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Identification and streamlining of climate Change Impact Indicators in Three Specially Protected Areas of Mediterranean Importance (SPAMI)

Executive Summary

The Mediterranean Sea is rapidly changing in response to the global warming of the world's atmosphere and oceans, and to the synergy of multiple local human disturbances. As a consequence, the Mediterranean is one of the most degraded basins in the world and its rich and diverse life, as well as the economic and social systems that depend upon it, are being threatened by phenomena predicted to increase.

To overcome the threats hanging over marine and coastal biodiversity, the Contracting Parties to the Barcelona Convention adopted in 2003 the Strategic Action Programme for the Conservation of Biological Biodiversity (SAP BIO) in the Mediterranean Region. Climate change issues, one of the priorities of the SAP stressed as a major hazard for biodiversity loss which must be assessed and mitigated, were updated into this Programme in 2009. It was supported by four subregional documents on climate change vulnerability and impact further compiled in a Regional synthesis to further facilitate the development of a climate change adaptation framework for Mediterranean countries to guarantee that adaptation considerations are addressed into the Region's management policies and governance.

The assessment of the status and trends in ecosystems and the potential climate change risks that may affect their structure and function is essential to the success of biodiversity management and conservation. This has been translated into policy implementation through the decision IG.22/1 of the Contracting Parties to develop a Mid-Term Strategy 2016-2021 (MTS), which includes the Strategic Theme "Climate Change Adaptation" as a "Cross-cutting Theme". The MTS aims to enable climate change monitoring and assesment as a main outcome, and it scopes for that purpose to achieve as a key output the consideration of climate change vulnerability issues in existing monitoring programes being undertaken by countries.

SPAMI and MPAs, as healthy coastal and marine areas that provide protection and increase the adaptive ability of populations to resist climate anomalies are one of the most effective tools to face the negative impacts of climate change on marine ecosystems and livelihoods. These areas serve as "sentinel sites" to detect and study the effects of climate change because they are fully or partially protected from local anthropogenic stressors and therefore, are less affected from other drivers of change.

Therefore, the development and implementation of a coordinated basin-wide monitoring of climate change impacts in SPAMI (Specially Protected Areas of Mediterranean Importance) and other MPAs (Marine Protected Areas) through indicators specific to the Mediterranean region becomes a defined SAP BIO and MTS valuable action to improve the understanding and management of climate change effects in the region.

The monitoring of major indicators of climate change impacts to marine ecosystems across SPAMI and MPAs in the Mediterranean Region needs however, common, widely accepted protocols to be established. Five indicators have been identified as of priority interest for putting into practice in protected areas – Sea surface temperature (SST) and thermal stratification, Mortality and bleaching events, Range shift of alien / temperature-sensitive species, Reproduction and breeding date of selected species, including flowering of *Posidonia oceanica*, and Episodic species outbreaks (blooms) - and their implementation has been recommended to managers and countries.

The report “Identification and streamlining of climate Change Impact Indicators in Three Specially Protected Areas of Mediterranean Importance (SPAMI)” is the result of a baseline work conducted to assess how these major climate change indicators are being studied and monitored in protected areas in the Mediterranean Basin, taking as pilot three SPAMI: 1) Mar Menor and Oriental Mediterranean Murcia coast, Spain, 2) Portofino, Thyrrenian Sea, Italy, and 3) Torre Guaceto, Adriatic Sea, Italy.

A detailed description of each major indicator is provided, and is also summarized in Data-sheets attached as Annexes at the end of the document. SST and thermal stratification are main temperature indicators related with thermal anomalies and their timespan. Mortality and bleaching events are large-scale phenomena that dramatically affect benthic organisms and may result in the partial or total mortality of colonies or populations. The range shift of alien / temperature-sensitive species indicator refers to changes in number/coverage and shifts in the geographical/depth distribution of species that are sensitive to temperature changes, including non-native species. The indicator “change in the reproduction and breeding date of key species” is related with earlier or delayed dates of the reproduction activities of species, as well as its duration and success. Finally, the episodic species outbreaks is the occurrence of sudden and abrupt increases in number of organisms, in a greater frequency, magnitude, and persistence than normally occurs; i.e. blooms of gelatinous zooplankton or algal populations.

Collecting existing data, graphs, photos, and information was crucial for developing this report. Information was obtained through consultation with expert colleagues and MPA managers, the reference lists of identified studies, and Internet searches.

Temperature data show a progressive warming of waters that affects the thermal stratification while deepening the thermocline. The monitoring of benthic communities evidences the recurrence of mass mortality events in at least two SPAMI, and how these phenomena have severely affected populations of the white gorgonian *Eunicella singularis*, yellow gorgonian *E. cavolini*, and red gorgonian sea fan *Paramuricea clavata*. Bleaching events have also hit benthic populations in these areas, affecting scleractinian species such as *Oculina patagonica* and *Cladocora caespitosa*. Overall, greater damage of colonies is likely related to prolonged warm conditions. The presence and increases in abundance of some thermophilous species within the SPAMIs has been reported for multiple species, such as for example the algae *Caulerpa cylindracea*, the coral *O. patagonica*, and the ragged sea *Bursatella leachii*. In addition, there is likely a correlation between increases in water temperature in the last years and changes in some fish abundance, such as the ornate wrasse *Thalassoma pavo*, bluefish *Pomatomus saltatrix,* Atlantic bonito *Sarda sarda* and yellowmouth barracuda *Sphyraena viridensis*. Moreover, data stress that the upper limit of some gorgonian populations and hydroid species has deepened. On the other hand, although highly variable in space and time, the flowering of the seagrass *P. oceanica* is positively related with increases in water temperature. Finally, increases in the frequency and intensity of both mucilage and blooming events are also apparent in these areas for gelatinous zooplankton and some microalgae, i.e. *Cotylorhiza tuberculata*, *Rizhostoma pulmo*, *Mnemiopsis leidyi,* and *Ostreopsis ovata*; temporal trends, however, should be carefully interpreted due to the sporadic nature of the sighting reports.

Results show how the monitoring of major climate change indicators in SPAMIs not only allows to follow temporal trends in the structure and composition of important coastal and marine communities, but also serve to better understand the dynamics and stability of ecosystems and to assess the effect of global warming on Mediterranean biodiversity. The data collected through monitoring are therefore key to guide countries over the following years in planning and implementing protection activities and to feed into current regional and international milestone commitments and agreements.

Results also highlight the great diversity of techniques and methods used among Mediterranean SPAMIs to survey/monitor climate change indicators. In an effort to contribute to the current need of common monitoring practices along the Mediterranean basin and to guide regional monitoring, this document describes a detailed framework and a series of standard protocols for five major indicators considered of priority interest to monitor and understand the impact of climate change on Mediterranean MPAs biodiversity. Thus, managers and other MPA's personnel can select the best monitoring option to be implemented in their area, depending on the MPA's necessities and possibilities. All the protocols include easy and cost-effective methodologies, include citizen science, are able to build a Mediterranean view, and guarantee that the data collected can be integrated to existing basin-scale, national, or international networks.

This document stresses the great potential of citizen science to support and increase the scope of monitoring activities in SPAMI, MPAs. Data collected by adequately trained volunteers can also help surpass the overall difficulty of sharing data among scientists, and both scientists and managers can use them. In addition, citizen science serves as a sensitizing and divulgation tool to highlight the increasing evidence of climate change and its effects on the marine environment.

The document arrives to several conclusions as follows:

- The five major climate change indicators assessed in this work are appropriate proxies for monitoring climate change impacts on biodiversity in SPAMI, MPAs at a Mediterranean scale.

- The current monitoring of major climate change impact indicators in SPAMI, MPAs in the Mediterranean basin is limited and the monitoring methodologies used vary greatly among SPAMIs. The further implementation of common climate monitoring activities in the entire region is needed. For this, it is proposed that managers consider the different monitoring options provided in this report, none requiring expensive equipment or highly technical abilities, and decide which protocols and recommendations should be implemented in their areas.

- Results of climate change monitoring programs enhance our understanding of how marine communities and ecosystems respond to climate change and help managers to assess the condition of their sites and the environmental changes that are occurring there. Results serve therefore to better understand how SPAMIs, MPAs and protected areas networks can cope with the effects of climate change and to indicate management options to be taken in order to increase ecosystem resilience.

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I. INTRODUCTION

Mediterranean Biodiversity

The Mediterranean Sea, with a small surface area of only 0.82% of the world ocean, is considered a global hotspot of endemism and biodiversity (Myers et al. 2000) because it shelters an unusually large number of species that represent between 4% and 18% of the world's marine species, i.e. more than 17,000 species (Bianchi and Morri 2000; Coll et al. 2010). The semi-enclosed nature of this sea, as well as its smaller inertia due to the relative short residence time of its water masses (70 years), make it highly reactive to external forcings. For this reason the Mediterranean region is predicted to be particularly sensitive to current and future climatic and anthropogenic impacts and has been identified as a hotspot for climate change. The Mediterranean has been inhabited and negatively impacted by humans for long and is currently one of the most degraded basins in the world. The synergistic interaction of climate change impacts with many other antropogenic disturbances, such as for example the excesive urbanisation of the littoral zone, pollution, overfishing, habitat destruction, and species introductions have disrupted the natural balance of coastal and marine ecosystems, resulting in habitat degradation and the extensive loss of biodiversity (Benoit and Comeau 2005).

Climate Change Impacts

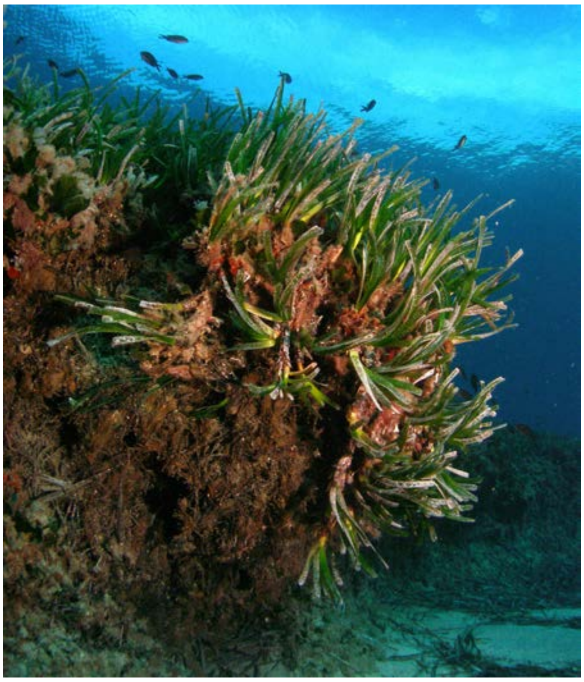
Climate change is a major pressure that threatens the biological diversity and ecosystem structure in the Mediterranean, as well as the economic and social systems that depend upon them. The warming of the Mediterranean Sea in the last three decades (Bethoux et al. 1990; Astraldi et al. 1995; Bethoux and Gentili 1996; Walther et al. 2002), likely faster than in other marine regions (Trenberth et al. 2007), with on average a +0.66° C increase in the Western basin and a +1.1° C in the Eastern basin, has already caused important ecological changes in marine ecosystems. Species response to changing environments is determined largely by population responses. While some organisms can adapt their physiology to increasingly warmer conditions, others can get weaken and become more vulnerable to sickness, and others may die causing the extirpation of populations. Heat stress may also favor the growth of virulent pathogens (Bally and Garrabou 2007), and increase the frequency of parasite infestations and the appearance of new diseases, all of which may increase mortality risks. Multiple mass mortalities events associated with increases in pathogens have already been reported in the Mediterranean, especially during unusually hot summers. The continuation of these trends may represent a danger of species extinctions, and therefore a loss in the Mediterranean biodiversity.

The effects of climate change on marine habitats are very wide and affect the trophic net at every level, from microrganisms to plants and megafauna, both in the benthic and pelagic realm. The effects on all the organisms can be recorded at physiological level, affecting their distribution and asking for quick acclimation, which will depend on the particular capacities of species, their phenological plasticity and genotipyc variability. These effects can be very impressive on some shallow benthic species, leading to local extictions in some cases (Cerrano et al. 2009). The correct monitoring of marine habitats requires baselines that are often not available, it needs "before" and "after" data taken with the correct sampling strategy.

Another clear indication of the biological effects of the Mediterranean warming are the shifts in the distribution of species and changes in population densities. Rises in water temperature have resulted in species migration: new "tropicalized" species are arriving (Bianchi 2007), extending their distributional ranges to the Mediterranean basin (tropicalization), while native cold-affinity species are moving northwards and deeper towards colder waters to escape thermal stress. Eventhough the success of tropicalized species in establishing new populations in the Mediterranean is uncertain, it is clear that communities are shifting, drawing new patterns in community composition or migration (Chevaldonné and Lejeusne 2003; Rosenzweig et al. 2007).

Changes in temperature have also affected the reproduction capacity of certain species. Planktonic populations, for example, are sensitive to climate warming. Temperature rises affect the development of these populations by changing the duration of growth phases, and the frequency and intensity of reproduction. For instance, some phytoplanktonic species are blooming earlier in the year in certain places in the Mediterranean, and have shifted the distribution of these blooms northwards. Thus, new phytoplankton species are appearing in some regions, while some local species are dissapearing (e.g. the dinoflagellate genus *Ceratium*). Because several of the new species are harmfull, increasing numbers of harmful phytoplankton blooms are appearing in coastal regions (for example, *Fibrocapsa japonica*) with deleterous consequences to other species and several socio-economic implications.

In addition to direct temperature effects, changes in the phytoplanktonic populations can severely alter the composition of higher trophic levels' community. For instance, a shift in the dominant phytoplanktonic species can induce phenological changes in copepods' abundance, which will ultimately affect fish recruitment and fisheries.



On the other hand, *Posidonia oceanica* (L.) Delile is a endemic seagrass species representing the most extensive seagrass meadows in the Mediterranean Sea of high ecological importance (Boudouresque et al. 2006). This seagrass is distributed on sandy beds, where they are the dominant ecosystem, and also on rocky sea beds, from the surface to approximately 40 m depth. It is a clonal plant that spreads mostly vegetatively through very slow vegetative growth (Marba and Duarte 1998) and, less frequently, by sexual propagules (Díaz-Almela et al. 2007b). These features make this plant to require centuries to millenia to develop, and therefore, to recover from decline, rendering these ecosystems highly vulnerable to anthropogenic impacts (Marba and Duarte 2010), resulting in important plant losses (Holon et al. 2015).

Figure 1. Above: *P. oceanica* meadows are the most important carbon deposits of the Mediterranean coastal ecosystem. This species plays a key role in the resilience of the ecosystems to global climate change. Below left: The herbovorous fish *Sarpa salpa* grazing on *Posidonia* leaves. Below right: Mature fruits of *P. oceanica*, Spring of 2015, Cabo de Palos, 7m depth. Photo credit: Juan Manuel Ruíz.

During the last decades, *P. oceanica* meadows have experienced continuous losses mainly due to the impact of human activities (Boudouresque et al. 2009). As *Posidonia* beds usually shelter very important species richness, their losses are undoubtedly leading to a decline of the Mediterranean biodiversity. Recent studies have provided evidence indicating the existence of a relationship between seawater warming and both *P. oceanica* mortality (Marba and Duarte 2010) and flowering intensity and prevalence (Díaz-Almela et al. 2005; Diaz-Almela et al. 2007).

So, the Mediterranean warming is already and will in the next future continue altering Mediterranean ecosystems in the coming decades with negative effects such as species extinctions, migrations and replacements.

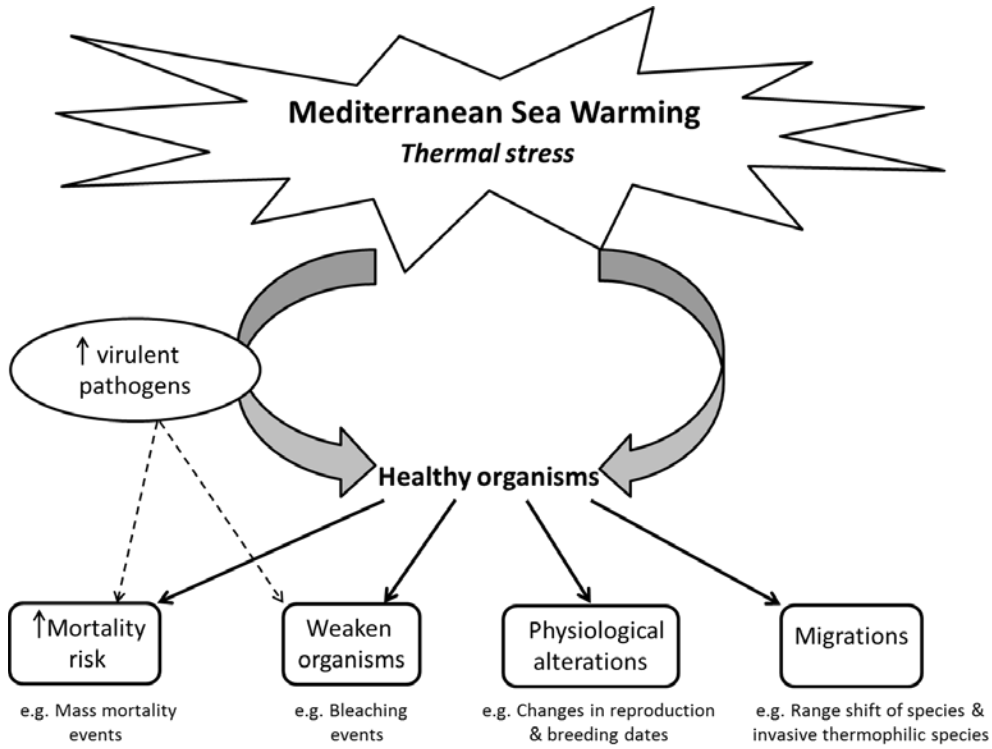


Figure 2. Diagram on the main impacts of climate change in the Mediterranean Sea.

Initiatives to Protect the Mediterranean biodiversity: MPAs and SPAMIs

Marine Protected Areas (MPAs) are supposed to alleviate local stressors, by providing protection in marine reserves, and therefore may increase the ability of populations to resist climate anomalies. Future climate scenarios include frequent and/or persistent disturbances, and the sinergy of multiple stressors (Figure 2), which is likely to increase the negative impacts. Eventhough the efforts towards a reduction of greenhouse emissions at global level are increasing, the most effective tool to face the negative impacts of global climate change on marine ecosystems and livelihoods may be the enhancement of resilience to climatic impacts through networks of marine reserves (Micheli et al. 2012). For this reason, a practical approach in the Mediterranean region to mitigate the harmful effects of climate change in coastal and marine systems is the establishment of MPAs, including Specially Protected Areas of Mediterranean Importance (SPAMI's).

Mediterranean MPAs aim to limit or reduce the pressure on habitats of great value, such as the *P. oceanica* meadows and the coralligenous assemblages. These areas are designed also to protect threatened (e.g. the red coral *Corallium rubrum*, the noble pen shell *Pinna nobilis*, the limpets *Patella ferruginea*) and endangered species (e.g. the monk seal *Monachus monachus*, the loggerhead turtle *Caretta caretta*, cetaceans, and several birds); and some of them provide refuge to heavily exploited species from fishing activities. Thus, MPAs directly protect the environments and species from multiple antropogenic stress factors but also act as buffers for climate change effects. By keeping the integrity of habitats and populations, MPAs foster marine ecosystems' resistance and resilience to local human impacts and global climate change, as stated above. Thus, MPAs may help minimize the risk of population collapses, community disruption, and biodiversity loss (Hughes et al. 2003). On the other hand, Mediterranean MPAs are sentinel areas where the effects of climate change can be studied, evaluated and monitored on the natural heritage, and where management strategies to adapt to, mitigate and, whenever possible, counter such negative effects can be developed.

Likewise, SPAMI are important sites for the conservation of the Mediterranean biodiversity, that contain ecosystems specific to the Mediterranean region or the habitats of endangered species. In order to promote the cooperation in the management and conservation of natural areas, as well as in the protection of threatened species and their habitats, the Contracting Parties to the Barcelona Convention established a list of SPAMI, through the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol). Since the 19th ordinary meeting of the Contracting Parties to the Barcelona Convention and its protocols in 2015, the SPAMI's List now includes 34 sites, one of which encompasses an area established on the high seas, including deep seas: the Pelagos Sanctuary for marine mammals. The protection and management measures applying in the SPAMI are those prescribed by the States proposing them but all parties are to comply with such measures. The Ligurian Sea Cetacean Sanctuary is the largest of the SPAMI sites.

Climate Change Impact Indicators in SPAMI's

The Strategic Action Programme for the Conservation of Biological Diversity in the Mediterranean Region (SAP BIO) was adopted in 2003 by the Contracting Parties to the Barcelona Convention to overcome the complex threats hanging over marine and coastal biodiversity in the Mediterranean. After extensive consultations at country level a diagnosis of the state of marine and coastal biodiversity was made and the national priorities were identified. One of those needs is to implement a monitoring network of climate change impacts in MPAs through indicators specific to the Mediterranean region. For this, a team of RAC/SPA appointed experts identified a set of 13 indicators of climate change for putting into practice in MPAs (UNEP(DEPI)/MED WG.382/Inf.13 2013), from which five were considered of priority interest, so as to advice their implementation to MPA managers and countries. These top indicators are the following: 1) SST and thermal stratification, 2) Mortality and bleaching events, 3) Range shift of alien / temperature-sensitive species, 4) Reproduction and breeding date of selected species, including flowering of *P. oceanica* where present, 5) Episodic species outbreaks (blooms). A detailed description of each of these climate change indicators is developed below, and summarized in Data-sheets attached as Annexes at the end of this document.

1. SST and thermal stratification

One of the main effects of climate change is the increase of the sea temperature which is expected to be a continual and gradual warming of temperatures. The relatively small volume of the Mediterranean Sea predicts a faster reaction to climate warming than the open oceans (Lejeusne et al. 2010), and for this reason it is considered a good model to forecast global change effects. Currently, it is already acknowledged that climate change-induced temperature variations have altered biological patterns and biodiversity (Gambaiani et al. 2008; Bensoussan et al. 2010).

Historical satellite data of the Mediterranean Sea confirms the rise of the sea surface temperature (SST) in the recent years (Nykjaer 2009). Rises in SST are linked with alterations of the vertical thermal structure and deepen thermocline which has potential bio-ecological effects on deeper areas than the first meters (Danovaro et al. 2001; Harley et al. 2006). However, the effect of the thermal anomalies along the water column on marine ecosystems are complex (Rodolfo-Metalpa et al. 2006; Ferrier-Pagès et al. 2013) and only high resolution temperature series collected consistently in space and time would allow the evaluation of the role and the impact of thermal anomalies on marine communities.

At present, long term sea temperature series (>30 years) have only been collected in a few coastal sites mainly in the north-western Mediterranean. Large scale SST obtained through satellite images and the use of few time-resolved T series allow the characterization of the spatial and inter-annual variability of the coastal water column in only a few Mediterranean sites. With the knowledge gathered from the analysis of these series, a clear warming trend in the NW Mediterranean (ca. 0.3º C per decade) is already visible, demonstrating the warming trend at different depths. However, there is still a lack of information for the rest of the Mediterranean Sea and more particularly in the first 50m of the water column where variability in the temperature regimes can have strong effects on the biological communities.

Therefore, the acquisition and analysis of high resolution temperature series in the coastal areas is crucial to manage climate change impacts on marine biodiversity. Temperature series will allow the characterization of thermal regimes (T_{max}, mean temperatures, stratification patterns, etc…), detect temperature anomalies and contribute to the tracking of warming trends in Mediterranean coastal areas.

Figure 3. Underwater sensor for the continuous measurement of water temperature in Grosa Island, Spain (5m depth). Photo credit: Juan Manuel Ruíz.

2. Mortality and bleaching events

Climate change affect benthic species more dramatically then pelagic ones, because they are not able to escape from harsh environmental conditions. Massive mortalities and bleaching events lead to the disappearance of a very important component of benthic assemblages, both under a structural and functional point of view. Usually they are long-lived sessile epibenthic species, predominantly filter feeders. Other organisms involved in bleaching and disease events are coralline algae (Hereu and Kersting 2016).

Figure 4. Partial (left) and total (right) bleaching of *Cladocora caespitosa* colonies in Portofino, 2010. Photo credit: Massimo Ponti.

Among the species having a structural role, playing as ecosystem engineers, one of the most important are the big anthozoan colonies building typical and unique seascapes, especially in coralligenous habitats and in the mesophotic zone. The presence of gorgonian forests reduce water flow velocity, facilitating the sedimentation of fine particles and limiting resuspension processes, as described also for other ecosystem engineers, for example sea-grass meadows (Gacia and Duarte 2001). As a consequence, bottom sediments are stabilized, increasing the quantity of sediment organic matter (Cerrano et al. 2010). Organisms living in "engineered" habitats are in a ''buffer zone'', where environmental modifications occur with a higher inertia respect to the surrounding ambient. Anthozoans forests plays a key role in the trophodynamics and biodiversity of surrounding habitats (Cerrano *et al.* 2010) but unfortunately they are also among the most affected taxa by climate anomalies. The local loss of these important habitat formers can negatively affect the resilience of the habitat, triggering an ecolgical shift, which will facilitate the development of photophilous assemblages instead of skiophilous ones (Scinto et al. 2010; Ponti et al. 2014). This scenario can open the door to invasive non indigenous species, as it is happening for the fast-spreading green alga *Caulerpa cylindracea* (Marín-Guirao et al. 2015; Montefalcone et al. 2015b; Bernardeau-Esteller 2015). Moreover big anthozoans play a very important role in the benthic-pelagic coupling (Coma et al. 1995) and their impressive and sudden decrease during the last ten years has an impact on trophic chain that still need to be evaluated (Linares et al. 2005). The recovery of the affected population can be very heterogenous on a regional scale (Cerrano et al. 2005; Linares et al. 2005a; Cupido et al. 2008, 2009) owing to the complex patterns of larval supply (Mokhtar-Jamai et al. 2011) and the presence of different stressors with different intensity level (Vezzulli *et al.* 2013).

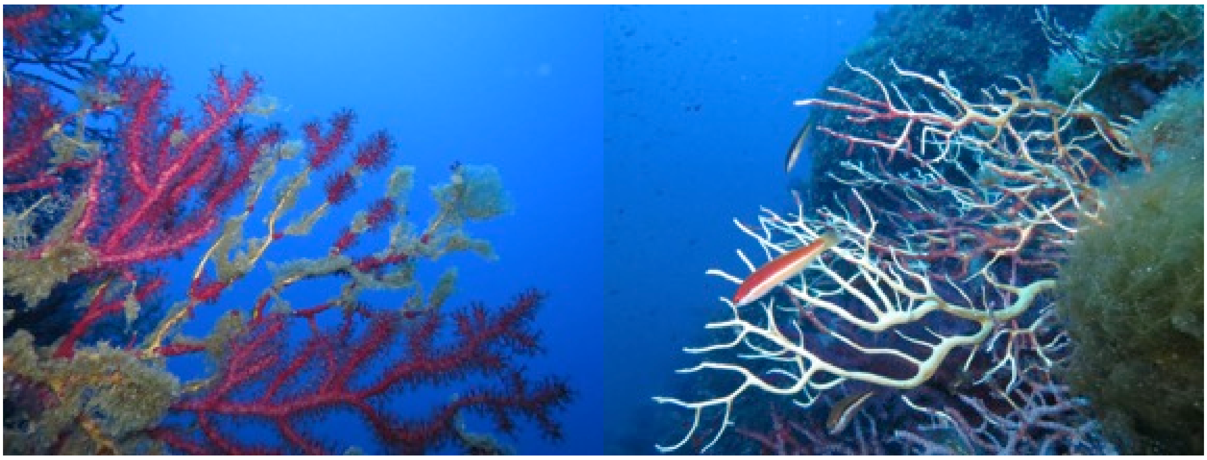


Figure 5. *Paramuricea clavata* with very recent (2-3 days) necrosis of tissue. Portofino MPA, 30 m depth, August 2015 (left) and August 2011 (right). Photo credit: Carlo Cerrano.

Considering the unpredictable cascade effects triggered by the loss of gorgonian colonies, the careful monitoring of these dynamics is even more important if we consider the high number of benthic species involved in these phenomena. Massive mortalities can be difficult to detect when sponges or criptic species are involved. Sponges skeletons rapidly dissolve in the case of tissue necrosis so that observers (recreational divers or scientists) can detect these events only if present during the phenomenon. While the hard proteinaceous scleraxis of gorgonians can stay *in situ* for 4-5 years after the mortality, sponges dissolve in few days. Even if reports on sponge mortalities related to climate change are available (Maldonado et al. 2010; Di Camillo and Cerrano 2015), the huge impact of their loss on benthic-pelagic coupling (Maldonado *et al.* 2012) and on the structure of benthic assemblages is strongly underestimated. In particular, sponges can represent important recruitment and refuge areas for several invertebrated taxa, thus their disappearance can compromise, at local level, the survivor of a lot of species (Heldt 2015).

In the infralitoral zone, the most important engineered habitat are the dense seagrass canopies of *P. oceanica*, which have been shown to be vulnerable to heat stress caused by heat waves, at least in some regions of the Western Mediterranean Sea. Several studies in North-western Mediterranean - Southern France (Mayot et al. 2005), Cabrera National Park (Marbà and Duarte 2010) and Majorque (Díaz-Almela et al. 2007b) document high mortalities of *P. oceanica* meadows likely related to extreme thermal anomalies in summer. Thus, the observed reduction in the areal extent of *P. oceanica* meadows in the Mediterranean (13-38% in surface since 1960, and 50% in density or biomass over the last 20 years) could be attributed mostly to the effects of climate change and other anthropogenic impacts (e.g. eutrophication, physical impacts and biological agents; (Boudouresque et al. 2009; Marbà et al. 2014; Pergent et al. 2015; Telesca et al. 2015).

Losses, in turn, could be leading to the degradation of the functioning of coastal ecosystems (Montefalcone et al. 2015b). Nevertheless, this effect of climate change on *P. oceanica* meadows can not be extrapolated to all Mediterranean coastal areas since in some regions (e.g. Spanish peninsular coast of Catalonia, Valencia and Murcia) seagrass populations have shown to be stable or even progressive, and reductions in the structure and surface of *P. oceanica* beds are rather attributable to local disturbances (Romero et al. 2012; Guillén et al. 2013; Roca et al. 2015; Ruíz et al. 2015). Therefore, the vulnerability of seagrass meadows to seawater warming could vary regionally. The hypothesis that regional climatic differences along the Spanish Mediterranean coast could be involved in this differential vulnerability is being evaluated (Ruiz, pers. com.). Nonetheless, despite this apparent discrepancy among geographical regions, the ecological relevance of this habitat makes crucial to monitor *P. oceanica* shoot densities in order to assess the frequency and intensity of warming-induced events of plant mortality (and hence in seagrass canopy structure and functions).

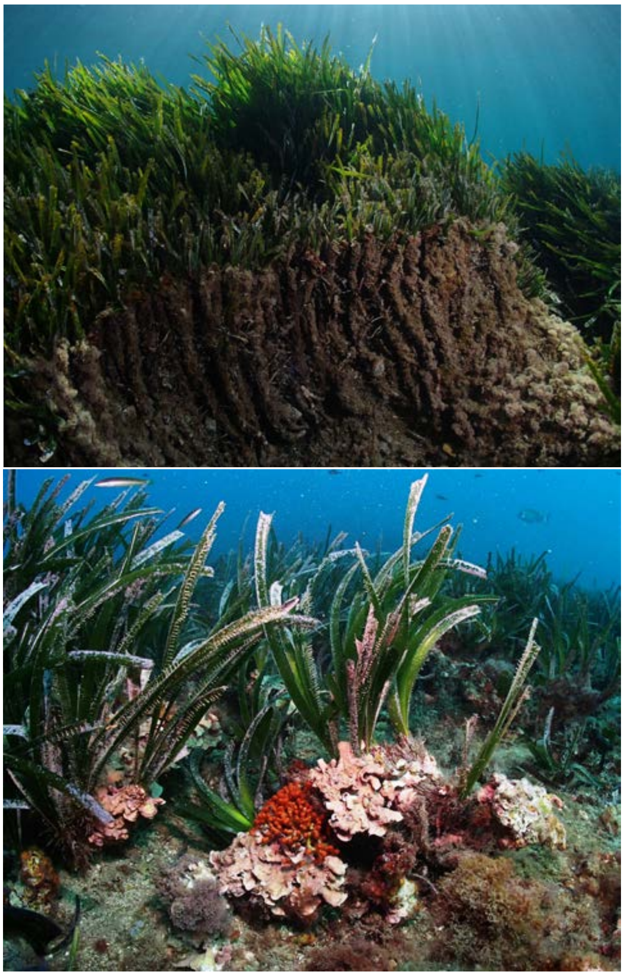


Figure 6. *P. oceanica* meadows in the Mediterranean Sea. Photo credit: Juan Manuel Ruíz.

3. Range shift of alien / temperature-sensitive species

Climate change in the Mediterranean Sea, is causing rapid shifts in the geographical and depth distribution ranges of many marine species (Lejeusne et al. 2010; Sorte et al. 2010; Smale and Wernberg 2013). Abrupt rising temperature since the end of the 1990s has increased the thermal habitat available for warm-water biota (Raitsos et al. 2010). This has resulted in new tropical/subtropical species, that were completely absent in the Mediterranean before, extending their distribution from nearby regions, in a phenomenon known as tropicalization (Bianchi 2007; Moschella 2008; Raitsos et al. 2010; Coll et al. 2012). Examples include barracudas *Sphyraena spp.*, the blue spoted cornetfish *Fistularia commersonii* (Azzurro et al. 2011)*,* and the rabbitfish *Siganus luridus* (Hassan et al. 2003; Golani 2013).

The Mediterranean warming is also favouring native warm-water species that mostly occurred in the southern, warmer parts of the basin until recently, which are moving northwards and increasing in number. For example, the round sardinella *Sardinella aurita* (Sabates et al. 2006), the dusky grouper *Epinephelus marginatus*, the ornate wrasse *Thalassoma pavo*, and the Mediterranean parrotfish *Sparisoma cretense* (Bianchi and Morri 2000; Sabates et al. 2006; Coll et al. 2012). Northwards migrations coupled with increasing loss of cold habitats is causing a harsh competition for limited resources (Otero et al. 2013), which usually results in warm-water species replacing cold-water species. A clear example are the cave dwelling mysids *Hemimysis margalefi* and *H. speluncola*, the former replacing the latter and likely driving it to extinction (Chevaldonné and Lejeusne 2003). Other cold-water species like the anchovy *Engraulis encrasicolus* (Bombace 2001), whiting *Merlangius merlangius*, spratt *Sprattus sprattus*, and the slender goby *Gobius geniporus*, limited to northern coldest areas such as the Gulf of Lions, North Aegean Sea, and North Adriatic, have also dramatically declined (Ben Rais Lasram et al. 2010), without any apparent evident link with overfishing (Boero et al. 2008). There is a rising concern about the fate of these species, for which it is impossible to migrate further north to escape thermal stress. Some cold-water species instead, have retreat into deeper habitats in search of colder waters (Moschella 2008; Puce et al. 2009).

Seawater warming will also favor the spread of thermophilic macroalgae species, both native (e.g. *Anadyomene stellata* and *Digenea simplex*) and introduced (*Lophocladia lallemandii* and *Asparragopsis taxiformis*) (Rodríguez Prieto *et al.* 2013). Little is known about thermal tolerance of Mediterranean macroalgal species and assemblages, but the few available evidence suggest that their distributional patterns will be affected by warming. Thus, (Samperio-Ramos et al. 2015) demonstrated that optimal ecophysiological temperatures of invasive species of tropical origin (e.g. 29ºC for *L. lallemandii*) are higher than those of temperate seas (e.g. 27ºC for *C. cylindracea*) and hence, sustained warming in future scenarios could even lead to the spread of the most thermophilic species but the regression of the less thermophilic ones.

The disappearance of endemic *Cystoseira* forests in some parts of the Eastern Mediterraneas has been attributed to recent changes in seawater temperatures and currents (Rodríguez Prieto *et al.* 2013).

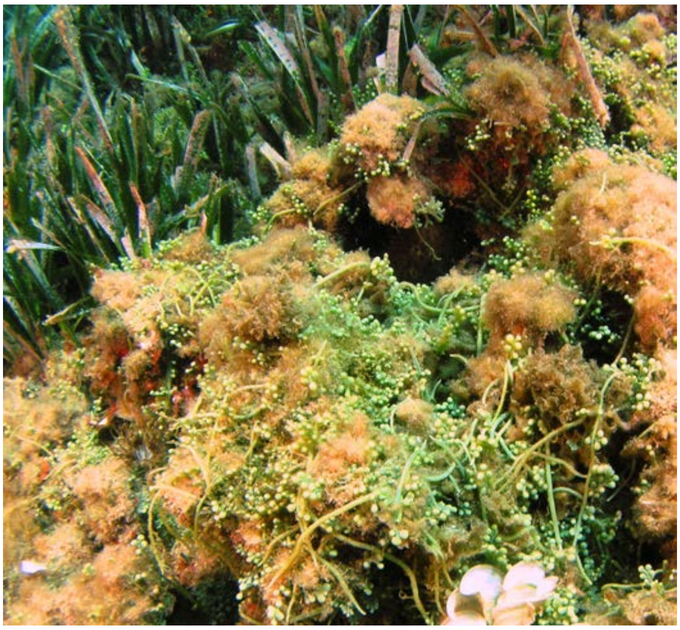


Figure 7. *C. cylindracea* colonizing photophyllic, native algal assemblages' characteristic of Mediterranean infralitoral bottoms. Photo credit: Juan Manuel Ruíz.

The success of species in establishing new populations in a new location is at two extremes: most of them are destined to local extinction, while others thrieve in the new area, even replacing indigenous species. Marine invasive non-indigenous species, for example, once established, rapidly spread and out-compete native species. Both the Mediterranean warming and antropogenic disturbances likely favor these tropical species over the native biota, making them one of the main causes of biodiversity loss in the Mediterranean (Coll et al. 2012). Ocean warming is therefore threat for the persistance of Mediterranean endemics (Ben Rais Lasram et al. 2010).

Shifting geographical and depth distributions of native and non-indigenous species might modify species interactions, and cause major changes in community structure (Chevaldonné and Lejeusne 2003; Rosenzweig et al. 2007; Dulvy et al. 2008; Simpson et al. 2011) with unexpected and harmful impacts on the biodiversity (e.g. loss of species, including endemic and protected species), on ecosystem functioning, as well as in economy and human health (Gomez and Claustre 2003; Galil 2008). All these factors emphasize potential conservation concerns, especially with the greater velocities of climate change and seasonal shifts in the Mediterranean, and therefore clearly pose new challenges for MPA managers in this region.

Changes in biota's distribution are thus reflecting changes in oceanographic conditions and can be an indicator of the effects of the Mediterranean warming on marine communities. Long-term monitoring can detect the arrival and spred of new tropical/subtropical species into the Mediterranean Sea and reveal substantial regressions or extensions in the ranges of indigenous species. If data gathering and monitoring is applied at a broad scale, such as promoted for Mediterranean MPAs by the CIESM Tropical Signals Programme, the outcomes may improve our understanding of how the changing distribution of species may affect the Mediterranean biodiversity (Otero et al. 2013).

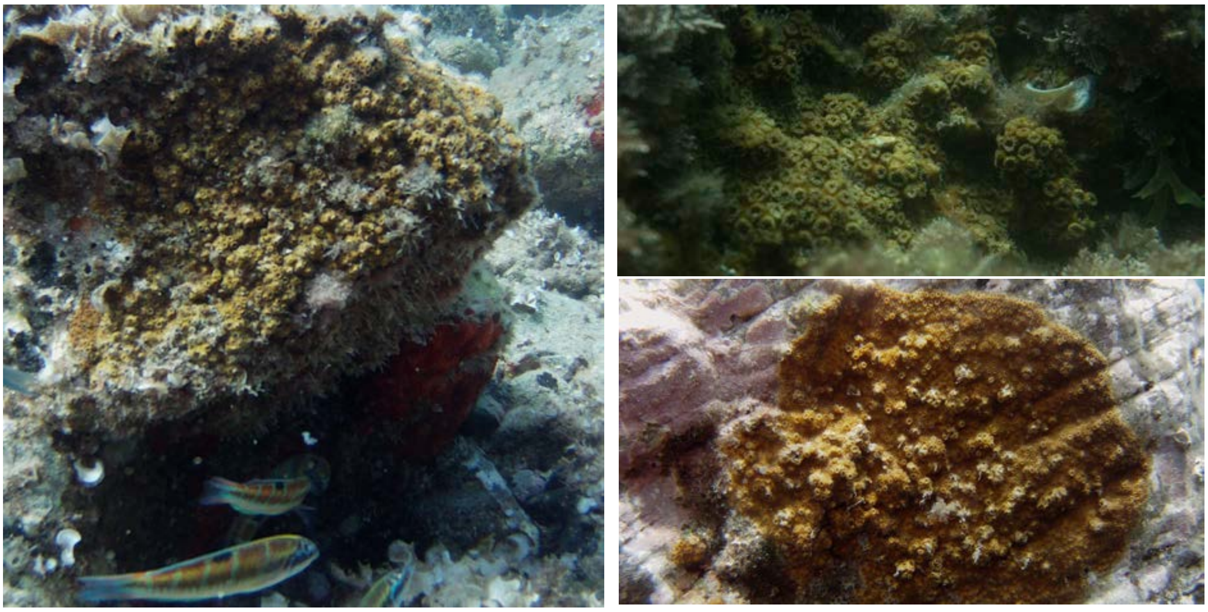


Figure 8. Colonies of the alien invasive hermatypic coral *Oculina patagonica* in Cabo de Palos, Murcia, Spain. Photo credit: Adrián Aguilar-Rodríguez.

