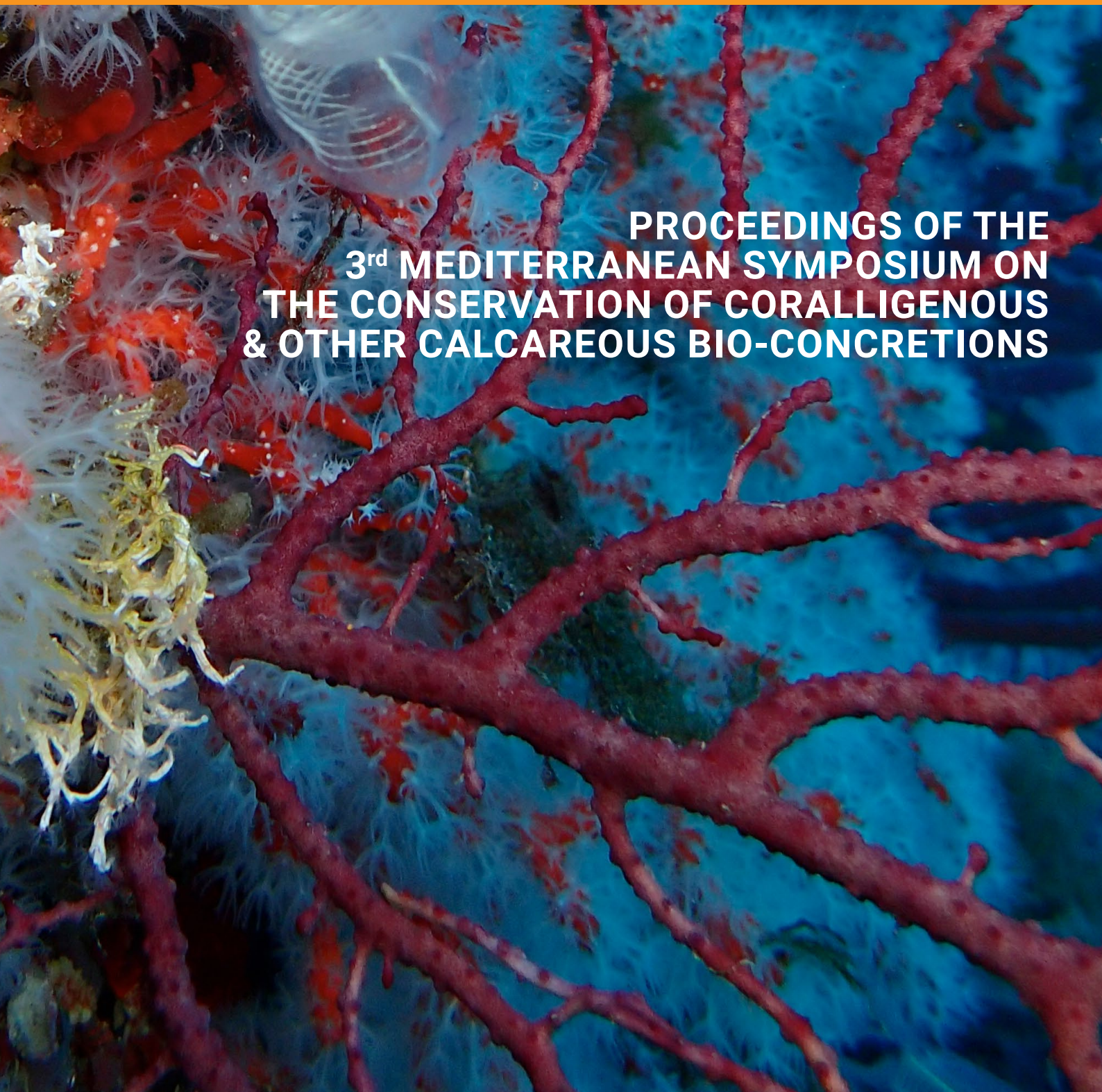




The Mediterranean
Biodiversity
Centre



PROCEEDINGS OF THE 3rd MEDITERRANEAN SYMPOSIUM ON THE CONSERVATION OF CORALLIGENOUS & OTHER CALCAREOUS BIO-CONCRETIONS

ANTALYA, TURKEY, 15-16 JANUARY 2019

Technical partner



Financial support



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**Proceedings of the
3rd Mediterranean Symposium on
the conservation of Coralligenous
& other Calcareous Bio-Concretions**

FORWARD

Dear Friends and Colleagues,

Following the recommendations of:

- the **Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea** (adopted by the Contracting Parties to the Barcelona Convention in 1999 and updated in 2012)
- the **Action Plan for the Conservation of Coralligenous and other calcareous bio-concretions of Mediterranean** (adopted by the Contracting Parties to Barcelona Convention in 2008 and updated in 2016)
- the **Action Plan for the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea** (Action Plan for Dark Habitats adopted by the Contracting Parties to the Barcelona Convention in 2013), and
- the **Action Plan concerning Species Introduction and Invasive Species** (Adopted by the Contracting Parties to the Barcelona Convention in 2003 and updated in 2016)

a series of scientific symposia, dedicated to these habitats and NIS, was initiated in 2000 by organising the first Mediterranean Symposium on Marine vegetation. These initiatives aimed essentially to take stock of the recently available scientific data and to promote the cooperation between specialists and key actors working in the Mediterranean.

The Mediterranean Symposia on Marine Key Habitats are an important output, not only of the UNEP/MAP Mid-Term Strategy for the period 2016-2021 (Decision IG.22/1)¹, but also for MedKeyHabitats II Project² "Mapping of marine Key habitats and assessing their vulnerability to fishing activities in the Mediterranean" financed by MAVA foundation under its Mediterranean Strategy.

The "Mediterranean Symposia on Marine Key Habitats and NIS" will also provide an opportunity to discuss best practices in the monitoring of marine key habitats and non-indigenous species and provide elements to further improve the **Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and related Assessment Criteria (IMAP)**, of the Ecosystem Approach (EcAp) in the Mediterranean.

The organization of the "Mediterranean Symposia on Marine Key habitats and NIS" together back to back in Antalya from 14 to 18 January, is a joint collaboration among **UNEP/MAP-SPA/RAC** and the **Turkish Ministry of Environment and Urbanization**, as follows :

- 6th Mediterranean Symposium on Marine vegetation (from 14 to 15 January 2018)
- 3rd Mediterranean Symposium on the Conservation of Coralligenous and other Calcareous Bio-Concretions (From 15 to 16 January 2018)
- 2nd Mediterranean Symposium on the Conservation of the Dark Habitats (17 January 2018)
- 1st Mediterranean Symposium on the Non-Indigenous Species (From 17 to 18 January 2018)

The Turkish Marine Research Foundation, **TUDAV**, as **SPA/RAC** partner will support the local organization of this event.

This edition will also be a good opportunity to discuss new topics such as monitoring and definition of Good Environmental Status (GES) in the Mediterranean and so strengthen links between scientists and scientific institutions.

Khalil ATTIA
SPA/RAC Director

¹ https://wedocs.unep.org/bitstream/handle/20.500.11822/6071/16ig22_28_22_01_eng.pdf?sequence=1&isAllowed=y

² <http://www.rac-spa.org/medkeyhabitats2>

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PROGRAMME

Tuesday 15 January 2019

- 14:00-14:15 Opening of the Symposium**
- 14:15-14:45 Keynote conference: "Mediterranean gorgonian forests: distribution patterns and ecological roles" by Massimo PONTI, TURICCHIA E., COSTANTINI F., GORI A., BRAMANTI L., DI CAMILLO C.G., LINARES C., ROSSI S., ABBIATI M., GARRABOU J., CERRANO C.**
- 14:45-15:15 Keynote conference: "Mediterranean bioconstructions: the case of Italy" by Gianmarco INGROSSO, DE LEO F., FRASCHETTI S., BOERO F.**
- Session 1: Knowledge of the Coralligenous communities and other calcareous bioconstructions**
Chair : Joaquim GARRABOU, **Rapporteur :** Atef OUERGHI
- 15:15-15:30 "Food web structure of a Mediterranean coralligenous ecosystem" by Bruno BELLONI, SARTORETTO S., CRESSON P., BOUCHOCHA M., GUILLOU G., LEBRETON B., RUITTON S., HARMELIN-VIVIEN M.**
- 15:30-15:45 "Community ecology of coralligenous assemblages using a metabarcoding approach" by Aurélien DE JODE, DAVID R., DUBAR J., ROSTAN J., GUILLEMAIN D., SARTORETTO S., FERAL J.-P., CHENUIL A.**
- 15:45-16:00 "Advances in the conservation of red coral populations: long-term demographic data, management tools and restoration activities" by Cristina LINARES, MONTERO-SERRA I., ASPILLAGA E., CABRITO A., CAPDEVILA P., LEDOUX J.B., LÓPEZ-SANZ A., LÓPEZ-SENDINO P., ROVIRA G., GARRABOU J.**
- 16:00-16:15 Discussion**
- Session 2: Impacts on the Coralligenous communities and other calcareous bioconstructions**
Chair : Monica Montefalcone, **Rapporteur :** Asma YAHYAOU
- 16:15-16:30 "Ghost Med: Assessment of the impact of lost fishing gear in the French Mediterranean Sea" by Sandrine RUITTON, BELLONI B., MARC C., BOUDOURESQUE C.F.**
- 16:30-16:45 "A mass mortality event of the sponge *Cliona viridis* in the coralligenous outcrops in the Côte Agathoise MPA (Gulf of Lion, Northwestern Mediterranean)" by Mathieu FOULQUIE, BLOUET S., CHERE E., DUPUY DE LA GRANDRIVE R., GARRABOU J.**
- 16:45-17:00 "Bleaching of crustose coralline algae in the Mediterranean Sea" by Bernat HEREU, MATAMALAS N., ASPILLAGA E., CAPDEVILA P., ROVIRA G.**
- 17:00-17:15 "Demography and disturbance levels of the coral *Astroides calycularis* (Pallas, 1766) in the Tunisian Marine Protected Area Of Zembra (Central Mediterranean)" by Raouia GHANEM, SOUFI-KECHAOU E., BEN SOUISSI J., LINARES C., LEDOUX J.B., GARRABOU J.**
- 17:15-17:30 Discussion**

Wednesday 16 January 2019

Session 3: Monitoring and quality assessments of the Coralligenous communities and other calcareous bioconstructions

Chair : Leonardo TUNESI, **Rapporteur :** Vasilis GEROVASILEIOU

8:30-8:45 "Effective SFM-Based methods supporting coralligenous benthic community assessments and monitoring" by **Marco PALMA**, PAVONI G., PANTALEO U., RIVAS CASADO M., TORSANI F., PICA D., BENELLI F., NAIR T., COLETTI A., DELLEPIANE M., CALLIERI M., SCOPIGNO R., CERRANO C.

