laboratories that allow for the ecological study of extreme environments. Further investigations on the Faanu Mudugau Blue Hole are needed to better understand the structure and functioning of this peculiar ecosystem.

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FINAL REMARKS

The global trend of change of natural ecosystems makes increasingly necessary to assess their ecological status and change over time to promote effective biodiversity conservation plans (Díaz et al., 2019). The present work addressed this need for Mediterranean rocky reefs and Maldivian coral reefs, which are considered biodiversity hotspots (Birkeland, 1997; Duffy et al., 2013) and are subjected to several local and global human pressures (Halpern et al., 2007).

Different methodological approaches can obviously be applied to evaluate ecological status of marine ecosystems. In this context, Marine Protected Areas (MPAs) are considered valid field laboratories to assess the effects of global warming and multiple stressors. In fact, in MPAs effects of natural changes can be discriminated from those of local human pressure. The present work firstly highlighted how, due to continuous changes occurring in marine ecosystems, the constant updating of species inventory and surveillance is still necessary in MPAs, also after their establishment (Bianchi et al., 2022).

Monitoring of target species is a valuable tool for the conservation of the environment they live in (Siddig et al., 2016). However, a complete knowledge about many of these is still lacking, especially for those that are not considered ‘flagship species’ and do not arise the so-called 'Walt Disney effect' (Bianchi and Morri, 2000). For instance, this is the case for *Axinella polypoides* in the Ligurian Sea, which was considered a thermophilic species spreading in response to sea water warming (Parravicini et al., 2015). Surprisingly, data collected since the 1950s revealed that the abundance of *A. polypoides* in the Ligurian Sea is actually comparable to that observed in the warm south-eastern Mediterranean Sea (Idan et al., 2018; Azzola et al., 2021), confirming its ‘Least Concern’ status already defined for the species in Greece (Gerovasileiou et al., 2018). Moreover, the zonation of existing Ligurian MPAs does not involve many habitat where *A. polypoides* is generally present (Azzola et al., 2021). In fact, less than 10% of the potential habitats of some major invertebrate phyla is protected within MPAs (Greathead et al., 2020). The present work has confirmed how this mismatch may relate to a lack of knowledge, and detailed information on species distribution is critical to develop effective environmental management plans (Gerovasileiou et al., 2019).

Visual methods are to be preferred when monitoring target species and valuable habitats, as they do not need the sacrifice of specimens and have the advantage of obtaining data immediately, without further time-consuming analyses (Bianchi et al., 2004). However, observer effect may hamper analyses of data, when collected by different diving scientist. This work has proven that visual methods are reliable and effective in monitoring coralligenous assemblages of the Portofino MPA (Ligurian Sea) and in assessing change over time in benthic communities of the Capo Carbonara MPA (Tyrrhenian Sea). In any case, when designing visual monitoring activities, variability amongst observers should always be fully taken into account to ensure the reliability of the information (Azzola et al., 2022a, b).

If it is true - as it is true - that knowledge is the first step for the development of effective conservation plans, characterization and evaluation of marine ecosystems at an ever-increasing spatial scale are of primary importance. An internationally coordinated network of MPAs in the Mediterranean Sea can represent the reference system for understanding the effects of global warming on marine ecosystems (Bianchi et al., 2022). In this context, the T-MEDNet network (Garrabou et al., 2009, 2019, 2022) provides protocols to monitor water temperature and the effects of climate change on benthic communities. Data on temperature, target species mortality, and thermophilic species occurrence collected in the Portofino MPA, at Elba Island (Ligurian Sea), in the Capo Carbonara MPA (Tyrrhenian Sea) and in the Plemmirio MPA (Ionian Sea) unveiled several effects of climate change in all areas, regardless of latitude and level of protection. These results demonstrated once again that the level of protection granted by the MPA cannot halt the effects of climate change. They also demonstrated that policy measures aimed at mitigating local human pressures shall go together with those that are in place for mitigating global pressures.

Climate change is affecting marine ecosystems in a multitude of ways promoting different effects such as the spread of alien species, the development of mucilage, and the increase in extreme events such as severe storm. Ecosystems are generally able to recover after a stress or a disturbance, but under the pressure of multiple stressors they may undergo irreversible change (Jaiswal et al., 2021). When multiple pressures act simultaneously, as observed in the Portofino MPA, each one may produce similar or distinct effects. Distinguishing the outcome of every single stressor is difficult (Turner et al., 2020). By studying short-term change in benthic communities, this work provided an example to tackle this difficulty. The analysis conducted at different depths (i.e., 10 m, 20 m, 30 m, and 40 m) in three different periods (i.e., June, October, and December) allowed to disentangle the effects of different stressors that the rocky reefs of Portofino MPA underwent in 2018. The results highlighted that the cumulative effect of multiple stressors is stronger than that of any individual stressor, no matter how intense it is. Interactions amongst multiple stressors are still poorly understood posing important challenges to researchers and conservationists (Gissi et al., 2021).

As a result of the cumulative effects of local and global human pressures, marine ecosystems have undergone important modifications in community composition and structure, namely 'phase shift' (Montefalcone et al., 2011). These changes can only be detected by analysing long-term data series (Gatti et al., 2015; Bianchi et al., 2022 and reference therein). When such data are absent, revisiting sites previously surveyed can prove successful when assessing change over time (Azzola et al., 2022b, and references therein).