4. Reproduction and breeding date of selected species, including flowering of *Posidonia oceanica* where present

Physiological performance is the principal determinant of a species' tolerance to environmental variability and change (Pörtner 2002; Pörtner et al. 2008; Payne et al. 2016). In recent years, numerous studies focus on the potential effects of global warming upon organisms and biological processes (Huey et al. 2012). As a result, a link between global warming and changes in life cycles, physiology and behaviour for a variety of organisms has been established (Somero 2010). As climate or other conditions shift, organisms initially respond based on physiological and behavioural adaptations molded through their evolutionary history; new conditions may be physiologically tolerable, allowing acclimatization (an adjustment of morphology or/and physiology within individuals) or adaptation (increased abundance and reproduction of tolerant genotypes over generations) (Somero 2010; Huey et al. 2012), or may be intolerable, thus promoting habitat or geographical shifts (of individuals or/and populations) (Robinson *et al.* 2009) and changes in phenology (timing of annual events) (Thackeray et al. 2010; Diez et al. 2012). Among these activities, those related to reproduction are ideal candidates to serve as indicators of seawater warming (Sheaves 2006; Pankhurst and Munday 2011). Seasonal reproductive events susceptible to serve as indicators of response to seawater warming could be related to the timing of seasonal migrations from or to estuarine or lagoonal systems, and to earlier dates of spawning, nesting, hatching or settlement of marine animals (fish, marine turtles, seabirds), as well as modifications of the reproductive phenology of *P. oceanica*, as briefly developed below.

4.1. Reproductive phenology of littoral fishes

Temperature at the time of spawning is likely to influence the starting date of reproduction, although other factores are also important (e.g. population density, habitat availability and quality, weather conditions, etc.). Therefore, climate-induced changes in the timing and duration of spawning in fishes and other marine organisms should be explored as a possible indicator of seawater warming. In the case of fishes, for this to be true the selected species should have easily recognisable spawning features (such as distinct reproductive coloration of males or females, nest-building, conspicuous courtship, visible parental care behavior, etc.) (DeMartini and Sikkel 2006; Wootton and Smith 2014) as well as relatively narrow temporal spawning periods (Tsikliras et al. 2010). Attending to these spawning features, specially suitable candidate species for this purpose in Mediterranean coastal areas are groupers (*Epinephelus marginatus, E. costae, Mycteroperca rubra*) (Zabala et al. 1997a, 1997b; Harmelin and Harmelin-Vivien 1999; Louisy and Culioli 1999; Pelaprat 1999; Marino et al. 2001; Hereu et al. 2006; Aronov and Goren 2008; Reñones et al. 2010), small labrids (*Symphodus ocellatus, S. roissali, S. cinereus, S. mediterraneus, Thalassoma pavo*) (Warner and Lejeune 1985; Taborsky et al. 1987; Wernerus and Tessari 1991; Ylieff et al. 1998; Ylieff 2000; Raventós 2004, 2006; Sara et al. 2005; Lipej et al. 2009; Raposeiro and Azevedo 2009; Rodrígues et al. 2015; Sinopoli et al. 2015), the damselfish *Chromis chromis* (Picciulin et al. 2004, 2006; Félix-Hackradt et al. 2013) and the painted comber *Serranus scriba* (Havelange and Voss 1993; Tuset et al. 2005; Zorica et al. 2005a, 2005b; Petersen 2006; Alós et al. 2010).

4.2. Marine turtles

Marine turtles are generally viewed as vulnerable to climate change because of the loss of nesting habitats through sea level rise and the role that temperature plays in the sex determination of embryos, their long life history, long age-to-maturity and their highly migratory nature (Hawkes et al. 2007, 2009; Poloczanska et al. 2009; Witt et al. 2010), and particularly in their reproductive performance (Broderick et al. 2003; Mazaris et al. 2008; Witt et al. 2010; Dudley et al. 2016). Nevertheless, the biological and ecological features of these animals, together with their adaptive capabilities, could make them less threatened by climate change than previously expected (Hays et al. 2010; Abella Perez et al. 2016). Marine turtles have been extensively studied in the Mediterranean (e.g. *Margaritoulis* et al. 2003; Margaritoulis 2005; Garofalo et al. 2009; Schofield et al. 2009, 2013; Casale and Margaritoulis 2010; Clusa et al. 2014; Mazor et al. 2016). In a recent global survey (Wallace et al. 2011) this region has been considered to be of low risk (i.e. high population viability), but high threat (i.e. subject to direct and indirect anthropogenic impacts) for marine turtles. Three species of marine turtles frequent the Mediterranean: the leatherback turtle (*Dermochelys coriacea*), the green turtle (*Chelonia mydas*), and the loggerhead turtle (*Caretta caretta*) (Carreras et al. 2014; Revuelta et al. 2015); only the last two breed in the basin and their Mediterranean populations have only a limited gene flow with those of Atlantic (Casale and Margaritoulis 2010; Carreras et al. 2011; Clusa et al. 2013, 2016).

Nesting sites of these two species are concentrated in the Eastern and Central basins, while it occurs only sporadically in the Western basin (Margaritoulis et al. 2003; Casale and Margaritoulis 2010; Maffuci et al. 2016; Mazor et al. 2016). It has been estimated that that the number of nests in the Mediterranean ranges between 3,375 and 7,085 (Margaritoulis et al. 2003; Casale and Margaritoulis 2010; Casale 2015). Nesting phenology, further from constituting an adaptive response to climate change, could be used as indicator of biological effects of climate change (Mazaris et al. 2008, 2009; Dalleau et al. 2012; Pike 2013; Neeman et al. 2015, Maffuci et al. 2016).

4.3. Seabirds

Seabirds are threatened worlwide by different anthropogenic disturbances, such as incidental catches by commercial fisheries, marine pollution, non-indigenous invasive predators, habitat degradation and others (Croxall et al. 2012). MPAs has been observed to protect seabird populations (Péron *et al.* 2013). The influence of climate change on bird phenology (particularly on the timing of migration and of nesting) has been the object of recent reviews (e.g. Crick 2004; Robinson et al. 2005; Visser and Both 2005; Wormworth and Mallon 2006; Grémillet and Boulinier 2009; Dunn and Winkler 2010; Dunn et al. 2010) and specific studies (e.g. Both and Visser 2001; Frederiksen et al. 2004; Moller et al. 2006; Votier et al. 2008; Jenouvrier et al. 2009; Reed et al. 2009; Sydeman and Kitaysky 2012). These features make seabirds excellent candidate sentinel indicators of global change (e.g. Diamond and Devlin 2003; Piatt et al. 2007; Parsons et al. 2008; Einoder 2009; Mallory et al. 2010; Wolf et al. 2010; Wormworth and Sekercioglu 2011, but see Durant et al. 2009; Grémillet and Charmantier 2010).

4.4. Flowering of Posidonia oceanica

Díaz-Almela et al. (2007b) found a significant, positive relationship between the prevalence and intensity of flowering of *P. oceanica* meadows (thus the flowering probability for any one shoot) and the annual maximum sea-surface temperature, applicable across the entire Mediterranean, as well as the individual basins. This suggests an effect of extreme warming events on flowering in *P. oceanica* meadows, a mechanism driven by both genetic and ecological causes (Jahnke et al. 2015).

Figure 9. Inflorescences of *P. oceanica*, Winter 2015, Cabo de Palos, 7 m depth. Photo credit: Juan Manuel Ruíz.

Nevertheless, other studies suggest that peaks of solar activity, and not warmer temperature *per se*, could be the main trigger of massive *P. oceanica* flowering events (Montefalcone et al. 2013). Hence, systematic records of *P. oceanica* flowering may provide evidence of the effects of temperature anomalies (should or not be a footprint of global warming) on the life history and ecosystems in the Mediterranean Sea. Whereas *P. oceanica* flowering is difficult to observe, compared to that of terrestrial plants, the growing effort to monitor these fragile ecosystems is enhancing the empirical basis to assess flowering events and, therefore, also providing an alert to the consequences of the climate change on Mediterranean coastal ecosystems. Furthermore, flowering stalks remains attached to the rhizomes once the inflorescence decay so that its precise datation is possible thanks to the so called 'lepidochronological analysis' (Pergent and Pergent-Martini 1991) and 'reconstructive techniques' (Duarte et al. 1994). Therefore, in those areas where time series of temperature exist could be possible to infere about the relationship between this factor and *P. oceanica* flowering events. The observation of pseudoviviparous plantlets in *P. oceanica* in Formentera island (i.e. floral peduncles without the participation of sexual reproduction) (Ballesteros et al. 2005) rises the possibility of new reproductive and dispersal mechanisms as a result of climate change, whose adaptive significance, however, are still to be dilucidated. Nonetheless, the observation of this kind of reproduction has not been reported again in other areas even in years with massive flowering events.

5. Episodic species outbreaks (blooms)

Episodic species outbreaks, i.e. pulses of primary and secondary production that result from the sudden increase in the population size of some key species (e.g. jellyfish, ctenophores, hydrozoans, tunicates, salps, algae) are part of the natural trends in marine ecosystems. As reviewed by Boero (2013), planktonic populations use to vary much in abundance over an annual cycle in a relatively predictable manner, tending to peak following the regular sequence of the phytoplankton spring pulse. Blooms take place when the expected population peak vastly exceeds the usual level, or when sudden outbreaks of a particular species comes to dominate the plankton for a period, and then resumes its normal seasonal abundance.

As the oceans get warmer, overfished and more disturbed, population blooms are becoming more common and persistent in coastal areas at a local and regional scale, and are changing in composition (Mills 2001; Brotz 2012; Acevedo et al. 2013; Wells et al. 2015; Kersting 2015). Although the environmental degradation due to both direct human impacts and climate change is leading to overall species and habitat loss, some factors such as warmer waters, eutrophication, pollution (e.g. fertilizers), water masses stratification (slow-moving water), changes in salinity, and the oceans lose of oxygen encourage the spread and massive increase in number of some species (Purcell 2005, 2012; Prieto et al. 2010; Canepa et al. 2014).

Massive swarms of gelatinous zooplankton, for example, formed by representatives of Cnidaria (true jellyfish, e.g. the box jellyfish *Carybdea marsupialis*) (Figure 10), Ctenophora (comb jellies) and Tunicata (salps), have became a frequent sight in the Mediterranean coasts in the last decades (Bordehore et al. 2011; Otero et al. 2013).

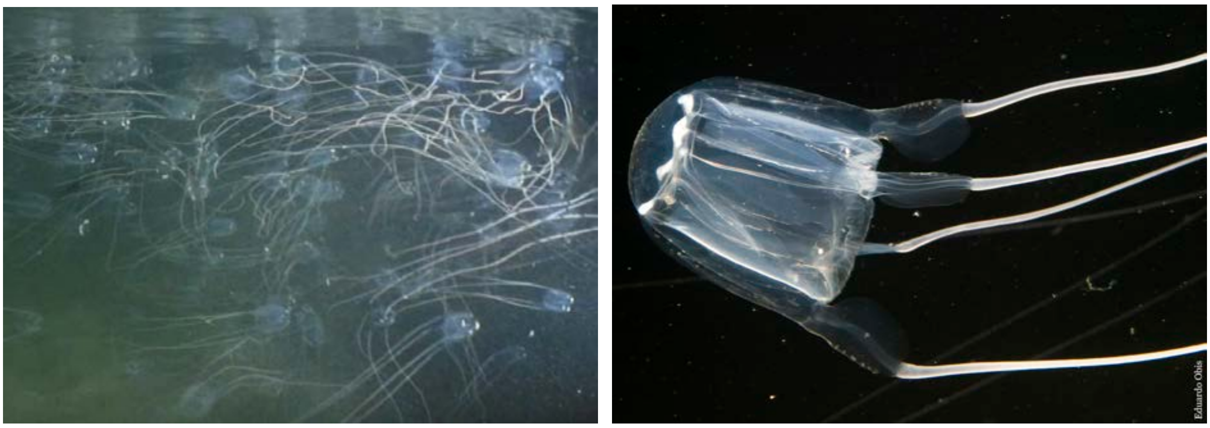


Figure 10. The box jellyfish *Carybdea marsupialis* is present in low abundances in coastal waters of the Mediterranean but in some areas bloom to high abundances (>5 inv m3). Above: night bloom of

this species in the Mediterranean, Spain. Below: adult box jellyfish. Photo credit: Eduardo Obis and LIFE Cubomed Project.

Warmer water amps up the metabolism of these organisms so they grow faster, eat more, breed more and live longer (Purcell 2005; Prieto et al. 2010; Canepa et al. 2014). These organisms have unique life cycles where they can tolerate harsh conditions and then rapidly thrive when conditions are favorable. Gelatinous zooplankton is capable of rapid population growth by cloning and generating millions of copies of themselves extremely fast. Thus, when conditions are favorable and a niche opens up for them they rapidly proliferate. So an increase in regional ocean temperature may make gelatinous zooplankton blooms a more common occurrence. Jellyfish and ctenophores blooms persist longer than previously also because their predators (top predators) have been wiped out in most oceans due to overfishing.

Likewise, the combined effects of increasing ocean temperature, nutrients, light, and ocean water acidity, is influencing the frequency, severity, and composition of algal blooms. Warmer water favors some algae reproduction, e.g. toxic green-blue algae, and also prevent water from mixing, allowing algae to grow thicker and faster. Besides, algal blooms absorb sunlight, making water even warmer and promoting more blooms. On the other hand, the increasing frequency of droughts and intense storms may cause more nutrient runoff into coastal areas, leading to more severe blooms that occur more often.

Bloom events may endanger the environment, biodiversity, human health and economies of a region. Gelatinous zooplankton blooms may harm the fisheries by clogging fishing nets, keeping fish away, and consuming fish larvae (Graham et al. 2003). As jellyfishes and ctenophores feed on the same prey as do many adult and larval fishes, they are both predators and potential competitors of fish (Purcell and Arai 2001; Purcel et al. 2007). In recent years, gelatinous zooplankton blooms have caused a number of power plants to shut down by clogging seawater cooling systems, and have killed important amounts of fish in aquatic farms by clogging and stinging their gills or by restricting the flow of water when trapped against a net (Purcel et al. 2007). These blooms have also negatively interfere with tourism as beachgoers can be stung by jellyfish floating in the water or stranded on the beach (Bordehore et al. 2015).

Phytoplankton blooms, on the other hand, although normally fuel productive ecosystems, may get to create very low oxygen concentrations in bottom waters (dead zones), killing or driving out marine fish or benthic organisms. In addition, some "harmful" algal blooms, previously known as red tides, blue-green algae or cyanobacteria produce potent neurotoxins able to sicken or even kill people and animals, and therefore can damage marine communities, and raise the treatment costs for drinking water while hurting industries that depend on clean water, such as aquaculture. The toxic benthic dinoflagellates of the genus *Ostreopsis* are examples of these harmful organisms. Increasingly frequent massive blooms (Rhodes 2011; Parsons et al. 2012), mostly *O. cf. ovata* (Battocchi et al. 2010; Perini et al. 2011), have been associated with both noxious effects on human health (Gallitelli et al. 2005; Del Favero et al. 2012) and mass mortalities of benthic marine invertebrates and macroalgae (Shears and Ross 2009; Gorbi et al. 2013).

Similarly, outbreaks of mucilaginous aggregates (Figure 11), resulting from the proliferation of different phytoplankton species can lead to the mortality of several benthic organisms or communities, like e.g. seagrass meadows or gorgonian forests (Danovaro et al. 2009) by suffocation, ripping and damaging of living macro-structures, light-limitation, and alteration of the physical and chemical environment (Rinaldi et al. 1995; Giuliani et al. 2005; Cibic et al. 2009). Therefore, both gelatinous zooplankton and algae blooms can cause important ecological, social and economic damage in a region.

In the short term, the sporadic nature of blooms reinforce the importance of long-term monitoring efforts to provide a better understanding of the Mediterranean ecosystem, and how it may be changing with climate change. Currently, bloom episodes have increased their frequency and there is an actual concern owing to the impossibility to predicit believable future scenarios due to the lack of sound long term data.

Figure 11. Left: Mucilage massive event at Targhetta (Portofino Promontory), July 2013, 20m depth. Right: Mucilage covering a violuminiscent sea-whip (*Paramuricea sp.*), Punta Vessinaro (Portofino Promontory), July 2003, 35m depth. Photo credit: Stefano Schiaparelli.

This document reports on how the five climate change indicators previously prioritized, are being studied and monitored in three Specially Protected Areas of Mediterranean Importance (SPAMIs), two in Italy (Portofino, Thyrrenian Sea and Torre Guaceto, Adriatic Sea) and one in Spain (Mar Menor and oriental Mediterranean Murcia coast). Based on the experience cumulated in these three sites, and in the knowledge of the authors of this report, a detailed framework and diverse cost-effective methodologies are proposed to support the implementation and establishement of the monitoring of climate change indicators in MPAs or SPAMIs in the Mediterranean and other marine ecoregions.

II. METHODS

II.1. Study areas

The SPAMIs selected are placed in key locations to asses climate change, including surroundings of Alboran Sea, subject to a dynamic interlinked with the Atlantic and hence easier arrival of Atlantic thermophilic species; the northern limits of the Mediterranean, where species needing colder waters concentrate; and the Adriatic area, where singular Mediterranean biotopes and biocoenoses exist.

II.1.1. The SPAMI Mar Menor and Oriental Mediterranean zone of the region of Murcia coast, Spain:

Selected to document the climate change dynamics at the Western Mediterranenan. This SPAMI, declared in 2001, is located in the Murcia region (SE Spain), and occupies a surface of 27,500 ha (Figure 12). Its marine limits are the border of Alicante province to the North, and the cape Negrete to the South, spanning 59 km of coastline. It includes the Mar Menor coastal lagoon and adjacent wetlands, the marine area in front of La Manga sandbar separating this coastal lagoon from the Mediterranean, the Cabo de Palos - Islas Hormigas marine reserve, and the waters washing the shores of Calblanque Regional Park; these areas coincide in part with the Natura 2000 Sites of Community Importance (SCIs) ES6200029 (Submerged littoral fringe of the Region of Murcia), ES6200030 (Mar Menor) and the easternmost part of ES6200048 (Submarine Canyons of Mazarrón Esparpment).

The Mar Menor (Pérez-Ruzafa et al. 2007) is a hypersaline coastal lagoon spanning an area of 135 km^2, with a mean and maximum depth of 3.6 m and 6.1 m, respectively. Five volcanic islands and small outcrops constitute the only natural rocky sustrata inside the lagoon. La Manga, a sandy bar 22 km long, acts as a barrier between the lagoon and the Mediterranean Sea. It is crossed by five inlets, called “golas”, of which the three most northern are natural and they were the only ones to communicate with the Mediterranean until the mid-nineteenth century. The other two, El Estacio, in the central area of La Manga, and Marchamalo or La Constanza in the south, were artificially constructed in 1860 for fisheries. Superimposed to the peculiar physico-chemical characteristics of the lagoon, the environmental heterogeneity present is very high: soft (muddy and sandy) bottoms alternate with hard ones (rocky reefs and artificial substrates) and consequently the assemblages living in each zone; on the other hand, the distance to the exchange inlets propitiate an inner-outer gradient of species richness and abundance. The wetlands surrounding the coastal lagoons are of extreme importance for aquatic birds. All these features made that

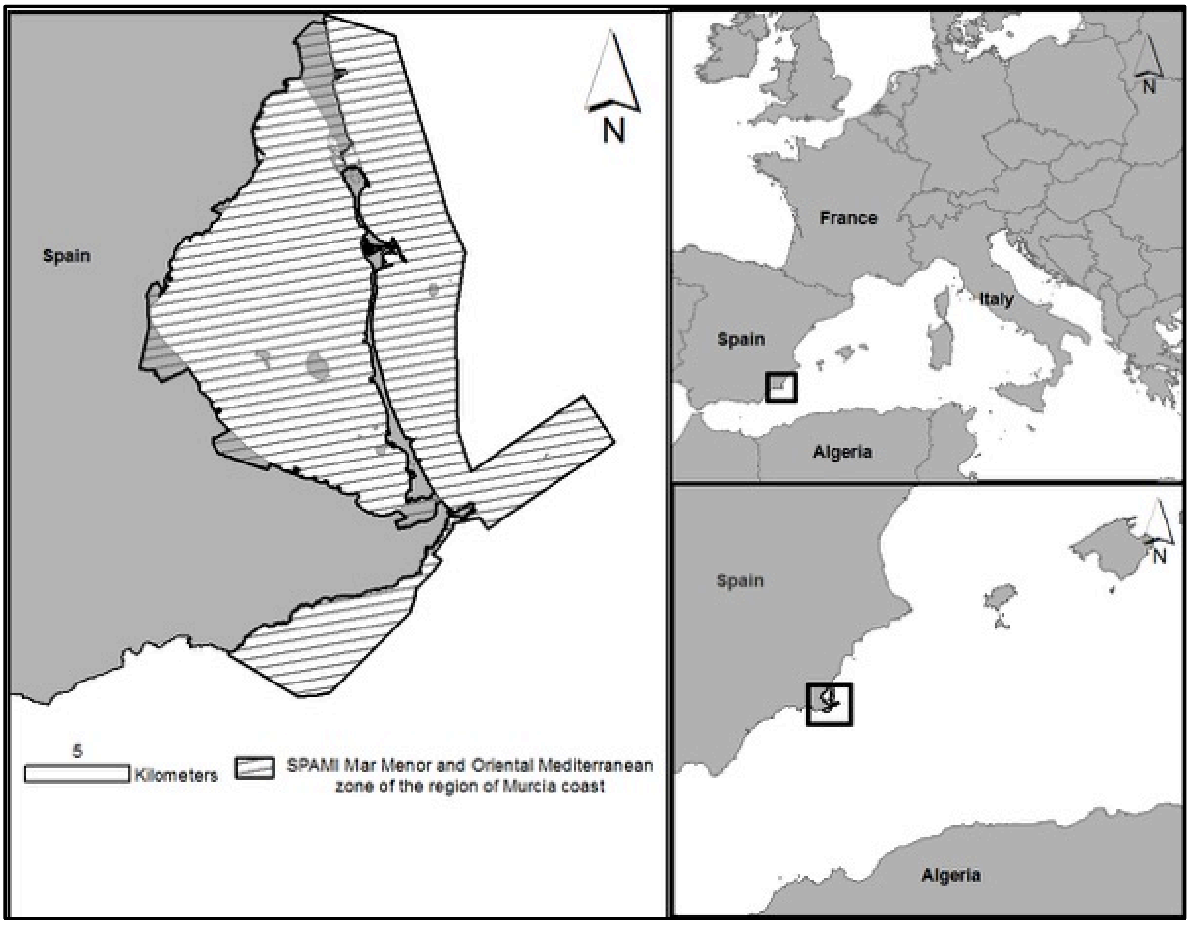


Figure 12. Location of the SPAMI Mar Menor and Oriental Mediterranean zone of the region of Murcia coast, Spain.

this lagoon deserved its designation as Ramsar Zone, SCI, Special Protection Area (SPA) for birds, SPAMI, etc. The lagoon has been subject to intense modifications during the last decades, which caused intense environmental shifts[1], and thus profound biological and

[1] Some of the changes are the result of coastal works to develop tourism facilities (land reclamation, the opening, deepening or extension of channels, urban development and associated wastes, marinas, artificial beaches, etc.), while others are related with agricultural practices in the watershed, which have changed from extensive dry crop farming to the cultivation of intensively irrigated crops, with the subsequent increase in the amount of agricultural wastes and nutrients received by the lagoon.

ecological changes in the species assemblage (Pérez-Ruzafa et al. 2005, 2006, 2007, 2015; Pérez-Ruzafa and Marcos 2012). Regarding the area adjacent to La Manga (in the northern part) and Calblanque regional park (southwards), they are both characterized by vast and well-preserved *P. oceanica* meadows (Ruiz et al. 2015). These seagrass meadows have been monitored since 2004 with the aim of determine long-term trends in meadow structure parameters and, more recently flowering events and the abundance of the invasive alga Caulerpa cylindracea (Ruiz et al. 2013). In all the area a series of small islands appear, which are of major interest for the presence of marine birds, so that the marine area of this SPAMI is part of an Important Bird Area for seabirds (marine IBA) ES0000508 (Tabarca-Cabo de Palos marine area). The marine reserve of fisheries interest of Cabo de Palos - Islas Hormigas was established in 1995 under fisheries legislation. It occupies 1931 ha, from which 270 ha form the core no-take area. The bottom in shallow areas is formed by rocky boulders interspersed with extensive patches of *P. oceanica* forming a narrow belt following the coast, while at deepest portions (>16 m) detritic formations predominate, after a series of steep rocky shoals and small islands with extensive algal plains covering the infralittoral zone, while the circalittoral zone is dominated by gorgonians and other coralligenous formations (Calvín *et al.* 1999). This MPA has been the object of scientific monitoring since the very inception of protection measures, and huge response of commercial fish species (groupers, brown meagres, common dentex, etc.) in terms of abundance, biomass and average individual size (García-Charton et al. 2015), which is reflected in increases in fishing catches by artisanal fisheries. In addition, several cetaceans and marine turtles appear regularly througout the entire SPAMI (Gómez de Segura et al. 2004, 2006).

II.1.2. The SPAMI Portofino, Italy

Selected to document the climate change dynamics at the North-Western Mediterranenan. The Marine Protected Area of Portofino was established in 1999, by decree of the Ministry of Environment (Law_979/1982). It was the first MPA in Italy to achieve the status of a SPAMI (Specially Protected Areas of Mediterranean Importance), in 2005. The entire MPA (Eastern Ligurian Sea 44°18.18′N; 09°12.83′E) covers about 346 ha and contains a “no entry-no take” area (A zone or integral reserve, 10 ha), two “entry-regulated” and “take-regulated” zones (B zones) and two buffer zones (C zones). Since November 2007, Portofino became part of the international Long Term Ecological Research Network (LTER), to investigate ecological processes over long temporal and broad spatial scales with the proposal to understand the influence of global change, and the decrease of biodiversity and pollution.

This MPA includes the municipalities of Camogli, Portofino and Santa Margherita Ligure, and covers about 346 ha., including 10 ha of “no entry-no take” area, two “entry regulated-take regulated” zones and two buffer zones. The Portofino Promontory is about 25km E of Genoa and, developing perpendicularly to the coast for more than 3km into the Ligurian Sea, breaks the continuity of the coastline and of the main westward Ligurian stream. It has a coastline of about 15 km (Figure 13). The vertical cliff of the Promontory is composed by pudding stone, a peculiar Oligocenic conglomerate. The Promontory of Portofino MPA is marked by steep walls, down to 50-70 m depth, with small submarine caves and boulders.

The shelf-break runs at an average distance of 10 km from the waterline and at a depth of 115-150 m. These features create many different niches and habitats. In particular, the area's rocky substratum and the hydrodynamic conditions allow establishment of a rich and varied sessile biota (Salmona and Verardi 2001; Cattaneo-Vietti et al. 2011). Below the photophylous biocoenoses, rich in *Cystoseira* spp. meadows, between 10 and 25 m depth, the typical biocenoses of the Promontory is the precoralligenous. It is characterised by the sponges *Axinella damicornis* and *A. verrucosa*, the zoanthid *Parazoanthus axinellae,* the scleratinian *Cladocora caespitosa,* and the gorgonian *Leptopsammia pruvoti*. Increasing the depth, coralligenous outcrops are present showing their peculiar organization with parallel horizontal rims. It presents different facies, characterized, according to the local conditions, by different species of gorgonians *(Paramuricea clavata, Eunicella cavolini)* and red coral *(Corallium rubrum).*

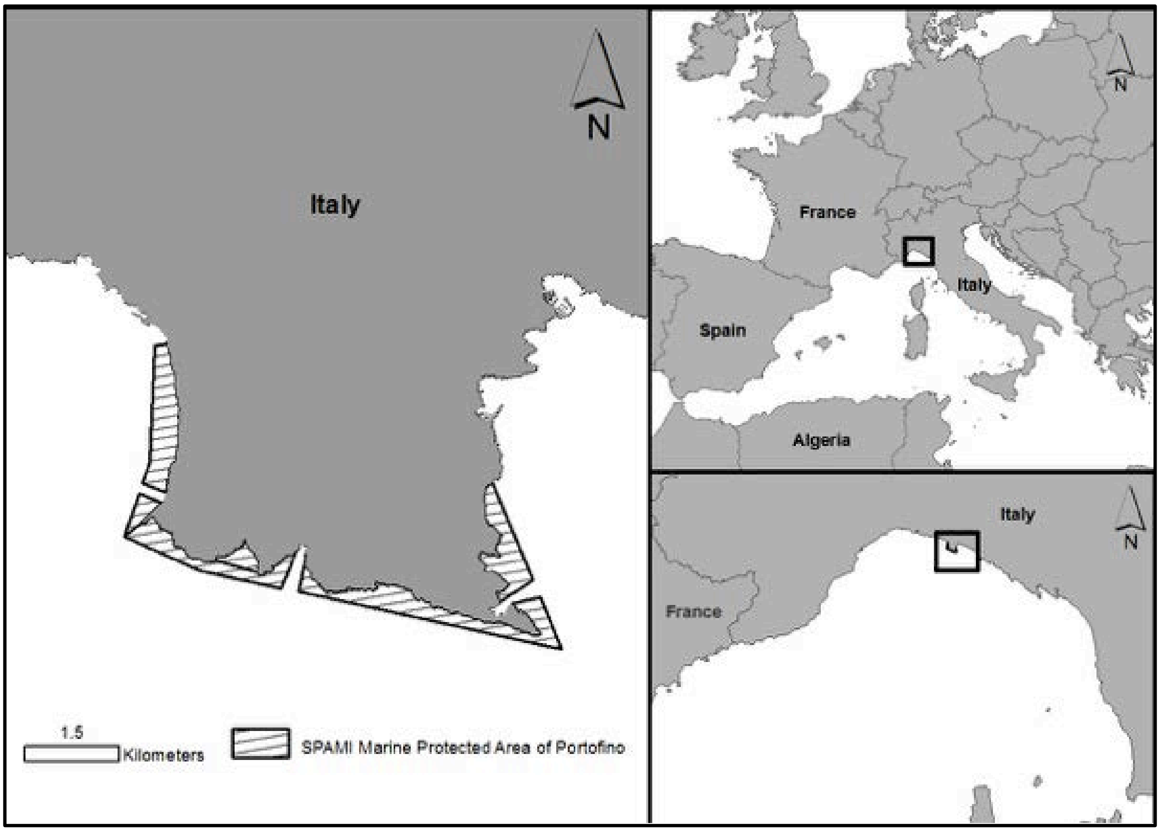


Figure 13. Location of the SPAMI Portofino, Italy.

Between the 20 and 45 meters of depth, suspension feeders find the ideal conditions for their development and reach very high densities. The seabed is characterized near the coast by the gorgonian *Leptogorgia sarmentosa* forming sparse beds at about 15 m of depth. Below 70 m depth, on scattered rocky outcrops, colonies of the gold coral *Savalia savaglia*, the black coral *Antipathella subpinnata,* the hydroid *Lytocarpia myriophyllum*, are occasionally present.

All the marine invertebrate species listed in the habitat Directive (*Pinna nobilis*, *Lithophaga lithophaga*, *Centrostephanus longispinus*, Annex IV) and around the 46% of those listed in Annexes II and III of the Barcelona Convention are here represented. Portofino MPA is of high environmental value, and hosts a significant amount of socio-economic activities (Markantonatou et al. 2016). Artisanal and recreational fisheries and diving activities are among the main pressures insisting on the area.

II.1.3. The SPAMI Torre Guaceto, Italy

Selected to document the climate change dynamics at the Adriatic Sea. The marine protected area of Torre Guaceto was established in 1990, and covers 2200 ha in the Apulia

Region, South-east of Italy, within the municipalities of Brindisi and Carovigno. It is about 8 km long from the zone of Apani till the littoral of Punta Penna Grossa.

The knowledge of the MPA is high, considering the main ecological processes, habitat distribution, inventories of species and socio-economic factors. This is due to the numerous research programmes that have been carried out, with the production of the biocenotic map.

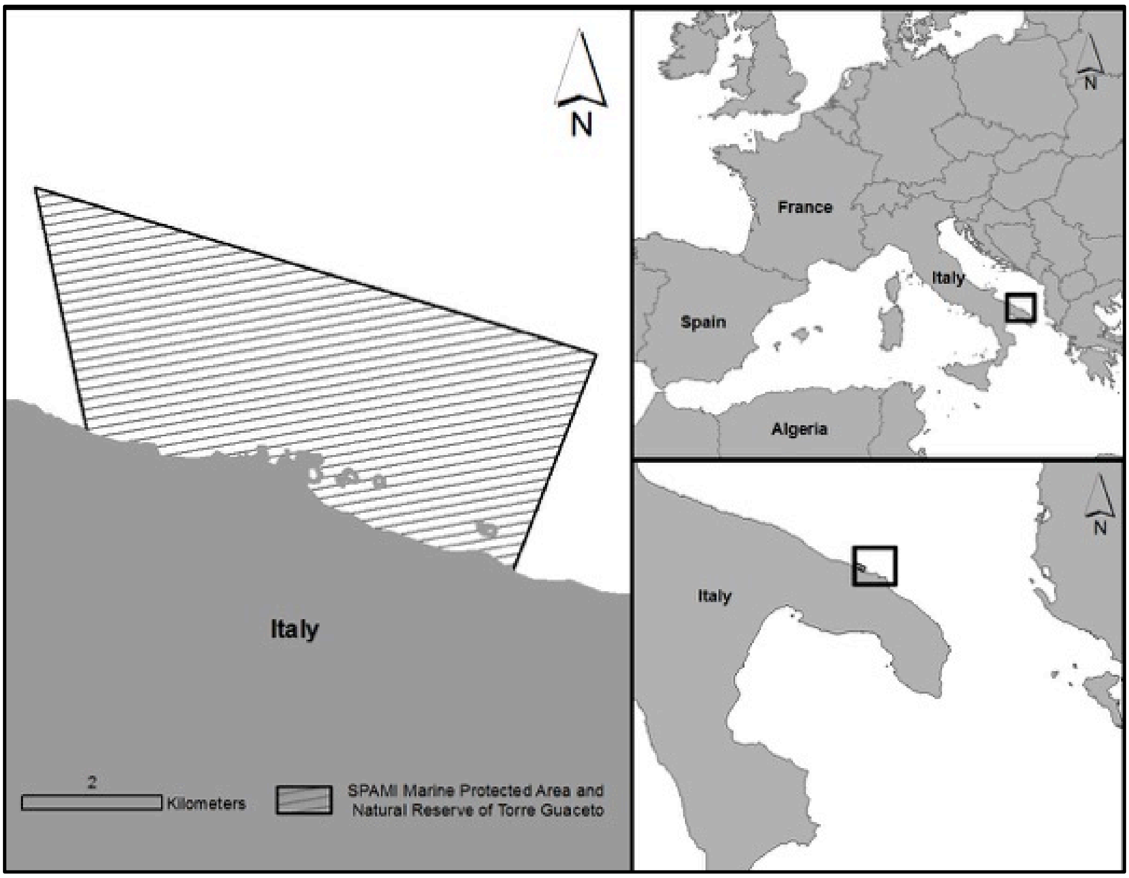


Figure 14. Location of the SPAMI Torre Guaceto, Italy

The western sector is characterized by a coastline with a series of small coves with pocket beaches. The eastern coastal sector is mainly sandy, with reduced rocky formations characterized by a regular, sinuous coastline. The sea floor develops with two depressions, creating parallel submarine cliffs, at two different depth ranges. The areas with brackish water are characterized by the presence of the marine phanerogam *Zostera noltii*. The rocky intertidal areas are covered by red algae such as *Laurencia* sp, and *Corallina elongata* and by a Cystoserietum belt along the rocky littoral.

The rocky infralittoral is characterized by dense populations of sea urchins that create barrens. *Cladocora caespitosa*, an important zooxanthellate hard coral of the Mediterranean Sea (Petrosillo et al. 2007) is well represented in the area. The sandy infralittoral is characterized by *P. oceanica* meadows. The dim light conditions created by leaf stratum allow the creation of skiophilous habitats, providing the optimal environmental conditions for numerous organisms, as sponges, hydroids, anthozoans, bryozoans, and bivalve mollusks, crustaceans and fish, often with high commercial value.

The rocky coast is characterized by a gently sloped rocky plateau, declining from the water surface to ~10–12 m depth over coarse sand. Rocky bottoms alternate with sand and *P. oceanica* seagrass beds at about 12–20 m depth. From about 25 to 35–40 m depth, coralligenous formations alternate with sand, and at deeper stands sandy–muddy bottoms widely dominate (Guidetti et al. 2010). Some of the precoralligenous formations, mostly localized in front of the Tower of Guaceto, at 15-17 meter depth, are characterized by patches of high density of gorgonians of the species *Eunicella cavolini* and *E. singularis*.

The invasive species *Caulerpa cylindracea* is very common. Source of local pollution are organic and inorganic discharges coming from cultivated internal lands, and organic charge that is transported along the littoral from the Northern Adriatic and from Albania due to winds and currents. Artisanal fishery is very well regulated with effective positive effects on fish biomasses.

II.2. Data compilation

Compiling existing data, graphs, photos, and information was an important part of this report. Published studies and publicly available reports concerning the study/monitoring of these five main climate change indicators in each of the three SPAMIs were collected and compiled. Information was obtained through internet searches, the reference lists of initially identified studies, and consultation with colleagues and MPA's managers.

III. RESULTS

III.1. Mar Menor and oriental Mediterranean Murcia coast, Spain

III.1.1. SST and thermal stratification

III.1.1.1. Methodological description

Since 2011, a local network of underwater sensors was set for continuous registration of seawater temperature (Figure 15). This SPAMI uses temperature sensors, HOBO Water Temp Pro v2 Data Logger, programmed to obtain data every 30 minutes. The battery life of these sensors is one year but the sensors are exchanged every 5-6 months to avoid losses and to actualize the temporal series of temperature

At present, 9 sensors are installed in 3 different locations, 4 at Isla Grosa, 4 at Isla Hormiga (Cabo de Palos and Isla Hormigas Marine Reseve) and 1 at the inner, northern basin of the Mar Menor Sea. In the first two locations, sensors are deployed at 4 different depths (5, 15, 25, and 35 meters) to characterize thermal anomalies both in time as in the water column (thermal stratification). The one deployed at the Mar Menor Sea is at 1,5 meter depth.

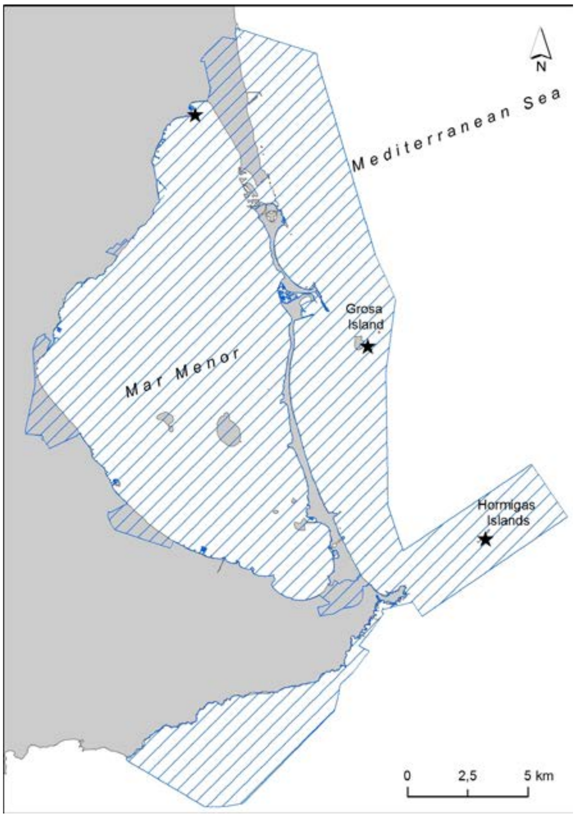


Figure 15. Local network of underwater temperature sensors in the SPAMI Mar Menor and oriental Mediterranean Murcia coast.