8:45-9:00 "Assessment of the conservation status of coastal detrital sandy bottoms in the Mediterranean Sea: An Ecosystem-Based Approach in the framework of the ACDSea Project" by **Patrick ASTRUCH**., GOUJARD A., ROUANET E., BOUDOURESQUE C.F., VERLAQUE M., BERTHIER L., DANIEL B., HARMELIN J.G., PEIRACHE M., PETERKA A., RUITTON S., THIBAUT T.

9:00-9:15 "Assessing the sensitivity of marine habitats to anthropogenic pressures: a key tool in evaluating risks to Mediterranean benthic habitats" by **Marie LA RIVIERE**, MICHEZ N., AISH A., BELLAN-SANTINI D., BELLAN G., CHEVALDONNE P., DAUVIN J.-C., DERRIEN-COURTEL S., GRALL J., GUERIN L., JANSO A.-L., LABRUNE C., SARTORETTO S., THIBAUT T., THIEBAUT E., VERLAQUE M.

9:15-9:30 Discussion

9:30-10:15 **Poster Session**

Session 4: Restauration of the coralligenous habitats

Chair : Hocein BAZAIRI, **Rapporteur :** Vasilis GEROVASILEIOU

10:15-10:30 "Restoration protocols for the Mediterranean coralligenous habitats" by **Carlo CERRANO**, BAKRAN-PETRICIOLI T., CARONI J., FERRETTI E., GARI A., GOMEZ-GRAS D., GORI A., HEREU B., KIPSON S., MILANESE M., LEDOUX J.B., LINARES C., LÓPEZ-SANZ S., LÓPEZ-SENDINO P., MONTERO-SERRA I., PAGÈS M., PICA D., SARÀ A., TORSANI F., VILADRICH N., GARRABOU J.

10:30-10:45 "Enhancing the effectiveness of restoration actions in coralligenous habitats: insights from a transregional thermotolerance experiment" by **Jean-Baptiste LEDOUX**, GOMEZ GRAS D., CRUZ F., ALIOTO T.S., BAKRAN-PETRICIOLI T., BOAVIDA J., del CAMPO J., CERRANO C., FERRETI E., GÓMEZ-GARRIDO J., GUT M., KIPSON S., LINARES C., LOPEZ-SENDINO P., LOPEZ-SANZ A., MASSANA R., MILANESE M., MONTERO-SERRA I., PAULO D., SERRAO E., GARRABOU J.

10:45-11:00 "Can facilitation processes enhance the effectiveness of restoration actions in the coralligenous habitat?" by **Silvija KIPSON**, CERRANO C., LINARES C., BAKRAN-PETRICIOLI T., FERRETTI E., GOMEZ-GRAS D., LEDOUX JB., LÓPEZ-SANZ A., MONTERO-SERRA I., PAGÈS M., SARÀ A., TORSANI F., GARRABOU J.

11:00-11:15	"Active restoration across marine coastal habitats: a focus on the Mediterranean Sea" by Giuseppe GUARNIERI , MCOWEN C., PAPA L., PAPADOPOULOU N., BILAN M., BOSTRÖM C., CAPDEVILA P., CARUGATI L., CEBRIAN E., DAILIANIS T., DANOVARO R., DE LEO F., FIORENTINO D., GAGNON K., GAMBI C., GARRABOU J., GEROVASILEIOU V., HEREU B., KIPSON S., KOTTA J., LEDOUX J.B., LINARES C., MARTIN J., MEDRANO A., MONTERO-SERRA I., MORATO T., PUSCEDDU A., SEVASTOU K., SMITH C., VERDURA J., FRACHETTI S.
11:15-11:30	Discussion
11:30-12:00	Awards for best poster
12:00-12:15	Closure of the Symposium
12:15-14:00	<i>Lunch</i>

KEYNOTE CONFERENCES

**Massimo PONTI, TURICCHIA E., COSTANTINI F., GORI A., BRAMANTI L.,
DI CAMILLO C.G., LINARES C., ROSSI S., ABBIATI M., GARRABOU J.,
CERRANO C.**

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MEDITERRANEAN GORGONIAN FORESTS: DISTRIBUTION PATTERNS AND ECOLOGICAL ROLES

Abstract

*Healthy coralligenous habitats may host dense populations of gorgonians, like *Paramuricea clavata* and *Eunicella cavolini* that build marine animal forests. According to recent studies, these forests appeared able to increase the resilience of coralligenous habitats and to enhance the structural complexity and bioconstruction processes. They are also able to increase species diversity and limit the invasion of alien species. The major limitation in the conservation of these forests is the lack of knowledge on their actual distribution and the extents of their ecological roles. Nowadays, by combining information from scientific literature, citizen science projects and the World Wide Web it is possible to fill part of these gaps and draw a more comprehensive picture for the Mediterranean Sea. This knowledge represents the baseline to address effective conservation measures on gorgonian forests and coralligenous accretions.*

Key-words: Suspension feeders; habitat complexity, biodiversity, coralligenous, habitat mapping

Introduction

Healthy coralligenous habitats (i.e. biogenic reefs growing in dim light condition, Ballesteros, 2006) may host dense populations of gorgonians that build marine animal forests (Rossi *et al.*, 2017). According to recent studies, these forests may increase the resilience of coralligenous habitats and to enhance the structural complexity and bioconstruction processes by favouring the settlement and development of encrusting calcareous algae and by limiting the growth of erect algae under different study conditions and in many investigated sites down to 40 m depth (Ponti *et al.*, 2014; Ponti *et al.*, 2018). They are also able to increase species diversity and limit the invasion of alien species (Ponti *et al.*, 2018). Moreover, by entrapping benthic mucilaginous aggregates with their branches, gorgonians risk topical necrotic lesions but may reduce the suffocation risks for understorey organisms (Piazzi *et al.*, 2018; Ponti *et al.*, 2018).

Integrity of gorgonian forests, however, is threatened by fishing lines and nets, anchors and recreational divers causing mechanical damage (Bavestrello *et al.*, 1997; Linares & Doak, 2010; Tsounis *et al.*, 2012), suffocation by mucilaginous benthic aggregates (Giuliani *et al.*, 2005; Mistri & Ceccherelli, 1996), invasion from non-indigenous species (Cebrian *et al.*, 2012), and increase in water turbidity and sedimentation rates due to run-off as a result of bad land management (Mateos-Molina *et al.*, 2015). At the same time, gorgonian forests are also heavily threatened by global climate change-related disturbances such as increased frequency of exceptional storms (Teixidó *et al.*, 2013) and thermal anomalies (Cerrano & Bavestrello, 2008; Cerrano *et al.*, 2000; Garrabou *et al.*, 2009) that may induce physiological stress and increase their susceptibility to pathogens

(Calvo *et al.*, 2011; Rivetti *et al.*, 2014). The latter, coupled with reduced food availability due to the stratification of the water column in summer, seem to lie at the basis of the gorgonian mass mortality events recorded in recent decades in the north-western Mediterranean Sea (Calvo *et al.*, 2011; Cerrano *et al.*, 2000; Crisci *et al.*, 2011; Garrabou *et al.*, 2009; Huete-Stauffer *et al.*, 2011; Linares *et al.*, 2005; Marbà *et al.*, 2015; Martin *et al.*, 2002; Rivetti *et al.*, 2014). The crises have not spared even the most remote gorgonian forests, far from direct anthropic disturbances (Turicchia *et al.*, 2018).

Currently, as a result of both local and global disturbances, many gorgonian forests are fragmented and considered in strong regression. Concerns for gorgonian forests loss and the related consequences (e.g. impairment of fertilisation and larval connectivity, shifts in the structure of benthic assemblages and the related ecosystem functioning) are rising. The major limitation in the conservation of these forests is the lack of knowledge on their distribution patterns and the extents of their ecological roles. The present study aims to summarise the currently available opportunities to lay the basis for a comprehensive database on the gorgonian forests distribution in the Mediterranean Sea, building-up a shared and reliable baseline for the implementation of effective conservation and management strategies.

Materials and Methods

In order to build a baseline on the spatial and depth distribution of the three main species that form extensive gorgonian forests in coastal bottoms of the Mediterranean Sea (*Eunicella cavolini* (Koch, 1887), *Eunicella singularis* (Esper, 1791) and *Paramuricea clavata* (Risso, 1826)), a multi-source approach was applied. It combines information from scientific literature (SCI), marine citizen science (CS) projects and the so-called Web Ecological Knowledge (WEK, Di Camillo *et al.*, 2018b).