Revisiting 25 years later the bed of the coral *Cladocora capitosa* at Bocca di Magra (Ligurian Sea), first studied in 1994, revealed that the population structure has changed with a significant increase in the frequency of small colonies and a decrease in the frequency of larger ones. Moreover, field observations in the Eastern Ligurian Sea between 2017 and 2021, showed that mortality affected predominantly the largest colonies (Azzola et al., 2022c). These findings are consistent with the five major mortality events of *C. caespitosa* occurred in the last decades (Rodolfo Metalpa et al., 2005; Garrabou et al., 2009; Kersting et al., 2013; Jiménez et al., 2016), most of which were observed together with with positive anomalies in SST maxima. Bianchi (2007) hypothesized that tropicalization of the Mediterranean Sea might favour the growth of *C. caespitosa* beds, as happened in warmer phases of the Quaternary (Peirano et al., 2004, 2009). Nonetheless, the results of the present work showed an opposite trend, casting doubts about the future constructional capacity of this coral. While it can be assumed that in its evolutionary history *C. caespitosa* has adapted to the temperate Mediterranean climate, the speed of present warming rate might not allow this species to acclimatize to the new conditions (Azzola et al., 2022c).

In contrast to a decrease in abundance of native species, the spreading of alien species is an increasing and recurrent phenomenon. The increased expansion rate of the species *Lamprohaminoea ovalis* recorded after 2014 in correspondence with an increase in temperature provides further evidence that global warming favors the spread of invasive species (Azzola et al., 2022d). The recorded presence of this species in the Ligurian Sea, one of the northernmost portions of the Mediterranean, confirmed the ongoing global water warming trend of even the coldest sectors, where thermophilic species are becoming more and more frequent (Parravicini et al., 2015; Bianchi et al., 2018).

In the face of a decrease in richness of native species and the spread of alien species, the biogeographic setting of the Mediterranean Sea is changing. Within benthic communities, a change in species composition results in a different community structure, as observed for the Portofino MPA, Elba Island, and Capo Carbonara MPA rocky reefs. Resurveying 20 years later five sites of the Capo Carbonara MPA investigated in 2000 allowed to detect the effects of the ongoing water warming: mucilaginous aggregates and thermophilic species (both alien and native) have increased, while the species vulnerable to heatwaves (such as gorgonians) have decreased. None of the species sensitive to local human impacts has decreased, while species subjected to fishing, protected species, and species usually impacted by divers have increased, indicating that the protection measures enforced by the MPA in these two decades have been effective (Scianna et al., 2019). Thus, this work conducted within an MPA made possible the discrimination of the effects of climate change from those of anthropogenic pressures, excluding the confounding effect.

The significant changes reported in the present work for the Mediterranean benthic communities of rocky reefs are consistent with what observed in other regions of the world ocean (de Young et al., 2008; de Bakker et al., 2017; Edmunds et al., 2018; Montefalcone et al., 2018b). In Huvadhoo Atoll (Maldives, Indian Ocean), when revisiting coral reefs 11 years after the first studies in 2009, a better recovery rate (i.e. higher resilience) of lagoon reefs compared to oceanic reefs in the face of the 1998 and 2016 massive bleaching events was observed. This trend is opposite to that observed in the central atolls of Maldives, where corals proved to be more resilient to the 2016 bleaching event in ocean reefs than in lagoon reefs (Montefalcone et al., 2020) probably due to the proximity of the former to more remixed waters (Muir et al., 2017). Compared to Huvadhoo, the central atolls of the Maldives are much more anthropized, and lagoon sites are subjected to a combination of global and local human pressures that can affect the recovery capacity of reefs (Montefalcone et al., 2020; Pancrazi et al., 2020). Huvadhoo is a remote atoll where local human pressures have not increased significantly in the past two decades. In addition, the topography of Huvadhoo lagoon, which is the deepest of the Maldives, may provide better environmental conditions in terms of water quality. This work, comparing atolls subjected to different level of anthropization provided further insights to better understand the recovery capacity of coral reefs under a climate change regime.

To the question posed at the beginning of this work, whether biodiversity can provide a buffer to maintain the ecosystem functioning in the face of environmental changes, many researchers answer positively (Hong et al., 2021; Loreau et al., 2021). A high degree of biodiversity can buffer the effects of environmental change, since tolerant species have higher chances to survive and thrive, and therefore are more likely to be present (Bianchi et al., 2022). The present work showed that in the case of the Punta Mesco coralligenous (Ligurian Sea) the functionality was maintained over time despite changes in community composition. However, functional redundancy has significantly decreased for all functions analysed (trophic guilds, growth forms and calcification), suggesting the risk of reduced ecosystem resilience.

The only ecosystem where changes over time were not observed by the present work was the Blue Hole, where, although extreme, environmental conditions are stable. But in a world of constant change, environmental factors cannot be kept stable, and change paradoxically promotes stability (Lewin, 1986).

To effectively tackle questions and challenges in ecology, it is essential to increase the resolution of empirical observational capacities over relevant spatial, temporal, and taxonomic scales. Supporting international collaborative research programs offers an effective approach to this goal (Garrabou et al., 2022). Global warming, and the resulting changes in marine ecosystems, are occurring at an unprecedented rate, and it is difficult to hypothesize what their future will be. Only through an increasingly cooperative view between research and international environmental policy will it be possible to develop more effective management decisions in the face of ongoing climate change.

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