Once in the lab, sensors are cleaned and a *HOBO* BASE-U-4 *Optic* USB Base Station is used to download data from the loggers. The new data collected are, for each place and depth, integrated with previous data to build annual series. Data management, analysis and graphical representations are produced with an script of MATLAB software designed ad hoc.

III.1.1.2. Data (Graphs or Figures)

The figure below shows an example of the daily temperature raw data registered from one data logger at Isla Grosa (Figure 16).

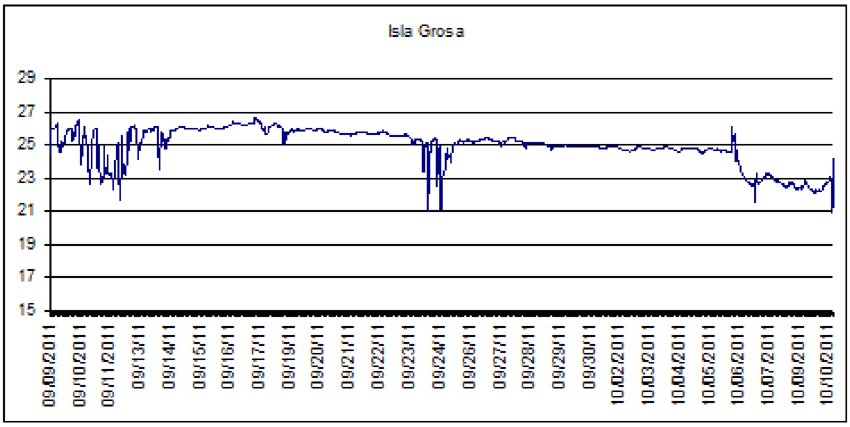


Figure 16. Example of temperature raw data as discharged from the underwater sensor.

Another example of temperature data collected in Isla Grosa, is shown in Figure 17 (below). Note the temporal variability, on an annual basis, at each depth of measurement.

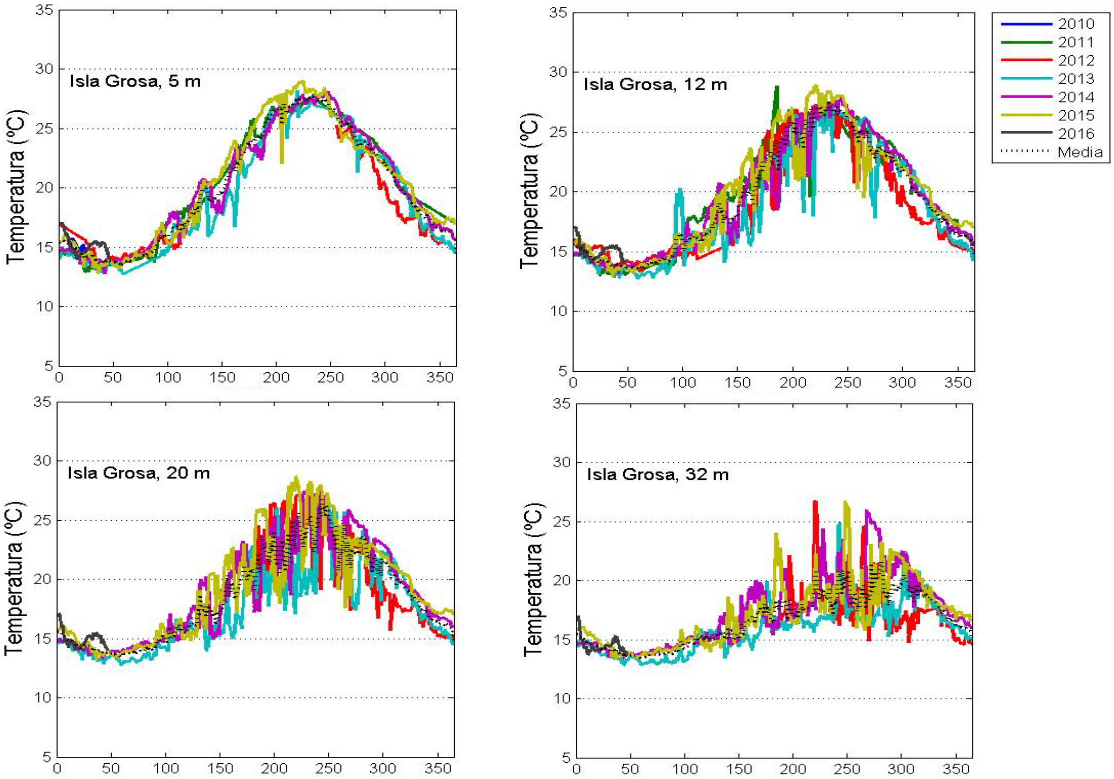


Figure 17. Annual cicles of temperature (ºC) obtained in the period 2011-2015 at 5, 12, 20 and 32 m depth in Grosa Island. The X-axis represent the number of days since 1th January.

From these data, the number of days beyond a critical temperature threshold can be estimated for a given year and depth (Figure 18). For instance, in Grosa Island at 5 meters depth is appreciated how the number of days beyond temperature thresholds of 26 and 27ºC increased between 2013 and 2015. The number of days with temperatures of 28ºC or higher were zero in 2013, 1 day in 2014 and 27 days in 2015. These data reveals that in the SPAMI area, surface waters showed a progressive warming during this period.

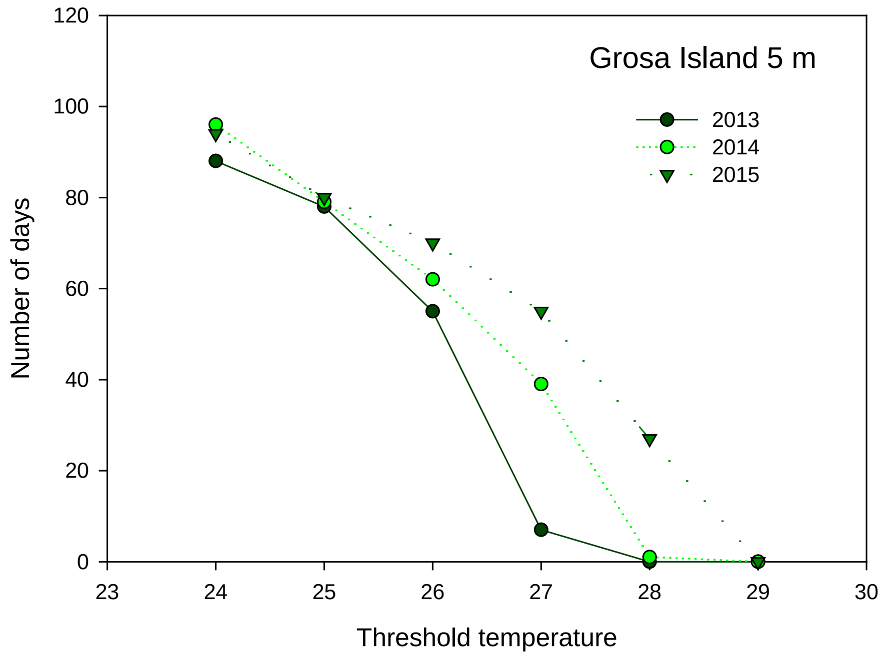


Figure 18. Number of days (Y-axis) above specific temperature thresholds (ºC, X-axis) obtained from annual cycles recorded in Grosa Island in 2013, 2014 and 2015 t 5 meters depth.

The Figure 19 also shows how this warming event is transmitted to deeper zones of the water column, then affecting thermal stratification. In 2014 and 2015 deeper zones (30m depth) experienced also warmer conditions than in the precedent years. In July 2015 a very intense hot wave affected the area, as can be seen in Figure 19.

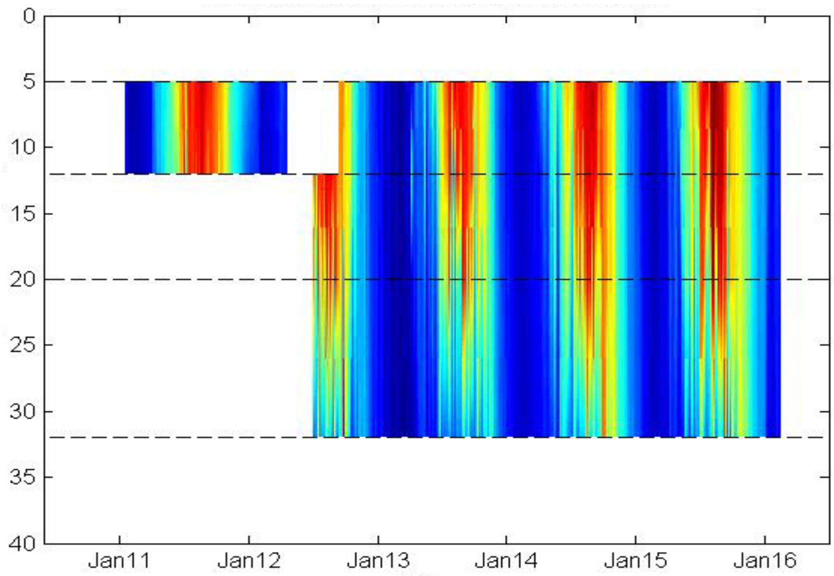


Fig 19. Temperature variability with depth (Y-axis, meters) along time (X-axis, days). Warmer colours represent higher temperatures and show the thermocline formation. Red colours are more intense in summer 2015 due to a very intense hot wave in July.

III.1.2. Mortality and bleaching events

In the Cabo de Palos e Islas Hormigas Marine Protected Area a long term study monitored white gorgonian *Eunicella singularis* populations from 2004 to 2015 (Figure 20), to investigate the potential impacts of human and environmental disturbances on the benthic communities of this area. White gorgonians have a slow population dynamic, and therefore are particularly susceptible to both human threats and environmental disturbances, especially in the light of arising climate changes.

Figure 20. Above: Sampling of *Eunicella singularis* colonies with quadrats, Cabo de Palos. Below: Nudibranch (*Cratena peregrina*) in a colony of *E. singularis* (left) and colony skeleton of *E. singularis* after mortality. Photo credit: José Pereñiguez.

In addition, a photographic survey of bleaching of the invasive scleractinian coral *Oculina patagonica* has been undertaken in 2012, in the framework of the EU project IRSES-RECOMPRA. Moreover, an assessment of the spatial distribution, density and cover of *O. patagonica* in 2014 was done as part of the regular monitoring of the Cabo de Palos - Islas

Hormigas marine reserve, during which this phenomenon has been repeatedly observed (Figure 21) (Pers. comm. Aguilar-Rodríguez, 2015).

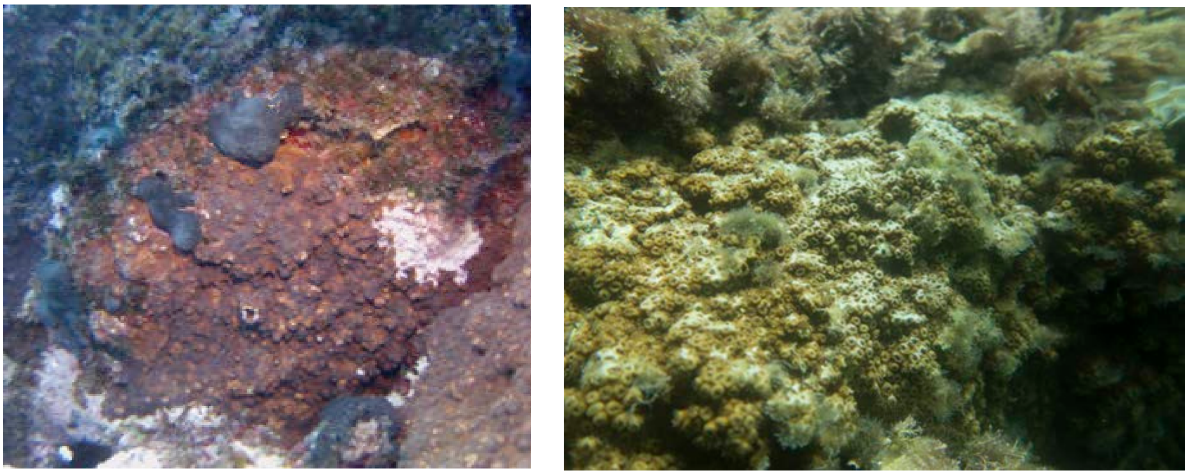


Figure 21. Partially bleached colonies of the invasive scleractinian coral *Oculina patagonica* at <5m depth, in El Hormigón, Cabo de Palos e Islas Hormigas Marine Protected Area, October 2014. Photo credit: Adrián Aguilar-Rodríguez.

In this SPAMI, a monitoring network of 10 *P. oceanica* stations are being followed up since 2004 by the Spanish Institute of Oceanography, in order to analyze the long-term dynamics and trends of the meadow population and structure in relation to natural and altered conditions. This monitoring programme is designed to detect mortality events of the *P. oceanica* meadow shoot population as well as changes in the habitat distribution.

III.1.2.1. Methodological description

E. singularis data were collected from 2004 to 2015 in two locations with different protection level: the integral reserve (Isla Hormiga e Islote del Hormigón) and the parcial reserve (bajo de Dentro). The monitoring was conducted at 22 and 31m depth, with scuba diving. Eight randomly distributed quadrats (of 1m^2) were assessed in two sites within each location. In each quadrat the number of dead and alive colonies was registered, as well as each colony's density (individuals/m^2), height (cm), and mortality coverage (%). The last two, were taken in 5-10 colonies ramdonly selected in the right corner of the quadrat. Maximum height, i.e. maximum distance between the base of the colony and the tips of the heigher axes, was measured with a ruler (precision ± 1 mm).

P. oceanica sampling stations consist in six permanent markers (metallic bars) deployed every 5m. In each station and marker, measurements of seagrass meadow cover (%) and shoot density (shoots/m^2) are obtained every year. These are the most important and widely used seagrass variables employed for the characterization of habitat structure and seagrass population. A measurement of the meadow cover is obtained in each marker by visual estimation of the percentage of substrate occupied by living seagrass patches within ten 40x40cm quadrats, deployed every meter along a 10m transect. The transect is through following a fixed, predetermined direction using a compass. Along each transect, three measurements of shoot density are also taken by counting the total number of shoots within 400cm^2 quadrats; these three measurements are made in random positions of the transect, but always within living *Posidonia* patches with a 100% substrate coverage.

With this sampling design, a total number of 6 replicates are obtained for meadow cover and 18 replicates for shoot density. Furthermore, close to each marker, a permanent 50x50cm quadrat is installed on a *P. oceanica* patch for precise censuses of shoot numbers with time. These quadrats (n = 6 replicates) allow us to estimate a net population change rate on an annual basis, which take negative values when a mortality event occur.

III.1.2.2. Data (Graphs or Figures)

Monitoring data on *E. singularis* reflected the impact of a positive thermal anomaly reported for the Cabo de Palos- Islas Hormigas MPA in 2007. The low percentages of death tissue in 2005 and 2006, markedly increased in 2007, affecting more than half of all the colonies (57 ± 27,2 %), and about 12,4 ± 20,2 % of each colony (Figure 22). Most colonies recovered in the following years of this thermal event, though the coverage of death tissue were always higher than before 2007. Moreover, monitoring data show an increase of the percentages of death tissue since 2007.

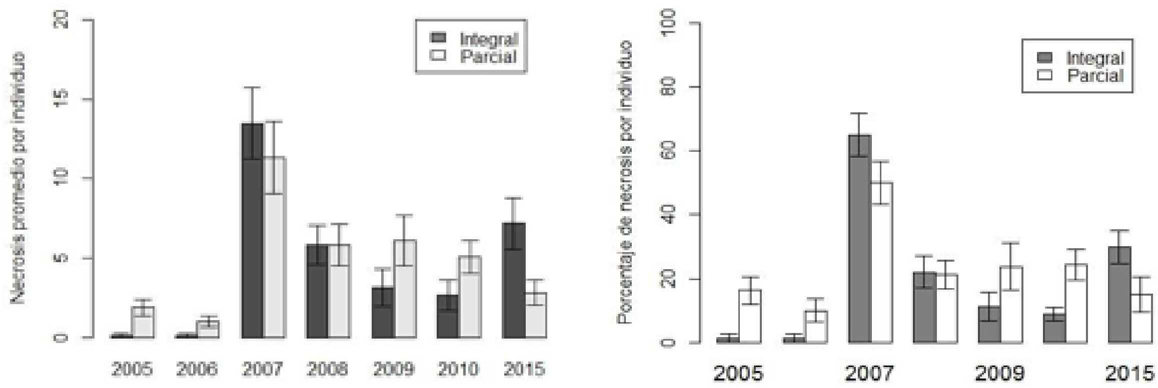


Figure 22. Average values (± DE) of the variables "average necrosis per colony" (left) and "percentage of necrotic colonies" (right) for *E. singularis* by year and protection level.

The response of *E. singularis* may reflect that of other benthic communities to the predicted more frequent occurrence of thermal anomalies in the future, associated with climate change. Thus, the occurrence of exceptional environmental perturbations (e.g. a strong and prolonged increase of sea water temperature) can affect the health and endanger the population dynamics of Mediterranean benthic communities.

With regard to the *P. oceanica* meadow, all monitoring stations in the SPAMI area except one, followed an stable or progressive evolution in the last 12 years (2004-2015). The one that followed a regressive dynamics is a site under the influence of the Mar Menor Sea, with a high level of environmental pressure due to human activities. Therefore, we can conclude that no massive mortality events of *P. oceanica* have occurred in this area and period.

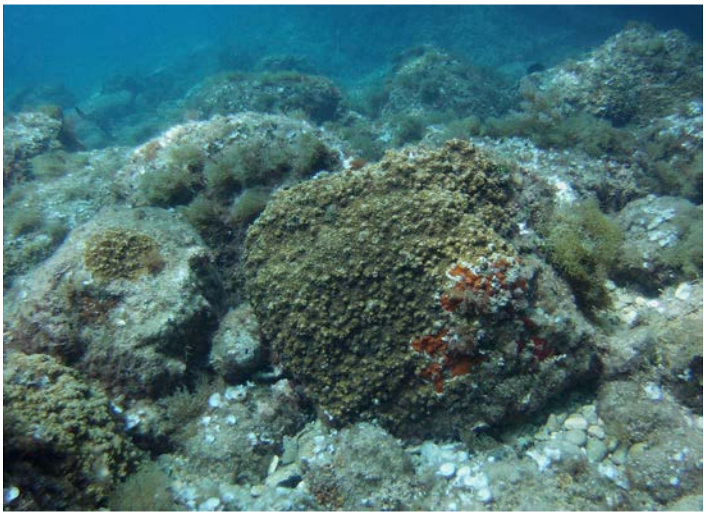
III.1.3. Range shift of alien species/Temperature-sensitive species

In the Mar Menor, the distribution and ecology of the tropical non-indigenous ragged sea hare *Bursatella leachii* has been studied by the Bursatella project since 2013. This mollusc entered the Mediterranean Sea in the 40’s and then became abundant in the eastern Mediterranean.

Figure 23. The non-indigenous ragged sea hare *B. leachii*, Mar menor 2013. Photo taken from: http://proyectobursatella. blogspot.com.es/p/ introduccion.html

First report in Spain was in Baleares, in 1996, and in the Mar Menor in 2008. Since then, this species has adapted to the high salinities and temperatures of the Mar Menor.

On the other hand, the presence and abundance of non-indigenous thermofilic species in the Cabo de Palos e Islas Hormigas Marine Protected Area has been assessed in several studies performed in the area. For instance, the density and cover of the invasive scleractinian coral *O. patagonica* in the reserve was recently studied by Aguilar-Rodríguez, 2015 (Figure 23).



Likewise, the distribution and abundance of the green algae *Caulerpa cylindracea*, one of the main invaders of the Mediterranean Sea, in this MPA and the rest of the SPAMI area is reported in Ruíz et al. (2011, 2013); and the spatial distribution of the sally lightfoot crab *Percnon gibbesi* is described in Félix-Hackradt et al. (2010).

Figure 24. Colonies of the non-indigenous invasive hermatypic coral *Oculina patagonica*, Cabo de Palos. Photo credit: Adrián Aguilar-Rodríguez

Moreover, the periodic monitoring of fish populations in the Cabo de Palos e Islas Hormigas MPA, conducted since 1990, has detected the presence and abundance of some non-indigenous thermofilic fish species in the area. In addition, fishermen (both professional and recreational) and divers use to informally inform the MPA, trough emails with photos or oral descriptions, when they capture/observe a non-indigenous species (or any species they don’t know) within the MPA.

III.1.3.1. Methodological description

For the monitoring of *Bursatella* in the Mar Menor the total area of the Mar Menor lagoon was divided into 2x2km sampling squares (Figure 25). At each square, the species is actively searched. Sampling squares located shallower than 2m depth were assessed using an underwater viewer (bathyscope) over a side of a boat at low speed (Figure 25), while those located at deeper depths were assessed diving. For the last, the species is searched in a series of transects, using a metric tape. Large areas are also sampled by active search performed by a diver towed by the boat and near the bottom. In addition, the presence /cover of benthic organisms are also noted, as well as water temperature and salinity, and sediment samples.

The assessment of *C. cylindracea* spread and abundance have been performed since its introduction in the region in 2005 (Ruiz et al. 2011).

The Bursatella project has implemented a system that allows any person to contribute to the data collection. Anyone who spot the species can report its observation on the webpage of the project. In addition, in partnership with the diving centers of the area the project has succesfully involved the local comunity in the monitoring activities of this species.

Figure 25. Monitoring of *Bursatella* in the Mar Menor. Left: Mar Menor lagoon area divided in 2x2km sampling squares. Right: Active search of Bursatella with a bathyscope. Images taken from the Bursella project webpage: http://proyectobursatella.blogspot.com.es/p/ metodologia.html

The relative abundance of this exotic invader has been quantified every year in each of the sampling stations of the existing *P. oceanica* monitoring network (see section III.1.2.) in order to assess its temporal dynamic and possible interactions with *P. oceanica* meadows (Marín-Guirao et al. 2015; Bernardeau-Esteller 2015). The relative abundance is measured using a 50x50cm quadrat subdivided in 5x5cm quadrats. Algal abundance is estimated as the percentage of sub-quadrats with presence of the alga relative to the total number of subquadrats. This measurement is performed totally at random and a total number of 10 replicates are necessary. Data are expressed in algal biomass (g DW/m^2) by using a relationship between relative abundance and biomass obtained at the beginning of the study.

The monitoring of fish communities in the Cabo de Palos e Islas Hormigas MPA has been performed since 1990, with scuba diving, in three different sectors. Species composition and abundance is registered in at least 3 transects of 50x5m^2, randomly placed in rocky bottoms, at about 10m depth. Special atention is put into non-indigenous termofilic species, in order to be able to report their presence within the MPA, and estimate its abundance, temporal dynamic, and spatial distribution.

III.1.3.2. Data (Graphs or Figures)

Results on *Bursatella leachii* have shown the uneven distribution of this species along the Mar Menor, with major aggregations in the central-North area (Figure 26).

On the other hand, *C. cylindracea* has spread over most of the SPAMI area, although the degree of colonization largely depends on the locality, depth, year, season, and type of substrate and habitat. *P. oceanica* meadows have act as very effective ecological barriers against the spread of the invasive alga. Figure 27 shows the intra- and interannual variability of this alga in unvegetated, sedimentary bottoms and within an adjacent *P. oceanica* meadow.

In early colonization stages, algal abundance was considerably higher in the shallower sites (e.g. Grosa Island) than in the deeper ones (e.g. Calblanque), although the temporal evolution was very similar in both cases, as can be seen in Figure 27.

Intra-annual variability is very high mainly due to the winter abundance drop, as described for this species in other Mediterranean sites.

Since 2012, the abundance of the alga has dramatically declined to very low values or disappearance. By contrary, between 2012 and 2015 the alga remains more or less abundant in the deepest sites, reaching mean values similar to those observed in the initial colonization phase. It is difficult to explain this spatio-temporal patterns, but based on temperature data available for this area (see section III.1.1.), we hypothesize that recent warming events could be involved in drastic drop of the algal abundance in the shallower areas. Nonetheless, a more in deep analysis of these data should be necessary to support such hypothesis.

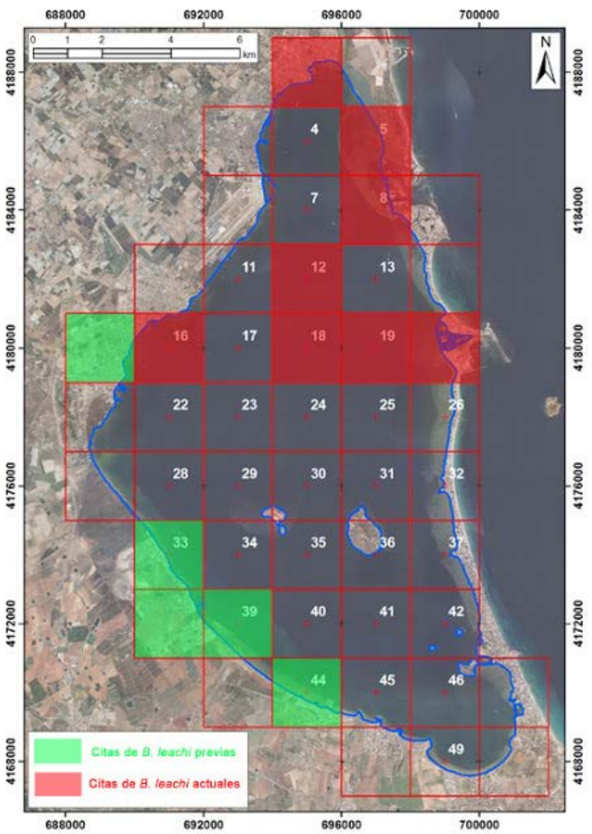
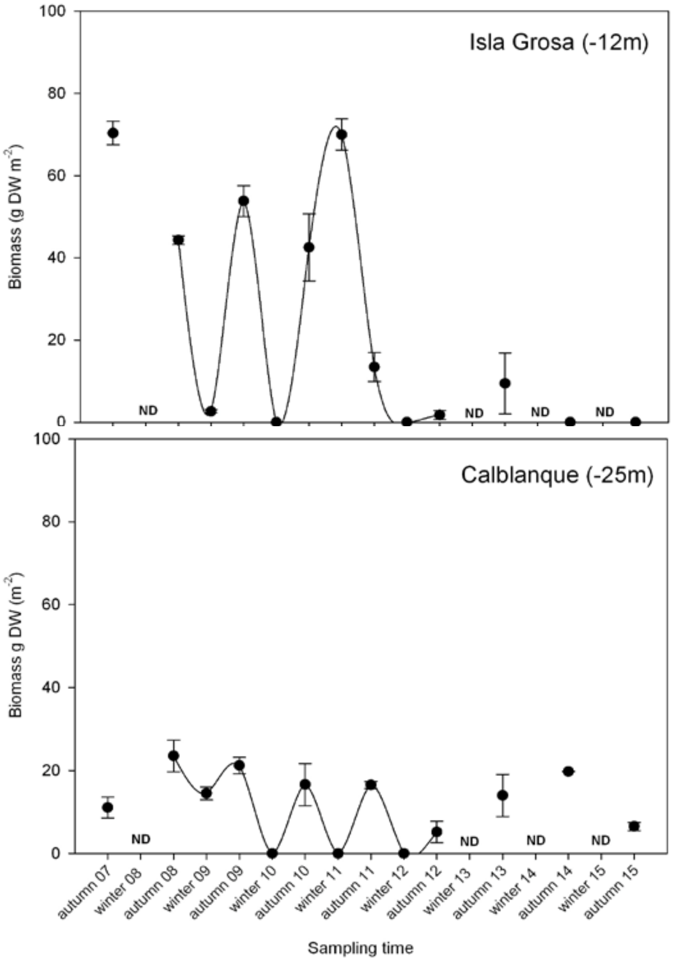


Figure 26. Map of the distribution of *Bursatella* in the Mar Menor lagoon, Murcia, showing areas where the species has been previously (in green) and recently (in red) cited. Image from Bursatella project webpage: http://proyectobursatella.blogspot.com.es/p/resultados.html



Both the periodic monitoring of fish communities in the Cabo de Palos e Islas Hormiga MPA and informal communications of stakeholders, e.g. fishermen and divers of the area, allowed to detect the presence of nine non-indigenous termophilic fish species in the area (Perez-Ruzafa et al. 2004).

The species observed were: the saltón *Gymnammodytes semisquamatus*, the serrano imperial *Serranus atricauda*, the mero blanco *Epinephelus aeneus*, the jurel dentón *Pseudocaranx dentex*, the burro listado *Parapristipoma octolineatum*, the roncador *Pomadasis incisus*, the sucla *Spicara melanurus*, the pargo real *Pagrus auriga*, the vieja o pez loro *Sparisoma cretense*, and the poyo *Scorpaena maderensis*.

Figure 27. Temporal variability of *C. cylindracea* biomass within the SPAMI: (Above) Grosa Island, at 12m depth, (Below) Calblanque, at 25m depth. Mean values and error bars are shown for each sampling event performed in winter (invierno) and autumm (otoño) every year since 2007.

In addition, research on the distribution and abundance of the ornate wrasse *Thalassoma pavo*, a highly thermophilic species, found this species to be abundant in the Cabo de Palos e Islas Hormiga MPA (Figure 28). This confirms a distributional range shift of this species which is becoming more frequent and abundant in the Mediterranean likely due to the tropicalization of this Sea.

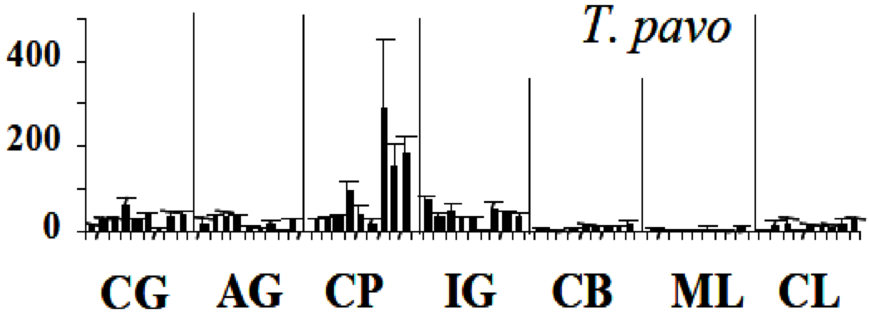
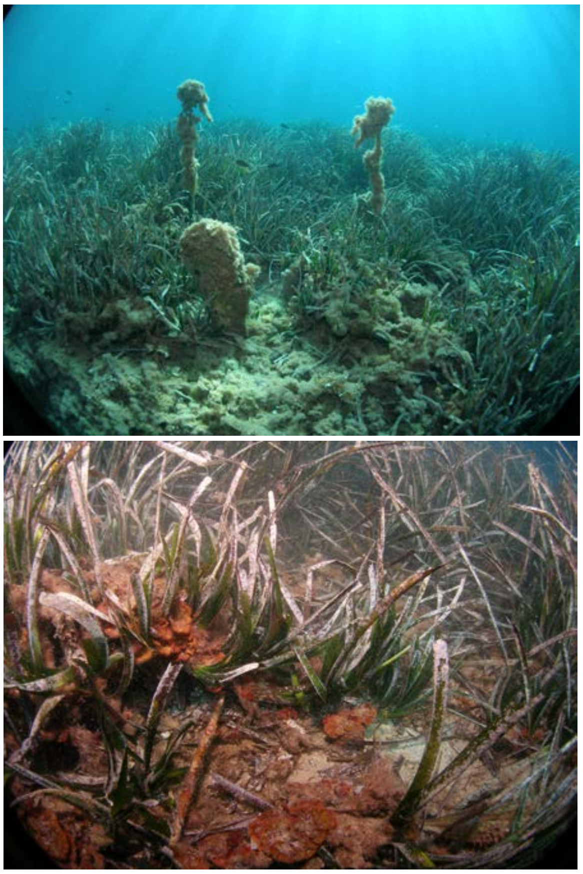


Figure 28. Mean abundance (± SE) (N_o of individuals*250m^{-2}) of the ornate wrasse in multiple west Mediterranean locations. Summer 1996. Order from South towards North (CG = Cabo de Gata; AG = Aguilas; **CP = Cabo de Palos**; IG = Isla Grosa; CB = Cabrera; ML = Mallorca; CL = Columbretes) [Figure taken from García Charton *et al.* 2004].

III.1.4. Reproduction and breeding date of selected species, including flowering of *Posidonia oceanica* where present

III.1.4.1. Methodological description

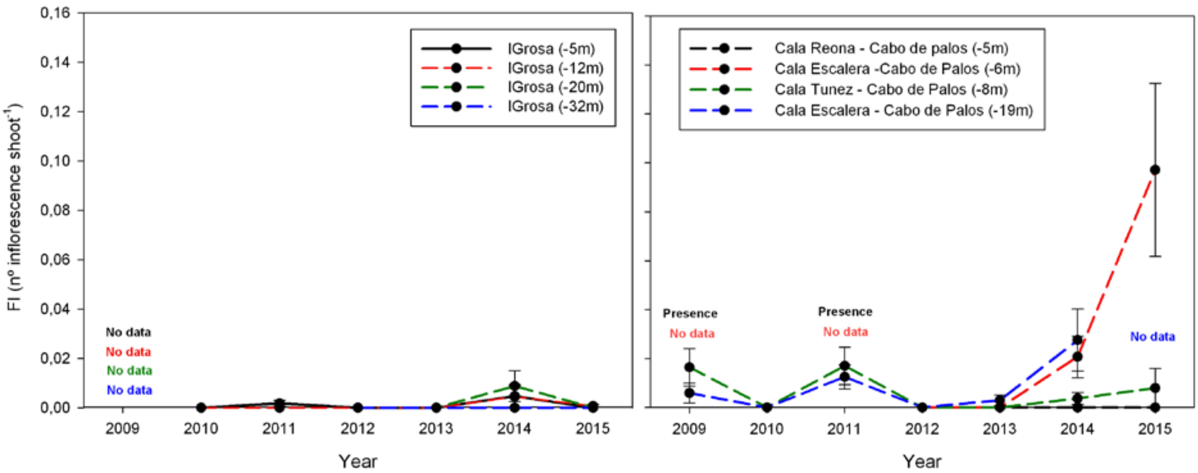


The quatification of the *P. oceanica* flowering events in a meadow basically consist in the determination of the Intensity of Flowering Index (IF, number of inflorescences per shoot; (Díaz-Almela et al. 2005)). To this end the number of inflorescences and shoots are simultaneously counted in 18 400cm^2 quadrats placed within living patches of *P. oceanica*. In our case, the number of inflorescences are counted every year in the same sampling quadrats used for the determination of shoot mortality events in the existing P. oceanica monitoring network (see section III.1.2.)., Because flowering usually occur at the end of September or in October in this area, all samplings are performed in this months.

Figure 29. Above : Sampling station of the P. oceanica monitoring network of the SPAMI area installed in 2004 (Cabo de Palos). Below : Permanent 50x50cm quadrats installed in the SPAMI area for the long-term monitoring of *P. oceanica*. Photo credit: Juan Manuel Ruíz.

III.1.4.2 Data (Graphs or Figures)

The graphs below show the interannual variability of the flowering intensity index (FI) of *Posidonia* meadows located at different localities and depths in the SPAMI area (P: presence of flowers). Only in one station (Cabo de Palos, Cala Escalera) the FI shows a



<u>Figure 30.</u> Flowering intensity index of *P. oeanica* meadows located at different depths, in the SPAMI. Left: Isla Grossa. Right: Cabo de Palos.

progressive and significant increase between 2013 and 2015 (Figure 30, left). During this period, the area has experienced an increasing warming of seawater (see results in section III.1.1.). Therefore, these results suggest that: a) the flowering process has a very high temporal and spatial heterogeneity, and b) the relationship between *P. oceanica* flowering and temperature is much more complex than previously proposed due in part to the influence of other environmental factors but also to genetic factors within the meadow population. Results also demonstrate that the measurement of this descriptor must be based on monitoring networks rather than in few, isolated sampling stations.

III.1.5. Episodic Species Outbreaks (blooms)

In the Mar Menor and Islas Hormigas SPAMI, gelationus zooplankton outbreaks have been identified from jellyfishes (*Cotylorhiza tuberculata* and *Rizhostoma pulmo*) and a ctenophores (*Mnemiopsis leidyi*) since the late 20th Century. Massive blooms of *C. tuberculata* and *R. pulmo* in the Mar Menor lagoon began manifesting in the early 1990s. These scyphomedusae are frequently present on the Mediterranean coast in pelagic and polyp phases during warm and cold months respectively. The connection between climatic forcing and population response is straightforward in *C. tuberculata* and can be summarized with the simple rule “the warmer the better”; mild winters and long summers facilitate its blooms. In the Mar Menor lagoon both species have shown annual blooms from 1993 to 2001 and again in 2006 (Mas 1999; Pérez-Ruzafa et al. 2002). Results suggest that the frequency of blooms has increased with time, especially in the late 20th Century; however there might be some bias on the sporadic nature of sighting reports. First reports on *M. leidyi* outbreaks date from the summer of 2012 in the Mar Menor lagoon (Marambio et al. 2013).

III.1.5.1. Methodological description

Since 1988, jellyfish populations have been studied in the Mar Menor and Islas Hormigas SPAMI. In 1997, (Pérez-Ruzafa et al. 2002) estimated the density of adults of *C. tuberculata* and *R. pulmo* in 20 sampling stations in the Mar Menor lagoon (Figure 31), combining simultaneous visual census performed from the boat and towed nets at a velocity of 1knot. The maximum value obtained for each sampling was considered as the more realistic data of the population. In the summer of 2012, (Marambio et al. 2013) studied the blooms of *M.*

leidyi (Figure 22). A first sampling in early July 2012 confirmed the presence of the species in the Mar Menor lagoon. The sampling continued in 24 stations on the 1st and 18th August and 1st September. A 40cm diameter bongo net (235μm mesh size) equipped with a flowmeter was towed horizontally at 1m depth. Densities of collected ctenophores were estimated according to the corresponding filtered volumes, that ranged between 22.8 and 36.21m^3.

III.1.5.2. Data (Graphs or Figures)

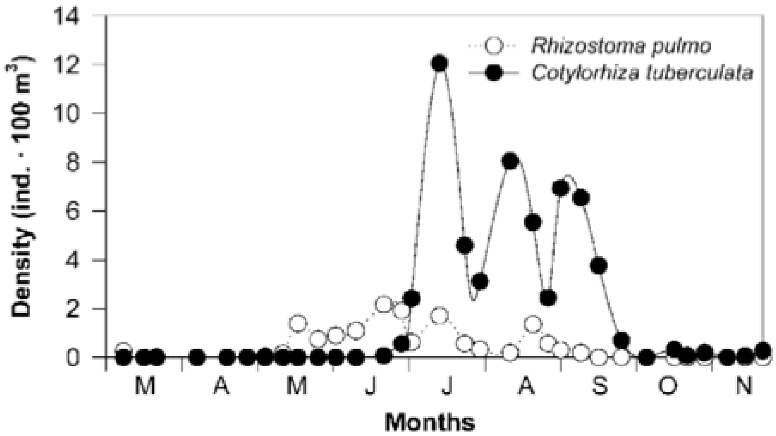


Figure 31. Temporal variation in the mean density of the jellyfishes *Rhizostoma pulmo* and *Cotylorhiza tuberculata* in the Mar Menor lagoon, from March to November 1997. Taken from Pérez-Ruzafa et al. (2002).