Data from SCI were originally being collected by scientific scuba divers or using remotely operated underwater vehicles (ROV), dredging, and fishing nets, and published in peer review journals. Relevant papers were retrieved from all database indexed in ISI Web of Science by searching for the words combination “Mediterranean AND (*Eunicella* OR *Paramuricea*)” in titles, abstracts, and keywords. Some other less accessible documents, including reports from environmental agencies and marine protected areas (MPAs), were also included.

CS data come from the Reef Check web based geographic information system (Web-GIS) for the Mediterranean Sea (RCMed, www.reefcheckmed.org), the Seawatchers platform (www.observadoresdelmar.es), iNaturalist (www.inaturalist.org) and other observational networks indexed by the Global Biodiversity Information Facility (GBIF, www.gbif.org). In particular, RCMed is the largest Web-GIS of underwater observations carried out according to a standard protocol (which includes abundance class estimations and absence reports) by trained volunteers in the Mediterranean Sea (Cerrano *et al.*, 2017). Seawatchers platform collates photographic records that are identified by a pool of experts. iNaturalist is a social network of nature enthusiasts that share and cross-validate photographic observations.

Information coming from the WEK included non-professional underwater videos (mostly published on the YouTube website), and pictures from websites and Facebook pages of dive centres or photographers. Following the procedure suggested by Di Camillo *et al.* (2018b), authors of videos and pictures published on the world wide web were contacted by phone or email to ask for further details as locality, depth and date of the images. Records lacking this information have not been considered.

While nautical positioning may rely on the few-meter accuracy of the enhanced satellite system (WAAS/EGNOS-enabled GPS, Witte & Wilson, 2005), underwater positioning is hampered by the difficulty of penetrating radio waves into the water. Although there are underwater acoustic positioning systems (Meidinger *et al.*, 2013) and new photographic methods of three-dimensional mapping of gorgonian forests are developing (Palma *et al.*, 2018), their use is still limited to specialists. Therefore, species distributions and depth range were analysed by pooling together multiple observations within a 15" grid (= 0.00417°, i.e., ¼ of nautical mile in latitude), which is comparable to the area explorable by scuba divers around mooring buoys and anchors. Whenever both presence and absence records fallen within the same grid cells, the latter were discarded. Vertical distributions were calculated by summing the observations from all grid cells and species, between the minimum and maximum-recorded depths (one-meter step).

Spatial analysis and mapping were done using the free software environment for statistical computing and graphics R ver. 3.4.2 (www.r-project.org) and the free and open source geographic information system QGIS ver. 2.18 (qgis.org).

Results

Search in the databases accessible through ISI Web of Science (last access October 1, 2018) returned 228 papers, many of which provided useful information on various study sites, but only a few specifically concerned the species distributions (e.g. Bo *et al.*, 2012; Di Camillo *et al.*, 2018b; Ghanem *et al.*, 2018; Gori *et al.*, 2011; Grinyo *et al.*, 2016; Kipson *et al.*, 2015; Linares *et al.*, 2008; Ponti *et al.*, 2018; Salomidi *et al.*, 2009; Sini *et al.*, 2015; Vafidis *et al.*, 1994).

RCMed Web-GIS includes 1624 records of *E. cavolini*, 1470 of *E. singularis* and 1605 of *P. clavata* containing visual observations ranked on 7 abundance classes, from no colonies to more than 50 colonies sighted during the searching time (30 minutes on average). Often the observations are accompanied by the minimum and maximum depth to which the colonies have been sighted, allowing for depth range analysis. However, data is still mainly limited to Italy, Croatia and France where the application of the protocol is better enforced. Seawatchers platform provides 28 records of *E. cavolini*, 109 of *E. singularis* and 92 of *P. clavata* substantially contributing in filling gaps in the north-western Mediterranean Sea.

GBIF provides many additional punctual observations from different sources under the category 'human observation': 1276 records of *E. cavolini*, 987 of *E. singularis* and 846 of *P. clavata* (GBIF, 2018). Most of the downloaded data belong to BioObs, a participative network of scuba divers in France. Not surprisingly most of the records come from southern France and Corsica. Unfortunately, depth is not provided, and coordinate uncertainty associated to these observations is often higher than the precision adopted in this study; therefore they can be useful only for studies at a regional scale that does not require local analysis. The only exceptions, found in the GBIF collection, are represented by few records (9 *E. cavolini*, 2 *E. singularis*, 13 *P. clavata*) coming from iNaturalist with high position accuracy (ca. 100 m) and depth reported. Some records from Diveboard (www.diveboard.com) and MNHN-SPN (inpn.mnhn.fr) were also retained.

WEK data available to date provide further insight especially for the central-eastern Mediterranean Sea (46 *E. cavolini*, 2 *E. singularis*, and 51 *P. clavata*). Data available on the presence of *E. cavolini*, *E. singularis*, and *P. clavata* are summarised in Fig. 1.

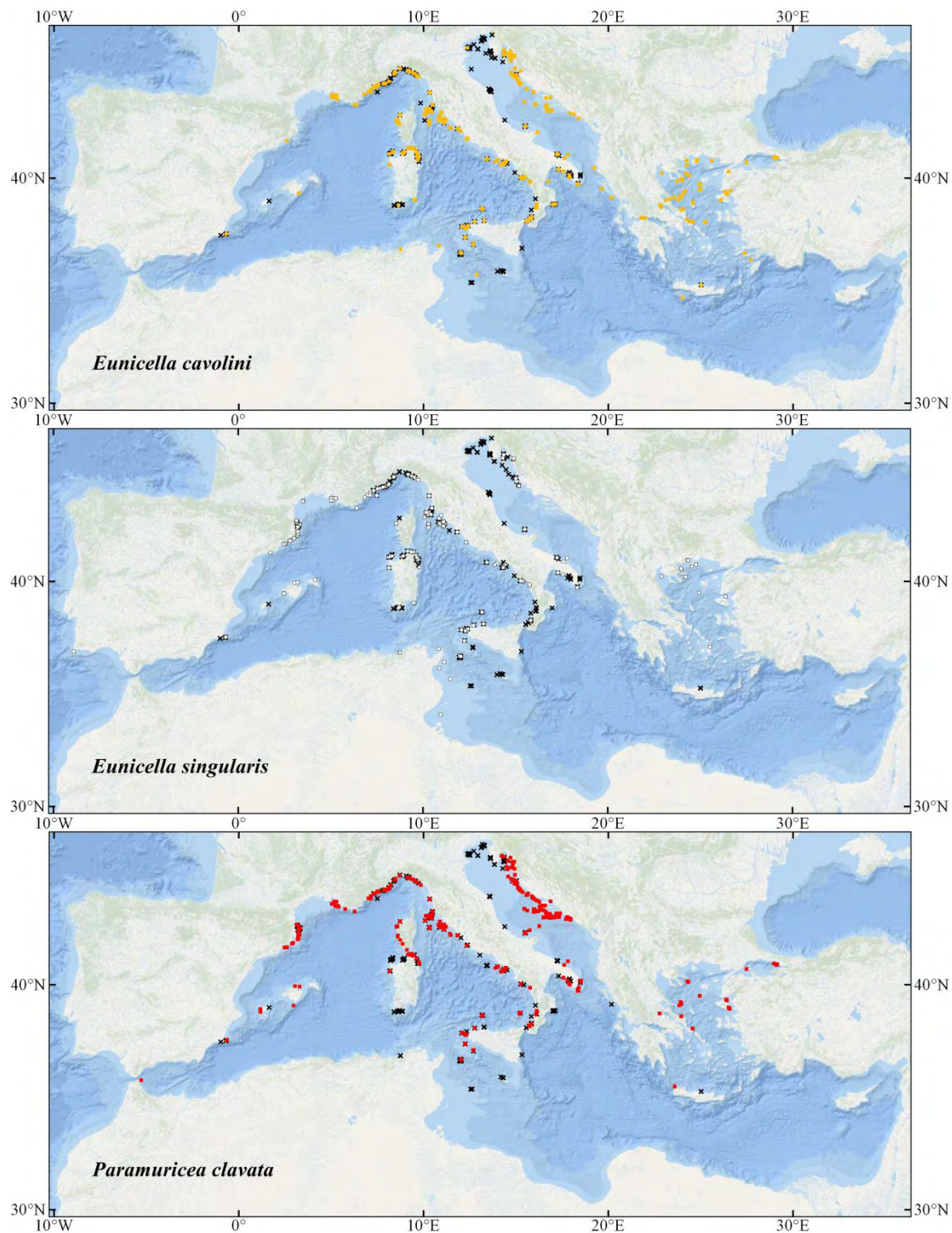


Fig. 1: Presence (filled cells) and absence (cross symbols) of *Eunicella cavolini*, *Eunicella singularis*, and *Paramuricea clavata* (Mercator projection; ESRI Ocean base map).

Discussion and conclusions

Despite technology innovation and developing biodiversity information facilities, collating together information on species occurrence and their fine-scale distribution analysis remain a not easy task, even dealing with iconic species like gorgonians.

Although many sources provide data on the presence of species, only specific studies or the use of dedicated protocols can provide information on their abundance. It is also difficult to know where the gorgonians are absent despite habitats suitable for their establishment exist. Overall, it is particularly difficult to retrieve information from many areas of the southern Mediterranean Sea.

Major limits in retrieving distribution data from the scientific literature are due to the still reduced propensity of authors to provide raw data and to adopt standards in taxonomy and coordinate systems (Di Camillo *et al.*, 2018a). The opportunity to publish 'data paper', as well as the request to supply the datasets from many scientific journals, especially those 'open access', and the development of biodiversity infrastructures (e.g. GBIF, EUNIS, EMODnet, Obis, LifeWatch-ERIC) fortunately counteract this tendency.