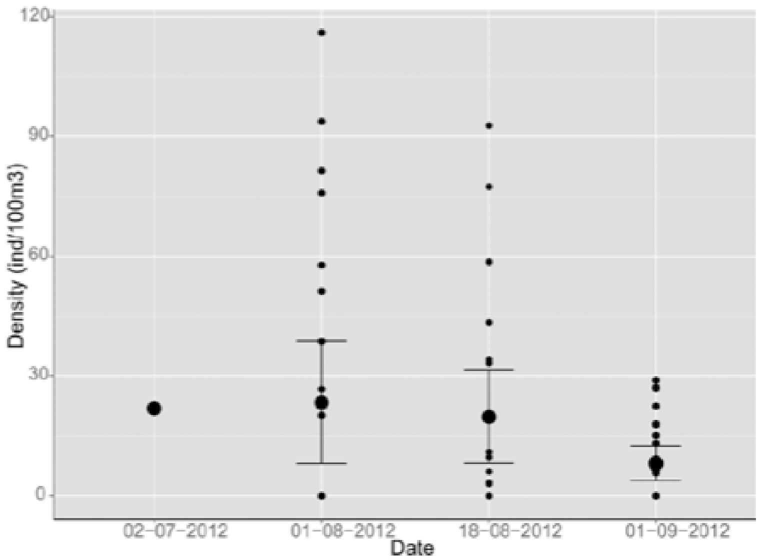


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Figure 32. Abundance of the ctenophore *Mnemiopsis leidyi* in the Mar Menor lagoon, from 2nd July to 1st September 2012. Mean (±SE) are shown for each sampling date. Taken from Marambio et al. (2013)

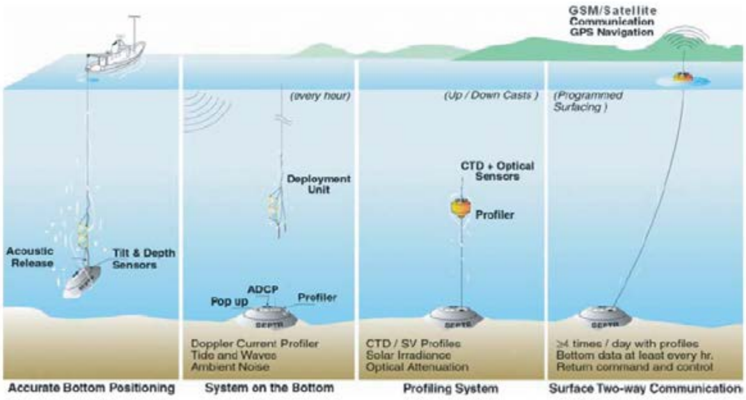
III.2. Portofino, Italy

III.2.1. SST and thermal stratification

III.2.1.1. Methodological description

Since 1985, Portofino MPA has been collecting temperature data with traditional methods and collaborating with several research organizations to develop new systems to monitor temperature continuously. The integration of the temperature data collected by different methodologies allowed the inclusion of this MPA in the LTER Italian network since 2007; nowadays it is part of the LTER international network. From 1985 to 1996, the University of Genova (DISTAV) collected monthly measurements of water temperature, which were continued from 1997 to 1999 but with a varying frequency. Since 1999, DISTAV monitores sea temperature in two specific spots of the MPA: Punta Faro and Cala Oro, using a CTD profile. Measurements are taken every 15 days.

The Portofino MPA was included in the T-Mednet network in June 2015. Since then, temperature data-loggers placed in the water column, from sea-surface to 40m depth (every 5m), are collected twice a year to gather the data recorded and make it available directly on the collaborative platform T-MedNet.



Sea temperature in Portofino MPA is also monitored with an in situ SEPTR (Shallow water Environmental Profiler in Trawl-safe Real-time configuration) (Figure 33), located at 30m depth. This tool provides a very detailed description of the water column temperature profile by continuous data acquisition, which makes it a great tool for monitoring marine coastal areas.

Figure 33. SEPTR operational scenario deployment and operations. Taken from Ruggieri *et al.* 2005.

III.2.1.2. Data (Graphs or Figures)

The integration of temperature data collected in Portofino MPA has produced historical temperature series very valuable to this MPA (Figure 34). For example, the data set of July and August 2003 show the evolution of thermic anomalies in surface waters, even during the day. Temperature profiles in August evidenced a progressive warming of seawater (even >28°C) and a deepening of the thermocline.

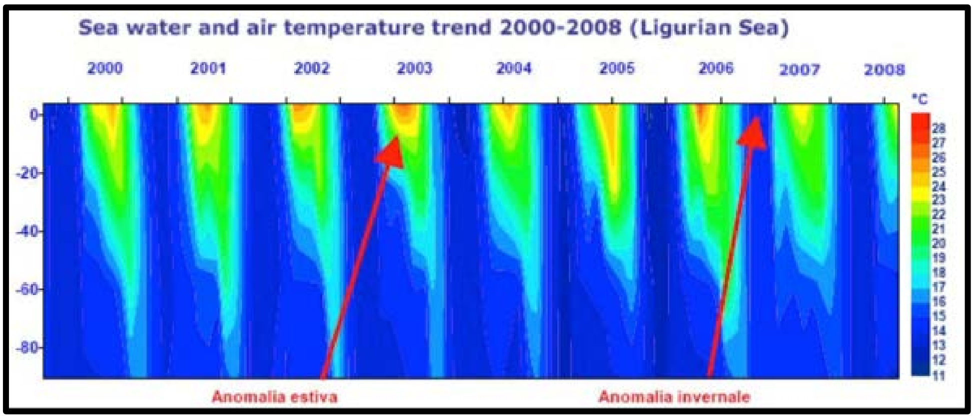


Figure 34. Data integration of the time series of water temperature profiles in the Portofino MPA. Taken from LTER Italy network.

Two examples of temperature data used to produce the historical temperature profiles for the Portofino MPA are those obtained with the SEPTR method (Figure 35) and with the T-MEDNET methodology (Figure 36).

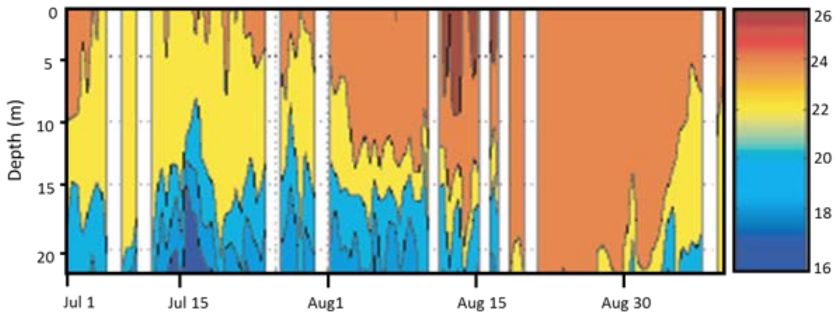


Figure 35. Sea temperature data collected in Portofino MPA, in 2004, with the SEPTR method. Taken from Ruggieri *et al.* 2005.

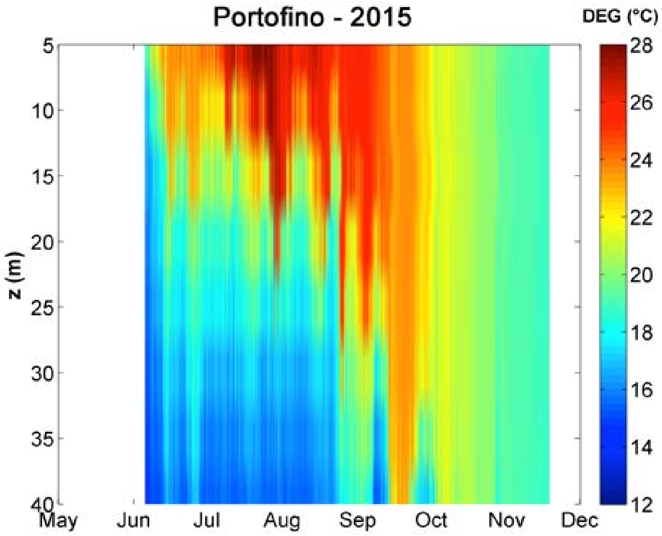


Figure 36. Sea temperature data collected in Portofino MPA, in 2015, with the T-MEDNET methodology. Taken from T-mednet webpage: www.t-mednet.org

III.2.2. Mortality and bleaching events

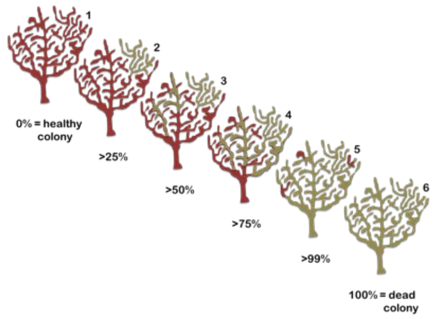
Massive mortalities in the MPA of Portofino were reported for the first time in 1986 (Bavestrello and Boero, 1986), when the yellow gorgonian *Eunicella cavolini* was affected. After that event, an unusual and sudden episode was detected during the fall of 1993. This event was likely due to a cold water mass floating along the coast (Bavestrello et al. 1994). Then, during summer 1999, occurred the biggest mass mortality ever reported in the Mediterranean, modifying for ever the seascapes of the NW Mediterranean Sea from the surface to 40m depth (Cerrano et al. 2000). In next years these episodes continued to be reported in the Mediterranean but generally on a more local scale. Anyway in 2003, Europe was strucked by an impressive heat wave, the hottest summer on record in Europe since at least 1540, causing the death of thousands of people. Likewise, underwater, this anomaly led to a widespread massive mortality that involved tens of species down to 30 m depth (Garrabou et al. 2009) and resetted the recovery and the recruits of previous events. One of the most affected species in these events was the violescent sea-whip *Paramuricea clavata*.

III.2.2.1. Methodological description

A group of researchers of the Polytechnic University of Marche, the University of Genoa, and UBiCa srl worked for several years with the Portofino MPA to examine the threats and changes of the benthic assemblages in this MPA related to climate change impacts. A population of the sea-fan *Paramuricea clavata,* was yearly monitored in the Portofino SPAMI, from 1999. In the meanwhile, other occasional studies have been performed in the same area to get more details on the population dynamics (Cerrano et al. 2005) and ecological function of gorgonian colonies (Scinto et al. 2010; Vezzulli et al. 2013; Ponti et al. 2014).

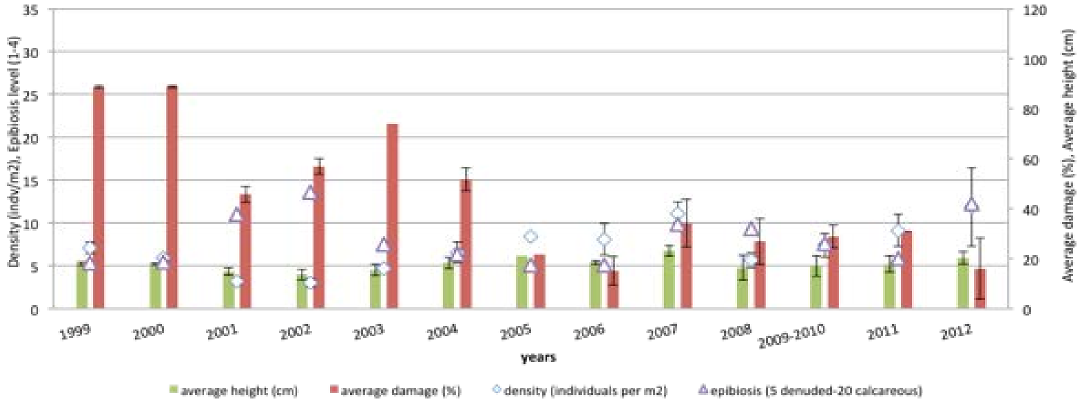
The long term study is following a standard methodology set out after several *ad hoc* studies (Coma et al. 2006; Linares et al. 2008; Huete-Stauffer et al. 2011; Kipson et al. 2015), having the aim to monitor gorgonian population dynamics. A minimum of four quadrats (50x50cm) are randomly sampled from 40 (below the average depth of the thermocline) to 10m depth (tentatively the upper limit of the gorgonian community) every 5m, taking into account the geomorphology of the sites. This mean, considering exposition and inclination of the substrate that should be kept similar and comparable for every quadrat. Within each quadrat the number of colonies (converted to colony density), colony heights, colony health (defined as the percentage of colony with injured coenenchyme), and the number of fishing lines or nets wrapped around the colonies are recorded (table 1). Furthermore, for each colony the epibiosis level is recorded (according to methods provided by Bavestrello et al. (1997)) and the epibiontic organisms are identified. On the basis of organisms that had settled on the scleraxis five different temporal phases are defined. The epibiosis is a consequence of tissue injuries. These injuries can be due to fishing lines or thermal stress, both leading to the loss of tissue, which leaves an axial skeleton available for fouling organisms.

Table 1. Information collected for each gorgonian colony within the quadrats:

Species name	*Paramuricea clavata* (Pc), *Eunicella cavolinii* (Ec), *E. verrucosa* (Ev), *E. singularis* (Es), *Corallium rubrum* (Cr), *Lophogorgia sarmentosa* (Ls), or others.
Height	in cm
Damage: • Percentage of damage (0-100%) • Range of damage (1-6)	0% (healthy), 100% (totally damaged)
	
Epibiosis level	0) no epibiosis 1) denuded skeleton or recent damage 2) first colonizers are algae and hydrozoans 3) second colonizers are some hydrozoans, eventually traces of initial stages of calcareous settlement 4) late successional stages, mainly colonized by calcareous organisms e.g. coralline algae, sponges and bryozoans * When the type of epibiosis level is mixed is reported to the damage percentage (e.g. out of the 50% damage of a colony, 15% corresponded to epibiosis level 2 and 35% to level 4)
Presence and N_o of fishing gears	Presence/absence and number

III.2.2.2. Data (Graphs or Figures)

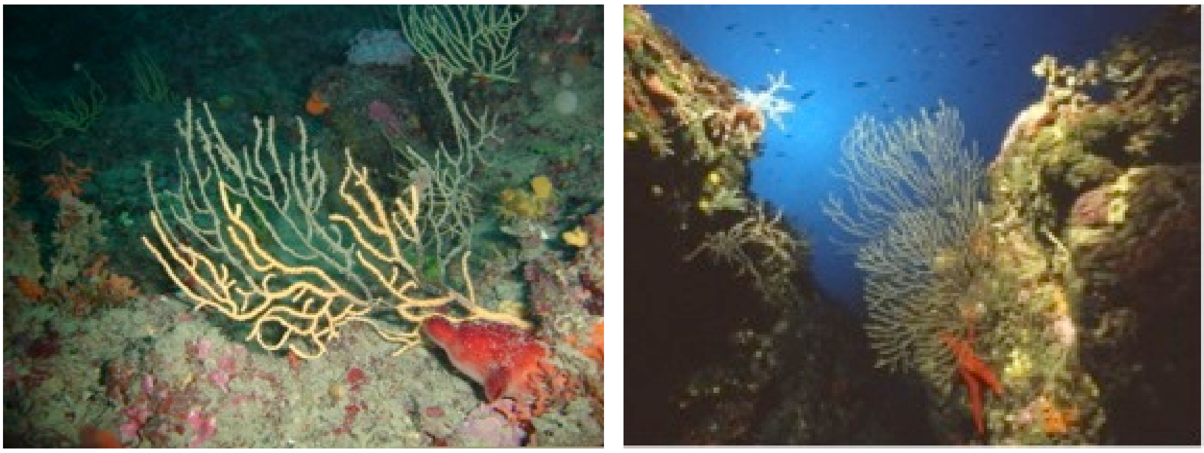
Time series on the structure of a *P. clavata* population within the Portofino SPAMI coupled with temperature profiles of the monitored years show that an elevated damage of the colonies coincide with prolonged summer conditions in 2007, 2009 and 2011 (Figure 37).



<u>Figure 37.</u> Long term monitoring of *Paramuricea clavata* population at 25m depth in Portofino considering height (cm), damage (%), epibiosis level (1-4) and density (N_o of colonies/m).

The monitoring also shows that no additional evident mass mortalities have occurred since 2008 within this MPA, and that a slow recover of the population might be occurring.

In addition, the monitoring shows that the upper limit of the gorgonian populations in the Portofino SPAMI has deepened, likely due to a temperature increase, allowing a more extended algal growth and becoming more susceptible to the arrival of allochthonous species.



<u>Figure 38.</u> *E. cavolini* with recent necrosis of tissue in Portofino MPA. Left: partial necrosis of tissue of 1-3 months old, at 27 m depth, September 2007. Right: Necrosis of tissue of 2-3 weeks old, 30 m depth, August 1999. Photo credit: Carlo Cerrano.

<u>Figure 39.</u> Partial bleaching of a *Cladocora* colony in Portofino MPA, 2010. Photo credit: Massimo Ponti.

III.2.3. Range shift of alien species/Temperature-sensitive species

The Portofino SPAMI is part of two large scale projects aimed at establishing a long-term, basin-scale network to detect and monitor environmental and biological signals of climate change in the Mediterranean Sea. Both projects assess in different ways the geographic shifts and changes in abundance of non-indigenous and indigenous temperature-sensitive species, which are used as proxies to assess the effect of global warming on Mediterranean biodiversity. One of these projects is the LTER Italia Network. To which Portofino SPAMI belongs since 2004. Data on yields and fish compositions are collected, allowing to follow temporal trends in the composition of the catches and detect the arrival of new species to the Mediterranean Sea. The Portofino SPAMI is also part of the MedPAN North project, since 2011, which aims the implementation of a regional monitoring programme on climate change impacts on MPA biodiversity in the Mediterranean. As such, this MPA continuously monitores key species inside the MPA, following the CIESM Tropical Signals protocol.

The Portofino SPAMI has also contributed to the awarenes of the arrival of new species to MPAs by producing a poster for reporting the presence of Alien Invasive Species (AIS) in Ligurian MPAs (Figure 40) and distributing it to the dive centers that operate in the region. Other additional efforts to monitor potential changes in the communities in the Portofino SPAMI include the assessment and comparison of the hydroids populations present on the rocky cliff of this MPA, performed in 1980 and 2004 by the University of Genoa, in collaboration with the Polytechnic University of Marche. Moreover an agreement with the not-for-profit organization Reef Check Italia onlus (RCI) is allowing, since 2006, the creation of a very detailed data set about the distribution of more then 40 species. The organization (RCI) trains and involves volunteer divers to collect data, offering the opportunity to develop spatial and temporal trends that could contribute to MPA monitoring (Cerrano et al. 2016). All these works will help to predict future trends in the biodiversity of Portofino SPAMI and examine future options for its efficient management.

Figure 40. Poster for the identification on invasive species in the Ligurian MPAs. Source: V. Cappenera, Portofino MPA. MedPAN North project.

III.2.3.1. Methodological description

Since 2004, fishermen have provided the Portofino SPAMI with data on their daily catches, which they have always registered in logbooks. In this way, Portofino SPAMI collects important data on yields and fish compositions dating from 1950 to today, which coupled with information on the physical properties of the seawater conditions, allow to follow temporal trends in the composition and number of catches and assess possible relationships with specific environmental variables related with climate change. In addition, the capture of rare species, e.g., sharks, marine turtles, and non-indigenous species, helps keep an updated record on the changes in the biodiversity of the Ligurian Sea, and the entire Mediterranean Sea.

As part of the MEDPAN North project, the Portofino SPAMI monitors both native and exotic cold-water fish, following the CIESM Tropical Signals program protocol. This monitoring is conducted in permanent sites, located within each zone of the SPAMI and outside the SPAMI, and characterized by having a rocky bottom with gentle slope, small coverage of *Posidonia* and no sand. At each site, visual censuses are conducted in two transects 25m long, parallel to the coast, and at two depth ranges: between 1 and 3m and between 12 and 15m. Each site is repeated twice. Transects are surveyed in approximately 5 minutes, with an average speed of 10m/min., using snorkelling and scuba diving. During the census, the presence/abundance of key indigenous and non-indigenous termifilic fish within an imaginary rectangle of 25x25m (total area 125m^2) is recorded. Sampling is carried out in November, January and March, twice a month, between 10.00 and 16.00 hour. Data is recorded on a table that follows the format of the CIESM Tropical Signals program (Figure 41).

The poster created by the Portofino SPAMI for reporting invasive alien species in MPAs of Liguria shows images of invasive alien species that have already been reported in other places of the Ligurian Sea (Figure 40). Copies of the poster were distributed at multiple dive centers in Portofino, so that customers and instructors of several diving centers may provide direct support to these monitoring activities while diving. When a diver detects any of the species shown in the poster, he/she should take a photograph of the organism(s) should be taken and provided to the Portofino MPA, with basic information (e.g. the date, name and surname, site of sighting and depth). This poster has been disseminated at least in the other two national MPAs of Liguria, "Cinque Terre" and “Bergeggi Island".

Observer	**Date...** ... .
Locality	**Hour** ... .

	1-3m (Snorkeling)						12-15m (SCUBA)					
(Transects)	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Epinephelus marginatus												
Caranx crysos												
Coris julis												
Thalassoma pavo												
Sparisoma cretense												
Serranus scriba												
Serranus cabrilla												
Sarpa salpa												
Sphyraena viridensis												
Siganus luridus												
Fistularia commersonii												

Figure 41. An example of the data-sheet format used to monitor native and exotic fish in MEDPAN North project. Adapted from CIESM Tropical Signals programme.

Changes in the hydroids populations present on the rocky cliff of the Portofino MPA were assessed by comparing the results of a first sampling in 1980 with a second one in 2004. Hydroids populations were monthly sampled, along transects from the surface to 20 m deep. Both samplings were conducted in the same environment, by the same researchers and with the same procedures (Bavestrello et al. 2006; Puce et al. 2009).

III.2.3.2. Data (Graphs or Figures)

Data collected in the Portofino SPAMI for the LTER Network project shows a correlation between increases in the Mediterranean temperature in the last years and changes in some target fish species abundance, such as *Sarda sardinian* and *Sphyraena viridensis*. On the other hand, populations of key indigenous and non-indigenous termofilic fish species monitored following the CIESM Tropical Signals protocol are being successfully assessed within the Portofino SPAMI. Some results are shown below (Figures 42, 43).

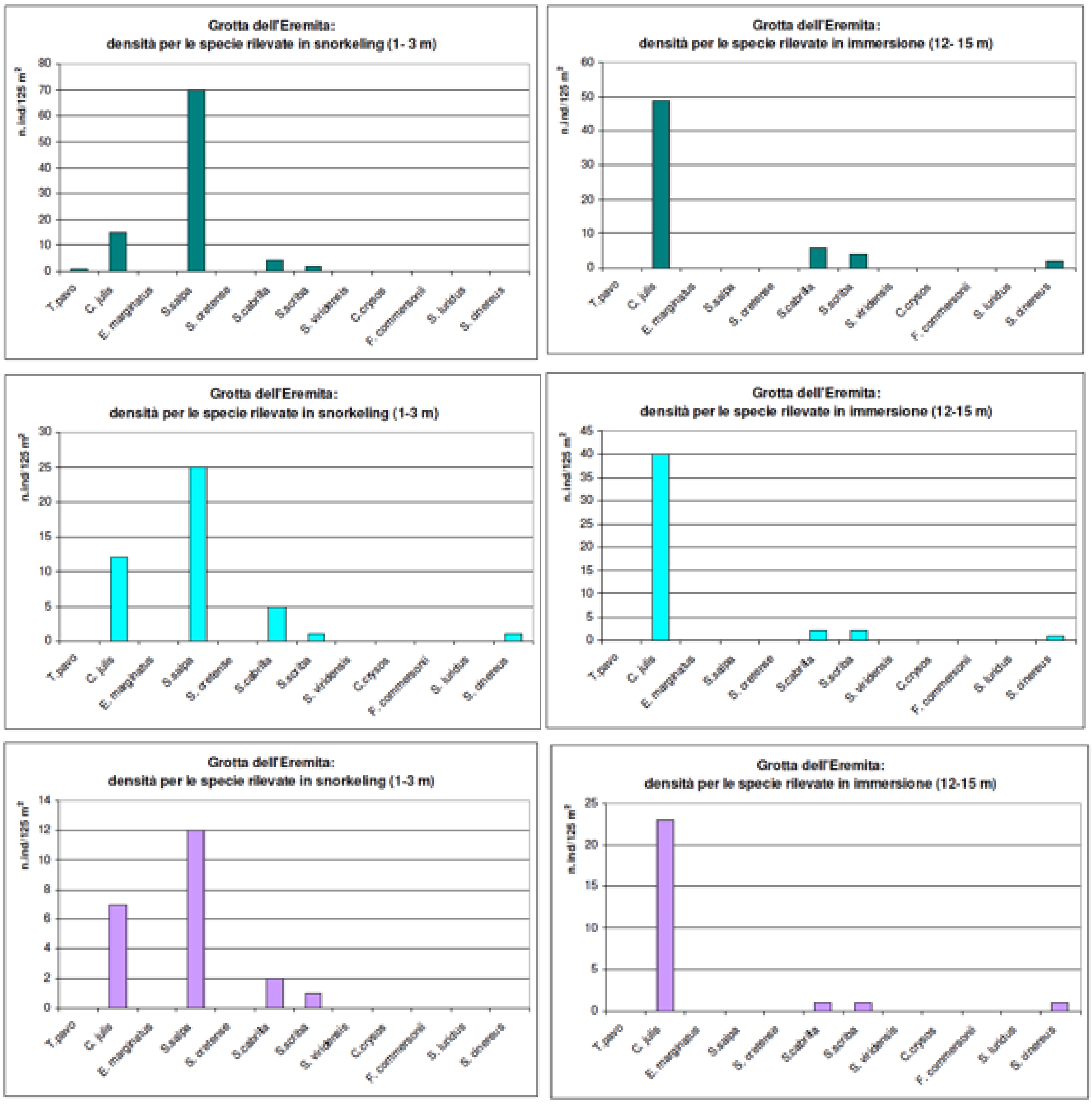


Figure 42. Key termophilic fish species observed in November 2012 (above), January 2013 (middle) and March 2013 (below) in Grotta dell' Eremita, at 1-3m depth (left) and 12-15m depth (right).

Censuses in the site of Lion's Head were carried out in November; the results are reported separately for snorkeling (1-3 m) and diving (12-15 m) surveys.

Figure 43. Key termofilic fish species observed in November 2012 (above), January 2013 (middle), and in March (below) in Testa del Leone, at 1-3 m depth (left) and 12-15m depth (right).

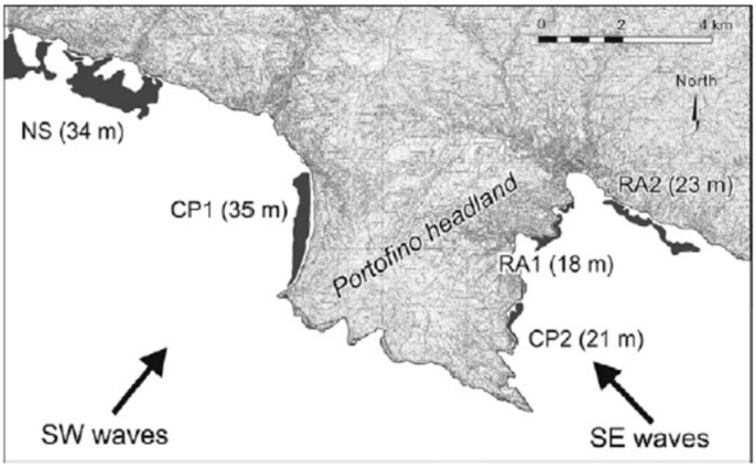
Regarding the changes in the composition and abundance of hydroids populations present on the rocky cliff of the Portofino MPA between 1980 and 2004, it was found that the number of cold-water species decreased, while that of thermophilic species increased; the period of activity of thermophilic species was extended and that of cold-water species decreased; there was a deeper displacement of all species observed as well. All these trends are a clear indication of sea warming over the past 25 years.

III.2.4. Reproduction and breeding date of selected species, including flowering of *P. oceanica* where present

III.2.4.1. Methodological description

The *Posidonia oceanica* meadows in the Portofino MPA are regularly monitored, and the flowering episodes are documented and recorded. However, no standardized protocols have been established at the moment to assess climate change effects on these habitats.

III.2.4.2. Data (Graphs or Figures)



P. oceanica meadows are well represented in the Portofino SPAMI, especially along the western coast of the Portofino Promontory, in the C zone, in an area sheltered from the Ligurian mainstream currents.

Figure 44. Distribution of *P. oceanica* meadows in Portofino, Italy (from Diviacco and Coppo, 2006 modified).

III.2.5. Episodic Species Outbreaks (blooms)

Positive thermal anomalies in the Portofino MPA have been associated with large-scale episodic species outbreaks (biological blooms), which in turn have resulted in mortality events (Cerrano et al. 2000). For instance, after the thermal anomaly recorded in 1999 there was a strong bloom of cyanobacteria in the reserve. Cyanobacteria covered many benthic species, some of which were unable to react, such as for example the yellow cluster anemone *Parazoanthus axinella*e, which ended up with most colonies covered by fungus and later dying (Cerrano et al. 2006).

Likewise, in the summer of 2003 took place the greatest explosion of mucilage (bloom) along the entire Portofino MPA, causing an important massive mortality event in the benthic community. Previous mucilage blooms in the area were seen only as sporadic blankets covering benthic organisms (Schiaparelli et al. 2007). The summer of 2003 was characterized by extremely high temperatures and anomalous temperature increase of seawater. In the summer of 2009, another mucilage event was monitored in the same location (Misic et al. 2011). Mucilage blooms in the Portofino MPA are frequent and recurrent and may represent an important threat to the benthic communities in the reserve.

Harmful microalgal blooms are also common in the Mediterranean Sea. These events are of health, ecological and economic concern. In the Portofino MPA, the benthic and planktonic cell abundances, and the periodicity and intensity of the blooms of the microalgae *Ostreopsis ovata* was monitored during 2007 and 2008 (Mangialajo et al. 2011). Although no critical intoxication events were recorded in the area during these years, the monitoring of both planktonic and benthic cells in the reserve is important because blooms may be involved in severe invertebrates' mass mortalities, potential modifications at the ecosystem level and loss of resources.

III.2.5.1. Methodological description

The mucilage outbreak was monitored in the Portofino MPA since its very beginning in May 2003. Rocky and soft bottoms were studied separately, with scuba diving at both 12m and 20m depth. The benthic organisms affected by the mucilage persistence were studied by underwater photography at random sites of the study area every week starting with the beginning of June. In each site the mucilage carpet was removed in an area of one square meter, and the effect of mucilage on benthic organisms was documented through digital pictures. Another mucilage bloom was monitored in the same area in the summer of 2009. A promising new monitoring methodology using time-lapse images to study the dynamics of the mucilage in a yearly fashion and to relate it to main environmental variables will be assessed in the near future in the Portofino MPA (S. Schiaparelli, pers. com.).

The assessment of *O. ovata* blooms in Portofino MPA was developed from June to November in both 2007 and 2008. The abundances of planktonic cells of *O. ovata* were estimated from sea water samples (from 250 ml to 1 l) collected using a plastic flask, at approximately 0.5 m depth. To estimate benthic cells abundances, samples of the dominant species of macroalgae were collected using a plastic bag, putting it over the chosen macroalgae, at 0.5–1 m depth, and delicately picking it up with the surrounding water. Both planktonic and benthic samples were fixed with 2–4% neutralized formaldehyde, or with a 1–2% Lugol's solution and then kept in the dark at 4 °C before countings.

Planktonic cells concentrations in the water samples were evaluated with an inverted microscope using the Utermöhl method (settling a minimum of 50 ml of sea water). The abundance of benthic cells was evaluated after vigorously shaking the macroalgae samples, followed by rinsing and filtration through a 200–500 µm mesh. *Ostreopsis* cells were then counted following the Utermöhl method, or using a direct microscope with calibrated squared chambers. Results were expressed as the abundance of planktonic cells per liter and of benthic cells per gram of fresh weight of macroalgae.

III.2.5.2. Data (Graphs or Figures)

The abnormal mucilage event in 2003 was immediately monitored and detailed described (Schiaparelli et al. 2007), as well as another episode in 2009 (Misic et al. 2011). In 2003, the event affected the whole stretch of coast of the Portofino Promontory (about 4 km), completely covering all horizontal and not steep rocky surfaces, between 10 and 25m depth (figures 44, 45). No particular signs anticipated the mucilage outbreak and, in about 2 weeks, almost all rocky bottoms and most of the benthic organisms, such as *P. oceanica* and the octocoral *E. singularis,* were densely covered by a carpet of mucilage about 10cm thick and almost engolfed. Deep currents reduced the mucilage to a filamentous form, and a strong storm moved most of the mucilage from shallow to deep areas, where it covered and strongly affected the red sea-fan *Paramuricea clavata*.

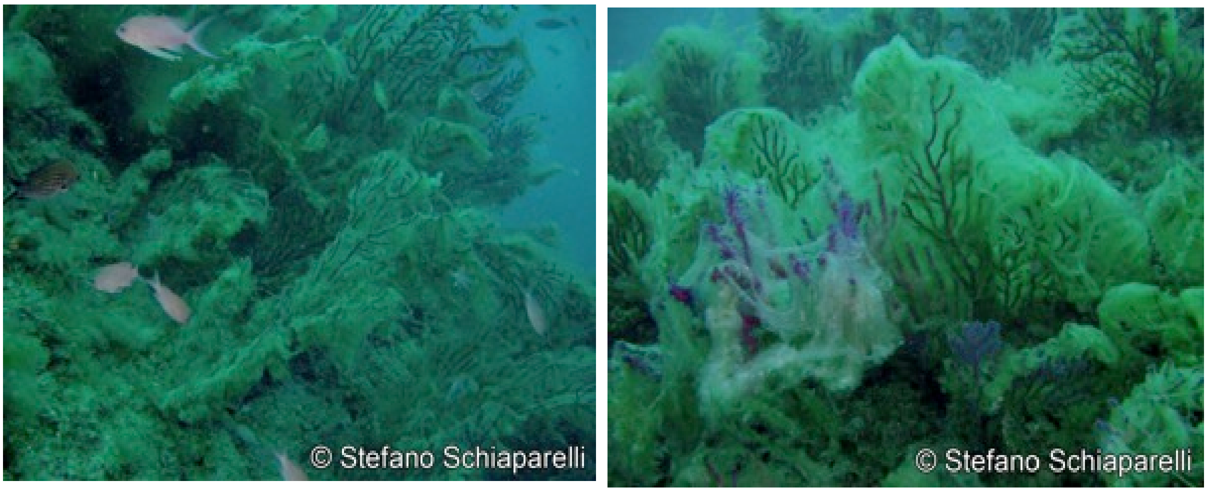


Figure 45. Mucilage aggregates covering benthic organisms in the Portofino promontory. Punta Vessinaro, 20 m depth, July 2003. Photo credit: Stefano Schiaparelli.

The persistence of mucilage took away the zooxanthellae of the tissues of the scleractinian coral *Cladocora caespitose* and caused its bleaching. Other organisms suffered necrosis (e.g. sponges), while others lose the integrity of their skeleton structure (e.g. corals). In addition, the degradation of the organic substance created anoxia conditions, which then led to the death of the entire community.

In 2009, the calm meteorological and sea conditions conducted to the thermal stratification of the water column, which in turn influenced the development of mucilage above the pycnocline. Similar to the event in 2003, the mucilage progressively moved from shallow depths to below the thermocline. The development and fate of the mucilage, as well as its interactions with the surrounding environment, were principally regulated by physical features. In the oligotrophic conditions of Portofino, as long as mucilage persistence doesn't cause severe oxygen depletion, certain compartments of the ecosystem are able to promptly respond and take advantage of the mucilage event (mucilage representing a new available trophic source). Therefore, while rocky-bottom communities are damaged by the mechanical forcing and experience loss of macroalgae biomass, this mucilage event seemed to have positively influenced the surrounding seawater and the soft-bottom biogeochemical processes. However, mucilage persistence may cause suffocation and macroalgae biomass depletion.

Figure 46. Mucilage aggregates covering benthic organisms in the Portofino promontory. Above: Targehetta, 20 m depth, July 2003. Photo credit: Stefano Schiaparelli. Below: Portofino MPA, 10 m depth, July 2015. Photo credit: Carlo Cerrano.

The monitoring of *O. ovata* found that the higher peak abundances of this benthic and planktonic microalgae occurs in mid-summer, by the end of July and early August. Benthic and planktonic cell abundances are correlated, so benthic cells can be considered as the stock of available biomass. By 2008, both benthic and planktonic cell abundances in Portofino MPA were low, never reaching 10,000 cells/l, quite far from harmful levels for human or ecosystems health.

Table 2. Synthesis of available data collected in the Portofino MPA in 2007 and 2008. EMV: Epibenthic max. value; PMV: Planktonic max. value. In bold the max. values for each year.

Site (macroalgal species)	Year	Month	Sampling dates (day of the month)	EMV	EMV date (T ^{O}C)	PM V	PMV date (Temp ^{O}C)
Portofino (*Stypocaulon scoparium*)	2007	June	7, 14, 21	1530	14 (21.8)	75	7 (20.6)
		July	17, 20, 26	**43,896**	17 (25.10)	**282 0**	20 (25.4)
		August	1, 7, 13, 24, 29	285	7 (25.2)	180	29 (23.0)
		September	7	4947	7 (22.2)	140	7 (22.2)
		October	2, 23	2036	23 (20.2)	70	2 (21.5)
		November	26	145	26 (16.0)	40	26 (16.0)
	2008	June	11, 24	248	24 (-)	20	24 (-)
		July	16, 28	**10,431**	16 (-)	**420**	28 (25.4)
		August	19, 27	3405	27 (25.8)	280	27 (25.8)
		September	-	-	-	-	-
		October	-	-	-	-	-
		November	-	-	-	-	-

III.3. Torre Guaceto, Italy

Below is a detailed map of habitat distribution in Torre Guaceto SPAMI that has been recently produced (Figure 47).

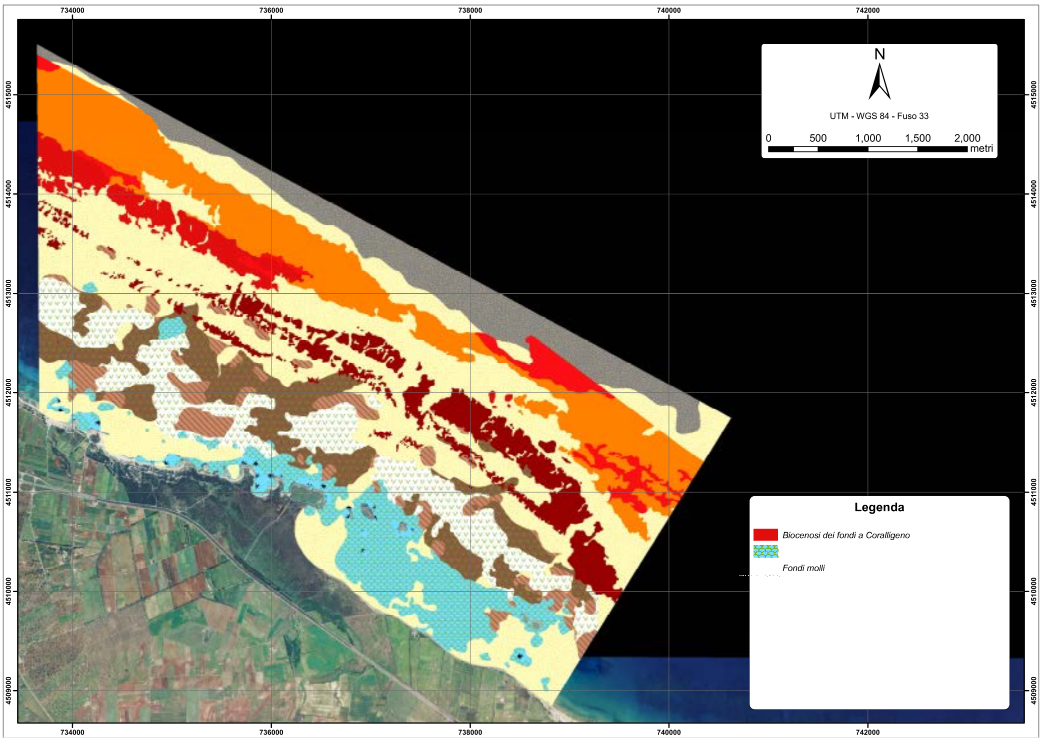


Figure 47. Biocenotic map of Torre Guaceto SPAMI. From: Fraschetti et al. (2014)

III.3.1. SST and thermal stratification

III.3.1.1. Methodological description

Water temperature (Figure 48) and other main environmental parameters are regularly collected by the local Regional Agency for Environmental Protection (ARPA) and provided to the Torre Guaceto MPA staff.

III.3.1.2. Data (Graphs or Figures)

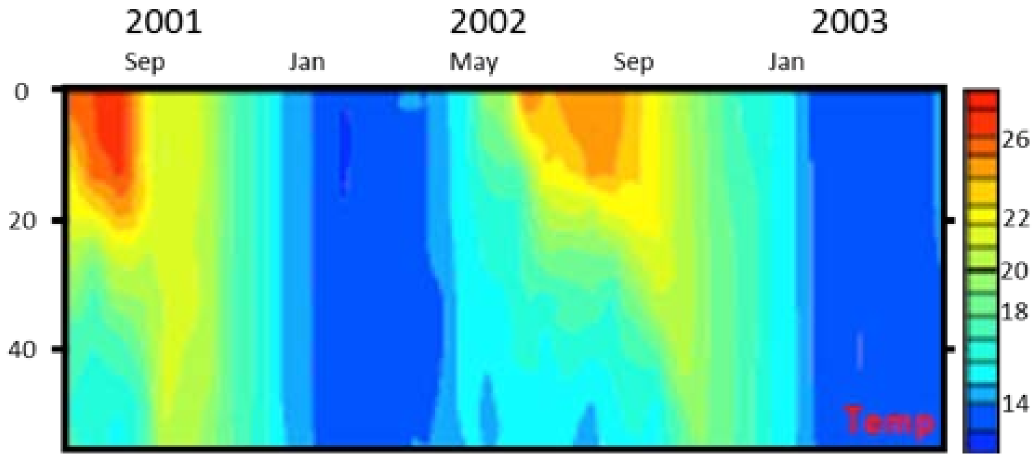


Figure 48. Sea-temperature profile data collected in Torre Guaceto SPAMI.

III.3.2. Mortality and bleaching events

III.3.2.1. Methodological description

The health conditions of coralligenous is occasionally monitored evaluating gorgonian colonies as indicators and comparing protected to not-protected sites.

Considering the limited depth of the area, coralligenous hosts mainly *Eunicella cavolini*, *E. singularis,* and *Cladocora caespitosa. Paramuricea clavata* and *Corallium rubrum* are not present in the MPA.

Four study sites located in Torre Guaceto SPAMI were assessed along 25m long transects. For each transect, density and height/diameter of colonies was recorded. For each colony (both *Eunicella* spp and *C*. *caespitosa*) the health status was detected considering 5 categories: 1. healthy (no sign of necrosis or epibiosis), 2. Necrotic (15-20% of damaged scleraxis), 3. Dead colony, 4. Detached colony (broken or laying on the bottom), 5. Colony with epibiosis.

III.3.2.2. Data (Graphs or Figures)

The comparison between protected and unprotected sites highlighted important differences in health conditions of the considered species. Colonies under protection were more healthy compared to colonies in not-protected sites (Figure 49). Considering the small size of the MPA, thermal anomalies can be considered similar in protected and unprotected sites. Therefore, differences in health conditions could be due to different level of antropic pressure (local effects). As reported in Vezzulli et al. (2013), fishing lines entangled in the colonies or the mechanical stress resulting from intense diving activities could negatively affect both resistence and resilience of coral colonies to thermal stress.

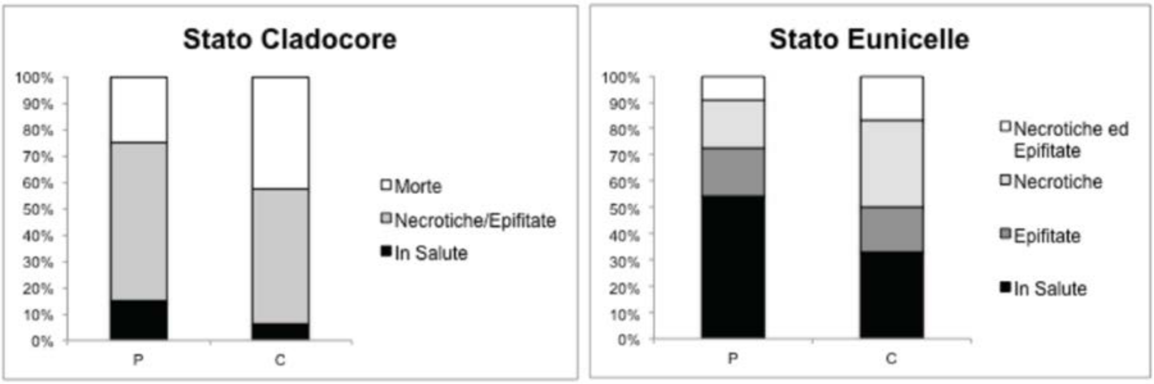


Figure 49. Relative percentage of the health conditions of the coral colonies inside and outside, respectively, of Torre Guaceto MPA. Left: coral *Cladocora*. White bars represent death, gray necrosis and epiphytes, and black healthy. Right: coral *Eunicella*. White bars represent necrosis and epiphytes, light gray necrosis, dark gray epiphytes, and black healthy. P = inside the MPA, C = outside the MPA. From: Fraschetti et al. (2014).

III.3.3. Range shift of alien species/temperature-sensitive species

Marine alien species in the Torre Guaceto SPAMI, and in other MPAs in Liguria, have been monitored by volunteer university students and members of the public since 2009, by the NGO For-Mare. The abundance and distribution of alien species in the reserve have been assessed through a series of summer training courses on Marine Applied Ecology, between June and September (Otero et al. 2013).

On the other hand, the non-indigenous thermophilic invasive green algae *Caulerpa cylindracea* has been extensively studied in the Torre Guaceto SPAMI. The species was reported for the first time in the Mediterranean in 1926, along the Tunisian coast (Hamel, 1930), and then, since the early 90's, it widely spreaded along the Italian coast. This invasive species can affect native ecosystem by decreasing biodiversity (Ceccherelli et al. 2002). It has the potential, for example, to invade seabeds of seaweds, changing the structure of the habitat and the distributional patterns of the organisms that live there.

The distribution of *C. cylindracea* in the Torre Guaceto SPAMI has been assessed in several studies, such as for example Fraschetti et al. (2005) and Ceglie et al. (2008). The spatial distribution of this alga is included in the biocenotic map of the reserve (Figure 47), and its presence and abundance is considered in the periodic assessment that this MPA develops on the main pressures acting in the reserve. Moreover, the effects of the presence of *C. cylindracea* on the foraging habits of the larger bream (Diplodus sargus), and its consequences on the fish fisiology have been assessed in the Torre Guaceto and Porto Cesareo MPAs by researchers of the University of Salento and the National Inter-University Consortium for Marine Science.

Finally, local knowledge is an important alternative information source in this SPAMI. Fishermen and divers use to inform on the presence and dynamics of non-indigenous or indigenous thermophilic species.

III.3.3.1. Methodological description

Volunteers from the NGO For-mare are provided with underwater digital cameras and guided by teachers on a brief snorkelling tour in the study area, where they can learn and gain confidence with species identification before starting any survey. Visual censuses are performed with both diving and snorkeling techniques. Volunteers play a leading role in data collection and interpretation in strict collaboration with instructors and experts.

The distribution of benthic assemblages, including *C. cylindracea* in the Torre Guaceto MPA was mapped in Fraschetti et al. (2005). Data on the distribution of dominant habitats were collected at depths up to 50m depth, with visual censuses conducted by snorkelling (at depths shallower than 15m) and SCUBA diving (between 15 and 50m depth). Between May and October 2002, three sites were sampled at each of four locations. At each site, ten 20x20cm random quadrats were used to evaluate in situ the percentage cover of sessile organisms.

The invasive green algae *C. cylindracea* was surveyed in Torre Guaceto SPAMI in Ceglie et al. (2008). Surveys were conducted with scuba diving, from the surface down to 35m depth, between April and October 2007. In addition to the presence of the algae, the type of substrate (rock, sand, mud, etc.), exposure, depth and colonization mode (patchy, sporadic rhizoids, lawn) were registred.

III.3.3.2. Data (Graphs or Figures)

In 2002, *C. cylindracea* was present in the shallow subtidal habitats of the Torre Guaceto SPAMI, with a percentage cover of up to 12% (+- 5.4) (Figure 50). By 2007, this species had widely colonized a large variety of substrates including rocky beds of organic origin (e.g. coralligenous biocenosis, dead Posidonia mats), sandy and muddy bottoms, and artificial substrates (e.g. breakwaters, wood, PVC) within and outside the reserve. *C. cylindracea* is very abundant in the Torre Guaceto SPAMI, where its distributed from surface to at least 35 m depth. In gentle hydrodynamic conditions this algae form large and dense meadows, while in superficially exposed areas it is present in small scattered patches (Ceglie et al. 2008).

The presence of *C. cylindracea* in the reserve has changed the foraging habits of breams, as it has become one of the main foods in the diet of this important fish (up to 88% of the stomach content). Breams feeding on this algae accumulate caulerpina in their tissues, which could lead to a state of health detriment and altered behaviors, potentially preventing the reproductive success of fish populations. Thus, *C. cylindracea* can alter food webs and may cause changes in fish stocks, and also, in the effectiveness of the biodiversity protection. *C. cylindracea* is one of the threats that persist in this SPAMI.

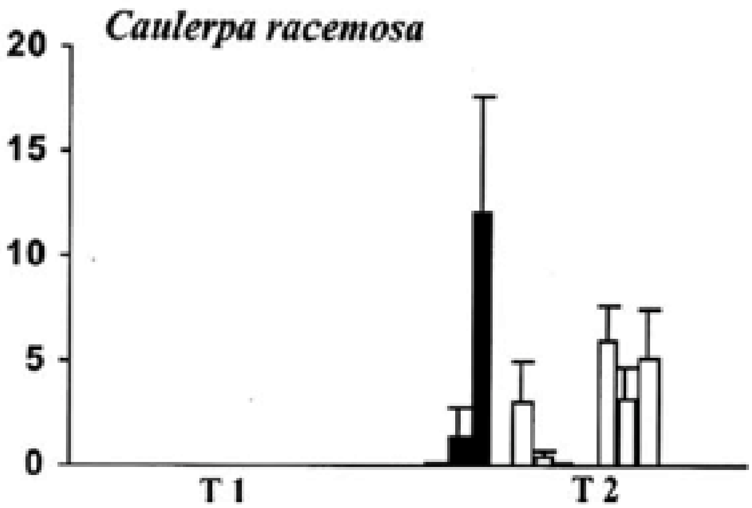


Figure 50. Mean percentage cover (SE, n . 10) of *C. cylindracea* in the shallow subtidal area. Data are shown for three sites (black bars) and controls (white bars) in two sampling times (T1, T2). From: Fraschetti et al. (2005).

Fishermen and divers have informally informed on the presence and dynamics of non-indigenous or indigenous thermophilic species, such as for example the Bluefish *Pomatomus saltatrix* and *C. cylindracea*. The Bluefish is currently perceived by artisanal fishermen operating in the AMP as a significant threat to the local fishing communities and to their own economy.

III.3.4. Reproduction and breeding date of selected species, including flowering of *P. oceanica* where present

The Torre Guaceto SPAMI monitors several aspects of *P. oceanica* meadows, such as for example the density, fenology and lepidocronology, but not the flowering events of this plant.

III.3.5. Episodic Species Outbreaks (blooms)

In Torre Guacerto SPAMI, blooms of jellyfish and other gelatinous creatures are commonly sighted in summer. Torre Guaceto SPAMI has become part of a big international “Cross-Border Cooperation” project aimed at the monitoring of jellyfish outbreaks under anthropogenic and climatic impacts in the coastal Mediterranean Sea, The MED-JELLYRISK project. This project includes 5 partner institutes from Italy, Malta, Spain and Tunisia, and a large network of experts on jellyfish proliferations.

The implementation of the MED-JELLYRISK project in the Torre Guaceto SPAMI is being done through annual summer trainings of volunteers. With the guidance of qualified instructors, the volunteers learn how to review, catalog and study the behavior of jellyfish encountered during exploration activities off the coast at sea.

IV. PROTOCOLS

The assessment of the status and trends in populations and ecosystems in MPAs and the potential climate change risks that may affect their structure and function is essential to the success of biodiversity management and conservation. At present, a great variety of techniques, tools, and technologies are used to monitor major indicators of climate change and detect impacts to marine ecosystems. The sampling frequency, complexity, spatial context, and duration usually vary between different countries within the Mediterranean basin, and also among MPAs. A shared, common vision is essential for a Mediterranean network of MPAs to be a reality. Establishing common, widely accepted protocols for the monitoring of climate change indicators across Mediterranean MPAs, starting from SPAMIs, could contribute to this effort and help MPA’s personnel, scientists, locals, and others determine the biological condition and trends in these areas in a consistent, verifiable way. The data collected through monitoring can be useful for developing plans to protect the ecosystem’s biological capacity. However, the availability of secure funding to build and maintain a common long-term database is critical.

This document describes below a series of standard monitoring protocols for five major indicators considered of priority interest to monitor and understand the impact of climate change on Mediterranean MPAs biodiversity (UNEP(DEPI)/MED WG.382/Inf.13 2013)). Because one single monitoring protocol will not fit the necessities of all MPAs, different commitment levels are provided so that each MPA can choose the best monitoring option depending on its own possibilities. All the protocols include easy and cost-effective methodologies, involve citizen science, are able to build a Mediterranean view, and guarantee that the data collected can be integrated to existing basin-scale, national, or international networks.

IV.1 Sea Surface Temperature and thermal stratification protocol

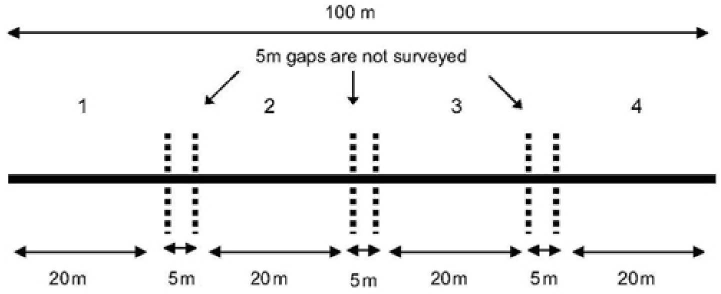
<table>
<tr><td>PROTOCOL 1</td><td colspan="2">Indicator: Sea Surface Temperature and Thermal Stratification</td></tr>
<tr><td>Problem / scientific question</td><td colspan="2">Sea Surface Temperature (SST) has increased in the recent years and is linked with alterations of the vertical thermal structure and deepens thermocline.
Only high resolution temperature series allow the evaluation of the impact of thermal anomalies on marine communities</td></tr>
<tr><td>Scientific rationale (hypotheses)</td><td colspan="2">• The small volume of the Mediterranean Sea predicts a faster reaction to climate warming than the open oceans
• Potential bio-ecological effects on deeper areas are linked with alterations of the vertical thermal structure and deepen thermocline
• Large scale mass mortality events of long-lived benthic species have been related to the anomalous temperature conditions
• Growth, distribution, composition and diversity of marine species are being altered.</td></tr>
<tr><td rowspan="6">Sampling program</td><td>Metrics (variable / indicator)</td><td>Temperature measurements at different depths (^{0}C)</td></tr>
<tr><td>Type of survey</td><td>Scientific – technical survey</td></tr>
<tr><td>Sampling technique</td><td>Autonomous temperature data loggers</td></tr>
<tr><td>Sampling unit (SU)</td><td>Temperature Data logger (Degrees Celsius)</td></tr>
<tr><td>Spatio-temporal distribution of SUs and sampling size</td><td>- Sampling sites: inside the SPAMI, or ideally inside and outside the MPA.
- The number of sampling sites will depend on the extension and oceanographic features of the MPA.
- 1 Sampling point per sampling site.
- At each sampling site: 1 sensor every 5 meter along a slope, from sea surface to 40-50 m max. depths.
- Continuous monitoring: every hour, recovering and deploying back the data-loggers 2 times/year.</td></tr>
<tr><td>Data analysis</td><td>T-MedNet web application for uploading and verifying the data and securely backing up all these records. Semiautomatic routines are set up in the web and are very efficient at producing summary reports on temperature conditions, including figures and temperature descriptors (mean, coefficient of variation, etc.).</td></tr>
<tr><td>Detailed procedure</td><td>Before</td><td>Site selection:
- Sensors can be fixed to rocky substrates or along buoy lines or chains.
- if geography allows, select sites exposed to dominant winds and currents
- In seagrass meadows, loggers can be installed within the canopy.</td></tr>
</table>

		- On beach environments, sand temperature can be measured with similar devices buried at different sites (i.e. nesting beaches) Prepare data loggers: - Label each logger with its depth and location - Replace the top cover - Using the software, check: Battery level, fill the File name (site name_date_depth), set up the data logging interval (1hour), launch time Before the field trip: Watch the video on setting up new fixation points from http://www.t-mednet.org/observation-system/video Prepare the fixation plan (3 options): - Option 1: Using putty in natural holes to fix plastic screws or clips where attach the logger. - Option 2: Using spits in drilled holes to fix plastic screws or clips where attach the logger. - Option 3: Using tight raps to fix the logger to surface or subsurface moorings (buoys), on ropes, chains.
	During	Deploying data loggers: - On the boat, plan of recollection and fixation at each site with divers before the dive. - Recollect and attach data loggers from deeper to shallower depths. - At each depth, collect old data logger and replace it with a new one - Check the fixation and attach a mark for rapid finding - Collection: Cut the plastic tights and take the loggers (remember they float)
	After	- Clean the data-loggers - Download the data collected - Upload data in T-Mednet web page. - Analyze data
List of material needed	Setting permanent sites: • scuba diving gear • GPS • Putty • Tight raps • Data loggers • Tape • Handle bag net mesh	
Personnel needed (specialization	*Note that one person may do several of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • Boat Captain	

level)	• Scientific personnel to prepare, set up, recover and clean the data loggers • Scientific personnel to upload the data collected • Scientific personnel to analyze the data • Divers with advanced diving license to recover and to fix the loggers	
Budget (tentative)	Personnel (permanent or temporary)	1 coordinator (volunteer) for all the Mediterranean MPA. Every 6 months, 2 full-months salary for 2-3 scientist(s) for each MPA to coordinate the activity (recover and install new data loggers), upload and analyze the data
	Durable equipment	GPS, Data loggers
	Consumables	Putty, Tide-wrap
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem
	Other costs (including overheads)	Trainings for data analysis or software Loss of data logger Webpage maintanace
	Subcontracting	
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Contribute to the T-MEDNET regional database creating high resolution data series of temperature in the Mediterranean Sea.	

IV.2 Mortality and bleaching events protocols

PROTOCOL 2	**Indicator:** Mortality and bleaching events- Snorkeling and Skin diving
Problem / scientific question	Temperature anomalies trigger a series of cascade effects leading to the tissue necrosis and mortality of several benthic species. The most visible effects can be dectected on sponges, gorgonians, corals, mollusks, bryozoans, echinoderms and tunicates. The disappearance of these organisms can be locally very important and dramatically affect the ecosystem functioning. Unfortunately the monitoring of these events is discontinuous both spatially and temporally. The standardization of underwater observations in each MPA will greatly improve knowledge o these phenomena and suggest management measures to mitigate their negative effects.
Scientific rationale	- Temperature anomalies trigger mortality and bleaching events - Areas damaged by fishing lines or impacted by massive diving activities are more vulnerable to massive mortality

(hypotheses)	- Occurrence of these events is seasonal and related to the depth of thermocline and to the sea conditions.	
Sampling program	Metrics (variable / indicator)	presence and abundance of damaged organisms (# individuals/20m transect) Level of damage Level of epibiosis (only for gorgonians)
	Type of survey	Option 1: Volunteers – citizen survey Option 2 : Scientific – technical survey
	Sampling technique	Snorkeling (transects looking for damaged/dead or bleached organisms) and Scuba diving
	Sampling unit (SU)	Snorkeling: four 20 m long transects with 5 m intervals (100 m long, parallel to the coast) (Annex 2)  Diagram of a transect line. This 100 m line is divided into four 20 m segments with a 5 m gap in between them to ensure sample independence. Scuba diving: four quadrats (50 x 50 cm) are randomly sampled from 40 (below the average depth of the thermocline) to 10 m depth (tentatively the upper limit of the gorgonian population) every 5 m, keeping the same exposition (Annex 3)
	Spatio-temporal distribution of SUs and sampling size	- Sampling locations: Protected areas of the MPA, or ideally in protected and unprotected areas of the MPA 3 sampling sites per location (1-5 km apart from each other) - # SUs per site: Snorkeling: 3 transects/site; Scuba diving: 4 quadrats per depth interval - Frequency: Min. 2 times/year: after summer (Sept/Oct) - late spring (Apr/May) Or 4 times/year: late spring (Apr/May), hottest period (Jul/Aug), end of summer (Sep/Oct), and coldest period (Feb/Mar)
	Data analysis	Basic descriptive statistics (e.g., mean, standard deviation, range), ANOVA. For quadrat photos-CPCe point-counting software or photoquad (http://www.mar.aegean.gr/sonarlab/photoquad/index.php)
Detailed procedure	Before	<u>Choose sampling locations</u>: Protected areas of the MPA, or protected and unprotected areas of the MPA <u>Select sites</u>: Include most and less human disturbed areas. Should be representative of the diversity of

		habitats present in the MPA Determine sampling frequency: Minimal: twice/year - after summer/late spring Optimal: four times/year Train participants: - Organize activities to disseminate and explain the project through direct involvement of dive centers. - Develop training sessions to introduce participants with species identification, the recognition of the tipology of tissue lesions or bleaching events, the monitoring methodology: • provide copies of the data-sheets (Annexes 2 and 3) and explain in detail how to properly fill them in. • trainees should practice the recognition of the species of concern (directly in the field or seeing diverse images,), and try the surveys with the quadrats. • trainees should learn how to share their data with managers of MPAs Better to familiarize the observers with the methods and tools to be utilized. For example, adjust observer's ability to recognize healthy from damaged colonies and their percentage of damage.
	During	• General information to be gathered: Name of Observer, Date, Name of site, Geographical coordinates, exposition • General observations to be gathered on other climate change indicators: Sea temperature (if possible) Detailed description of the survey: - Work in pairs, each person develops one survey - Fill in the upper portion of the data-sheet (General information) Note: * If the observer is not sure of species ID, reference photographs of the species should be taken and, if possible, a sample (plastic bag). * Photographs must be catalogued: date, depth, site location.
	After	- Create a database where collected information will be registered (baseline). - Introduce collected data into baseline. - Analize the data. - Create e-tools to facilitate citizens to report disease/mortality episodes. Or upload data in existing tools and networks, e.g. www.climcares.medrecover.org, http://www.observadoresdelmar.es/projecte-1-que-pots-fer-tu.php, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016). - Divulge results in both the science and citizen environment
List of material needed	For the surveys: • scuba diving gear	

<table>
<tr><td></td><td colspan="2">• GPS
• underwater photo camera (if available)
• 50x50 cm PVC quadrat
• 1 meter to mesure colonies height
• plastic sheets to write observations and measures

For the training:
• Images of the species involeved in massive mortalities
• Samples of the monitoring data-sheets (one per person)
• Underwater photo camera
• All needed for each monitoring sampling (see below), but adjusting the quantities of material to the N_o of trainees)

For each sampling (and pre-survey):
• snorkeling/scuba diving gears
• submersible watch (one per observer)
• GPS
• plastic board (one per observer)
• pencils (one per observer)
• underwater data-sheets to register observations (one set per observer)
• underwater photo camera (if available; ideally one per pair of observers)
• plastic bags to collect samples
• metric tape or rope (>100m long) (one per transect)
• permanent marker
• PVC quadrat</td></tr>
<tr><td>Personnel needed (specialization level)</td><td colspan="2">• Divers to locate and shoot photos to benthic quadrats
• Scientific personnel to identify species (in field, from photos, videos, or samples collected)
• Scientific personnel to lecture on species identification and monitoring methodology
• People to preserve the collected samples and take reference photos
• People to introduce the data collected into database
• Scientific personnel to analize the data (statistics, CPCe point-counting software)
• Scientific personnel to divulge results in both the science and citizen environment</td></tr>
<tr><td>Budget (tentative)</td><td>Personnel (permanent or</td><td>* Note that one person may do more than one of the different tasks, e.g. a scientific diver could do both the tasks for a scientist and the tasks for a diver.</td></tr>
</table>

	temporary)	Snorkeling: 2 full days salary for two professional divers to collect data along horizontal transects. Scuba diving (for each sampling period): • 4-6 full days salary for two professional divers to collect data along vertical transects by quadrats * professional diving license may be required in some countries. • 1 week/year salary for two lecturers on species identification/monitoring methodology in the training • 2 weeks salary/year for one person to divulge the project/results among scientists and stakeholders • 1 month salary/year for one data analyst (statistics, CPCe point-counting software) * boat captain (in some cases)
	Durable equipment	GPS, Scuba diving gear, Fins, mask and snorkel, Photo camera (underwater if available), Submersible watch (one per observer), Computer, Boat
	Consumables	Plastic boards and pencils, 50x50cm PVC quadrat, Images of the target species, Copies of the monitoring data-sheets, Underwater monitoring data-sheets to register observations (one set per observer), Plastic bags to collect samples, Permanent marker
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Varing, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	Scuba tanks, Scuba tanks filling, * boat
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers	

*** See protocol 4a for the monitoring of mortality events of *P. oceanica.***

IV.3 Range shift of temperature-sensitive species protocols

PROTOCOL 3a	**Indicator:** Range shifts of alien/native temperature sensitive species – Snorkeling and Skin diving
Problem /	Alien/thermofilic species are extending their geographic range in response to a higher availability of temperate waters in the

scientific question	Mediterranean Sea. Indigenous cold-water species, in turn, are contracting their distributional range and moving to deeper, colder waters to escape termal stress, at least temporarly.	
Scientific rationale (hypotheses)	- Native warm-water species are extending their range distributions northwards. - A higher number of tropical/subtropical thermophilic species are arriving the Mediterranean (some of them highly invasive). - Arriving species may occupy indigenous species' niche and drive them to local extinction. They may represent new fishery resources, though. - Cold-water environments in the Mediterranean are becoming scarce. - Cold-water species are compiting with thermophilic arriving species for resources. - Cold-water species are moving northwards and deeper, declining in number, and may become locally extinct. - All these may result in important lossess of biodiversity.	
Sampling program	Metrics (variable / indicator)	presence and abundance of thermophilic (alien/native) and cold-water species (# individuals/100m transect)
	Type of survey	Option 1: Volunteers – citizen survey Option 2 : Scientific – technical survey
	Sampling technique	Snorkeling and Skin diving
	Sampling unit (SU)	Transect 100 m long, parallel to the coast If possible, also quadrat 40*60cm
	Spatio-temporal distribution of SUs and sampling size	- Sampling locations: in protected areas of the MPA, ideally in protected and unprotected areas of the MPA - 3 sampling sites per location (1-5 km apart from each other) - Depth strata per site: 0-3m - # SUs per site: 2 transects/site and 3 quadrats/transect - Min. 2 times/year: after summer (Sept/Oct) - late spring (Apr/May), ideally 4 times/year: late spring (Apr/May), hottest period (Jul/Aug), end of summer (Sep/Oct), and coldest period (Feb/Mar)
	Data analysis	Basic descriptive statistics (e.g. mean, range), ANOVA. For quadrat photos-CPCe point-counting software.
Detailed procedure	Before	<u>Choose sampling locations</u>: Protected areas of the MPA, or protected and unprotected areas of the MPA <u>Select sites</u>: Include most and less human disturbed areas. Should be representative of the diversity of habitats present in the MPA <u>Determine sampling frequency</u>: Twice/year - after summer/late spring or four times/year <u>Set up permanent monitoring tools:</u> • Set up two 100m permanent transects per site: - Nail a short rod (about 25cm long) into substrate until firmly fixed. Swim 10m parallel to the coast and nail a second rod (can use a metric tape to measure the distance between rods. Make sure to keep a

		depth 0-3m). Repeat the process 10 times (the last rod will be 100m apart from the first one). - Then firmly tie a flagging tape to each rod. - Mark the transect location with a GPS or drawing a detailed map showing the starting and end point of the transect. You can also photograph a peculiar, permanent feature of the shore from the first rod. - With a rope, firmly tie a small buoy to the first rod (it should float 20-30cm above the rod). Repeat for the last rod. - Repeat the process to set another additional transect at 0-3m depth. Each transect should be located 1-5km apart from each other. • Set up permanent benthic photoquadrats (if possible): - Select at least three areas along each transect to place the permanent benthic photoquadrats (focus on areas where invasive species may be present, or where different benthic communities grow). - Permanently mark the location of each of these areas, guided with a 60x40cm PVC quadrat, by nailing a stake and a plastic mark with an ID number (e.g. livestock ear tags) at the left low corner of the quadrat (interior) and another one at the right up corner (interior). Register the distance of these points from the first rod in case taggs are lost or overgrowned by algae. Train participants: - Organize activities to disseminate and explain the project through direct involvement of dive centers and fishermen. - Develop training sessions to introduce participants with species identification, the monitoring methodology, and the upload of observations with the application MedMIS: • provide copies of the data-sheets (Annex 4) and explain in detail how to properly fill them in. • trainees should practice the recognition of the species of concern (directly in the field or seeing diverse images, e.g., the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)), and if possible, the estimation of abundances. • trainees should practice locating, placing and shoting photos of the photoquadrats • trainees should learn: how to download the application (for cell phones: App store, Google play) or access the MedMIS platform (for computers: http://www.iucn-medmis.org/?c=About/show), create an account in MedMIS, and report sightings in MPAs Conduct Pre-survey (if possible) To familiarize the observers with the methods and tools to be utilized. For example, adjust observer's swimming speed to cover 100m in approx. 15 min.
	During	Census methodology adapted from the CIESM tropical signals Programme • General information to be gathered: Name of Observer, Date, Name of site, Start and end time of survey, Sea condition (rough, calm,etc.), Underwater horizontal visibility (m), Shore exposure (exposed,

		moderately exposed, sheltered), Slope of seabed (steep shore, flat ledges), Type of substrata (rocky, boulders, sandy/ gravelly, mixture of soft and rock bottoms), Type of community (e.g. seagrass meadow) and dominant species if any. • General observations to be gathered on other climate change indicators: Sea temperature (if possible), *Posidonia ocenica* flowering, bleaching or mass mortality/necrosis, blooms (e.g. of jellyfish). Detailed description of the census: - Work in pairs, each person develops one survey - Locate at one of the permanent transects - Fill in the upper portion of the data-sheet (General information) - Tie the measuring tape/rope to the first rod of the transect - Extend the measuring tape/rope while swimming slowly and at constant speed for 15 min. along the entire transect, following the rods. - While swimming, look for the target species for 15 min. Assign to each species found a category of the ACFOR species abundance scale (see below), or record as P (present) or N (none, if apparently absent) - Time used during the survey to take photographs, collect samples or dive to have a closer look to the species, should be added to the standard total 15 minutes. - Before leaving, have a quick swim around the sampling area to confirm first impression of species presence and relative abundance. - Fill in the “General observations to be gathered on other climate change indicators” in the data-sheet. - Repeat the process with the other transects. Note: * If the observer is not sure of species ID, reference photographs of the species should be taken and, if possible, a sample (plastic bag). * Photographs must be catalogued: date, depth, site location. The ACFOR abundance scale has 5 categories: • **A=** Abundant – found almost everywhere, covering extensive areas in cover or in N_o of individuals; (approx. cover >40% or number of individuals >1000/m^2) • **C=** Common – found almost everywhere, but not dominant as category A (approx. cover 20-40% or N_o of individuals 100-1000/m^2) • **F**= Frequent – found in many places with little searching (approx. cover 10-20% or N_o of individuals 10-100/m^2) • **O**= Occasional – found in few places after accurate searching (approx. cover 5-10% or N_o of individuals

		1-10/m^2) • **R**= Rare – found in one or two places only after intense searching (approx. cover 1-5% or N_o of individuals 1-10/10m^2) In addition, the observer should record also the following: • **P**= Present –the species is present but the abundance is not meaningful (that is only one individual or only one micropatch of algae) • **N**= None – the species is apparently absent. <u>Please note (about the scale):</u> * Where two or more species exist, for instance foliose algae overgrowing crustose algae, total percentage cover can be over 100% and the abundance category will reflect this. * Use quadrats as reference frames for counting, particularly when density is borderline between two categories of the scale. * Some extrapolation of the scales may be necessary to estimate abundance for restricted habitats (e.g. rockpools).
	After	- Take reference photos and preserve the collected samples. - Provide samples to experts for identification as soon as possible. - Create a database where collected information will be registered (baseline). - Introduce collected data into baseline. - Analize the data. - Share data with CIESM tropical signals program. - Create e-tools to facilitate citizens to report incidental observations on alien/native termophilic or cold-water species, or upload data in existing e-tools and networks (e.g. www.observadoresdelmar.es, http://www.iucn-medmis.org, http://proyectobursatella.blogspot.com.es/p/colabora.html, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016). - Register an account in MedMIS and send an email to the MedMIS administrator (medmarine@iucn.org) stating your full name, your MedMIS account name, and the MPA that you manage. As a manager you will be notified by MedMIS of any activity related to your MPA and receive alerts when new sightings near your MPA are added. - Divulge results in both the science and citizen environment
List of material needed	Setting permanent sites: • scuba diving gear • GPS • 11 rods (25 cm long) per transect • hammer	

	• rope (1m per transect) • flagging tape • photo camera • underwater photo camera (if available) • 2 small buoys/transect • metric tape • plastic board and pencil • 60x40cm PVC quadrat • 6 stakes per transect • 6 plastic marks with number (e.g. livestock ear tags) per transect * Sites with difficult access from the coast may also need: boat and captain. For the training: • Images of the species of concern (for example, the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)) • Samples of the monitoring data-sheets (one per person) • Field guides-slates to identify species (if available) • Underwater photo camera • All needed for each monitoring sampling (see below); adjust the quantities of material to the number of trainees. * Sites with difficult access from the coast may also need: boat and captain For each sampling (and pre-survey): • snorkeling/skin diving gears • submersible watch (one per observer) • GPS • plastic board (one per observer) • pencils (one per observer) • underwater data-sheets to register observations (one set per observer) • underwater photo camera (if available; ideally one per pair of observers) • plastic bags to collect samples • metric tape or rope (>100m long) (one per transect) • permanent marker • PVC quadrat

<table>
<tr><td></td><td colspan="2">* Sites with difficult access from the coast may also need: boat</td></tr>
<tr><td>Personnel needed (specialization level)</td><td colspan="2">*Note that one person may do more than one of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA.
• Divers to set up the permanent transects and photoquadrats
• Observers able to recognize alien/thermophilic/cold-water species and estimate abundance using the ACFOR scale
• People able to locate and shoot photos to permanent benthic quadrats
• Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected)
• Specialist to lecture on species identification and monitoring methodology
• People to preserve the collected samples and take reference photos
• People to introduce the data collected into database
• People to share the data collected with the CIESM tropical signals program and other online network platforms/e-tools
• People to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts
• Data analyst (statistics, CPCe point-counting software)
• People to divulge results in both the science and citizen environment
* boat captain (in some cases)</td></tr>
<tr><td>Budget (tentative)</td><td>Personnel (permanent or temporary)</td><td>* Note that one person may do more than one of the different tasks, e.g. a scientific diver could do both the tasks for a scientist and the tasks for a diver.

• 4-6 full days salary for two professional divers- to set up the permanent transects and photoquadrats
* professional diving license may be required in some countries.
• 4-8 weeks/year salary for two lecturers on species identification/monitoring methods in the trainning
• 1 month salary/year for one person to divulge the project/results among scientists and stakeholders
• Volunteers or 10-20 full days salary/year for 2 observers to recognize thermophilic/cold-water species and estimate abundance using the ACFOR scale
• 2-4 full days salary/year for one person to preserve the collected samples, take reference photos, and contact specialists to identify species
• Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected) (usually this has no cost)
• 2-4 weeks salary/year for one person to introduce the data collected into a database and to share the data collected with the CIESM tropical signals program and other on-line network platforms or e-tools
• 1 month salary/year for data analyst (statistics, CPCe point-counting software)
• 1 month salary for one person to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts (optional)
* boat captain (in some cases)</td></tr>
</table>

	Durable equipment	GPS, Scuba diving gear, Fins, mask and snorkel, Photo camera, Underwater photo camera (if available), Submersible watch (one per observer), Computer, Boat
	Consumables	Hammer, Metric tape (>100m long) (one per transect), 2 small buoys/transect, 11 rods (25 cm long)/transect, Rope (1m/transect), Flagging tape, Plastic boards and pencils (one per observer) 60x40cm PVC quadrat, 6 stakes per transect, 6 plastic marks with number (e.g. livestock ear tags)/ transect, Images of the species of concern (e.g. the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)), Copies of the monitoring data-sheets (one/trainee), Field guides-slates to identify species (if available), Underwater monitoring data-sheets to register observations (one set per observer) Plastic bags to collect samples, Permanent marker
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Varing, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	Scuba tanks, Scuba tanks filling, * boat
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integration of data into existing international/basin-scale monitoring networks such as: The CIESM tropical signals programme, MedMIS, observadores del mar.	

PROTOCOL 3b	**Indicator:** Range shifts of alien/native temperature sensitive species - Scuba diving
Problem / scientific question	Alien/thermofilic species are extending their geographic range in response to a higher availability of temperate waters in the Mediterranean Sea. Indigenous cold-water species, in turn, are contracting their distributional range and moving to deeper, colder waters to escapetermal stress, at least temporarly.
Scientific rationale (hypotheses)	- Native warm-water species are extending their range distributions northwards. - A higher number of tropical/subtropical thermophilic species are arriving the Mediterranean (some of them highly invasive). - Arriving species may occupy indigenous species’ niche and drive them to local extinction. They may represent new fishery resources, though. - Cold-water environments in the Mediterranean are becoming scarce. - Cold-water species are compiting with thermophilic arriving species for resources. - Cold-water species are moving northwards and deeper, declining in number, and may become locally extinct.

	- All these may result in important lossess of biodiversity.	
Sampling program	Metrics (variable / indicator)	presence and abundance of thermophilic (alien and native) and cold-water species (# individuals/100m transect)
	Type of survey	Option 1: Volunteers – citizen survey Option 2 : Scientific – technical survey
	Sampling technique	Scuba diving
	Sampling unit (SU)	Transect 100m long, parallel to the coast If possible, also quadrat 40*60cm
	Spatio-temporal distribution of SUs and sampling size	- Sampling locations: in protected areas of the MPA, ideally in protected and unprotected areas of the MPA - 3 sampling sites per location (1-5 km apart from each other) - Depth strata per site: at least one (0-3m), ideally two (0-3m and 12-15m) - # SUs per site: 2 transects/site/depth and 3 quadrats/transect - Min. 2 times/year: after summer (Sept/Oct) - late spring (Apr/May), ideally 4 times/year: late spring (Apr/May), hottest period (Jul/Aug), end of summer (Sep/Oct), and coldest period (Feb/Mar)
	Data analysis	Basic descriptive statistics (e.g., mean, standard deviation, range), ANOVA. For quadrat photos-CPCe point-counting software.
Detailed procedure	Before	Choose sampling locations: Protected areas of the MPA, or protected and unprotected areas of the MPA Select sites: Include most and less human disturbed areas. Should be representative of the diversity of habitats present in the MPA Determine sampling depth(s): Shallow depth: between 0-3m, or shallow and deep depths: 0-3m, 12-15m Determine sampling frequency: Twice/year or four times/year Set up permanent monitoring tools: • Set up two 100m permanent transects per site, per depth (0-3m, 12-15m): - Nail a short rod (about 25 cm long) into substrate until firmly fixed. Swim 10m parallel to the coast and nail a second rod (can use a metric tape to measure the distance between rods. Make sure to keep a constant depth). Repeat the process 10 times (the last rod will be 100m apart from the first one). - Then firmly tie a flagging tape to each rod. - Mark the transect location with a GPS or drawing a detailed map showing the starting and end point of the transect. You can also photograph a peculiar, permanent feature of the shore from the first rod. - With a rope, firmly tie a small buoy to the first rod (it should float 20-30 cm above the rod). Repeat for the last rod. - Repeat the process to set another additional transect at 0-3m depth, and **(**given the case**)** another two

		transects at 12-15m depth. Each transect should be located 1-5 km apart from each other. • Set up permanent benthic photoquadrats (if possible): - Select at least three areas along each transect to place the permanent benthic photoquadrats (focus on areas where invasive species may be present, or where different benthic communities grow). - Permanently mark the location of each of these areas, guided with a 60x40cm PVC quadrat, by nailing a stake and a plastic mark with an ID number (e.g. livestock ear tags) at the left low corner of the quadrat (interior) and another one at the right up corner (interior). Register the distance of these points from the first rod in case taggs are lost or overgrowned by algae. Train participants: - Organize activities to disseminate and explain the project through direct involvement of dive centers and fishermen. - Develop training sessions to introduce participants with species identification and the monitoring methodology: • provide copies of the data-sheets (Annex 4) and explain in detail how to properly fill them in. • trainees should practice the recognition of the species of concern (directly in the field or from diverse images, e.g., the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)), and if possible, the estimation of abundances. • trainees should practice locating, placing and shoting photos of the photoquadrats • trainees should learn: how to download the application (for cell phones: App store, Google play) or access the MedMIS platform (for computers: http://www.iucn-medmis.org/?c=About/show), create an account in MedMIS, and report sightings in MPAs Conduct Pre-survey (if possible) To familiarize the observers with the methods and tools to be utilized. For example, to adjust each person swimming speed to cover 100m in approx. 15 min.
	During	Census methodology adapted from the CIESM tropical signals Programme • General information to be gathered: Name of Observer, Date, Name of site, Start and end time of survey, Sea condition (rough, calm,etc.), Underwater horizontal visibility (m), Shore exposure (exposed, moderately exposed, sheltered), Slope of seabed (steep shore, flat ledges), Type of substrata (rocky, boulders, sandy/ gravelly, mixture of soft and rock bottoms), Type of community (e.g. seagrass meadow) and dominant species if any. • General observations to be gathered on other climate change indicators: Sea temperature (if possible), *Posidonia ocenica* flowering, bleaching or mass mortality/necrosis, blooms (e.g. of jellyfish) • Detailed description of the census:

- Work in pairs, each person develops one survey
- Locate at one of the permanent transects
- Fill in the upper portion of the data-sheet (General information)
- Tie the measuring tape/rope to the first rod of the transect
- Extend the measuring tape/rope while swimming slowly and at constant speed for 15 minutes along the entire transect, following the rods.
- While swimming, look for the target species for 15 min. Assign to each species found a category of the ACFOR species abundance scale (see below), or record as P (present) or N (none, if apparently absent)
- Time used during the survey to take photographs, collect samples or dive to have a closer look to the species, should be added to the standard total 15 min.
- Before leaving, have a quick swim around the sampling area to confirm first impression of species presence and relative abundance.
- Fill in the “General observations to be gathered on other climate change indicators” in the data-sheet.
- Repeat the process with the other transects.