Citizen Science projects are a very promising source of substantial information on the distribution of conspicuous species, especially when they are based on robust, effective and easy to apply protocols, well addressed to specific goals and with a strong recognition to participants (Thiel *et al.*, 2014). RCMed provides the largest dataset on gorgonians abundance estimation, and it is to date the only source that considers the 'absence' data explicitly. Data consistency is ensured by multiple observations simultaneously carried out by different trained observers and often reiterated in time (Cerrano *et al.*, 2017). On the other end, platforms like Seawatchers and iNaturalist require no training to be approached by nature enthusiasts because species identification relies on the involvement of experts and cross-validations. In both cases, useful complementary information (e.g. depth, temperature, estimated forest densities and extension) can be asked to the participants, through a dialogue with scientists, as in Seawatchers, or by specific project launched on the iNaturalist platform (i.e. the project 'Gorgonians and other corals of the Mediterranean and Black Seas'). However, redistributors, like the GBIF backbone, are not able to provide the full range of data possibly associated with each observation and stored in the original databases.

Thanks to the diffusion of social networks and new technologies to capture and share underwater images, WEK appears a very promising source of ecological information and species-distribution data. The main advantages consist of no costs in promoting initiatives and in training participants and the accuracy of species identification provided by scientists involved in retrieving data. However, high efforts are necessary to integrate this data into standardised databases.

In the present study, retrieved data largely vary in reliability and accuracy of location and depth, according to their primary source. Data providers should always supply information on data quality and credits to the primary data producers, which also imply the adoption of more or less robust protocols able to ensure taxonomic consistency and the desired positioning accuracy. This study highlighted the importance of using different data sources datasets to provide complete information on the distribution of gorgonian forests. We contend that thematic web-based collaborative databases able to upload information from different platforms are promising tools to facilitate "near real-time" comprehensive datasets. Besides, these initiatives will help the adoption of standardised surveying protocols, to avoid redundant data gathering efforts and identify geographic gaps for future research and provide unrestricted, open-source and easy access to data. For instance, all the data collated on the distribution of gorgonian species in the Mediterranean Sea could be made available, together with their metadata, through CORMedNet (cormednet.medrecover.org) a collaborative initiative that specifically aims to gather information on distribution, population dynamics and genetics of key species

dwelling in the Mediterranean coralligenous assemblages. Data quality control and a continuous data flow should be assured in order to feed informed ecosystem-based management and transboundary conservation policies. Conservation strategies should reduce the risk of mechanical damage by regulating fishing activities, anchoring and scuba diving behaviour where gorgonian forests are present. Moreover, when evident alterations are documented, restoration actions should be implemented to recover gorgonian forests integrity.

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MEDITERRANEAN BIOCONSTRUCTIONS: THE CASE OF ITALY

Abstract

Marine bioconstructions are three-dimensional structures generated by the superposition of the remains of benthic organisms that persist on site, do not decay and are overgrown by other organisms, either of the same species or of other species. Tropical coral reefs are the most famous bioconstructions, but other types of biogenic formations are also present in temperate seas. Coralligenous formations are the most prominent bioconstructions in the Mediterranean Sea together with vermetid reefs, cold water corals, Lithophyllum byssoides trottoirs, banks formed by the corals Cladocora caespitosa or Astroides calycularis, and sabellariid and serpulid worm reefs. Bioconstructions modify the primary substrate where they settle upon, creating new habitats for organisms that do not occur on the primary substrates, so enhancing biodiversity. A recent review on the diversity and the spatial distribution of bioconstructions in Italian waters highlighted the importance of this suite of habitats that, together, make up the core of the “reef” habitat type in the Habitats Directive of the European Union. Anthropogenic pressures (i.e. pollution, organic enrichment, fishery, coastal development, direct physical disturbance), climate change, and the spread of invasive species impact on the integrity of these habitats, whose management and conservation require holistic approaches at basin scale, within ecologically coherent units based on connectivity: the cells of ecosystem functioning.

Key-words: bioconstructions, habitat formers, ecosystem engineers, biodiversity, anthropogenic pressures.

Introduction

Marine bioconstructions, or bioherms, are made of dead benthic organisms whose remnants (usually skeletons or shells) are colonized by other benthic species that will, in their turn, elevate the substrate, to be then overgrown by other organisms after their death. Bioconstructions occur from the sea surface to the deep sea, some are ephemeral, whereas others persist for centuries or even millennia.

Bioconstructors modify primary (i.e. geological) substrates and provide secondary (i.e. biogenic) substrates for new bioconstructors and for non-bioconstructors who simply inhabit them. Habitat formers modify primary substrates with their presence, but most of them (e.g. erect algae) are ephemeral and must renew with each generation. Bioconstructors also form new habitats and are, thus, habitat formers but, contrary to ephemeral habitat formers, they are mostly perennial and result from centuries or even millennia of biological activities. Due to their importance and their “history”, perennial bioconstructions require the highest attention in conservation measures since they are not easily replaceable.

The European Union (EU) Habitats Directive 92/43/EEC identifies the category “reefs” as being of community importance, deserving protection in the Natura 2000 network. Reefs comprise both biogenic and geogenic formations that arise from the sea bed. The rhizomes of *Posidonia* meadows, a distinct habitat type from reefs in the Habitats

Directive, have a continuous vertical growth, entrap sediments and elevate the substrate. Thus, *Posidonia* is a bioconstructor and might be considered as a simple subcategory of “reefs”. Both *Posidonia* meadows and other biogenic “reefs”, as bioconstructions, promote high levels of biodiversity and enhance ecosystem functioning.

In the Mediterranean Sea, Natura 2000 networks mostly protect *Posidonia* meadows, whereas other types of bioconstructions have received scant attention as a result of the application of the Habitats Directive. The understanding of the status of most Mediterranean bio-constructions is poor.

The management of bioconstructions, just as that of all components of the natural capital, is based on three pillars:

- a) Patterns—assessing the distribution of habitats and their conservation status.
- b) Processes—understanding the drivers that determine the patterns and identifying stressors and their impacts.
- c) Measures—assessable management actions based on solid scientific evidence.

The most important biogenic habitats along the 8500 km of the Italian coast can be considered as being representative of the possible situation in the whole basin. Their distribution has been assembled from 468 documents reporting spatial information and unpublished in situ observations from various sources, all validated and georeferenced, allowing to make digital maps integrating all the available knowledge on the distribution of these habitats.

Results

The main biogenic structures of the Mediterranean Sea, besides the well-known *Posidonia* meadows, are: *Lithophyllum byssoides* concretions/trottoirs, *Astroïdes calycularis* formations/reefs, coralligenous assemblages, *Cladocora caespitosa* formations/reefs, vermetid reefs, sabellariid reefs, cold-water coral frameworks, serpulid reefs, including biostalactites.

They all are important in generating and maintaining marine biodiversity, increasing spatial complexity and settlement opportunities to a vast array of species. The distribution pattern of these bioconstructions along the Italian coast is reported in Fig. 1.

Fig. 1 shows that, as a whole, bioconstructions occur along the whole Italian coast. The bioconstructions formed by *Posidonia* meadows are relevant along the whole coast too, with the exception of the central and northern Adriatic Sea. Hence, bioconstructions are of paramount importance in almost all Italian coastal habitats, reaching the deep sea in the form of cold coral banks.

If, as a whole, bioconstructions are ubiquitous, the various types are less evenly distributed. Coralligenous assemblages are the most widespread bioconstruction type, and they are widely distributed whenever substrates are rocky. Even on soft bottoms, however, platform coralligenous can thrive and elevate the substrate. Vermetid reefs can be very prominent, but they are mostly confined to southern Italy, together with *Astroïdes calycularis* bioconstructions. Biostalactites are even more restricted in occurrence, but they might prove more widespread than presently known, when marine caves will be better explored. *Lithophyllum* rims appear mostly widespread in the western basin, even though they are very prominent along the coast of the Salento Peninsula (Apulia). Sabellarian reefs, an ephemeral bioconstruction, are widespread along the Adriatic coast and mostly occur on soft substrates. They are also widespread along the southern coast of Sicily, and occasionally occur in the Tyrrhenian and Ligurian seas.