Note:

* If the observer is not sure of species ID, reference photographs of the species should be taken and, if possible, a sample (plastic bag).

* Photographs must be catalogued: date, depth, site location.

- The ACFOR abundance scale has 5 categories:

- **A=** Abundant – found almost everywhere, covering extensive areas in cover or in N_o of individuals; (approx. cover >40% or number of individuals >1000/m^2)
- **C=** Common – found almost everywhere, but not dominant as category A (approx. cover 20-40% or N_o of individuals 100-1000/m^2)
- **F**= Frequent – found in many places with little searching (approx. cover 10-20% or N_o of individuals 10-100/m^2)
- **O**= Occasional – found in few places after accurate searching (approx. cover 5-10% or N_o of individuals 1-10/m^2)
- **R**= Rare – found in one or two places only after intense searching (approx. cover 1-5% or N_o of individuals 1-10/10m^2)

In addition, the observer should record also the following:

- **P**= Present –the species is present but the abundance is not meaningful (that is only one individual or only one micropatch of algae)

		• **N**= None – the species is apparently absent. Please note (about the scale): * Where two or more species exist, for instance foliose algae overgrowing crustose algae, total percentage cover can be over 100% and the abundance category will reflect this. * Use quadrats as reference frames for counting, particularly when density is borderline between two categories of the scale. * Some extrapolation of the scales may be needed to estimate abundance for restricted habitats (e.g. rockpools).
	After	- Take reference photos and preserve the collected samples. - Provide samples to experts for identification as soon as possible. - Create a database where collected information will be registered (baseline). - Introduce collected data into baseline. - Analize the data. - Share data with CIESM tropical signals programme. - Create e-tools to facilitate citizens to report incidental observations on alien/native termophilic or cold-water species, or upload data in existing e-tools and networks (e.g. www.observadoresdelmar.es, http://www.iucn-medmis.org, http://proyectobursatella.blogspot.com.es/p/colabora.html, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016). - Register an account in MedMIS and send an email to the MedMIS administrator (medmarine@iucn.org) stating your full name, your MedMIS account name, and the MPA that you manage. As a manager you will be notified by MedMIS of any activity related to your MPA and receive alerts when new sightings near your MPA are added. - Divulge results in both the science and citizen environment
List of material needed	Setting permanent sites: • scuba diving gear • GPS • 11 rods (25 cm long) per transect • hammer • rope (1m per transect) • flagging tape • photo camera • underwater photo camera (if available) • 2 small buoys/transect • metric tape	

	• plastic board and pencil • 60x40cm PVC quadrat • 6 stakes per transect • 6 plastic marks with number (e.g. livestock ear tags) per transect * Sites with difficult access from the coast may also need: boat and captain. For the training: • Images of the species of concern (e.g. the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)) • Samples of the monitoring data-sheets (one per person) • Field guides-slates to identify species (if available) • Underwater photo camera • All needed for each monitoring sampling (see below); adjust the quantities of material to the number of trainees. * Sites with difficult access from the coast may also need: boat and captain For each sampling (and pre-survey): • scuba diving gear • submersible watch (one per observer) • GPS • plastic board (one per observer) • pencils (one per observer) • underwater data-sheets to register observations (one set per observer) • underwater photo camera (if available; ideally one per pair of observers) • plastic bags to collect samples • metric tape or rope (>100m long) (one per transect) • permanent marker • PVC quadrat * Sites with difficult access from the coast may also need: boat and captain
Personnel needed (specialization level)	*Note that one person may do more than one of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • Scuba divers to set up the permanent transects and photoquadrats • Scuba divers able to recognize alien/thermophilic/cold-water species and estimate abundance using ACFOR scale • Scuba divers able to locate and shoot photos to permanent benthic quadrats

	• Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected) • Specialist to lecture on species identification and monitoring methodology • People to preserve the collected samples and take reference photos • People to introduce the data collected into database • People to share the data collected with the CIESM tropical signals program and other online network platforms/e-tools • People to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts • People to analize the data collected (statistics, CPCe point-counting software) • People to divulge results in both the science and citizen environment * boat captain (in some cases)	
Budget (tentative)	Personnel (permanent or temporary)	Note that one person may do more than one of the different tasks, e.g. a scientific diver could do both the tasks for a scientist and the tasks for a diver. • 4-11 full days salary for two professional divers to set up the permanent transects and photoquadrats * professional diving license may be required in some countries. • 4-8 weeks/year salary for two lecturers on species identification/monitoring methodology in the trainning • 1 month salary/year for one person to divulge the project/results among scientists and stakeholders • Volunteers or 4-8 weeks salary/year for 2 divers to recognize thermophilic/cold-water species and estimate abundance using the ACFOR scale • 2-4 full days salary/year for one person to preserve the collected samples, take reference photos, and contact specialists to identify species • Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected) (usually this has no cost) • 2-4 weeks salary/year for one person to introduce the data collected into a database and to share the data collected with the CIESM tropical signals program and other on-line network platforms or e-tools • 1 month salary/year for one data analyst (statistics, CPCe point-counting software) • 1 month salary for one person to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts (optional) * boat captain (in some cases)
	Durable equipment	GPS, Scuba diving gear, Fins, mask and snorkel, Photo camera, Underwater photo camera (if available), Submersible watch (one per observer), Computer, Boat
	Consumables	Hammer , Metric tape (>100m long/transect), 2 small buoys/transect, 11 rods (25cm long/transect), Rope (1m/transect), Flagging tape, Plastic boards and pencils (one per observer), 60x40cm PVC quadrat, 6 stakes/transect, 6 plastic marks with number (e.g. livestock ear tags)/transect, Images of the species of concern, Copies of the monitoring data-sheets (one/trainee), Field guides-slates to identify species (if

		available), Underwater monitoring data-sheets to register observations (one set per observer), Plastic bags to collect samples, Permanent marker
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	Scuba tanks, Scuba tanks filling, * boat
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integration of data into existing international/basin-scale monitoring networks such as: The CIESM tropical signals programme, MedMIS, observadores del mar.	

PROTOCOL 3c	**Indicator:** Range shifts of alien/native temperature sensitive species – Intertidal walk	
Problem / scientific question	Alien/thermofilic species are extending their geographic range in response to a higher availability of temperate waters in the Mediterranean Sea. Indigenous cold-water species, in turn, are contracting their distributional range and moving to deeper, colder waters to escapetermal stress, at least temporarly.	
Scientific rationale (hypotheses)	- Native warm-water species are extending their range distributions northwards. - A higher number of tropical/subtropical thermophilic species are arriving the Mediterranean (some of them highly invasive). - Arriving species may occupy indigenous species' niche and drive them to local extinction. They may represent new fishery resources, though. - Cold-water environments in the Mediterranean are becoming scarce. - Cold-water species are compiting with thermophilic arriving species for resources. - Cold-water species are moving northwards and deeper, declining in number, and may become locally extinct. - All these may result in important lossess of biodiversity.	
Sampling program	Metrics (variable / indicator)	presence and abundance of thermophilic (alien and native) and cold-water species (# individuals/100m/15 min.)
	Type of survey	Option 1: Volunteers – citizen survey Option 2 : Scientific – technical survey
	Sampling	Intertidal walk, Snorkeling

<table>
<tr><td></td><td>technique</td><td></td></tr>
<tr><td></td><td>Sampling unit (SU)</td><td>100m/15 minutes</td></tr>
<tr><td></td><td>Spatio-temporal distribution of SUs and sampling size</td><td>- Sampling locations: in protected areas of the MPA, ideally in protected and unprotected areas of the MPA
- 3 sampling sites per location (100m apart from each other)
- Depth strata per site: between 0-0.5m
- # SUs per site: 3 surveys/site
- Min. 2 times/year: after summer (Sept/Oct) - late spring (Apr/May), ideally 4 times/year: late spring (Apr/May), hottest period (Jul/Aug), end of summer (Sep/Oct), and coldest period (Feb/Mar)</td></tr>
<tr><td></td><td>Data analysis</td><td>Basic descriptive statistics (e.g., mean, standard deviation, range), ANOVA.</td></tr>
<tr><td>Detailed procedure</td><td>Before</td><td><u>Choose sampling locations</u>: Protected areas of the MPA or protected and unprotected areas of the MPA
<u>Select sites</u>: Include most and less human disturbed areas. Should be representative of the diversity of habitats present in the MPA
<u>Determine sampling frequency:</u> Twice/year or four times/year

<u>Train participants</u>:
- Organize activities to disseminate and explain the project through direct involvement of volunteers.
- Develop training sessions to introduce participants with species identification and monitoring methodology:
• provide copies of the data-sheets (Annex 4) and explain in detail how to properly fill them in.
• trainees should practice the recognition of the species of concern (directly in the field or from diverse images, e.g., the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40)), and if possible, the estimation of abundances.
• trainees should practice how to do the monitoring survey.
• trainees should learn: how to download the application (for cell phones: App store, Google play) or access the MedMIS platform (for computers: http://www.iucn-medmis.org/?c=About/show), create an account in MedMIS, and report sightings in MPAs

<u>Conduct Pre-survey (if possible)</u>: To familiarize the observers with the methods and tools to be utilized.</td></tr>
<tr><td></td><td>During</td><td><u>Census</u> methodology adapted from the CIESM tropical signals Programme
• General information to be gathered: Name of Observer, Date, Name of site, Coordinates of starting point and end point (Lat; Long), Starting and end time of survey, Sea condition (rough, calm,etc.), Shore exposure (exposed, moderately exposed, sheltered), Slope of seabed (steep shore, flat ledges), Type of substrata (rocky, boulders, sandy/ gravelly, mixture of soft and rock bottoms), Type of community (e.g. seagrass meadow) and dominant species if any.
• General observations to be gathered on other climate change indicators: Posidonia ocenica flowering,</td></tr>
</table>

bleaching or mass mortality/necrosis, blooms (e.g. of jellyfish), mortality of organisms.

- Detailed description of the census:
  - Work in pairs, each person develops one survey
  - In a monitoring permanent site, identify the area to be sampled (approximately 500 m along the shore)
  - Mark the survey’s starting point location using a GPS, or photograph a peculiar, permanent feature of the shore from where you start the survey
  - Fill in the upper portion of the data-sheet (General information)
  - Nail a stack in the starting point and tie the extreme of a metric tape
  - Take note of the starting time of the survey
  - Extend the measuring tape while walking slowly and at constant speed to cover an area of approx.100m. While walking, look for the target species for 15 minutes. Assign to each species found a category of the ACFOR species abundance scale (see below), or record as P (present) or N (none, when apparently absent)
  - Make sure to look in all shore features (crevices, pools, underneath rocks)
  - Photograph specific features of interest and any rare organisms/new records
  - Time used during the survey to take photographs, collect samples or get closer to have a better look to the species, should be added to the standard total 15 minutes.
  - Register the ending time of the survey
  - Mark the survey’s end point location with a GPS, or photograph a peculiar, permanent feature of the shore from where you end the survey
  - Before leaving, have a quick walk around the sampling area to confirm first impression of species presence and relative abundance.
  - Fill in the section “General observations to be gathered on other climate change indicators” in the data-sheet.
  - Walk a further 100 m and start another timed search of 15 minutes along 100m shore. Then repeat the same for a third survey.

<u>Note</u>:

* If the observer is not sure of species ID, reference photographs of the species should be taken and, if possible, a sample (plastic bag).

* Photographs must be catalogued: date, depth, site location.

- The ACFOR abundance scale has 5 categories:

		• **A=** Abundant – found almost everywhere, covering extensive areas in cover or in N_o of individuals; (approx. cover >40% or number of individuals >1000/m^2) • **C=** Common – found almost everywhere, but not dominant as category A (approx. cover 20-40% or N_o of individuals 100-1000/m^2) • **F**= Frequent – found in many places with little searching (approx. cover 10-20% or N_o of individuals 10-100/m^2) • **O**= Occasional – found in few places after accurate searching (approx. cover 5-10% or N_o of individuals 1-10/m^2) • **R**= Rare – found in one or two places only after intense searching (approx. cover 1-5% or N_o of individuals 1-10/10m^2) In addition, the observer should record also the following: • **P**= Present –the species is present but the abundance is not meaningful (that is only one individual or only one micropatch of algae) • **N**= None – the species is apparently absent. Please note (about the scale): * Where two or more species exist, for instance foliose algae overgrowing crustose algae, total percentage cover can be over 100% and the abundance category will reflect this. * Use quadrats as reference frames for counting, particularly when density is borderline between two categories of the scale. * Some extrapolation of the scales may be necessary to estimate abundance for restricted habitats (e.g. rockpools).
	After	- Take reference photos and preserve the collected samples. - Provide samples to experts for identification as soon as possible. - Create a database where collected information will be registered (baseline). - Introduce collected data into baseline. - Analize the data. - Share data with CIESM tropical signals program. - Create e-tools to facilitate citizens to report incidental observations on alien/native termophilic or cold-water species, or upload data in existing e-tools and networks (e.g. www.observadoresdelmar.es, http://www.iucn-medmis.org, http://proyectobursatella.blogspot.com.es/p/colabora.html, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016). - Register an account in MedMIS and send an email to the MedMIS administrator (medmarine@iucn.org) stating your full name, your MedMIS account name, and the MPA that you manage. As a manager you will be notified by MedMIS of any activity related to your MPA and receive alerts when new sightings near your

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- Divulge results in both the science and citizen environment</td></tr>
<tr><td>List of material needed</td><td colspan="2">For the training:
• Images of the species of concern (e.g. the poster for reporting of invasive alien species in MPAs of Liguria (Figure 40))
• Samples of the monitoring data-sheets (one per person)
• Field guides-slates to identify species (if available)
• Photo camera
• All needed for each monitoring sampling (see below); adjust the quantities of material to the number of trainees
* Sites with difficult access may also need boat and captain

For each sampling (and pre-survey):
• mask and snorkel (one per observer)
• watch (one per observer)
• GPS
• plastic board (one per observer)
• pencils (one per observer)
• data-sheets to register observations (one set per observer)
• photo camera (if available; ideally one per pair of observers)
• plastic bags to collect samples
• metric tape (>100m long) (one per survey)
• permanent marker
• PVC quadrat (optional)
• stacks (1 per survey)
• hammer
* Sites with difficult access may also need boat</td></tr>
<tr><td>Personnel needed (specialization level)</td><td colspan="2">*Note that one person may do more than one of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA.
• People able to recognize alien/thermophilic/cold-water species and estimate abundance using the ACFOR scale
• Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected)
• Specialist to lecture on species identification and monitoring methodology
• People to preserve the collected samples and take reference photos
• People to introduce the data collected into database</td></tr>
</table>

	• People to share the data collected with the CIESM tropical signals program and other on-line network platforms or e-tools • People to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts • People to analize the data collected (statistics, CPCe point-counting software) • People to divulge results in both the science and citizen environment * boat captain (in some cases)	
Budget (tentative)	Personnel (permanent or temporary)	Note that one person may do more than one of the different tasks, e.g. a scientific diver could do both the tasks for a scientist and the tasks for a diver. • Volunteers or 1 week salary/year for 2 observers to recognize thermophilic/cold-water species and estimate abundance using the ACFOR scale • 4-8 weeks/year salary for two lecturers on species identification/monitoring methodology in the trainning • 1 month salary/year for one person to divulge the project/results among scientists and stakeholders • 2-4 full days salary/year for one person to preserve the collected samples, take reference photos, and contact specialists to identify species • Specialists to identify the alien/thermophilic/cold-water species (in field, from photos, videos, or samples collected) (usually this has no cost) • 2-4 weeks salary/year for one person to introduce the data collected into a database and to share the data collected with the CIESM tropical signals program and other on-line network platforms or e-tools • 1 month salary/year for one person to analize the data collected (statistics) • 1 month salary for one person to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts (optional) * boat captain (in some cases)
	Durable equipment	GPS, Scuba diving gear, Fins, mask and snorkel, Photo camera, Watch (one/observer), Computer, * Boat (in some cases)
	Consumables	Hammer , Metric tape (>100m long/transect), Plastic boards and pencils (one/observer), 60x40cm PVC quadrat, 1 stake per transect, Images of the species of concern, Copies of the monitoring data-sheets (one/trainee), Field guides-slates to identify species (if available), Underwater monitoring data-sheets to register observations (one set/observer), Plastic bags to collect samples, Permanent marker
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including	Variable, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution

	overheads)	
	Subcontracting	Scuba tanks, Scuba tanks filling,* boat
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integrate data into existing international/basin-scale monitoring networks such as: The CIESM tropical signals programme, MedMIS, observadores del mar.	

PROTOCOL 3d	**Indicator:** Range shifts of alien/native temperature sensitive species – Questionnaires	
Problem / scientific question	Alien/thermofilic species are extending their geographic range in response to a higher availability of temperate waters in the Mediterranean Sea. Indigenous cold-water species, in turn, are contracting their distributional range and moving to deeper, colder waters to escapetermal stress, at least temporarly.	
Scientific rationale (hypotheses)	- Native warm-water species are extending their range distributions northwards. - A higher number of tropical/subtropical thermophilic species are arriving the Mediterranean (some of them highly invasive). - Arriving species may occupy indigenous species' niche and drive them to local extinction. They may represent new fishery resources, though. - Cold-water environments in the Mediterranean are becoming scarce. - Cold-water species are compiting with thermophilic arriving species for resources. - Cold-water species are moving northwards and deeper, declining in number, and may become locally extinct. - All these may result in important lossess of biodiversity.	
Sampling program	Metrics (variable / indicator)	presence and abundance of thermophilic (alien and native) and cold-water species
	Type of survey	Volunteers – citizen survey (fishermen, divers)
	Sampling technique	Interview, adopted as part of the CIESM Tropical Signals programme
	Sampling unit (SU)	Standard questionnaire
	Spatio-temporal distribution of SUs and sampling size	- Sampling sites: - harbors, disembarking areas (e.g. beaches), and dive centers that operate in the MPA. - # SUs per site: Option 1: local/recreational fishermen and divers with more than ten years of experience in the MPA Option 2: local/recreational fishermen and divers with more than five years of experience in the MPA - Sampling frequency: at least once a year, ideally in Summer (Jun-Sept)

	Data analysis	multivariate and univariate analysis. ANOVAs.
Detailed procedure	Before	<u>Select sites</u>: All harbors, disembarking areas (e.g. beaches) and dive centers that operate in the MPA Or major harbors and disembarking areas and larger dive shops that operate in the MPA <u>Determine sampling frequency</u>: At least once a year, or all year round, randomly. <u>Develop a questionnaire</u>: Include relevant questions about the observation/capture, abundance, appearance/disappearance of alien/native temperature sensitive species in the MPA in the last decade. - Design it so that filling it in should take less than 30 min. - Ask respondents to provide a qualitative ranking of the abundance of the species through time, on an annual basis, according to 6 different grades: • 0 =ABSENT • 1 =RARE (once in a year) • 2= OCCASIONAL (sometimes in a year) • 3=COMMON (regularly in a year) • 4 =ABUNDANT (regularly in captures/dives and abundant) • 5=DOMINANT (always in captures/dives and with great abundances). At the end of the questionnaire ask respondents to provide an abundance trend factor for each recorded species: species that ‘‘INCREASE’’ (level ‘‘I’’) species that ‘‘DECREASE’’ (level ‘‘D’’) species that ‘‘FLUCTUATE (level ‘‘F’’) over the respondent experience period. - Fishermen and divers can be met during their land activities, while for example cleaning their equipments in the harbors/stores. - Begin a conversation with a fisherman/diver without formalities and focus on which species may have appeared or disappeared in the last decades. *Special attention should be placed to the approach, since fishermen and divers generally mistrust fisheries researchers and managers. - Once they show interest in the subject through discussion then the interview may formally begin.
	During	Questionnaire adapted from the CIESM Tropical Signals programme and Azzurro *et al.* 2011 • General information to be gathered: Date, name of site, name of fisherman/diver, age, fishing gear, years of experience diving/fishing in the SPAMI. • General observations to be gathered on other climate change indicators (images as examples may help): Indicate if have recently seen any of the following, and approx. when and where: *Posidonia ocenica* (seaweeds) flowering, bleaching or mass mortality/necrosis, blooms (e.g. of jellyfish), mortality of organisms

		• Detailed description: - Start the interview and fill in the questionnaire in max. 30 min. - Showing images (e.g. photographs) may help to match local fish names with taxonomic ones. - Only the species mentioned by the fisherman/diver should be registered. - Clearly explain the six different ranks of the scale of abundance (0 =ABSENT; 1 =RARE; 2= OCCASIONAL; 3=COMMON; 4 =ABUNDANT; 5=DOMINANT). - Clearly explain how to assign an abundance trend factor for each species (INCREASE: level I; DECREASE: level D; FLUCTUATE: level F)
	After	- After finish the interview, ask for the possibility of being introduced to other fishermen/divers. - Create a presence/absence and semiquantitative abundance database with the data collected (baseline). - Analize the data (multi and univariate analyses: e.g. ANOVA, Similarity Percentages Procedure (SIMPER), Non-metric Multi Dimensional Scaling (nMDS). - Create e-tools to facilitate citizens to report incidental observations on alien/native termophilic or cold-water species, or upload data in existing e-tools and networks (e.g. www.observadoresdelmar.es, http://www.iucn-medmis.org, http://proyectobursatella.blogspot.com.es/p/colabora.html, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016). - Divulge results in the science environment and among fishermen and divers
List of material needed	• Images of the species of concern (e.g., photographs from a field guide, or the poster for reporting invasive alien species in MPAs of Liguria (Figure 40)) • Images with examples of bleaching, mortality events, *Posidonia* flowering, jellyfish/mucilage blooms • plastic boards (one per observer) • pencils (one per observer) • data-sheets with questionnaires	
Personnel needed (specialization level)	*Note that one person may do more than one of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • People to make the interviews • People to introduce the data collected into database • People to analize the data collected (statistics: ANOVA, SIMPER, nMDS) • People to create e-tools to facilitate casual/sporadic observation/capture of alien/native termophilic or cold-water species (optional) • People to upload and share the data collected with existing networks (CIESM Tropical Signals programme) • People to divulge results in both the science and citizen (fishermen/divers) environment	
Budget (tentative)	Personnel (permanent or	Note that one person may do more than one of the different tasks, e.g. one single person could do both interview people and introduce data into database

	temporary)	• Salary for interviewers (N_o of interviewers and full days depend on the N_o of people to be interviewed) • 2-4 weeks salary/year for one person to introduce the data collected into a database and to share the data collected with the CIESM tropical signals program and other on-line network platforms or e-tools • 1 month salary/year for one data analyst (statistics ANOVA, SIMPER, nMDS) • 1 month salary for one person to create e-tools to facilitate citizens to report on alien/native termophilic or cold-water species range shifts (optional) • 1 month salary/year for one person to divulge the project/results among scientists and stakeholders
	Durable equipment	1 Computer
	Consumables	Images of the species of concern, Images with examples of bleaching, mortality events, *Posidonia* flowering, jellyfish/mucilage blooms, mortality of organisms; Plastic boards (one/observer) pencils (one/observer), Data-sheets with questionnaires
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for people interviewed (if possible), Overheads-variable rates depending on the institution
	Subcontracting	
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integrate data into existing international/basin-scale monitoring networks such as: The CIESM tropical signals programme, MedMIS, observadores del mar.	

IV.4 Reproduction and breeding date of selected species protocols, including flowering of P. oceanica

PROTOCOL 4a	**Indicator:** Mortality and bleaching events: mortality of *Posidonia oceanica* Reproduction of selected species: flowering of *P. oceanica*
Problem / scientific question	The rapid warming of the Mediterranean Sea is affecting seagrass physiologically. This seawater warming has shot up the mortality of *P.oceanica* in the last decades and has altered the intensity and frequency of *P.oceanica* flowering.

<table>
<tr><td>Scientific rationale (hypotheses)</td><td colspan="2">Global warming will affect P. oceanica meadows structure (shoot density and meadow cover), shoot population dynamics and trends.
Reproductive phenology in P. oceanica meadows is being altered by extreme warming events.
The vulnerability of seagrass meadows to seawater warming may vary locally and by other factors (e.g. depth)
Changes across years are not significant or don’t show a clear temporal pattern.
Peaks of solar activity could also be involved.</td></tr>
<tr><td rowspan="5">Sampling program</td><td>Metrics (variable / indicator)</td><td>Meadow structure and population dynamics:
Shoot density: N_o of shoots in $0.04m^2$ placed within living P. oceanica patches with 100% cover.
Meadow cover: proportion of substrate occupied by seagrass living patches. Unit: %.
Rate of shoot population net growth: relative amount of change in shoot numbers within 50x50cm quadrats on a per unit time basis. Unit: % / t. Time series of a minimum of 8 years are necessary.
Temporal trends of meadow structure variables.
Presence and quantification of P.oceanica inflorescences (Díaz-Almela et al. 2005).
Flowering intensity index (FI, N_o of inflorescences per shoot). N_o of inflorescences divided by the N_o of shoots counted within sampling quadrats (both 0.04 and $0.25m^2$).
Flowering Prevalence (FP, N_o of meadows flowering in a given year). Proportion of sites with flowering meadows, relative to the total N_o of sampling sites which compose the monitoring network.
Flowering frequency (FF, N_o of years that a given meadow has flowered, relative to the total N_o of years of the time series).
Flowering probability (Pf, fraction of flowering stalks appeared per annual segment). Only when reconstructive techniques are applied.</td></tr>
<tr><td>Type of survey</td><td>Scientific – technical survey
Volunteers – citizen survey (divers)</td></tr>
<tr><td>Sampling technique</td><td>Diving and snorkeling. Sampling stations placed at fixed sites that are repeatedly visited each year.</td></tr>
<tr><td>Sampling unit (SU)</td><td>Shoot density and flowering indexes: Quadrats (20x20cm, $0.04m^2$)
Shoot net growth: quadrats (50x50cm, $0.25cm^2$)
Meadow cover: quadrats (40x40cm, $0.16m^2$) and transects (10m)</td></tr>
<tr><td>Spatio-temporal distribution of SUs and sampling size</td><td>- Sampling sites: inside the SPAMI, ideally inside and outside the SPAMI.
- Sampling stations at three different depths: shallow, intermediate and deep.
- Each sampling station has 6 fixed markers (e.g. irong pegs; sampling points) separated 5-7m between them.
- For shoot density and flowering: 3 random $0.04m^2$ quadrats placed at each sampling point (always within living seagrass patches with 100% cover).</td></tr>
</table>

		- For meadow cover: 10 measurements of % cover in each sampling point obtained by visual estimation within 0.16m^2 quadrats placed each meter along a 10m tape, thrown from the marker towards a predetermined direction. - Each sampling point has a 0.25m^2 permanent quadrat for exact counts of P. oceanica shoots. - The presence of inflorescences outside of the quadrats is registered qualitatively. - The presence of inflorescences in sites out of the monitoring network can be registered by divers (both SCUBA and snorkel) qualitatively. - Monitoring frequency: Once a year between September and December (sampling months can vary depending on the region, see previous flowering events in the region).
	Data analysis	0.04 and 0.50 quadrats allows counting the number of shoots and inflorescences and characterizing quantitatively the shoot density (shoot/m^2), the flowering intensity index (FI, number of inflorescences per shoot) and the shoot net population growth (% per unit time). To estimate shoot density the 3 measurements performed in each sampling point are random replicates nested within each sampling point. To estimate meadow cover, the 10 measurements performed in each sampling point (i.e. each 10m transect) is averaged to obtain the true replicate so that a total N_o of replicates of 6 are available for analyses. IF is estimated from all quadrats and averaged. Trends in meadow variables are obtained from the value, sign and significance of Kendall-tau analyses performed on variable's time series. Other alternative methods are possible depending upon the nature of the data available. Analysis of variance and multivariate analyses.
Detailed procedure	Before	In case of volunteers participation: - Dissemination of the project and invitation letters to participate. - Calendar establishment (trainings for volunteers and tentative field trips), and meeting with diving centers to calendar approval, coordination and organization. Preparation of sampling material. Site selection: - Choose the stations at different depths and locations using geographic information system and benthic map if they are available.

		- Each station will have six fixed (marked and numbered) metallic sticks (see above). Close to each of these markers a 50x50 cm^2 quadrat is installed using 4 short iron pegs in the corners of the quadrat and a rope for the perimeter of the quadrat. All structures must be close to the bottom to avoid physical interaction between the seagrass leaves and the artificial structure. - To make a detailed diagram or map of the position of markers and permanent quadrats, exact distances and directions between them, seagrass limits, etc. - Before the sampling trips, preliminary trips are necessary. The new sampling stations need to be prepared (marked, labeled). The old sampling stations should be revised (sticks, markers and labels) to restore possible flaws caused by weather conditions during the year.
	During	- Connect the six metallic bars using a 50 m plastic tape. - At each station, 18 quadrats of 0.04 m^2 will be sampled to count the number of shoots and inflorescences. Three quadrats per sampling point. - At each fixed 0.25 m^2 quadrat (6), all shoots and inflorescences are counted. - At each sampling point the 10 m transects are throw from the base of the iron peg swimming towards a predetermined direction using an underwater compass
	After	- Introduce data collected in an EXCEL datasheet. - Analyze and graph the data - Create e-tools to facilitate citizens to report incidental observations on flowering of *Posidonia* and on mortality or bleaching events, and upload data in existing e-tools and networks (e.g. www.observadoresdelmar.es, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016).
List of material needed	• Scuba diving gear • GPS • Handle bag net mesh • Markers • Iron Stakes • PVC 20x20 cm and 40x40 cm quadrats • PVC underwater slate (20x30cm) and pencil • PVC data sheets • Underwater compass • 10 m plastic tapes • 50 m plastic tapes • Ropes, small buoys, plastic tags • Underwater camera and video camera	

Personnel needed (specialization level)	• Boat Captain • Scientific personnel to do the sampling • Scientific personnel to analyze the data • Trained divers with demonstrated diving experience to do the seagrass sampling.	
Budget (tentative)	Personnel (permanent or temporary)	3 full time scientists at each MPA
	Durable equipment	Diving equipment, underwater camera, GPS, underwater compass
	Consumables	Markers, Iron Stakes, 10 m plastic tapes, 50 m plastic tapes Ropes, small buoys, plastic tags
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Dissemination campaigns,
	Subcontracting	Rent of boat for field trips, etc.)
Exploitation of results	Scientific teams responsible of existing *P. oceanica* monitoring programmes in each region. Coordination with regional authorities, diving centers, etc.	

PROTOCOL 4b	**Indicator:** Reproductive activity of selected fish species
Problem / scientific question	The timing of reproductive events (e.g. spawning, nesting, hatching, settlement, etc.) could be changing - by occurring earlier in the year, due to seawater warming, although other environmental factors could be acting synergistically (e.g. population density, habitat availability and quality, weather conditions, etc.). The present protocol would help to define proper CC indicators based on the timing and duration of fish reproductive events.
Scientific rationale (hypotheses)	• Timing and duration (i.e. phenology) of reproductive events in reef fishes is likely to be driven in part by changes in seawater temperature. • Small labrids (*Symphodus* spp., *Thalassoma pavo*), damselfishes (*Chromis chromis*), and both small (*Serranus scriba*) and big serranids (groupers), display conspicuous reproductive behaviour, easily recognisable by divers • The record of reproductive events at regional scale could enlighten the influence of seawater temperature on them and

	thus their usefulness as climate change indicator	
Sampling program	Metrics (variable / indicator)	Date of first record of conspicuous spawning activities in fish species: • *Symphodus* spp. (*S. ocellatus, S. roissali, S. mediterraneus, S. cinereus*): spawning, courtship, non-aggressive interactions between males and females, nest construction, broodcare, nest defence behaviour • *Thalassoma pavo*: gathering, priming, ascent behaviour, spawning • *Chromis chromis*: nest establishment and defence, settlement • *Serranus scriba*: inviting display, agonistic behaviour, spawning • Groupers (*Epinephelus* spp., *Mycteroperca rubra*): spawning aggregations, territory defence, courtship, false rises, spawning [Note: other species could be included after suggestions from other specialists and/or local knowledge]
	Type of survey	Volunteers – citizen survey Scientific – technical survey
	Sampling technique	SCUBA diving and snorkeling
	Sampling unit (SU)	Underwater roaming surveys of undetermined time duration in selected sampling stations
	Spatio-temporal distribution of SUs and sampling size	- Haphazard sampling effort allocation, adapted to local conditions
	Data analysis	Earlier dates of reproductive activities of selected species to be recorded in a regional database at a pluriannual basis
Detailed procedure	Before	Identification of a regional coordinator, and a group of persons responsible of coordinating the gathering of information in each specific MPA. <u>Site selection</u>: - Choose the stations suitable for the observation of the selected species at different depths and locations, based on the local ecological knowledge (with the participation of both scientists and habitual divers, and using geographic information system and benthic habitat maps (when available). <u>Training sessions to participants</u>: - Dissemination of the project and invitation letters to diving centers, associations and private citizens to participate. - Calendar establishment (trainings for volunteers and tentative field trips), and meeting with diving centers

		to calendar approval, coordination and organization. - Develop training sessions to introduce participants to species identification, recognition of reproductive displays to be followed, etc.: o design proper field-sheets, provide copies to participating organisations o trainees should practice the recognition of species involved (both in pictures and directly in the field) o conduct pre-surveys (if possible) to know better the protocol to be developed, the species to be monitored, and the habitats of the selected sites.
	During	**Option 1**: autonomous work by volunteer or scientific divers.- trained divers (i.e. having participated in training sessions), working in pairs at minimum, record their observations independently from the others, in their regular (recreational and/or scientific) diving activity: - selected sites are preferentially chosen for diving, coordinated as possible with the general calendar established previously. - general information to be gathered: name of observer, date, name of site, start and end time of survey, sea condition (rough, calm, etc.), estimated underwater horizontal visibility (m), shore exposure (exposed, moderately exposed, sheltered), slope of seabed (steep shore, flat ledges), type of substrata (rocky, boulders, sandy/ gravelly, mixture of soft and rock bottoms, etc.), type of habitat (e.g. seagrass meadow, rocky boulders, etc.), and dominant habitat-forming species (if any). - during diving walk, divers pay attention to reproductive events of selected species - observations are recorded in the field-sheet specifically designed - take photos / videos of the reproductive event if possible **Option 2**: organised field trips with volunteer divers.- diving centers / associations organise periodically specific dives to survey the different selected sites, as part of their commercial / amateur activity: - diving excursions are to be organised at the MPA level following the general calendar, to ensure that all selected sites and dates are covered - if divers were not part of the initial trainees, a specific, *ad hoc* abridged training session is to be organised with the group - during their diving walk, divers pay attention to reproductive events of selected species - observations are recorded in the field-sheet specifically designed - take photos / videos of the reproductive event if possible
	After	- In the case that positive observations had been made, field-sheet will be given to the person(s) responsible of coordinating the gathering of information; if divers are part of organised diving excursions, field sheets are to be collected by a person from each participating diving center / association. - In the case that photos/videos had been taken, provide a copy specifying: date, site, depth - A pluriannual dataset to be built in order to record all reproductive observations at the regional (Mediterranean) scale

		- Create e-tools to facilitate citizens to report incidental observations of reproductive events - Disseminate results in both scientific and general public environments
List of material needed	• For the traning: images of the species / habitats of concern to be used in the ppt presentations • Scuba diving gears (to be ceded by volunteer persons / organisations in their participation in the activity) • GPS (to position selected sites) • PVC underwater slates (25x30cm) • Polyester field-sheets • Pencils • Photo/video cameras (to be provided by volunteers)	
Personnel needed (specialization level)	*Note that one person may do more than one of the different tasks • Scientific personnel to organise and give the training sessions • Boat Captains and divemasters (volunteers) • Divers to do the visual surveys (volunteers) • A regional coordinator (to gather and analyse data)	
Budget (tentative)	Personnel (permanent or temporary)	1 regional coordinator (volunteer) 3 full-months salary for 2-3 scientist(s) at sub-regional (country or region including several MPAs) and/or local (each MPA) level to give the training sessions and coordinate the activity
	Durable equipment	1 computer
	Consumables	PVC underwater slates, Polyester field-sheets, Pencils
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, Images for training ppt presentation, Webpage to host specific e-tool to facilitate the participation of citizens, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	
Exploitation of results	Create a regional database in a pluriannual basis, to record and compare first dates of reproductive events of selected fish species	

PROTOCOL 4c	**Indicator:** Nesting phenology of marine turtles	
Problem / scientific question	The timing of nesting in marine turtles could be changing - by occurring earlier in the year, due to seawater warming, although other environmental factors could be acting synergistically. The present protocol would help to define proper CC indicators based on the timing and duration of marine turtle reproductive events.	
Scientific rationale (hypotheses)	• Timing and duration (i.e. phenology) of reproductive events in marine turtles is likely to be driven in part by changes in seawater temperature. • The record of reproductive events at regional scale coupled with SST measurements could enlighten the influence of seawater temperature on them and thus their usefulness as climate change indicator	
Sampling program	Metrics (variable / indicator)	- dates for the first and last emergence of a nesting female (not always resulting in nests), - date of first and last nest laid, - duration of the nesting season, measured as the duration between (i) the first recorded emergence onto the beach and the last nest and (ii) the first and last nest, - total number of emergences, - total number of nests laid - adult nesting success, defined as the proportion of emergences resulting in nest construction Depending on the logistic and funding availability, other variables can be followed: - mean clutch size, - hatching success, defined as the % of eggs that hatched, - hatchling emergence success, defined as the % of eggs that actually hatched and produced viable hatchlings that exit the nest, - hatchling mortality in nest, - total number of hatchlings produced
	Type of survey	Volunteers – citizen survey Scientific – technical survey
	Sampling technique	Beach surveys
	Sampling unit (SU)	Mean value per beach of the selected sampling sites
	Spatio-temporal distribution of SUs and sampling size	- Beach surveys in a daily basis (if possible), in all beaches where nesting is known to occur, starting once the first reproductive event has been observed in the location - When sampling periodicity is > daily due to logistics or adverse weather conditions, check whether female tracks had obliterated by weather conditions or human trampling, and then estimate lost emergences (and nests) by interpolating with previous 2 and next 2 surveys.