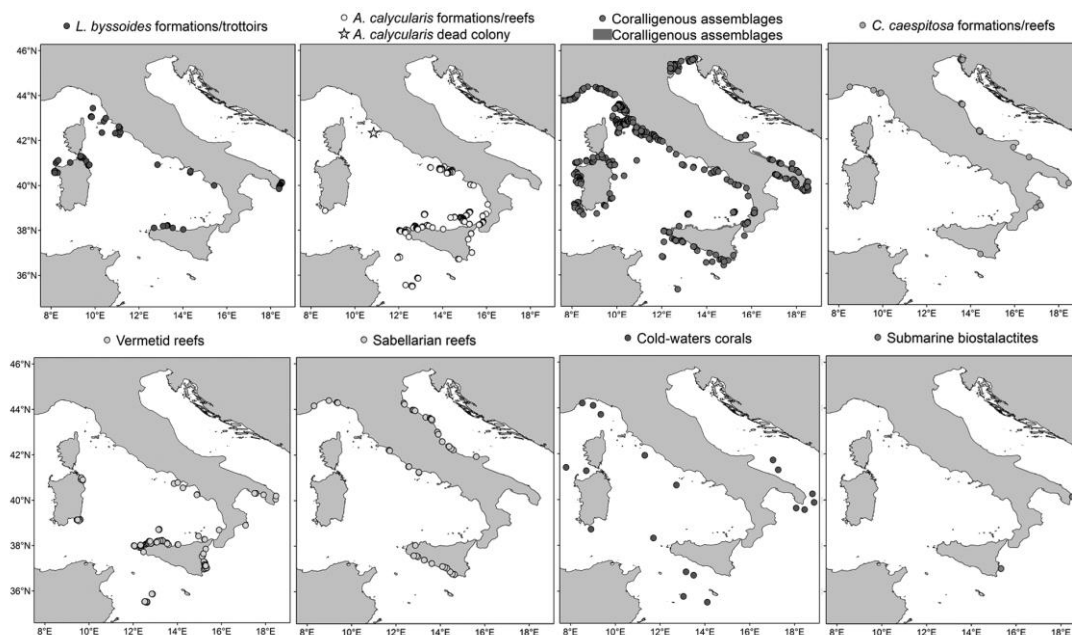


Fig. 1 Distribution pattern of marine bioconstructions along the Italian coast. Boundaries of point and polygon features of the data layers have artificially been enhanced for illustrative purposes, so they do not reflect the real habitat extent

Discussion

The age of bioconstructions - Coralligenous pinnacles dating back 6,207 years from the Ionian Sea showed a core partially made up by serpulid remains, a feature in common with other bioherms studied in the Southern Ionian Sea and with pseudo-stalactites found in several marine caves from the Aegean and Ionian Seas. The rims of *L. byssoides* are fairly persistent: in conditions of stable sea level they can grow for centuries, up to 900–1,000 years. *Madrepora oculata* and *Lophelia pertusa* can build huge bioconstructions that can exceed 1 m in height and width per colony. Dead colonies are an exploitable hard substrate for many associated species. These bioconstructions can produce carbonate mounds that sometimes endure for many hundred thousand to millions of years of discontinuous coral succession. Sabellarian reefs have much lower life expectancies, since they are produced by the amalgamation of sand grains and are, thus, rather unstable, over the long period.

Connectivity - Bioconstructors have a low dispersal capability compared to actively motile organisms, such as fish and marine mammals and reptiles and free-living invertebrates, so they should have low population connectivity. In fact, dispersal in bioconstructors relies exclusively on larvae and propagules that are liable of being transported by currents for limited periods. However, the larval/propagule biology of bioconstructors is usually poorly known. Knowledge on the connectivity of different bioconstructions is scant, though a variety of techniques have been developed to fill this gap such as genetic analyses, dispersal simulation models and β -diversity studies. Besides life cycle connectivity, also food web connectivity might be very important in defining homogeneous ecological spaces where their requirements are satisfied. The cold corals in the southern Adriatic and the Ionian sea probably take advantage (in terms of food and nutrients) of the current generated by the Northern Adriatic cold engine that sends newly formed deep water across the submarine plains of the Central and Northern Adriatic, then flowing towards the bottom through the Bari canyon, reaching the cold coral formations.

These, then, are trophically connected with the Northern Adriatic, even though they do not occur there.

Major threats to Mediterranean bioconstructions - The intensive human exploitation of the Mediterranean Sea has profound negative effects on marine biota. Industrial, urban and agricultural pollution, coastal development, climate change, increases in sedimentation, trawling and other types of industrial fisheries, anchoring, and the introduction of alien species represent the main threats for Mediterranean marine benthic communities. Almost all coastal biodiversity of the Mediterranean is affected by these threats that, in some cases, can impact also the deep sea due to downwelling currents and offshore activities such as deep sea drilling.

Current conservation measures - Although not legally binding, the Barcelona Convention's Action plan adopted in 2008 for the conservation of coralligenous outcrops and other calcareous bioconcretions in the Mediterranean Sea prescribes that "coralligenous/maërl assemblages should be granted legal protection at the same level as *P. oceanica* meadows". Coralligenous outcrops also appear in the EU Habitats Directive (under habitat type 1170 "Reefs"), and in the Bern Convention. Two maërl-forming Mediterranean species, *Lithothamnion corallioides* and *Phymatolithon calcareum*, are included in Annex V of the Habitats Directive. Finally, under European law, destructive fishing is prohibited over Mediterranean coralligenous and maërl bottoms. The lack of relevant geospatial data on these habitats, however, significantly hinders the effective implementation of these policies.

Guidelines and recommendations - The two main marine protection tactics enforced in European waters comprise nationally designated MPAs, and the Sites of Community Importance (SCIs) as part of the Natura 2000 network. Typically, MPAs should have a management plan, a president, a director, a staff and a budget. The SCIs, instead, in most cases have neither a management plan and budget nor a staff of any kind. The protection of the marine environment is further enforced through an entangled multitude of initiatives, usually termed as OECMs (Other Effective Area-based Conservation Measures). Currently, the harmonization of protection tactics into a single strategy involves the extension of MPAs so as to comprise most SCIs. This will allow for consistent management. The enforcement of protection, indeed, distinguishes "real" MPAs, where protection is enforced, from "paper parks", where measures are simply on paper. In general, many MPAs have been designated where seascapes are particularly attractive for scuba divers and "beauty" has been the main reason for their proposal. Since bioconstructions, and especially coralligenous ones, are particularly spectacular, many MPAs include bioconstructions. The designation of SCIs, instead, focused almost only on *Posidonia* meadows, but their attention is now turning towards bioconstructions as part of the "reef" category of the EU Habitats Directive. The two protection strategies (nationally designated MPAs and SCIs, together with OECMs, when possible) must be harmonized and integrated, considering also other principles and measures such as the Ecosystem Approach, Maritime Spatial Planning, Integrated Coastal Zone Management.

Conclusions

The basic principle of guidelines to preserve bioconstructions is very simple: impacts must be identified and removed. This can be straightforward for direct impacts, but global impacts such as climate warming are more difficult to manage. Extensive mass mortalities of important species that either form or grow on coralligenous formations, such as sponges and sea fans, occurred during periods of deepening of the mixed layer, but the

impact on the bioconstructors such as algae is still poorly known. The avoidance of direct stress on biogenic structures as a result of protection policies leads to healthier habitats that, hopefully, will better respond to global stressors such as warming and acidification. The impact of non-indigenous species is rather elusive, since it is not clear if they have a direct impact on resident species or if they simply replace indigenous species affected by temperature increases. Most aliens, in fact, are of tropical origin and are then pre-adapted to the environmental changes ensuing from global warming. The eradication of aliens is seldom effective and might even prevent the impacted systems to reach a new state, in harmony with the new conditions determined by global warming.

Industrial fisheries are the main direct threat to these benthic habitats: they can be regulated so as to reduce the impacts. Other impacts, however, originate from human activities that are carried out inland. Eutrophication, together with agricultural and industrial pollution are land-based and do not fall under the jurisdiction of the authorities entitled to manage the protected marine spaces.

In general, the Marine Strategy Framework Directive (MSFD), with the objective of Good Environmental Status (GES) in all EU waters, is the legal tool to prevent impacts on marine systems, including bioconstructions. The first descriptor of GES (Biodiversity is maintained) obviously covers also the biodiversity expressed by bioconstructions. The other descriptors list all possible impacts, from non-indigenous species to eutrophication, marine litter, overfishing, contaminants, alterations of hydrology, introduction of energy, and prescribe that they do not affect the ecosystems. The enforcement of the MSFD and the ensuing policy towards GES, thus, are very suitable to protect and manage also bioconstructions. This objective is not only valid within MPAs but also in the space in between, since they apply to all EU waters. Hence, the creation of networks of MPAs is the next logical step to protect marine biodiversity.

MPA networks are not simple bureaucratic objects as, at present, the Natura 2000 Network is. Instead, they should be nested into coherent ecological spaces where connectivity (both in terms of propagule dispersal and of nutrient distribution) define highly connected spaces. MPA networks, especially in the Mediterranean Sea, should often comprise waters under the jurisdiction of different states, not necessarily belonging to the European Union. The efficient enforcement of protection measures, hence, requires international agreements that cover wider spaces than those the MSFD is going to be implemented. The Ecosystem Approach (ECAP) is shared also by non EU countries and is the perfect tool to enforce such a management and conservation policy. In order to be effective, however, both the MSFD and the Ecosystem Approach are to be applied to spaces where ecosystem processes are consistent as a result of high connectivity: the Cells of Ecosystem Functioning. The definition of these cells is still very rough, and will require focused investigations that consider all the features of the environment, and that do not focus only on specific components of the ecosystems. A policy aimed at defending bioconstructions, hence, is to be nested into a much wider strategy. Under this respect, the MSFD is very suitable to reach this objective through the definition of the descriptors of GES, and should be shared also by non EU countries.

The protection of bioconstructions under the logic of the Habitats Directive implies direct measures regarding the habitat, focusing on the patterns of distribution of particular expressions of biodiversity. The protection of bioconstructions under the logic of the MSFD, instead, comprises not only the patterns of distribution of biodiversity, but also the processes (i.e. ecosystem functioning) that allow for their persistence and well-being.

Under this respect, thus, it is highly advisable that the logic of the MSFD is applied, replacing the logic of the Habitats Directive, since it incorporates it and expands it to the ecosystem, so operationally enforcing the Ecosystem Approach.

Since many habitats are impacted by human activities, it is also advisable to attempt restoration activities. In general, the options are: passive restoration, i.e., the reestablishment of natural conditions with no further intervention, and removal of impacts; active restoration that might be attempted if passive restoration does not occur, even if it is difficult to conceive a human-driven reestablishment of communities such as coralligenous ones, whose construction requires millennia. However, further research is needed to understand if for some bioconstructions active restoration actions might be a good solution to achieve recovery goals.

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ORAL COMMUNICATIONS

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ASSESSMENT OF THE CONSERVATION STATUS OF COASTAL DETRITAL SANDY BOTTOMS IN THE MEDITERRANEAN SEA: AN ECOSYSTEM-BASED APPROACH IN THE FRAMEWORK OF THE ACDSEA PROJECT

Abstract

Coastal detrital sandy bottoms are an ecosystem (CDE) that is widespread over the continental shelf from 30 down to 100 m depth in the Mediterranean Sea. Despite the extensive surface area it covers on the continental shelf, this key ecosystem has not been considered as a habitat of European interest by the Habitat Directive of the European Union (Natura 2000, 92/43/CEE). Since the 1960s, many studies have highlighted the high species diversity of its epiflora, epifauna and endofauna (macrophytes, including fucales and corallinales, bryozans, crustaceans, molluscs, porifera, annelids, etc.), its functional role (foraging and spawning area, nursery ground, carbon sink, key habitat for a variety of taxa) and its vulnerability to human activities and global change (trawling, dredging, anchoring, terrestrial and marine pollution, flooding, non-indigenous species, etc.). Here, a conceptual model of the functioning of the CDE is proposed. It is based on literature data, updated with the addition of recent field data collected within the new Port-Cros National Park (Provence, France, north-western Mediterranean Sea), along a gradient of human disturbance. Scuba diving (sampling and taxa identification) and an innovative tool, the BioCube (photo-quadrats, biotic covering analyses, etc.), have been implemented. The aim of the ACDSea project (Assessment of the conservation status of Coastal Detrital Sandy bottoms: an ecosystem-based approach) is to develop a method to assess the conservation status and quality of the CDE. The present study is a preliminary one, as more data, in a variety of ecoregions, is needed to improve the conceptual model and develop an Ecosystem-based Quality Index (EBQI). Subsequently, surveillance networks will have to be set up, at regional scale, enabling stakeholders and managers (MPAs, local and regional authorities) to better take into account this ecosystem in their management plans.

Key-words: Ecosystem-based approach, Coastal detrital, Human impact, Ecosystem management, Conservation

Introduction

The Coastal Detrital Ecosystem (CDE) is one of the most extensive ecosystems on the continental shelf of the Mediterranean Sea. It is a sandy bottom constituted by recent organogenous and bioclastic sediments, originating there or from adjacent ecosystems (e.g. coralligenous, photophilous infralittoral reefs). Thriving between 30 and 100 m depth, the CDE can vary widely, depending upon adjacent ecosystems and hydrodynamic conditions (Pérès and Picard, 1964; Johér *et al.*, 2015). A variety of associations and facies are found in the CDE (Michez *et al.*, 2014): (i) associations of free-living calcareous (e.g. mainly Corallinales and Peyssonneliaceae), perennial and seasonal macroalgae (e.g. *Cystoseira* spp., *Osmundaria volubilis*, etc.); (ii) facies of bryozoans (e.g. *Pentapora fascialis*). Several heritage value taxa are typical of the CDE: *Cystoseira* spp., maerl beds (Annexe 5 of the Habitat Directive Natura 2000). Other heritage value taxa can be found within the CDE such as *Pinna nobilis* (protected by the Bern convention, annexe 2). Important ecosystem services (Costanza *et al.*, 1997),

hitherto non-evaluated, are provided by the CDE: (i) the high diversity of macrophyte stand structure provides a key habitat for vagile invertebrates and fishes and a trophic resource. (ii) a spawning and nursery ground for fish and crustaceans including species of fishery interest (e.g. Sparidae, *Palinurus elephas*) (Harmelin and Harmelin-Vivien, 1976), (iii) a fishing ground for trawlers and artisanal fishers, (iv) a long term carbon sink, (v) primary production, (vi) recycling of necromass (mainly rhizomes and dead *Posidonia oceanica* leaves and drift macroalgae). A high vulnerability to human activities and global change is highlighted (trawling, dredging, anchoring, terrestrial and marine pollution, flooding, non-indigenous species, etc.). Current knowledge regarding the CDE is mainly centred on the description and taxonomy of assemblages (Jaquotte, 1962; Boudouresque and Denizot, 1973; Augier and Boudouresque, 1975; Laborel *et al.*, 1976; Harmelin, 1978; Bourcier, 1982, 1985, 1988; Fornos *et al.*, 1988; Astruch *et al.*, 2012; Joher *et al.*, 2012, 2015). Since the 1960s, many studies have highlighted the high species diversity of its epiflora, epifauna and endofauna (macrophytes, including fucals and corallinales, bryozans, crustaceans, molluscans, porifera, annelids, etc.). Despite its high ecological and heritage value and threats, this key ecosystem has not been considered as a habitat of European interest by the Habitat Directive of the European Union (Natura 2000, 92/43/CEE; Cahier Habitats Natura 2000, 2004). The extension of the Marine Protected Area surface area (national parks, Natura 2000 sites, etc.), since 2010, has incorporated vast expanses of seabed occupied by the CDE. In the light of these new management issues, it has become a challenge to better understand this wide spread ecosystem. The aim of the ACDSea project (Assessment of the conservation status of Coastal Detrital Sandy bottoms: an ecosystem-based approach) is to develop a method to assess the conservation status and quality of the CDE.

Material and methods

Study area: the new Port Cros National Park

The Port-Cros National Park (PCNP) was established in 1963. It encompassed the Island of Port-Cros and the neighbouring island and islets. Between 2012 and 2016, the PCNP was engaged in a major redefinition and extension of its territory; the new Port-Cros National Park (N-PCNP), established in 2016, includes the Port-Cros and Porquerolles Archipelagos as core areas (both terrestrial and marine), and a 120 000 ha Adjacent Marine Area (AMA) including the Gulfs of Hyères and Giens and extending seawards to the edge of the continental shelf (Figure 1) (Astruch *et al.*, 2018).

Sampling strategy

Macrophytes were sampled at 14 sites using scuba diving at the upper depth range of the CDE (35-45 m depth) (Figure 1). Three replicates per site were performed; 21 samples were randomly chosen for species identification at the lab. Data on cover or density of living organisms (flora and fauna) was sampled by implementing the 'Biocube'. This is a 1 m high device that enables the acquisition of 80 cm x 80 cm frame photoquadrats (Figure 2) without scuba diving. Photoquadrats were made with GoPro® Hero 4 with 30 second-time lapse triggering. Another GoPro linked to a screen at the surface is fixed to the Biocube to control the workflow and the position of the frame in real time. During the data acquisition, a third GoPro was filming the surrounding landscape for complementary information on demersal fish, extent of assemblages, etc. Field work was carried out from September to October 2015. Conservation status of the CDE has been assessed according to the definition of Lepareur (2011). The Biocube was implemented at 38 sites, at 35-80 m depth (30 photoquadrats per sites). A conceptual model of the functioning of the CDE is proposed, based on literature data, original data from the present work and expert judgement.

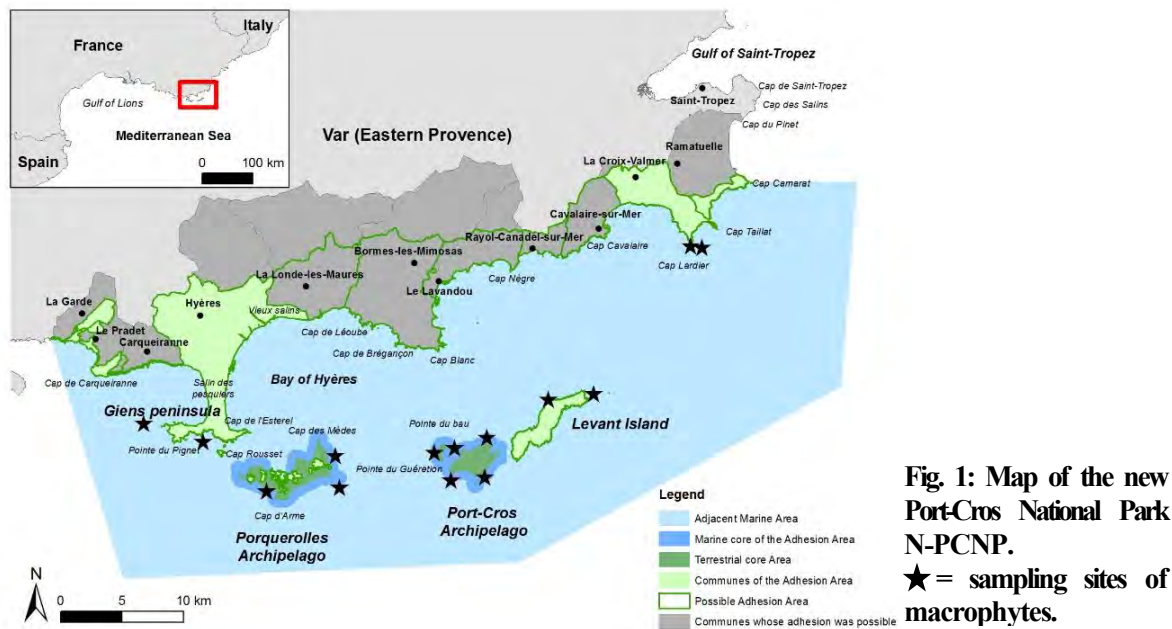
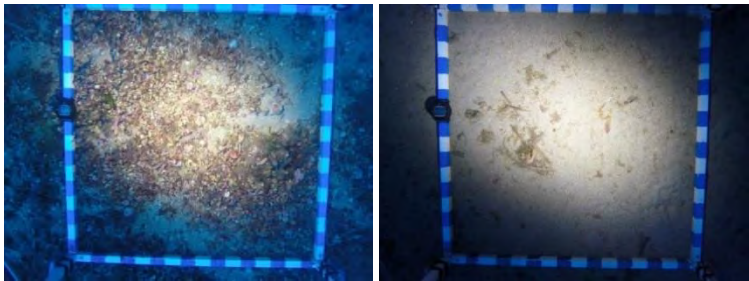


Fig. 1: Map of the new Port-Cros National Park N-PCNP.
★ = sampling sites of macrophytes.