	Data analysis	Earlier dates of reproductive activities of selected species to be recorded in a regional database at a pluriannual basis Relationships with temporal change in SST to be explored by linear regression.
Detailed procedure	Before	Identification of a regional coordinator, and a group of persons responsible of coordinating the gathering of information in each specific location Training sessions to participants: - Dissemination of the project and invitation letters to associations and private citizens to participate. - Develop training sessions to introduce participants to visual recognition of emergence and nesting events and features: o design proper field-sheets, provide copies to participating organisations o conduct first surveys by volunteers with the supervision of scientific specialists.
	During	- Organise volunteer personnel in groups of 2-3 people. - Patrol each beach at night (from 20:00 to 08:00 h), with groups separated by 15-30 minute intervals. - For each female encountered emerging from the sea, observe whether she lays the eggs and cover them with sand. - Take pictures and videos of the process. In the case that hatchling activity is to be monitored: - Record the position of each nest by triangulating to marker posts at 50-m intervals at the back of the beach, - place a numbered anti-predation wire screen to prevent predation by dogs or other animals. - After 40 days of incubation, place a circular wire-mesh corral around each nest to prevent emergent hatchlings from making their way to the sea, use the previous wire screen as cover of the cage to prevent predation, and leave the sand undisturbed. - Monitor constantly every night to check for hatchling activity. - Once hatchling detected, monitor the nest constantly, record the number of hatchlings and the time of emergence of each hatchling once it had completely emerged from the sand (leave them to emerge without assistance). - Prevent the hatchlings emerging in daytime (after 06:00 h) to keep enclosed in the corral by re-opening the cages forming a U-shape with the exit facing the sea*. - Once 48 h had elapsed with no hatchling emergence, excavate the nest to monitor the nest contents (measure the depth to the top and bottom of the egg chamber, enumerate the total number of unhatched eggs and hatched shell fragments).]
	After	- Field sheets and photos/videos to be gathered, treated and analysed by the regional coordinator. - A pluriannual dataset to be built in order to record all reproductive observations at the regional (Mediterranean) scale

<table>
<tr><td></td><td></td><td>- Organise annually a workshop to discuss results of surveys
- The work should be coordinated by (or done in agreement with) existing initiatives, such as the Marine Turtle Specialist Group (IUCN) and/or the experts involved in the updating and development of the Action Plan for the Conservation of Mediterranean Marine Turtles supported by RAC/SPA (UNEP-MAP).</td></tr>
<tr><td>List of material needed</td><td colspan="2">• For the training: images of the species / habitats / reproductive events of concern to be used in the ppt presentations
• GPS (to position selected sites)
• Handheld VHF radios
• Notebooks
• Pencils
• Photo/video cameras (also to be provided by volunteers)</td></tr>
<tr><td>Personnel needed (specialization level)</td><td colspan="2">*Note that one person may do more than one of the different tasks
• Scientific personnel to organise and give the training sessions
• Observers (volunteers)
• A regional coordinator (to gather and analyse data)</td></tr>
<tr><td rowspan="6">Budget (tentative)</td><td>Personnel (permanent or temporary)</td><td>1 regional coordinator (volunteer)
3 full-months salary for 2-3 scientist(s) at sub-regional (country or region including several MPAs) and/or local (each MPA) level to give the training sessions and coordinate the activity</td></tr>
<tr><td>Durable equipment</td><td>1 computer</td></tr>
<tr><td>Consumables</td><td>Notebooks, Pencils</td></tr>
<tr><td>Travel, accommodation and diets</td><td>Gas/diesel for transportation (from/to site): car and boat
Diet per diem
Accommodation allowance per diem</td></tr>
<tr><td>Other costs (including overheads)</td><td>Variable, but for example: Outreach (media), Banners, Posters, Images for training ppt presentation, Webpage to host specific e-tool to facilitate the participation of citizens, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution</td></tr>
<tr><td>Subcontracting</td><td></td></tr>
<tr><td>Exploitation of results</td><td colspan="2">Create a regional database in a pluriannual basis, to record and compare data on nesting phenology</td></tr>
</table>

PROTOCOL 4d	**Indicator:** Seabird migration phenology	
Problem / scientific question	The timing of migration in seabirds could be changing - by occurring earlier in the year, due to global warming, although other environmental factors could be acting synergistically. The present protocol would help to define proper CC indicators based on the timing of seabird populations phenology.	
Scientific rationale (hypotheses)	• Timing and duration (i.e. phenology) of migration events in marine birds is likely to be driven in part by changes in atmospheric temperature. • The record of migration at regional scale coupled with air temperature measurements could enlighten their usefulness as climate change indicator	
Sampling program	Metrics (variable / indicator)	- dates for migration of selected seabird species
	Type of survey	Volunteers – citizen survey Scientific – technical survey
	Sampling technique	Surveys from selected observatory migration points (ideally within or near MPAs) [at-sea censuses could be also used, but it is considered more difficult to co-ordinate at the regional scale]
	Sampling unit (SU)	Presence and number of seabirds (by species or higher taxonomic level) per day
	Spatio-temporal distribution of SUs and sampling size	Synchronized surveys from selected prominent observatories (capes, points, etc.) in a monthly basis across the region along the year, complemented with sparse and circumstantial observations from a network of authorized observers (with focus on migration months, namely February to May)
	Data analysis	- Earlier dates of occurrence of selected migrating seabird species to be recorded in a regional database at a pluriannual basis - Relationships with temporal change in temperature to be explored by appropriate statistical methods.
Detailed procedure	Before	Identification of a regional coordinator, and a group of persons responsible of coordinating the gathering of information in each specific location Convene an observation protocol at the regional scale: - Organise a regional workshop to agree on a common protocol - Design a field sheet and an online database Organise observer groups in selected observation points: - Select the observation points across the Mediterranean, and characterize them in a standardized table (e.g. geographical coordinates, height over the sea level, etc.)

		- Dissemination of the project and invitation letters to selected seabird networks and individual observers to participate. - Organisation of a monthly observation calendar (e.g. first Saturday of each month along the whole year) - Organisation of a protocol to gather sparse information from specialized observers throughout the year (outside of the "official" observation days), with emphasis in the months when migrants (both species starting their breeding seasons, and wintering species going back to summer areas) are more likely to be observed (i.e. from February to May). In the days immediately previous to each synchronic observation day: - Volunteer observers contact the local coordinator person to organise the work
	During	- Organise volunteers in groups of at least 2 (ideally 3 or more) people per observation point and day. Each observation day: - Establish a 4-h observation period (e.g. 8-12 h in winter, 7-11 h in summer) - Identify and count all observed seabirds (ideally at species level, at least at genus or family level) at 30-min intervals [use telescopes - min. 20×, and 8-10× binoculars: the telescope is fixed to cover 30-50% of sea fringe under the horizon sea fringe under the horizon, and binoculars are used to cover the rest of sea surface to the coast] - Cross-validate observations among observers in the migration point operating this day. - Include other bird species of potential interest (such as shorebirds, raptors, passeriformes, etc.) - For each observation, take notes on the behaviour of observed individuals (e.g. flying direction - N, S, E or W, occurrence of erratic flies, foraging behaviour, resting at sea, etc.) - Note down the observations to the field sheet, or use a portable digital recorder (in the later case, transfer the data immediately to the field sheet) - Other data to be recorded: observation conditions of each day (weather conditions, sea conditions, visibility, direction and intensity of the wind, etc.) - This protocol (and their accompanying material) should be made available online to other volunteer observers not participating in the "official" observation days, in order to allow gathering other observations throughout the year.
	After	- Field sheets to be sent immediately to the regional coordinator. - A pluriannual dataset to be built in order to record all observations at the regional (Mediterranean) scale - Organise (annually or longer periodicity) a workshop to discuss results of surveys - The work should be coordinated by (or done in agreement with) existing initiatives, such as BirdLife International (http://www.birdlife.org/europe-and-central-asia/news/birdlife-partners-working-across-mediterranean-protect-seabirds), EBCC (http://www.ebcc.info), RAM project (http://redavesmarinas.blogspot.com.es/), etc.
List of material	• For the training: images of the species to be used in the ppt presentations	

needed	• telescopes and binoculars (also to be provided by volunteers) • Notebooks • Pencils • Digital voice recorders • Photo/video cameras (also to be provided by volunteers)	
Personnel needed (specialization level)	*Note that one person may do more than one of the different tasks • Scientific personnel to organise and give the training sessions • Observers (specialists / volunteers) • A regional coordinator (to gather and analyse data)	
Budget (tentative)	Personnel (permanent or temporary)	1 regional coordinator (volunteer) 3 full-months salary for 2-3 scientist(s) at sub-regional (country or region including several MPAs) and/or local (each MPA) level to give the training sessions and coordinate the activity
	Durable equipment	1 computer, Several telescopes / binoculars, Several digital voice recorders
	Consumables	Notebooks, Pencils
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site) Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, Images for training ppt presentation, Webpage to host specific e-tool to facilitate the participation of citizens, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	
Exploitation of results	Create a regional database in a pluriannual basis, to record and compare data on seabird migration phenology	

IV.5 Episodic Species Outbreaks (blooms) protocols

PROTOCOL 5a	**Indicator:** Jellyfish (gelatinous zooplankton) and algal blooms - Episodic events – Questionnaires
Problem /	Jellyfish (gelatinous zooplankton) and algal blooms have become more frequent in the Mediterranean coastal areas in the

scientific question	last decade likely in response to the increasingly warmer conditions of the Mediterranean Sea and other human impacts. These events have important negative ecological and socio-economic impacts.	
Scientific rationale (hypotheses)	- Increases in water temperature are related with increases in the reproduction rate and therefore with increases in jellyfish and algae number. - Water quality loss (e.g. nutrient pollution) and overfishing are additional common pressures in coastal areas of the Mediterranean, which also help trigger blooming events. - Jellyfishes are important plankton predators. Extreme increases in jellyfish populations may result in changes in the trophic chain mostly driven by plankton depletion and important fish larvae reductions. - Jellyfish blooms can provide additional food and shelter for comercially and ecologically valuable marine species (e.g. fishes, sunfish, marine turtles), when present. - Blooms may affect the Mediterranean biodiversity	
Sampling program	Metrics (variable / indicator)	Presence and semiquantitative abundance of jellyfish and algal blooms
	Type of survey	Volunteers – citizen survey
	Sampling technique	Interview
	Sampling unit (SU)	Standard questionnaire
	Spatio-temporal distribution of SUs and sampling size	- Sampling sites: - harbors, nautical clubs, disembarking areas, beaches, and dive centers that operate in the MPA. - # SUs per site: as much as possible - Sampling frequency: at least once a year, better if in Summer (Jul-Ag). Ideally homogeneous/periodic sampling along the entire year.
	Data analysis	multivariate and univariate analysis. ANOVAs.
Detailed procedure	Before	- Properly disseminate the blooms monitoring program in the MPA (e.g. posters, talks) <u>Develop a questionnaire</u>: Include relevant questions about the observation of jellyfish or algae blooms in the SPAMI - Design it so that filling it in should take less than 15 min. - Include a question where respondents provide a qualitative ranking of the abundance of jellyfishes in the bloom, according to 4 different grades: • 0 = <50 • 1 = 50-100

		• 2= 100-500 • 3= <500 - Include a question where respondents provide an approximate area (m^2) covered by the algal bloom, and a general description of its aparience (e.g. color) - Include a question where respondents identify/recognize the jellyfish species of the bloom (using jellyfish identification guides) <u>Select sites</u>: Major harbors, disembarking areas, more popular beaches, nautical clubs, larger dive shops that operate in the MPA. <u>Determine sampling frequency</u>: One survey a year (Summer: Jul-Aug), Or one survey in summer (Jul-Aug), and random additional surveys year round. - Respondents may be met just after finishing their water activities, e.g. in the harbors or dive stores. - Begin a conversation with a stakeholder without formalities and focus on the recent sighting of blooms. *Special attention should be placed to the approach, since some stakeholders may mistrust fisheries researchers and managers. - Once they show interest in the subject through discussion then the interview may formally begin. Optional: - To ensure the widest possible dissemination, the questionnaire can be distributed, for example, through dive shops to divers coming out of the water. For this, provide a jellyfish identification guide to the store and leave clear instructions on how each participant should return the completed questionnaire (e.g. take a photo and send it to an email, leave the completed questionnaire in the store (MPA managers may collect (e.g. weekly/biweekly) all completed questionnaires left in the store)).
	During	Questionnaire: • General information to be gathered: Date of sighting, time of sighting, name of sighting site, distance of the bloom form shore (m), distance of bloom from observer (m), depth of the bloom (shallow/deep/both), observer activity (boat, land, diving/snorkeling, fishing), location of bloom (in a bay, off a headland, offshore, stranded on a beach), approximate jellyfish number in the bloom? (scale) or approximate area covered by the algal bloom? Specific weather conditions during blooms: e.g. wind, waves, water temperature. • General observations to be gathered on other climate change indicators (showing images as examples may help): Indicate if have recently seen any of the following, and approx. when and where: - *Posidonia ocenica* (seaweeds) flowering - bleaching or mass mortality/necrosis - alien - mortality of organisms • Detailed description:

		- Start the interview. Filling in the entire questionnaire should take max. 15 min. - Clearly explain the four different ranks of the scale of abundance (0 =<50 ind.; 1 = 50-100 ind.; 2=100-500 ind.; 3=>500 ind.) - Show the respondents the jellyfish identification guides for the identification of the species sighted. - Clearly explain respondents the different ways for their further participation in the monitoring of blooms by reporting their furture sights: directly to the MPA managers (email, phone number), or through e-tools and cell phone apps (e.g. Medjelly, Meteo Meduse), or webpages (e.g. www.observadoresdelmar.es, www.cubomed.eu).
	After	- After finish the interview, ask for the possibility of being introduced to other stakeholders to interview more people. - Create a semiquantitative abundance database file with the data collected (baseline). - Analize the data (multivariate and univariate analyses, ANOVA, interannual comparissons). - Create e-tools to facilitate further stakeholders' reports on incidental observations of blooms, or upload data in e-tools from existing networks (e.g. LIFE Cubomed Project, www.cubomed.eu, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016), www.observadoresdelmar.es, www.ciesm.org/marine/programs/jellywatch.htm) - Divulge results in the science environment and among stakeholders in the MPA (e.g. posters, talks, media)
List of material needed	• Jellyfish identification guides • Images of bleaching, mortality events, *P. oceanica* flowering, jellyfish/algal (e.g. mucilage) blooms • plastic boards • pencils • data-sheets with questionnaires	
Personnel needed (specialization level)	*Note that one person may do more than one of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • Interviewers • People to introduce the data collected into database • Scientists to analize the data collected (statistics: multivariate and univariate analyses) • People to create e-tools to facilitate stakeholders' reports on incidental observations of blooms, or upload data in e-tools from existing networks (CIESM Jellywatch programme) • People to promote and divulge the survey in both the science and citizen (stakeholders) environment	
Budget (tentative)	Personnel (permanent or temporary)	Note that one person may do more than one of the different tasks, e.g. one single person could do both interview people and introduce data into database • Salary for interviewers (N_o of people and full days will depend on the N_o of people to be interviewed)

		• 2-4 weeks salary/year for one person to introduce the data collected into a database and share data collected with the CIESM tropical signals program and other online network platforms/e-tools • 1 month salary/year for one data analyst (statistics: multi and univariate analyses) • 1 month salary for one person to create e-tools to facilitate reporting on blooms events (optional) • 1 month salary/year for one person to divulge the project/results among scientists and stakeholders
	Durable equipment	Computer
	Consumables	Jellyfish identification guides, Images with examples of bleaching, mortality events, *P. oceanica* flowering, jellyfish/mucilage blooms, mortality of organisms, Plastic boards and pencils, Data-sheets with questionnaires
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for people interviewed (if possible), Overheads-variable rates depending on the institution
	Subcontracting	
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integration of data into existing international/basin-scale blooms monitoring networks, such as: LIFE Cubomed Project, Medjelly, Meteo Meduse, Observadores del mar, Cubomed, Ciesm-jellywatch	

PROTOCOL 5b	**Indicator:** Jellyfish (gelatinous zooplankton) and algal blooms - Episodic events– Volunteer observers/etools & apps
Problem / scientific question	Jellyfish (gelatinous zooplankton) and algal blooms have become more frequent in the Mediterranean coastal areas in the last decade likely in response to the increasingly warmer conditions of the Mediterranean Sea and other human impacts. These events have important negative ecological and socio-economic impacts.
Scientific rationale (hypotheses)	- Increases in water temperature are related with increases in the reproduction rate and therefore with increases in jellyfish and algae number. - Water quality loss (e.g. nutrient pollution) and overfishing are additional common pressures in coastal areas of the Mediterranean, which also help trigger blooming events. - Jellyfishes are important plankton predators. Extreme increases in jellyfish populations may result in changes in the trophic chain mostly driven by plankton depletion and important fish larvae reductions.

	- Jellyfish blooms can provide additional food and shelter for comercially and ecologically valuable marine species (e.g. fishes, marine turtles), when present. - Blooms may affect the Mediterranean biodiversity	
Sampling program	Metrics (variable / indicator)	presence and semiquantitative abundance of jellyfish and algal blooms
	Type of survey	Volunteers – citizen survey
	Sampling technique	Interview
	Sampling unit (SU)	Online questionnaire
	Spatio-temporal distribution of SUs	- Sampling sites: online - Sampling frequency: all year round
	Data analysis	multivariate and univariate analysis. ANOVAs.
Detailed procedure	Before	- Organize activities to disseminate and explain among stakeholders the new blooms' monitoring to be implemented in the MPA through direct involvement of volunteers as observers (e.g. talks, posters, media). - Look for volunteers (stakeholders) willing to participate as observers, for example, in harbors, nautical clubs, beaches, disembarking areas, fish markets, dive centers that operate in the MPA. Talk with divers, fishermen, beach life guards, or people from other existing volunteer groups, which are usually highly sensitive to environmental problems. Train observers: - Develop training sessions to introduce participants with the different sightings reporting options and algal and jellyfish blooms identification: • If possible, provide each observer with a jellyfish identification guide • Clearly explain the multiple options that observers have to participate in the monitoring by reporting their sights: for example, directly to the MPA managers (emails, sms, phone calls), or through cell phone apps (e.g. Medjelly, Meteo Meduse) or e-tools/webpages (e.g. www.observadoresdelmar.es, www.cubomed.eu). • Trainees should learn how to register an account, properly complete the information requested on each site, and how to report their sightings in the MPA. - If existing internet platforms of other programs were suggested to volunteers as reporting options, contact the platform administrator (e.g. through email) stating your full name and the MPA that you manage and

		ask them for the possibility of keeping you notified of any activity related to your MPA and receive alerts when new sightings near your MPA are added.
	During/After	- Keep frequent and periodic track of the sightings reported by volunteers. - Make sure all reporting options are working properly and available at all times. - Create a database file with the data collected (baseline) and update it frequently/periodically. - Analize the data (multivariate and univariate analyses, ANOVA, interannual comparissons) periodically. - Frequently and periodically acknowledge volunteers work and divulge results in the science environment and among stakeholders in the MPA (e.g. posters, talks, internet, media).
List of material needed	• Jellyfish identification guides • Posters on the blooms' monitoring program • If possible provide each observer with: - a notebook containing general information on the jellyfish and algal blooms' monitoring - a set of fact sheets with photographs and detailed description of the species of jellyfish or the algal blooms most frequently sighted in your area - a shirt and a cap with the logo of the blooms monitoring program. This will help engaging volunteers by making them feel an important active part of the project.	
Personnel needed (specialization level)	*Note that one person may do several of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • People to divulge the blooms' monitoring and engage volunteers • People to train observers • People to create e-tools to facilitate observers' sightings or get info about sightings in the MPA from existing networks • People to keep track of the sightings reported and introduce the data reported into database • People to analize the data collected (multivariate and univariate analyses) • People to share info with existing blooms monitoring networks (e.g. CIESM Jellywatch programme) • People to thank, promote and divulge the survey in both the science and citizen (stakeholders) environment	
Budget (tentative)	Personnel (permanent or temporary)	Note that one person may do more than one of the different tasks, e.g. one single person could do both interview people and introduce data into database • 1 month salary/year for one person to thank, promote and divulge the blooms monitoring program in both the science and citizen (stakeholders) environment and engage volunteers • 1-2 weeks/year salary for two experts to train observers • 1 month salary for one person to create e-tools to facilitate observers' sightings (optional) or get info about sightings in the MPA from existing networks

		• At least 1 month/year salary for a person to keep track of the sightings reported and introduce the data reported into database • 2-4 weeks salary/year for one person to share the data collected with existing blooms monitoring networks (e.g. CIESM Jellywatch programme) • 1 month salary/year for one data analyst (statistics: multivariate and univariate analyses)
	Durable equipment	Computer
	Consumables	Jellyfish identification guides, Plastic boards and pencils, Data-sheets with questionnaires, Posters and banners on the jellyfish blooms' monitoring, A notebook containing general information on the jellyfish and algal blooms' monitoring (optional), A set of fact sheets with photographs and detailed description of the species of jellyfish most frequently sighted in the area
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Banners, Posters, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integration of data into existing international/basin-scale monitoring networks, such as: LIFE Cubomed Project, Medjelly, Meteo Meduse, Observadores del mar, Cubomed, Ciesm-jellywatch	

PROTOCOL 5c	**Indicator:** Jellyfish (gelatinous zooplankton) and algal blooms - Episodic events– Beach & coastal areas inspections
Problem / scientific question	Jellyfish (gelatinous zooplankton) and algal blooms have become more frequent in the Mediterranean coastal areas in the last decade likely in response to the increasingly warmer conditions of the Mediterranean Sea and other human impacts. These events have important negative ecological and socio-economic impacts.
Scientific rationale (hypotheses)	- Increases in water temperature are related with increases in the reproduction rate and therefore with increases in jellyfish and algae number. - Water quality loss (e.g. nutrient pollution) and overfishing are additional common pressures in coastal areas of the Mediterranean, which also help trigger blooming events. - Jellyfishes are important plankton predators. Extreme increases in jellyfish populations may result in changes in the trophic

	chain mostly driven by plankton depletion and important fish larvae reductions. - Jellyfish blooms can provide additional food and shelter for comercially and ecologically valuable marine species (e.g. fishes, marine turtles), when present. - Blooms may affect the Mediterranean biodiversity	
Sampling program	Metrics (variable / indicator)	presence and semiquantitative abundance of jellyfish and algal blooms
	Type of survey	Scientific – technical survey
	Sampling technique	Option 1: Intertidal/beach walk Option 2: snorkeling Option 3: drone/plane flight Option 4 : boat trip (e.g. ferrys or boats that periodically navegate in the area)
	Sampling unit (SU)	For intertidal/beach walk, drone/plane flights, and boat trip: Meters per minute For snorkeling: 100m transect/10 min
	Spatio-temporal distribution of SUs and sampling size	- Sampling locations: in protected areas of the SPAMI, or ideally in protected and unprotected areas of the MPA - For intertidal/beach walk, drone/plane flights, and boat trip: cover the largest area possible - For snorkeling: • 2 sampling sites/location (areas frequently showing jellyfish/algal blooms; each site located 100m apart from each other; sites exposed to different winds and currents (e.g. eastwards, northwards, etc). • Depth strata per site: depending on vertical visibility (e.g. between 0-10m) • # SUs per site: 3 surveys/site - At least once a year, in summer (Jul-Aug). Ideally, homogeneous/periodic sampling all year round
	Data analysis	Basic descriptive statistics, ANOVA.
Detailed procedure	Before	<u>Choose sampling locations</u>: In protected areas of the SPAMI Or in protected and unprotected areas of the SPAMI <u>Select sites</u>: - choose sites previously reported to present jellyfish or algal blooms events - cover most and less human disturbed areas - include sites differently exposed to main winds and currents (e.g. usually receiving eastern currents/winds) <u>Determine sampling frequency:</u> - At least once a year, ideally in summer (Jul-Aug)

		<u>Conduct Pre-survey (if possible)</u> To familiarize the personnel with the methods and tools to be utilized.
	During	General information to be gathered: Name of observer, date, coordinates of starting point and end point (Lat; Long), starting and end time of survey, sea condition (rough, calm,etc.), shore exposure (exposed, moderately exposed, sheltered), coordinates and time of sighting, name of sighting site, distance of the bloom from shore (m), distance of bloom from observer (m), location of bloom (in a bay, off a headland, offshore, stranded on a beach), approximate jellyfish number in the bloom (sea the scale below) or area covered by the algal bloom. • Detailed description of the census: <u>Intertidal/beach walk, drone/plane flights, and boat trips</u>: - Cover the largest area possible - Register the coordinates of starting point and end point (with a GPS, or photograph a peculiar, permanent feature of the shore from where you start the survey) - Fill in the upper portion of the data-sheet (General information) - For drone/plane flights: register the altitude of the survey - Take note of the starting and end time of survey - While walking/flying/navegating slowly and at constant speed, look for jellyfish/algal blooms along the largest area possible. - If a bloom is found, assign a jellyfish abundance category of the scale (see below) or estimate the approximated area covered by the algal bloom, or record as P (present) or N (none, if apparently absent) - Photograph the bloom and, if possible, zoom in for jellyfish identification. - If possible, identify the species present in the bloom - Before leaving, have a quick walk/fly/trip around the sampling area to confirm first impression of blooms presence and relative abundance/coverage. - Repeat the same procedure to sample other sites. - Photographs must be catalogued: date, site location, sampling technique, depth/altitude. The abundance scale for jellyfish blooms has 4 categories: • **0**=<50 ind**.** • **1=** 50-100 ind. • **2=** 100-500 ind. • **3=**>500 ind.

		In addition, the observer may record also the following: • **P**= Present –there is a bloom but organisms abundance is not clear • **N**= None – there is no apparent bloom <u>For snorkeling</u>: - Visit one permanent site and choose an area to sample. - Mark the survey’s starting point and end point locations using a GPS, or photograph a peculiar, permanent feature of the shore from where you start/finish the survey - Fill in the upper portion of the data-sheet (General information) - Take note of the starting and end time of survey - While swimming slowly and at constant speed to cover an area of approx.100m, look for jellyfish/algal blooms for about 10 min. - If a bloom is found, assign a jellyfish abundance category of the scale (see below) or estimate the approximated area covered by the algal bloom, or record as P (present) or N (none, if apparently absent) - Photograph the bloom and, if possible, zoom in for jellyfish identification. - If possible, identify the species present in the bloom - Before leaving, have a quick swim around the sampling area to confirm first impression of blooms presence and relative abundance. - Swim a further 100m and start another timed search of 10 min. along 100m transect. Then repeat the same for a third survey. - Repeat the same procedure to sample other sites. - Photographs must be catalogued: date, site location, sampling technique, depth. - The abundance scale for jellyfish blooms has 4 categories: • **0**=<50 ind**.** • **1=** 50-100 ind. • **2=** 100-500 ind. • **3=**>500 ind. In addition, the observer may record also the following: • **P**= Present –there is a bloom but organisms abundance is not clear • **N**= None – there is no apparent bloom
	After	- Create a database where collected information will be registered (baseline). - Introduce collected data into baseline. - Analize the data (statistics: multivariate and univariate analyses, ANOVA, interannual comparissons)

		- Share data collected with existing blooms monitoring programmes and etools (e.g. LIFE Cubomed Project, CIESM Jellywatch programme, www.observadoresdelmar.es, www.cubomed.eu, MedSeaObserverApp (beta version of this mobile app is ready to be launched in google market in 2016)). * Contact existing internet platforms of other blooms monitoring programmes, state your name and the name of your MPA, and ask for the possibility of keeping you notified of any activity related to your MPA and receive alerts when new sightings near your MPA are added. - Divulge results in both the science and citizen environment
List of material needed	• Posters on the jellyfish blooms' monitoring • Monitoring data-sheets • Field guides-slates to identify species • Mask, fins and snorkel • Underwater photo camera • watch • GPS • plastic board • pencil • boat/drone/plane	
Personnel needed (specialization level)	*Note that one person may do several of the different tasks, i.e. the number of personnel required will depend on the capabilities of each MPA. • People to monitor jellyfish/algal blooms in the SPAMI (census) • People to create a database and introduce the data collected into it • People to share the data collected with existing blooms monitoring networks (e.g. the CIESM Jellywatch programme) • People to analize the data collected (statistics) • boat captain, drone/plane pilot • People to divulge the project/results in both the science and citizen environment	
Budget (tentative)	Personnel (permanent or temporary)	Note that one person may do more than one of the different tasks, e.g. one single person could do both sample and introduce data into database • 1-2 full days/salary/year for an observer to monitor and estimate relative abundance of jellyfish or algal blooms in the SPAMI (census) • 1 month salary/year for one person to divulge the project/results among scientists and stakeholders • 1-2 weeks salary/year for one person to introduce the data collected into a database and to share the data collected with existing blooms monitoring networks (e.g. the CIESM Jellywatch programme) • 1 month salary/year for one person to analize the data collected (statistics)

		* 1-2 full day salary for boat captain, drone/plane pilot
	Durable equipment	Mask, fins and snorkel, Photo camera (Underwater photo camera if possible), Watch (underwater watch for snorkeling), GPS, Computer, * boat/drone/plane
	Consumables	Plastic boards and pencils (one/observer), Posters/banners on the blooms' monitoring program Monitoring data-sheets, Field guides-slates to identify species
	Travel, accommodation and diets	Gas/diesel for transportation (from/to site): car and boat Diet per diem Accommodation allowance per diem
	Other costs (including overheads)	Variable, but for example: Outreach (media), Posters/Banners , Batteries, T-shirts or other gifts for volunteers (if possible), Overheads-variable rates depending on the institution
	Subcontracting	* boat/drone/plane
Exploitation of results	Comparison among MPAs and identification of the most vulnerable areas to provide measures of mitigation to managers. Integration of data into existing international/basin-scale blooms monitoring networks, such as: Medjelly, Meteo Meduse, Observadores del mar, Cubomed, Ciesm-jellywatch	

V. LESSONS LEARNED AND RECOMMENDATIONS

Criteria for MPA CC monitoring programs

* Monitoring programs in MPAs should be planned to last several years (decades) and to collect great amounts of data. Efforts therefore should be made to guarantee a continued funding that allows both the establishment and continuity of monitoring programs. Budget is usually limited in Mediterranean MPAs, though, as well as staff/personnel (low capacities).

* Technology advances do not always guarantee the collection of better or more data. In some occasions, advanced equipments are expensive and therefore its use is restricted to only few MPAs. So, sometimes, at least at a Mediterranean scale, more may be done with less.

* Some sampling methodologies can be adapted to monitor more than one indicator and therefore to be more cost-effective. For example, permanent quadrat/transects to monitor mortality events can serve as well to monitor bleaching, and/or the distribution and abundance of benthic species.

* There is a need to develop a standardized method to train volunteers on common climate change indicators protocols, but it must be guaranteed even without specific funding, e.g. by asking volunteers to pay for their training and recognizing them a certification. This system allows to track the data coming from certified volunteers. This approach is well established in several international citizen science programmes and results can be very strong.

* Results of CC monitoring programs should be used at local and sub-regional level to understand how MPAs and MPA networks can cope with the effects of CC, and to indicate management options to be taken in order to increase ecosystem resilience.

* Monitoring programs should always provide feedback of the results to the stakeholders/volunteers. Volunteers appreciate being recognized for their job (e.g. public recognizion) and learning about the ocean environment.

The importance of citizen science

* The great potential of citizen science to support and increase the scope of monitoring programs in MPAs needs to be fully exploited. Not only citizen scientists can provide unique information based on their local ecological knowledge (LEK), but they also represent a great work force for the generation of scientific knowledge, i.e. observation, sampling, collection of data, and analysis of data, which end up directly supporting management decisions in MPAs.

* Colaborative monitoring efforts that involve several partners, e.g. the government agencies/institutions, NGOs, the academia (Universities) and the civil society (e.g. local operators, ecological groups, divers, fishermen), have greater acceptance by local communities and help create further community understanding of issues and develop effective partnerships for future actions.

The way forward

* Capacity-building initiatives should be planned regularly at regional level to keep assigned personnel updated on the principles, methods, main results and advances in knowledge of monitoring programmes and the effects of climate change on Mediterranean ecosystems.

* The integration of Mediterranean MPAs into existing large-scale (e.g. basin-scale) climate change monitoring projects/networks, which already use standard protocols, should be a more common and widely implemented practice.

* Sensitization and divulgation actions are important to highlight the increasing evidence of climate change and its effects on the marine environment.

VI. CONCLUSIONS

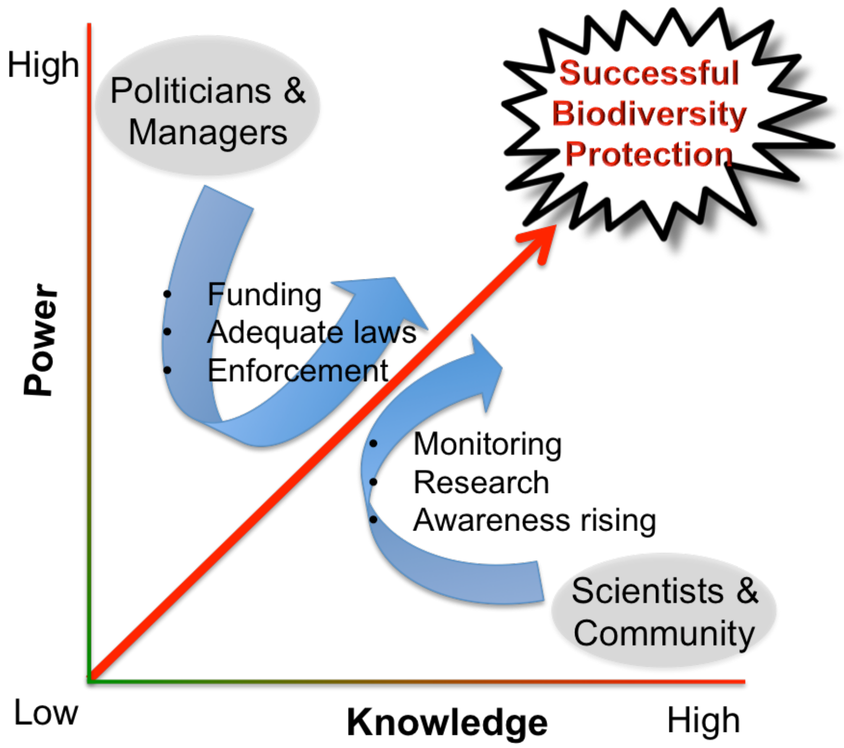

Figure 51. Building the capacity of MPAs, SPAMIs through data collection, monitoring and awareness-raising about climate change contributes to the efforts being made across the Mediterranean region to improve information and adapt to change.

As documented in biological studies, the passage from a simple to a complex system is generally based on an increase in the communication efficiency. Single elements of a system start to communicate and to exchange information among them allowing the evolution of a new system. This is what happened at every key step of evolution. If there is the willingness to build a working network of Mediterranean MPAs it is important to develop a common language to share experience and results. The data sharing is often still very difficult owing to a general little availability of scientisits to open their datasets. The opportunity offered by citizen science could strongly cover this gap in communication, sharing data collected by volunteers that both scientists and managers could exploit. The main typical criticism towards this approach is a general little consideration of the data coming from volunteers, usually considerd by certain scientists poor and unreliable data. Anyway there is a wide scientific literature on this topic, with several tests performed to compare data sets from volunteers and actual scientists, confirming the validty of data from volunteers.

All countries in the Mediterranean basin should unify their efforts towards a common conservation goal: to mitigate the impacts of climate change in the Mediterranean marine biodiversity. This will be only possible if each country, through its politicians and natural resources managers, find a way to support research and monitoring activities. Scientific results should support decision-makers actions and MPA's management plans, implementing both adequate laws and enforcement. Specific finantial programs and external sources of funding should be developed. Large basin-scale international networks for the monitoring of climate change in the Mediterranean already exists to facilitate the cooperation among all countries. If more countries and SPAMI's take part of these networks and provide their own data the success of such efforts would be much greater. Research and monitoring activities allow study the MPA and better understand the impacts of climate change on marine and coastal ecosystems. These data in turn will help determine funding for grants and programs. Monitoring programmes can furnish valuable information that can feed into current efforts to evaluate the impact of climate change by the end of the current century.

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Annex 1. Fact-sheets of Climate Change Indicators

CODE: A1	*Sea Surface Temperature and Thermal Stratification*	**Category(ies): Physical – chemical indicators**

Definition: Main temperature indicators: T anomalies, T_{max}, % of time above different temperature thresholds. These indicators can only be obtained from high resolution temperature series.

Rationale:

Due to the relatively small volume of the basin, the Mediterranean Sea is expected to react faster to climate warming than the open oceans (Lejeusne et al. 2010), and for this reason it is considered a good model to forecast global change effects. At present, it is already acknowledged that climate change-induced temperature variations have altered biological patterns and biodiversity (Gambaiani et al. 2008; Bensoussan et al. 2010).

Historical satellite data of the Mediterranean sea confirms the rise of the sea surface temperature (SST) in the recent years (Nykjaer 2009). Rises in SST are linked with alterations of the vertical thermal structure and deepen thermocline which has potential bio-ecological effects on deeper areas than the first meters (Danovaro *et al.* 2001; Harley *et al.* 2006). However, the effect of the thermal anomalies along the water column on marine ecosystems are complex (Rodolfo-Metalpa *et al.* 2006; Ferrier-Pagès *et al.* 2013) and only high resolution temperature series collected consistently in space and time would allow the evaluation of the role and the impact of thermal anomalies on marine communities.

At present, long term sea temperature series (>30 years) have only been collected in a few coastal sites mainly in the North-western Mediterranean. Large scale SST obtained through satellite images and the use of few time-resolved T series allow the characterization of the spatial and inter-annual variability of the coastal water column in only a few Mediterranean sites. With the knowledge gathered from the analysis of these series, a clear warming trend in the NW Mediterranean (ca. 0.3º C per decade) is already visible, demonstrating the warming trend at different depths. However, we are still lacking information for the rest of the Mediterranean Sea and more particularly in the first 50m of the water column where variability in the temperature regimes can have strong effects on the biological communities.

Therefore, the acquisition and analysis of high resolution temperature series in the coastal areas is crucial to manage climate change impacts on marine biodiversity. Temperature series will allow the characterization of thermal regimes (T_{max}, mean temperatures, stratification patterns, etc…), detect temperature anomalies and contribute to the tracking of warming trends in

Mediterranean coastal areas.

Sensitivity to climate change and other factors (essential information from scientists explaining why the indicator is relevant and important in assessing climate change impacts)

Temperature anomalies, even over a short period of time, can significantly affect Mediterranean ecosystems and biological diversity (Walther *et al.* 2002; Philippart *et al.* 2011) because of the sensitivity of keystone species. Thanks to the very few time-resolves temperature series, changes in species distribution and mass mortality events of keystone species have been reported during the last decades and related to the warming trend observed in the Mediterranean region. Some examples are:

- The large scale mass mortality events (MME) of long-lived benthic species during 1999 and 2003 in the NW Mediterranean, have been related to the anomalous temperature conditions registered in coastal waters (0-50 m depth) (Bensoussan et al. 2010; Crisci et al. 2011). The effect of successive heat waves with peaks in the water temperature and the position of the thermocline (Cerrano *et al.* 2000; Perez *et al.* 2000). In addition, niches resulting from these mortality events become available for new invasive colonizers (Occhipinti-Ambrogi 2007).
- The strong influence of the SST in changes in the growth, distribution, composition and diversity of anchovies (*Engraulis encrasicolus*) or sardine (*Sardinella aurita* and *Sardina pilchardus*) populations (Basilone *et al.* 2006; Sabatés *et al.* 2006)
- The changes suffered by alteration of the hydroclimatic factors and the nutrient ratio in the structure, distribution and phenology of Mediterranean plankton communities, the base of the food chain (Licandro and Ibanez 2000; Goffart *et al.* 2002; Mercado *et al.* 2005; Molinero *et al.* 2005; Fernández De Puelles and Molinero 2008).

However, in other areas the lack of data sets and information on temperature variability has prevented analysis that may lead to a direct correlation between temperature and impacts onto the marine communities.

In such context and given actual warming trends and projections, the acquisition of high resolution temperature series is crucial for increasing our detection, understanding and forecasting abilities of possible impacts. These abilities will be the key to implement appropriate conservation and management plans for the conservation of the rich Mediterranean biodiversity.

Methodology and sampling concerns:

Automatic measuring systems (cheaper and less person- demanding), such as data loggers or oceanographic buoys, should be used to reduce costs and efforts on this monitoring. There are several options depending on the budget. From the cheaper to more expensive:

Cheaper. Deploy temperature data loggers *(Stowaway Tidbits, Stowaway Tidbits v2 and HOBO Water Temp Pro v2)* every 5 m, from 5 to 50 m depth, fixing them to the rocky substrate or along the buoy lines/chains. They are usually used by *research institutions.* Temperature data loggers should be setup to collect hourly measurements and recovered annually or semi-annually by divers usually before and after the summer period. Specific information from these sensors is the collection of temperature profiles.

Expensive but less person-demanding. Moored or free-floating buoys which have the possibility to measure oceanographic, meteorological and water quality data. The efficiency of buoys has been considerably increased owing to advanced technology including solar storage batteries, data logging controller and environment-friendly antifouling coatings. Data can be transmitted in real-time to land-based observatories via satellite communications. Although there are more expensive, it could be an alternative source of information if there is a buoy close to the MPA.	
Representation (quantitative, graphical, cartographic, GIS): Online quantitative database, graphics, regional cartography on GIS	**Geographic relevance:** Mediterranean
Policy relevance: The acquisition of temperature records will allow assessment of the climate change risks for the different Mediterranean areas. This will permit adoption of management and conservation actions depending of the degree of the risk.	
Other data sources (existing Mediterranean/National monitoring programs): • National observatory services • http://www.t-mednet.org/ • http://www.moon-oceanforecasting.eu/index.php?option=com_frontpage&Itemid=1 • http://gnoo.bo.ingv.it/mfs/ • http://www.myocean.eu/ • http//www.ciesm.org/marine/programs/hydrochanges.htm	
Relevant modelling programs: • ClimCares project • Tmed-net	
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CODE: A2	***Mortality, necrosis and bleaching events***	**Category(ies): Population**

Definition: Occurrence and relative importance of partial or total mortality in benthic species

Rationale:

There is wide scientific evidence of the increased spread and intensity of massive mortalities and bleaching events in several areas of the Mediterranean, modifying in unpredictable ways the functioning of the ecosystems of the basin. Marine Protected Areas are hopefully monitored with major continuity and can be considered a warning system at basin level.

Two main quite recent events can be considered responsible for the huge regime shifts we are nowadays following in the Mediterranean sea. The 1980s regime shift may be the beginning of the acceleration of the warming shown by the IPCC. This event is far to be definitively understood even if a recent report suggests a volcanic eruption, occurred in Mexico in 1982, as triggering agent (Reid *et al.* 2015). In the same late 1980s period, regime shifts are reported in the North Sea, the Baltic Sea, the Black Sea, and the Adriatic Sea (Conversi *et al.* 2010). In this period in the Mediterranean Sea also wide massive mortalities were reported for commercial sponges (Gaino *et al.* 1992).

The El Nino occurred during 1998, is considered a second important event triggering worldwide regime shifts. Temperature anomalies in the Mediterranean led to a series of massive mortalities affecting several taxa, mainly benthic suspension feeders (Rivetti *et al.* 2014). These phenomena can affect indifferently species with or without autotrophic symbionts. They are often reported and described in Marine Protected Areas (Cerrano *et al.* 2000; Garrabou *et al.* 2009; Huete-Stauffer *et al.* 2011) but the etiological agents still remain uncertain. Neither temperature anomalies nor recognized microbial pathogens are solely sufficient to explain for the events. Anthropogenic influence may play a significant role in determining the coral health status by affecting the composition of the associated microbial community.

In case of species symbiotic with zooxanthellae, bleaching events are reported. *Cladocora caespitosa* (Rodolfo-Metalpa *et al.* 2014), *Balanophyllia europaea* (Kružić and Popijač 2014) and *Oculina patagonica* (Rubio-Portillo *et al.* 2014) are the species where this phenomenon has been described. As for tropical corals, youngest colonies are usually the most affected.

In recent years, the coralligenous community, one of the most diverse in the Mediterranean Sea, where suspension feeders are dominant, has been strongly affected by several mass mortality events. Lejeusne *et al.* (2010) reports the occurrence of bleaching and mortality in 38 Mediterranean species (13 sponges, 10 cnidarians, 5 bryozoans, 6 molluscs, 1 sea-urchin and 3 ascidians) and most of them live in coralligenous habitats. Engineer species, including sponges and gorgonians, have been the most evident affected taxa, down to depths of 45 m. Regarding gorgonians, massive mortalities affect mainly larger and female colonies.

The causes of mass mortality events in the Mediterranean is related to the synergy of several conditions, and our ability to predict the effects of these events depends on characterizing them and elucidating trends exhibited by potentially causative factors.

Sensitivity to climate change and other factors (essential information from scientists explaining why the indicator is relevant and important in assessing climate change impacts)

Current hypotheses about the causes of these events in the Mediterranean focus on their relationship with the occurrence of distinctive climatic anomalies in late summer and early fall. The occurrence of such climatic anomalies in late summer implies prolonged exposure of organisms dwelling above the thermocline to summer conditions (high temperatures and low food availability) (Vezzulli *et al.* 2010).

Where pressures are present the resilience of the communities towards climate anomalies can be lower respect the areas without any stressors (Micheli et al. 2012). There are no actual possibilities to modify the negative responses of marine species and habitats to climate warming. Anyway, considering that massive mortality events are enhanced in case of occurrence of multiple stressors, managers should limit or reset the presence of anthropogenic stressors in areas affected by massive mortalities. Stressors that can limit the resistance and the resilience of sensitive species can be both chemical and physical. In the first case, pollution coming from sewage and sediment delivery from rivers (Mateos-Molina *et al.* 2015) can compromise filtering activities of suspension feeders. Physical stressors can be fishery, anchorages, and diving activities. Regarding fishery, bottom trawling can directly affect sea floor integrity but can also increase sediment resuspension processes, compromising the survivor especially of

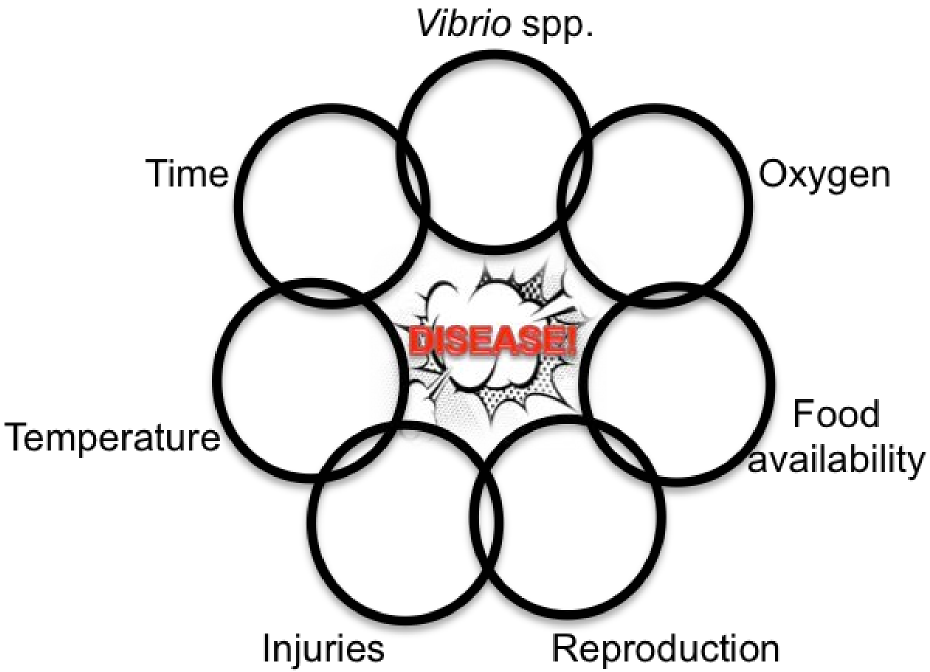

Figure of the conceptual multi-aetiology model of *P. clavata* in mass mortality events of the NW Mediterranean Sea (from Vezzulli et al. 2011, modified)

benthic sessile organsims. Artisanal and recreational fishing activities can cause direct damage to benthic species, causing the complete detachment of sessile organisms or tissue injuries. Tissue regeneration can modify the natural metabolic pattern of injured organisms addressing differently energy allocated for secondary production, and can alter the composition of the associated microbioma (Vezzulli *et al.* 2013), the main immune system of lower invertebrates.

Stressful events such as the occurrence of temperature anomalies and starvation periods as well as the presence of highly virulent microbial strains may be superimposed to compromise immunity and facilitate mortality outbreaks.

The indicator is relevant and important in assessing climate change impacts because it is responsible of the regime shifts that are documented in this last years for several marine habitats. As demonstrated in (Ponti et al. 2014) the gorgonian disappearances may cause a shift from complex assemblages characterised by skiophilous species to simple assemblages characterised by photophilous species, decreasing biodiversity and resilience of coralligenous bioconstructions.

Methodology and sampling concerns:

Qualitative surveys:

In each MPA, lists of species showing clear-cut signs of recent necrosis, should be reported, such as white patches in coralline algae, necrotic tissue in sponges, denuded skeletons in gorgonians, bleached scleractinian corals, empty valves in bivalves both attached to the substratum (e.g. *Spondylus gadaeropus*) or on the sea floor (e.g. *Arca noae*), dead portions of bryozoan skeletons, spine losing sea urchins, necrotic tissue in tunicates. These observations are often firstly furnished by recreational divers suggesting a potential way to exploit their experience.

Quantitative surveys:

Sponges: counting on standard areas the total number of specimens and those suffering partial or total mortality (Cerrano et al. 2010; Di Camillo et al. 2013; Di Camillo and Cerrano 2015). The shape (encrusting, massive, branching) and the size should also be recorded. Sponge morphology can affect the dynamics of pathogens spreading inside each specimen. Scleractinian corals: In case of zooxanthellate species, bleached colonies are easily detectable. Photographic monitoring on standard areas of a sufficient number of colonies. Gorgonians: random (or permanent, depending on the purpose) quadrats to monitor total density, total mortality, partial mortality, contribution of each of type of injury to total injury, and colony size (height). Quadrats (50 x 50 cm) randomly sampled every 5 m from the bottom to the top of each shoal or cliff, following standard methodology (Cerrano et al. 2005; Coma et al. 2006; Linares et al. 2008) and in relation to the geomorphology of the sites. Within each quadrat the number of colonies (converted to colony density), colony heights, colony health (defined as the percentage of colony with damaged coenenchyme: 0% was considered healthy, <25, <50, <75,<99 and 100%), and the number of fishing lines and/or nets wrapped around the colonies should be recorded. Furthermore, for each colony the epibiosis level should be recorded and the epibiontic organisms identified. On the basis of organisms that had settled on the scleraxis different temporal phases: (i) denuded (when the scleraxis is visible, with tissue on scleraxis), (ii) new (covered with filamentous green and/or red algae, and/or hydrozoans), (iii) medium (possessing a thick coat of algae, and/or sponges) and (iv) old (mainly colonized by calcareous organisms such as bryozoans). In all cases, photographic or video methods are also possible (see, e.g., Kipson *et al.* 2011, Teixidó *et al.* 2011).	
<u>Representation</u> (quantitative, graphical, cartographic, GIS): Online quantitative database, graphics, regional cartography on GIS	**<u>Geographic relevance</u>:** Mediterranean
<u>Policy relevance</u>: The assessment of the distribution and frequency of massive mortality events will prioritize areas where managers have to urgently limit several stressors. The impressive reduction of habitat complexity where massive mortalities recur can vanish many other management measures. The loss of complexity compromise the recruitment of several species, also of commercial interest, and open the door to non indigenous species.	
<u>Other data sources (existing Mediterranean/National monitoring programs)</u>: • http://climcares.medrecover.org • www.observadoresdelmar.es	
<u>Relevant modelling programs</u>: N/A	

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CODE: A3	*Range shifts of **alien/native** temperaturesensitive species*	**Category(ies): Population**
Definition: Occurrence, changes in abundance (e.g. number, coverage), and shifts in the geographical/depth distribution of native and non-native temperature sensitive species, i.e. warm-water species and cold-water species.		

Rationale:

Oceans' warming is causing important changes in the abundance and geographical/depth distribution ranges of many marine species. The higher availability of temperate environments coupled with the increasing contraction of cold-water habitats is resulting in a global trend of poleward migration (Parmesan and Yohe 2003; Chen et al. 2011; Sunday et al. 2012), which may potentially drive to widespread extinctions where dispersal capabilities get limited or suitable habitat unavailable.

In the Mediterranean, such trends are already apparent. Northwards shifts of isotherms (Burrows *et al.* 2011), with evident biotic consequences are documented for the whole basin (Lejeusne et al. 2010). Warm-water species are extending their geographic range to areas that used to be cold but have become warmer (tropicalization) (Bianchi 2007; Moschella 2008; Raitsos et al. 2010; Coll et al. 2012). Thus, several native warm-water species that occurred mostly in the southern parts of the basin, the warmest area in the basin, are increasing in number and moving to the north (Bianchi & Morri, 2000). For example, the round sardinella *Sardinella aurita* (Sabates et al. 2006), the dusky grouper *Epinephelus marginatus*, the ornate wrasse *Thalassoma pavo*, and the Mediterranean parrotfish *Sparisoma cretense* (Bianchi and Morri 2000; Sabates et al. 2006; Coll et al. 2012).

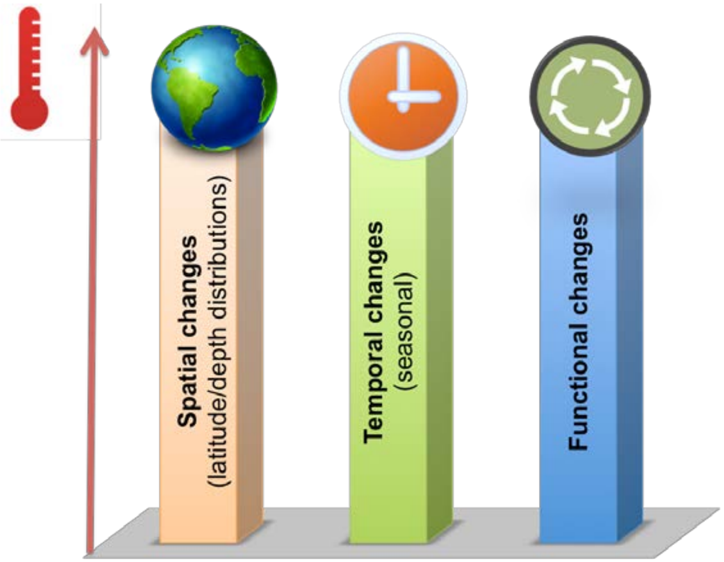
In addition, a larger number of tropical/subtropical thermophilic species are arriving to the Mediterranean from nearby regions (Bianchi 2007), such as for example, barracudas *Sphyraena spp.*, the blue spoted cornetfish *Fistularia commersoli* (Azzurro et al. 2011), and the rabbitfish *Siganus luridus* (Hassan et al. 2003; Golani 1993). Some of these non-native species are invasive and might thrieve in new areas with overwhelming success, getting to occupy native species' niche and driving them to important decreases or even local extinction. Because most of these species are tropical, the Mediterranean warming up favours them over the native biota. Anthropogenic disturbances, such as dredging, sewage and thermal discharges, eutrophication, hypoxia, fishing pressure and trawling, create additional opportunities for invader species. Marine bioinvasions are regarded as one of the main causes of biodiversity loss in the Mediterranean (Galil, 2007; Coll et al. 2010), and represent a growing problem extending to all its regions (Galil, 2007, Galil et al. 2009; Zenetos *et al.* 2010), with unexpected and harmful impacts on the biodiversity and ecosystem functioning, economy and human health (Galil, 2008, Gomez and Claustre 2003).

On the other hand, native cold-water species are constricting their distributional range to escape thermal stress by moving northwards and deeper (Sorte et al. 2010; Wernberg et al. 2011). However, cold-water species already trapped in the northernmost, coldest parts of the Mediterranean basin can not migrate further north, given the particular geography of this sea. Deeper, colder habitats are likely the last thermal refuge for these species, at least temporarily (Moschella 2008; Puce et al. 2009).

The shrinkage of cold habitat, coupled with important increases of warm-water species and their northwards migrations is resulting in cold-water species competing with thermophilic arriving species for limited resources (Otero et al. 2013). This may drive to the replacement of cold-water species by their warm-water counterparts and the collapse of cold-water populations (Quignard & Raibault, 1993), which may become locally extinct. The cave dwelling mysids *Hemimysis margalefi* and *H. speluncola* are good examples of species replacements due to climate change in the Mediterranean. The warm-water mysid *H. margalefi* is replacing the cold-water mysid *H. speluncola* and driving it to extinction (Chevaldonné and Lejeusne 2003). Other cold-water populations, like the anchovy *Engraulis encrasicolus* (Bombace 2001), whiting *Merlangius merlangius*, spratt *Sprattus sprattus*, and the slender goby *Gobius geniporus* have suffered drastic declines associated with maximal temperature anomalies in the basin (Ben Rais Lasram et al. 2010), without any apparent evident link with overfishing (CIESM, 2008). Therefore, there is a rising concern about the fate of cold-water species, which are indeed vulnerable to regional warming and potentially further threatened by the increasing relative dominance of warm-adapted ecological antagonists.

Shifts in the distribution of species modify species interactions, draw new patterns in community composition or migration (Chevaldonné and Lejeusne 2003; Rosenzweig et al. 2007), and may ultimately result in the loss of biodiversity. Also, major changes can occur in community structure (Dulvy et al. 2008; Simpson et al. 2011), ecosystem functioning, economy and human health (Gomez and Claustre 2003; Galil 2008). These factors emphasize potential conservation concerns, especially with the rapid climate change and seasonal shifts in the Mediterranean, and clearly pose new challenges for MPA managers in the region.

Sensitivity to climate change and other factors (essential information from scientists explaining why the indicator is relevant and important in assessing climate change impacts)



Climate change is modifying the oceans and driving to changes in the distribution of marine species. Shifts in the spatial and temporal distribution of species are one of the most commonly reported effects associated with global warming conditions. The range shift of species in the Mediterranean, i.e. spreading of warm-water species and contraction of cold-water species, is consistent with the rapid warming conditions of this basin, and therefore, is likely an indicator of the effects of the Mediterranean warming on marine communities (Otero *et al.* 2013). These spatial and temporal changes may cause profound functional changes in the marine and coastal ecosystems as they may result in the enhanced ability of certain arriving species to invade new regions, while simultaneously diminishing the competing capacity (resistance to invasion) of native communities by disturbing the dynamic equilibrium among them. As a result, important losses of biodiversity occur as few warm-water invasive species flourish while native cold-water species

decline or become locally extinct.
<u>Methodology and sampling concerns:</u> Long-term monitoring allows detect both the arrival/spread of warm-water species and the contraction/reduction of cold-water species in the Mediterranean Sea. If monitoring were applied at a basin-scale, e.g. Mediterranean MPAs (for example, the CIESM Tropical Signals Programme), the outcomes would provide a better understanding of how the shift of species ranges may threaten the Mediterranean biodiversity. **<u>Qualitative surveys:</u>** In each MPA, temperature-sensitive species (warm and cold-water species) should be listed, and if possible, include at least a gross abundance estimate and an indication of the date of first observation in the area. In addition, the temporal dynamics of selected seasonally migrating species could be registered yearly, e.g. the date of first observation at each year. Incorporating stakeholders in the monitoring could be very useful (e.g. through semi-structured interviews/questionnaires) (Azurro *et al.* 2011). Fishermen and scuba divers, for example, are often great vectors to report accurate first observations. **<u>Quantitative surveys:</u>** Routine surveys: Visual censuses conducted with snorkelling, skin/scuba diving, or intertidal walks, at least two times a year: after summer (Sept/Oct) - late spring (Apr/May). Abundance estimates in standard areas (100 m long) of all temperature-sensitive species, or a selected group of them (e.g. of fishes: *Pseudocaranx dentex, Acanthurus monroviae, Serranus atricauda, Sparisoma cretense, Thalassoma pavo, Epinephelus marginatus, Sphyraena viridensis*, and non-native species, e.g. of well established, potentially noxious species: *Caulerpa racemosa, Lophocladia lallemandii, Percnon gibbesi, Oculina patagonica, Microcosmus exasperatus, Lagocephalus sceleratus, Siganus rivulatus*). New occurrences of non-native and invasive species shall be noted. * Photographic monitoring in permanent areas (40x60cm quadrats) can be part of the routine surveys, to study potential changes in benthic communities. In addition, a cold/warm affinity ratio of species can be used, such as for example the LD index. By comparing the density of cold and warm-water species with this index Milazzo *et al.* (submitted) assessed the spatio-temporal patterns of the geographical and vertical distribution of *T. pavo* and *Coris julis* across the whole Mediterranean region.

<u>Representation</u> (quantitative, graphical, cartographic, GIS): Online quantitative database, graphics, regional cartography on GIS	**<u>Geographic relevance</u>:** Mediterranean

<u>Policy relevance</u>: The assessment and monitoring of the range shifts of temperature-sensitive species in MPAs allow managers to assess both the condition of their sites and the potential risks to community structure and function that may be occurring there due to climate

warming.

Losses of biodiversity in areas affected by non-native invasive species will prioritize areas where managers have to urgently limit several stressors.

Other data sources (existing International/Mediterranean/National monitoring programs):

- Mediterranean basin-wide biodiversity monitoring program ''CIESM Tropical Signals'' www.ciesm.org/marine/programs/tropicalization.htm
- Citizen science project for marine research. http://www.observadoresdelmar.es
- Mediterranean alien species dissemination project "Ojo invasoras". www.ojoinvasoras.info
- Online information system to monitor alien species in Marine Protected Areas (MPA) in the Mediterranean. www.iucn-medmis.org
- The DASIE project (Delivering Alien Invasive Species Inventories for Europe), an inventory for invasive species in Europe. www.europe-aliens.org
- Global alien species database by the ISSG-UICN (Invasive Species Specialist Group from UICN. www.issg.org/database/welcome/
- The atlas of exotic species prepared by the CIESM. An online reference to identify non-native flora species. www.ciesm.org/online/atlas/intro.htm
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A scientific article published in the journal **PlosOne** where data on fish species are obtained through the collaboration between fishermen and divers. www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0024885

Relevant modelling programs:

- Correlative species distribution models (SDMs)
- Free modeling software MAXENT

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<u>CODE</u>: A4	*Phenology of reproductive events (fishes, marine turtles, seabirds, flowering of P. oceanica)*	**<u>Category(ies)</u>: Individual / phenological**

<u>Definition</u>:

Fishes: Date of first record of conspicuous spawning activities (e.g. spawning aggregations, gathering, territory defence, courtship, non-aggressive interactions, inviting display, agonistic behaviour, false rises, ascent behaviour, nest construction, broodcare, nest defence, settlement...)

Marine turtles: Date, duration and success of nesting (first and last emergence of a nesting female, first and last nest laid, duration of the nesting season, total number of emergences, total number of nests laid, adult nesting success); depending on the logistic and funding availability, other variables can be followed (e.g. mean clutch size, hatching success, hatchling emergence success, hatchling mortality in nest, total number of hatchlings produced)

Seabirds: Dates for migration of selected bird species

Posidonia oceanica floration: Flowering citations and flowering intensity of a given meadows, annual flowering prevalence , flowering frequency, flowering probability

<u>Rationale</u>:

In recent years, numerous studies focus on the potential effects of global warming upon biological organisms and processes. As a result, a link between global warming and changes in life cycles, physiology and behaviour for a variety of organisms has been established. Phenology, defined as the timing of seasonal activities, has been suggested as an indicator of ecosystem responses to global climate change. Physiological performance is the principal determinant of a species' tolerance to environmental variability and change. As climate or other conditions shift, organisms initially respond based on physiological and behavioural adaptations molded through their evolutionary history. New conditions may be physiologically tolerable, allowing acclimatization (an adjustment of physiology within individuals) or adaptation (increased abundance and reproduction of tolerant genotypes over generations), or may be intolerable, promoting migration (by individuals or populations), change in phenology (timing of annual events), or death and local extinction if adaptation is not possible. Among these activities, those related to reproduction are ideal candidates to serve as indicators of seawater warming. Seasonal reproductive events susceptible to serve as indicators of response to seawater warming could be related to earlier dates of spawning, nesting, hatching or settlement of marine animals (fish, marine turtles, seabirds), as well as modifications of the reproductive phenology (occurrence, prevalence and intensity of flowering) of *P. oceanica*.

<u>Sensitivity to climate change and other factors (essential information from scientists explaining why the indicator is</u>

relevant and important in assessing climate change impacts)

Temperature at the time of spawning is likely to influence the starting date of reproduction, although other factors are also important (e.g. population density, habitat availability and quality, weather conditions, etc.). Therefore, climate-induced changes in the timing and duration of reproductive activities in fishes and other marine organisms (marine turtles, seabirds, seagrasses) should be explored as a possible indicator of seawater warming. As temperature affects gonadal development of species, seawater warming would result in spawning times occurring earlier than normal conditions. In the case of fishes, for this to be true the selected species should have easily recognisable spawning features (such as distinct reproductive coloration of males or females, nest-building, conspicuous courtship, visible parental care behavior, etc.). For marine turtles, nesting phenology, further from constituting an adaptive response to climate change, could be used as indicator of biological effects of climate change. The influence of climate change on bird phenology (particularly on the timing of migration and of nesting) has been observed in some cases, so that these features make seabirds excellent candidate sentinel indicators of global change.

Although the mechanisms responsible for an increased flowering subsequent to warming events, increased induction or increased development, remain unknown, the shoot decline associated with high temperature suggests that flowering in this clonal species could represent a response to heat stress. Flowering may be more responsive to seawater temperature anomalies than to the warming trend. However, these two effects may compound as the absolute SST reached in warm years is increasing over time, as a similar anomaly occurs over a shifting baseline. If flowering in *P. oceanica* was a stress response to high temperature, it could be expected that the absolute warming trend added to positive thermal anomalies could induce increased widespread and intense meadow flowering of this temperate species, and thus, less intense thermal anomalies would be needed to produce widespread flowerings. *P. oceanica* meadows appear to amplify the temperature footprint of global warming, as a difference of 1–2 ºC seems to induce widespread flowering throughout the Mediterranean coasts. Moreover, the prevalence and intensity of flowering increases with the amplitude of the anomaly, providing evidence of global sea climate change at a much longer scale than that possibly encompassed by the sparse or short seawater temperature time series in the Mediterranean.

Methodology and sampling concerns:

Fishes: Volunteers' campaigns and scientific surveys to be organised alternatively or complementarily. Detection of reproductive events for particular fish species (*Symphodus* spp., *Thalassoma pavo, Chromis chromis, Serranus scriba,* groupers - *Epinephelus* spp., *Mycteroperca rubra*) by underwater (SCUBA or skin diving) roaming surveys of undetermined time duration in selected sampling stations. Two non-mutually exclusive alternatives are possible: (i) autonomous work by volunteer or scientific divers, and (ii) organised field trips with volunteer divers

Marine turtles: Beach surveys in a daily basis (if possible), in all beaches where nesting is known to occur, starting once the first reproductive event has been observed in the location, for recording dates, duration and success of nesting; if possible (depending on the logistic and funding availability), other variables can be monitored, related to hatchling sucess. Beach parameters (sand Temperature, sand composition grain size, slope,…) for suitability for marine turtle nesting and appropriate sex ratio. Not only

success, but also sex ratio is important and must face population feminization or must be monitored in new colonizing areas with cooler temperatures producing only (or mostly) males. **Seabirds:** Synchronized surveys from selected prominent observatories (capes, points, etc., ideally within or near MPAs) in a monthly basis across the region along the year, complemented with sparse and circumstantial observations from a network of authorized observers, to record presence and number of migratory seabirds (by species or higher taxonomic level) per day **Flowering of *P. oceanica* meadows:** Volunteers' campaigns and scientific surveys to be organised alternatively or complementarily. Flowering intensity (FI) data is to be estimated directly in the field as percentages of flowering shoots or inflorescences densities. When records consist on inflorescence densities, FI values must be estimated dividing them by mean shoot densities. Past flowerings of a *P. oceanica* shoot can be detected because of the base of inflorescences stalks that remain attached to the shoots decades after flowering. Annual cycles of rhizome vertical growth, which can be detected by measuring the leaf sheaths thickness or the rhizome internodal, allow the past flowerings to be dated and their time series reconstructed.	
<u>Representation</u> (quantitative, graphical, cartographic, GIS): Online quantitative database, graphics, regional cartography on GIS	**<u>Geographic relevance</u>:** Mediterranean
<u>Policy relevance</u>: Further from the use of reproductive phenology of concerned species as indicator of CC, the information on reproductive success of fishes, marine turtles, seabirds and seagrasses within or in the vicinity of MPAs is of crucial importance for the establishment of MPA effectiveness in harbouring viable populations of emblematic species, or at least to serve as observation and monitoring reference point for the successful maintenance of key ecological processes to support marine coastal ecosystem functioning.	
<u>Other data sources (existing Mediterranean/National monitoring programs)</u>: • Groupement d'Étude du Mérou (GEM) - http://www.gemlemerou.org/cms/ • Science and Conservation of Fish Aggregations (SCRFA) - http://www.scrfa.org/ • IUCN Groupers and Wrasses Specialist Group (GWSG) - http://www.iucn.org/about/work/programmes/species/who_we_are/ssc_specialist_groups_and_red_list_authorities_directory/fishes/groupers_wrasses_sg/ • Action Plan for the Conservation of Mediterranean Marine Turtles - http://www.rac-spa.org/marine_turtles • IUCN SSC Marine Turtle Specialist Group (MTSG) - https://iucn-mtsg.org/ • BirdLife International / Europe and Central Asia / Mediterranean Partnership - http://www.birdlife.org/europe-and-central-	

asia/news/birdlife-partners-working-across-mediterranean-protect-seabirds

- BirdLife International / Europe and Central Asia / Seabirds and Marine - http://www.birdlife.org/europe-and-central-asia/programmes/seabirds-and-marine
- European Bird Census Council (EBCC) - http://www.ebcc.info
- Red de observación de Aves y Mamíferos marinos (RAM) - http://redavesmarinas.blogspot.com.es/
- Trektellen (Public database to share bird migration counts / ringing results) - http://www.trektellen.org/
- Spanish regional networks for the monitoring of *Posidonia oceanica* meadows (http://posimed.org)
- French regional network for the monitoring of *Posidonia oceanica* meadows (http://mio.pytheas.univ-amu.fr/gisposidonie/?p=1954)
- Action Plan for the conservation of marine vegetation in the Mediterranean Sea - http://www.rac-spa.org/vegetation
- Seagrasswatch

Relevant modelling programs:

- N/A

Relevant references:

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Flowering of *Posidonia oceanica*:

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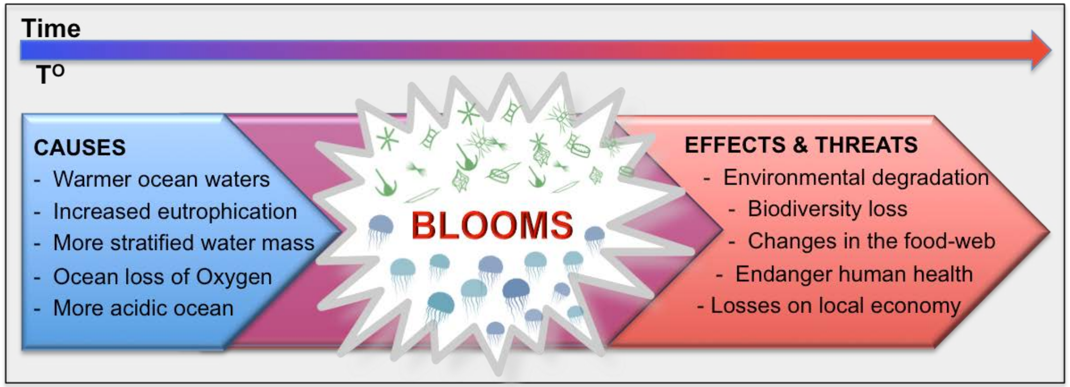
CODE: A5	*Episodic marine species outbreaks (blooms)*	**Category(ies): Population**

Definition: Occurrence, changes in composition, and increases in frequency, magnitude, persistence and relative importance of blooms (gelatinous zooplankton and algal populations outbreaks).

Rationale:

Gelatinous zooplankton (e.g. jellyfish, ctenophores, hydrozoans, tunicates) and algal (e.g. *Ostreopsis*, dynoflagellates) blooms have become more frequent in the Mediterranean coastal areas in the last decade in response to both climate change and increasingly disturbed waters and habitats (Mills 2001, Fuentes *et al.* 2010, Wells *et al.* 2015). Although planktonic populations naturally vary much in abundance over an annual cycle in a more or less predictable manner (Boero 2013), abundance peaks eventually vastly exceed the usual level in events known as blooms. Blooms also take place when sudden outbreaks of a particular species comes to dominate the plankton for a period, to latter resume back to its normal seasonal abundance.

Warming waters coupled with other degrading conditions such as eutrophication, pollution (e.g. fertilizers), water masses stratification (slow-moving water), and the oceans lose of oxygen are encouraging the spread and massive increase in number of some species in the Mediterranean Sea (Purcell 2005, Fuentes *et al.* 2010, Canepa *et al* 2014,). These organisms can tolerate harsh conditions (e.g. low oxygen levels) and are opportunistic, so they usually do great in degraded habitats and rapidly thrive when conditions are favorable. Likewise, warming waters combined with increasing nutrients, light, and ocean water acidity, are favoring algae reproduction and more often driving to more severe algal outbreaks events, e.g. toxic green-blue algal blooms, *Ostreopsis* blooms, or mucilage events. These outbreaks may persist longer than previously due to changes in the food-web and the overfishing of their predators.



Massive blooms can affect the environment and biodiversity, and can also threaten human health and local economies by interfering with human activities. Gelatinous zooplankton blooms impact fisheries when they clog fishing nets, keep fish away, and consume fish larvae (Graham et al. 2003). Jellyfishes and ctenophores are both predators and potential competitors of fish because they feed on the same prey as many adult and larval fishes (Purcell and Arai 2001). Both gelatinous zooplankton and algal blooms can cause important losses to aquaculture operations by killing important amounts of fish in aquatic farms; swarms of gelatinous zooplankton can clog and sting fish gills, or restrict the flow of water through nets, whereas some algal blooms produce potent neurotoxins able to poison and kill massive amounts of animals. In addition, gelatinous zooplankton can clog seawater cooling systems and even drive to the shut down of power plants, as well as hurt other industries that depend on ocean water, including tourism (swimmers and scuba divers can be stung by jellyfish). Algal blooms, on the other hand, can reach huge dimensions and cover areas of hundreds of kilometres of coastline causing mass mortalities of marine fish or benthic communities (Danovaro *et al.* 2009, Shears and Ross, 2009; Gorbi *et al.* 2013). Algal blooms can cause very low oxygen concentrations in bottom waters (dead zones), and harmful species, like those of the genus *Ostreopsis*, can produce toxic substances able to alter cellular processes of other organisms from plankton to humans (Gallitelli *et al.* 2005; Del Favero *et al.* 2012). Likewise, mucilaginous aggregates can sank to the bottom and coat and suffocate benthic communities and sediments while causing hypoxic and/or anoxic conditions.

Population blooms can be large-scale episodic events able to suddenly modify large areas of the seascape and to drive to catastrophic consequences. Given the increased frequency and persistence of these episodes in the Mediterranean Sea, and the current concern owing to the impossibility to predict believable future scenarios, studying and monitoring their occurrence in MPAs is important for the conservation of the Mediterranean biodiversity.

Sensitivity to climate change and other factors (essential information from scientists explaining why the indicator is relevant and important in assessing climate change impacts)

Over recent decades, the coastal and marine ecosystems of the Mediterranean basin have begun to show real change in response to human induced stress. Climate change and other anthropogenic impacts have been attributed to be the major driving forces of increasingly frequent and persistent blooms of both gelatinous zooplankton (Gili *et al.* 2010, Canepa et al 2014, Kersting 2015) and algae (Ramos *et al.* 2005, Glibert *et al.* 2005, Cloern *et al.* 2005, Schiaparelli *et al.* 2007, Granéli *et al.* 2011, Botana *et al.* 2013). These organisms have rapid responses to environmental changes given their short life cycles, and therefore, are considered useful indicators of the effects of climate change (Hays *et al.* 2005). Warm waters favor algae reproduction and increase the metabolism of gelatinous zooplankton promoting its faster growth, greater feeding, and longer live (Purcell 2005, Prieto 2010, Canepa et al. 2014). Recent studies demonstrate alterations in the seasonality of phytoplankton cycles, with spring blooms occurring earlier in the year and autumn blooms later (González-Taboada and Anadón 2013, Kersting 2015). Also, variations in zooplankton abundance have been reported closely related to ocean temperature anomalies (Fernández de Puelles y Molinero 2008). Being key elements of the food-web, substantial variations in the gelatinous zooplankton and algae abundance may affect the spatial distribution and abundance of many other marine organisms, lead to the loss of biodiversity, and affect the proper functioning of marine and coastal

ecosystems. Rises in sea temperature resulted by global warming likely play a crucial role inducing the frequency, geographical expansion, species composition and biomass accumulation by blooms of these organisms. Blooms, however, are complex, with interactions and synergies among multiple stressors (related to climate change, as well as other ones), which difficult robust associations with climate change (Purcell *et al.* 2007). Therefore, more research is needed to better characterize this link, and improve its understanding and forecasting.
Methodology and sampling concerns: Qualitative surveys: Noting the occurrence, timing and size of gelatinous zooplankton and algal (e.g. mucilage aggregates) blooms. Quantitative surveys: The estimated density/extension of jellyfish (gelatinous zooplankton) and algal blooms can be monitored periodically with visual censuses performed from a boat, drone, plane, or by snorkeling. Blooms affecting the beaches can be surveyed by intertidal walks along the coast. In addition, a large number of observations can be obtained from interviews/questionnaires (person to person, or on-line) completed by local communities and stakeholders. Mucilaginous events: Seawater and mucilage aggregates can be sampled by means of sterilized Niskin bottles and by 100 mL syringes operated by SCUBA divers, respectively. The mucilage present in a known surface (e.g. determined with the help of a metallic frame) can be collected in plastic bags, paying particular attention to preserve the original interstitial water within the aggregates, to characterise the algal species living within the mucilage. The effect of mucilage on benthic organisms can be documented through digital pictures, after removing the mucilage carpet in an area of one square meter in each site. In addition, the use of time-lapse images to study the dynamics of the mucilage in a yearly fashion and to relate it to main environmental variables (a method suggested by S. Schiaparelli) could be a great way to monitor these events in MPAs. Harmful algal blooms (e.g. *Ostreopsis*): Seawater samples (from 250 ml to 1 l) can be collected for the investigation of phytoplankton taxonomic structure, numerical abundance and biomass through microscopy, and toxin and pigment analyses. In addition, a qualitative and semi-quantitative PCR-based assay for the detection of several potentially harmful algal blooms species and genera belonging to Dinophyceae, Bacillariophyceae and Raphydophyceae, developed by Penna *et al.* (2007), could be useful for the monitoring of these blooms in MPAs.

Representation (quantitative, graphical, cartographic, GIS): Online quantitative database, graphics, regional cartography on GIS	**Geographic relevance:** Mediterranean

Policy relevance: The assessment of the distribution and frequency of gelatinous zooplankton and algal blooms in MPAs allow managers to assess both the condition of their sites and the potential risks to community structure and function that may be

occurring there due to climate warming. Losses of biodiversity in areas affected with blooms will prioritize areas where managers have to urgently limit several stressors.

Other data sources (existing Mediterranean/National monitoring programs):

http://www.ciesm.org/marine/programs/jellywatch.htm

Life Cubomed Project (http://www.cubomed.eu/en/)

http://www.perseus-net.eu/site/content.php?locale=1&sel=515

http://jellieszone.com/

http://jellyrisk.eu/en/links/#.VyIDf5OLQW0

http://www.jellywatch.org/blooms

http://www.m3-habs.net

Relevant modelling programs: Med-jellyrisk Jellyfish Dispersion Model http://193.188.45.234/jdm/index.php/login

Relevant references:

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Annex 2. Data-sheet for surveys on Mortality and bleaching events (scuba diving)

Data-sheet with the information to be collected for surveys on mortality/bleaching events using quadrats along vertical transects. If the damage is bleaching please add the symbol (B) to the damage category.

Quadrat	Depth	Species	Height	Damage category	Epibiosis category	Occurrence of fishing lines	Occurrence of mucilage

Information to be collected within 50x50 cm quadrats:

Depths: from 5, 15, 20 until 45m (5 m interval). A minimum of 4 quadrats per depth.

Damage categories:

1 affected 0%
2 1-25%
3 25-50%
4 50-75%
5 75-99%
6 affected 100%

Epibiosis categories:

1 Naked skeleton
2 Filamentous algae and small/pioneer hydroids
3 Filamentous algae and big/old hydroids
4 Old/calcified organisms (coralline algae, bryozoa, vermetids…)

Fishing lines: yes/no

Mucilage: yes/no

Annex 3. Data-sheet for surveys on Mortality and bleaching events (snorkeling/SCUBA)

Data-sheet with the information to be collected for surveys on mortality/bleaching events along horizontal transects (snorkeling/SCUBA) and/or along a vertical gradient using 50x50 cm quadrats (SCUBA).

If the damage is bleaching please add the symbol (B) to the damage category

Quadrat		Depth	Species	Height/diameter/ size	Damage category	Epibiosis category	Occurrence of fishing lines	Occurrence of mucilage
Transect	Distance							

Damage categories:

1 affected 0%

2 1-25%

3 25-50%

4 50-75%

5 75-99%

Epibiosis categories:

1 Naked skeleton

2 Filamentous algae & small/pioneer hydoids

3 Filamentous algae & big/old hydoids

4 Old/calcified organisms (coralline algae, bryozoan, vermetids,…)

Fishing lines: yes/no

Mucilage: yes/no

For 50x50 cm quadrats:

Depths: from 5, 15, 20 until 45m (5m interval). A minimum of 4 quadrats per depth.

Annex 4. An example of a data-sheet to monitor temperature-sensitive species.

Observer	Date... ... .
Locality	Hour ... .

	1-3m (Snorkeling)						12-15m (SCUBA)					
(Transects)	1	2	3	4	5	6	1	2	3	4	5	6
Epinephelus marginatus												
Caranx crysos												
Coris julis												
Thalassoma pavo												
Sparisoma cretense												
Serranus scriba												
Serranus cabrilla												
Sarpa salpa												
Sphyraena viridensis												
Siganus luridus												
Fistularia commersonii												

Annex 5. Jellyfish identification poster- From Meteo Meduse