Fig.2: Examples of photoquadrats used for CDE analyses.
(left: at 40 m depth;
right: at 80 m depth)



Results

Macroalgae diversity

A total of 108 taxa (alpha species diversity) were identified in 21 samples, mainly represented by Rhodophyta including 10 calcareous or encrusting taxa (Table 1). Among the most frequent species (Table 2), three are non-indigenous species: *Caulerpa cylindracea*, *Acrothamnion preissii* and *Womersleyella setacea*. Other observed species with less frequent occurrence play an important role in the functioning of the CDE: the perennial fucalae *Cystoseira montagnei* and *C. zosteroides*, the Rhodophyta *Osmundaria volubilis* and *Kallymenia* spp., and large Phaeophyceae as *Zanardinia typus*, *Arthrocladia villosa*, *Carpomitra costata*, *Nereia filiformis*, *Sporochnus pedunculatus*.

Tab.1: Calcareous or encrusting macrophytes of the Coastal Detrital Ecosystem (CDE) of Port-Cros National Park.

Order	Species
Corallinales	<i>Lithophyllum stictaeforme</i> (J.E. Areschoug) Hauck
	<i>Spongites fruticulosa</i> Kützting
	<i>Lithothamnion corallioides</i> (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan
	<i>Lithothamnion minervae</i> Basso
Peyssonneliales	<i>Peyssonnelia harveyana</i> P.L. Crouan & H.M. Crouan ex J. Agardh
	<i>Peyssonnelia rubra</i> (Greville) J. Agardh
	<i>Peyssonnelia squamaria</i> (S.G. Gmelin) Decaisne
	<i>Peyssonnelia</i> sp. Decaisne
	<i>Peyssonnelia rosa-marina</i> Boudouresque & Denizot
Nemaliales	<i>Tricleocarpa fragilis</i> (Linnaeus) Huisman & R.A. Townsend

Tab. 2: More frequent observed species of the Coastal Detrital Ecosystem (CDE) of Port-Cros National Park (N-PNPC) sorted by occurrence (Occ.) in 21 samples; *: non-indigenous species.

Occ.	Species	Occ.	Species
13	<i>Dictyota implexa</i> (Desfontaines) J.V. Lamouroux	8	<i>Sphacelaria plumula</i> Zanardini
12	<i>Caulerpa cylindracea</i> Sonder *	6	<i>Ceramium codii</i> (H. Richards) Mazoyer
12	<i>Polysiphonia subulifera</i> (C. Agardh) Harvey	6	<i>Eupogodon planus</i> (C. Agardh) Kützing
11	<i>Dictyota dichotoma</i> (Hudson) J.V. Lamouroux	6	<i>Jania adhaerens</i> J.V. Lamouroux
9	<i>Acrothamnion preissii</i> (Sonder) E.M. Wollaston *	6	<i>Pterothamnion crispum</i> (Ducluzeau) Nägeli
9	<i>Leptofaucheia cf. coralligena</i> Rodríguez-Prieto & De Clerck	6	<i>Sphacelaria cirrosa</i> (Roth) C. Agardh
9	<i>Womersleyella setacea</i> (Hollenberg) R.E. Norris *	5	<i>Aglaozonia chilosa</i> Falkenberg
8	<i>Brongniartella byssoides</i> (Goodenough & Woodward) F. Schmitz	5	<i>Arthrocladia villosa</i> (Hudson) Duby
8	<i>Cryptonemia lomation</i> (Bertoloni) J. Agardh	5	<i>Gracilaria dura</i> (C. Agardh) J. Agardh
8	<i>Halopteris filicina</i> (Grateloup) Kützing	5	<i>Myriactula stellulata</i> (Harvey) Levring
8	<i>Laurencia</i> sp. J.V. Lamouroux	5	<i>Peyssonnelia rubra</i> (Greville) J. Agardh
8	<i>Osmundea pelagosae</i> (Schiffner) K.W. Nam	5	<i>Sporochmus pedunculatus</i> (Hudson) C. Agardh
		5	<i>Valonia macrophysa</i> Kützing

The following metrics have been assessed from the photoquadrats: (i) bottom type (percentage of mud, sand and bioclastic sediment): an increase in the percentage of mud is observed according to a gradient from the east to the west, (ii) cover of dead leaves of *Posidonia oceanica*, (iii) cover of living organisms (percentage of Corallinales, Peyssonneliaceae, *Caulerpa cylindracea* and other organisms), (iv) point species diversity (mean number of species per photoquadrat), (v) mean density of macro-invertebrates (e.g. Holothuridae, Asteridae, Cnidaria, etc.). According to these metrics, the conservation status of CDE has been assessed at N-PCNP scale (Table 3).

Tab. 3: Conservation status of the Coastal Detrital Ecosystem (CDE). Sites are sorted by level of anthropogenic impact. See Figure 1 for sites localization and Lepareur (2011) for assessment method.

Site	State of conservation
Giens	Good
Porquerolles	Excellent
Cap Lardier	Good
Levant	Good to excellent
Port-Cros	Excellent

Conceptual model of the functioning of the Coastal Detrital Ecosystem (CDE)

The conceptual model of CDE (Figure 3) shows a dominance of primary producers (represented by macrophytes) in the functioning of the ecosystem. It is the basis of the trophic network from phytoplankton to calcareous, perennial and seasonal macroalgae. CDE functioning is strongly linked with adjacent ecosystems (exportation and importation of living or dead organisms).

Discussion

According to the conservation status assessment of the CDE of the N-PNPC, the human impact gradient is too weak and new data from more contrasted areas within the

Mediterranean are needed. The accuracy of the analysed metrics is linked to the definition of the photoquadrat and does not allow precise species identification. It does however allow comparison between sites. Improvement of the Biocube (*i.e.* homogenous lighting, automatized data analysis) is needed. The project ACDS_{Sea} will complete the data acquisition and will allow the implementation of a network survey of the CDE at French Mediterranean scale using a standardized method including an Ecosystem-based Quality Index (EBQI) based on the conceptual model presented here. An EBQI has been developed for 4 Mediterranean coastal ecosystems (Personnic *et al.*, 2014; Ruitton *et al.*, 2014; Rastorgeff *et al.*, 2015; Thibaut *et al.*, 2017). The goal, in the longer term, is to provide a useful tool for coastal managers (MPAs, local authorities) and argue for better consideration of this key ecosystem / habitat. Application out of the Mediterranean context (Eastern Atlantic, English Channel) is also expected.

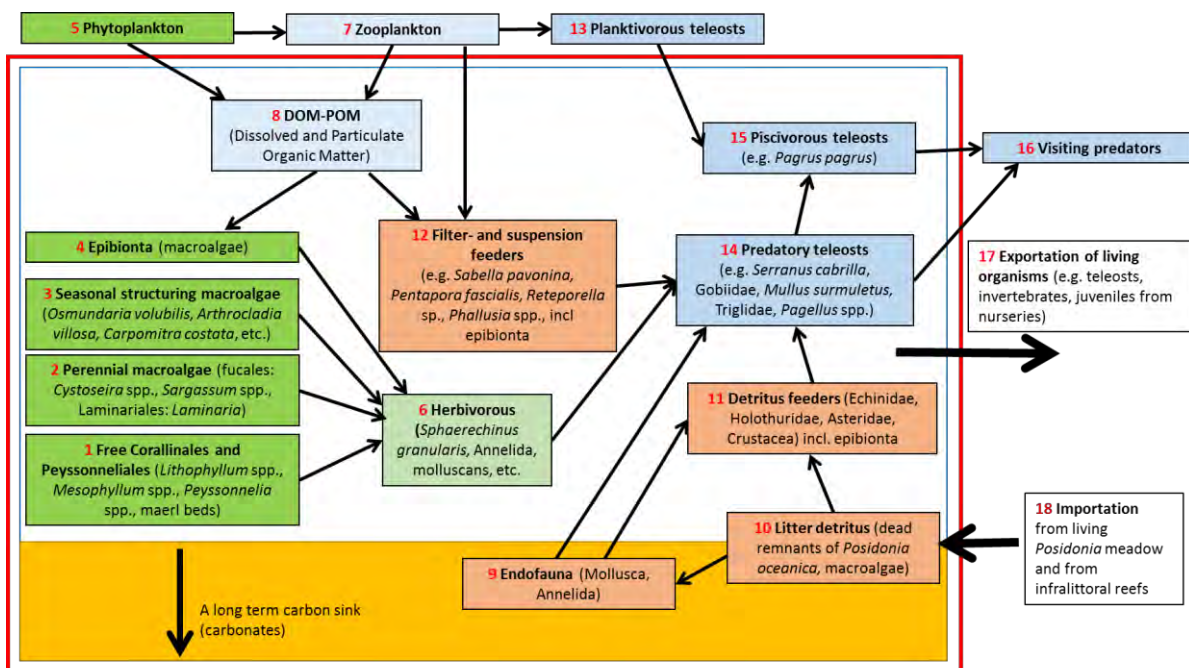


Figure 3. Conceptual model of the Coastal Detrital Ecosystem (CDE).

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FOOD WEB STRUCTURE OF A MEDITERRANEAN CORALLIGENOUS ECOSYSTEM

Abstract

The coralligenous ecosystem is one of the biodiversity hotspots in the Mediterranean Sea. This study aims at determining (i) the organic matter (OM) sources at the base of the food web in a coralligenous ecosystem near Marseille (Cap Caveau, Frioul Island) and (ii) the global functional structure of its community. For that purpose, C and N stable isotope analyses were performed on 78 compartments or species collected in this habitat between 30 and 40 m depth. Results suggested that the pelagic particulate organic matter (POM), mainly composed of phytoplankton, was the main OM source fuelling coralligenous food webs, followed by sedimented organic matter (SOM) and some macrophytes. At least three level of consumers were identified from plankton-feeding invertebrates to piscivorous teleosts. Coralligenous food webs seemed to be characterized by a high abundance and trophic diversity of plankton and filter feeders. Among them numerous sessile invertebrates, such as cnidarians, bryozoans and ascidians, were poorly directly consumed, but played an important role in POM transfer. Further studies on spatial and temporal variations of coralligenous communities would be necessary for providing a general trophic functioning model of this highly diversified ecosystem.

Key-words: food webs, coralligenous ecosystem, stable isotopes, carbon, nitrogen.

Introduction

Coastal areas shelter important marine ecosystems both in terms of production and biodiversity (Ray, 1988). They are subjected to high anthropogenic pressures related to fishing activities, urbanization of coastline and sewage outfalls leading to inputs of continental organic and inorganic matter. Other threats affect these marine ecosystems due to the global change affecting the oceans, warming and acidification of seawater in particular (e.g. Doney *et al.*, 2012). In the Mediterranean Sea, two coastal ecosystems constitute "hotspots" of biodiversity: *Posidonia oceanica* meadows (Boudouresque *et al.*, 2012) and coralligenous bioconstructions (Ballesteros, 2006). The latter is a natural biogenic hard substrate (bioherm) built, under dim light conditions, by the accumulation of calcareous organisms, mainly calcareous algae (Corallinales and Peyssonneliaceae). Since its description by Marion (1883), numerous studies have described the structure and dynamics of these bioconcretions and listed the associated biodiversity (see Ballesteros, 2006). More recently, studies focused on the impact of global warming on large sessile invertebrates present on coralligenous bottoms (Garrabou *et al.*, 2009; Lejeusne *et al.*, 2010; Rivetti *et al.*, 2014). By contrast, compared to other benthic ecosystems such as *Posidonia oceanica* meadows, few data is available on the functioning aspects of coralligenous bioconstructions, particularly their trophic structure (McClanahan & Sala, 1997). To fill this gap, using C and N stable isotope analyses we tracked the sources of organic matter (OM) at the base of the food web in a coralligenous ecosystem located close to Marseille (France) and describe its trophic structure.

Materials and methods

The study area is located in the southern Bay of Marseille (France) at Cap Caveau on the south-west part of Pomègues Island, Frioul archipelago (43°15'36.7" N; 5°17'23.4" E). The geomorphology of this coastline presents steep rocky cliffs extending underwater down to 47 m depth. Coralligenous concretions are well developed from 25 m to 45 m depth, with an erected facies of gorgonians and sponges. The sources of OM considered in this coralligenous ecosystem include the particular organic matter (POM) of seawater, the sedimented organic matter (SOM), all macrophytes and settled *Posidonia oceanica* detritus. These sources, plus a number of invertebrate and fish species belonging to several trophic functional groups, were collected by SCUBA diving between 30 and 40 m depth from October to December 2015 (Tab. 1). Large and high level vagrant piscivores (moronids, carangids) could not be sampled due to the high fishing pressure in this area.

Tab. 1: List of abbreviations used for the taxa analysed

Macrophytes		<i>Astrospartus mediterraneus</i>	Ame	<i>Schizoretepora serratimargo</i>	Sser
<i>Caulerpa cylindracea</i>	Ccy	<i>Echinaster sepositus</i>	Ese	<i>Myriapora truncata</i>	Mtr
<i>Codium bursa</i>	Cbu	<i>Marthasterias glacialis</i>	Mgl	<i>Salmacina</i> sp.	Salm
<i>Codium coralloides</i>	Cco	<i>Hacelia attenuata</i>	Hat	<i>Eunice</i> sp.	Eunice sp.
<i>Flabellia petiolata</i>	Fpe	<i>Arbacia lixula</i>	Ali	Lumbrineridae	} detritivorous
<i>Pseudochlorodesmis furcellata</i>	Pfu	<i>Paracentrotus lividus</i>	Pli	<i>Syllis</i> sp.	
<i>Halimeda tuna</i>	Htu	<i>Sphaerechinus granularis</i>	Sgr	<i>Harmothoe</i> sp.	Harmothoe
<i>Palmophyllum crassum</i>	Pcra	<i>Holothuria tubulosa</i>	Htub	<i>Goniadia</i> sp.	goniadia
<i>Posidonia oceanica</i>	Pocea	<i>Lithophaga lithophaga</i>	Llith	<i>Bonellia viridis</i>	Bvi
<i>Pseudolithophyllum cabiochae</i>	Pcab	<i>Lima lima</i>	Llim	<i>Halocynthia papillosa</i>	Hpa
<i>Peyssonnelia squamaria</i>	Psqua	<i>Hexaplex trunculus</i>	Htru	Teleosts	
<i>Peyssonnelia bornetii</i>	Pbor	<i>Cantharus</i> sp.	Cantha	<i>Anthias anthias</i>	Aan
<i>Sphaerococcus coronopifolius</i>	Sco	<i>Bittium reticulatum</i>	Bret	<i>Chromis chromis</i>	Cchr
<i>Cystoseira zosteroides</i>	Czo	<i>Clanculus corallinus</i>	Ccora	<i>Spicara maena</i>	Smae
<i>Halopteris filicina</i>	Hfi	<i>Jujubinus exasperatus</i>	Jex	<i>Apogon imberbis</i>	Aim
Dyctyotales	Dyct	<i>Felimare picta</i>	Fpic	<i>Coris julis</i>	Cjul
<i>Padina pavonica</i>	Ppav	<i>Felimare tricolor</i>	Ftri	<i>Symphodus mediterraneus</i>	Smed
Invertebrates		Hydrozoa	Hydr	<i>Labrus mixtus</i>	Lmi
Decapoda	Decapo	<i>Cribrinopsis crassa</i>	Ccra	<i>Mullus surmuletus</i>	Msur
<i>Palinurus elephas</i>	Pele	<i>Eunicella cavolini</i>	Eca	<i>Diplodus sargus</i>	Dsar
Paguridae	Pagu	<i>Leptogorgia sarmentosa</i>	Lsar	<i>Diplodus vulgaris</i>	Dvu
Galatheididae	Gala	<i>Paramuricea clavata</i>	Pcl	<i>Scorpaena porcus</i>	Spor
<i>Leptocheirus</i> sp.	} Amphipodes détritivores	<i>Corallium rubrum</i>	Cru	<i>Scorpaena scrofa</i>	Sscro
<i>Erichthonius</i> sp.		<i>Alcyonium coralloides</i>	Aco	<i>Serranus cabrilla</i>	Scab
<i>Leucothoe</i> sp.		<i>Leptopsammia pruvoti</i>	Lpr	<i>Muraena helena</i>	Mhel
zooplankton	zooplankton	<i>Parazoanthus axinellae</i>	Pax	<i>Phycis phycis</i>	Pphy
<i>Ophiotrix fragilis</i>	Ofrag	Sipunculidae	Sipuncle	<i>Zeus faber</i>	Zfab
<i>Ophioderma longicauda</i>	Olong				

All samples were frozen, freeze-dried and ground into a fine powder using a mortar and pestle. For samples containing carbonates (*i.e.* POM, SOM, corals, bryozoans), one subsample was treated with 1 % HCl in excess. Samples were then rinsed with deionized water and dried before measurement of carbon isotope composition (expressed as $\delta^{13}\text{C}$). The raw subsample was used for the measurement of nitrogen isotope composition (expressed as $\delta^{15}\text{N}$). Stable isotope analyses were performed at the LIENSs stable isotope facility University of La Rochelle, France, using a continuous-flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Bremen, Germany) coupled to an elemental analyser (Flash EA1112 Thermo Scientific, Milan, Italy). Results are expressed in the δ notation as deviations from international standards: Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ values and atmospheric N_2 for $\delta^{15}\text{N}$ values (Peterson & Fry, 1987). Isotope

compositions are expressed in parts per thousand (‰) according to the equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Calibration was done using reference materials (USGS-24, IAEA-CH6, IAEA-600 for carbon; IAEA-N2, IAEA-NO-3, IAEA-600 for nitrogen). For both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, measurement precision is < 0.15 ‰ based on the analyses of acetanilide (Thermo Scientific) and peptone (Sigma-Aldrich) used as laboratory internal standards. A total of 78 compartments were analysed: 76 taxa, POM and SOM (Belloni *et al.*, 2018). For each compartment or taxon, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calculated from 3 to 6 replicates. The isotope compositions measured in a consumer is close to its diet, taking into account a trophic fractionation factor (mean = 1 ‰ for $\delta^{13}\text{C}$ and = 3.4 ‰ for $\delta^{15}\text{N}$) (Peterson & Fry, 1987). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values provide information about the trophic level and the origin of the OM sources, respectively. Combining isotope analyses of C and N allows an efficient monitoring of the transfer of organic matter in food webs (Layman *et al.*, 2012). Data processing was carried out using R (v1.1.456).

Results

The food web of the coralligenous ecosystem presented a large range of $\delta^{13}\text{C}$ values (from -33.0 ± 0.2 ‰ to -10.6 ± 0.9 ‰), while $\delta^{15}\text{N}$ values ranged from 2.1 ± 0.3 ‰ to 11.4 ± 0.4 ‰ (Fig. 1).

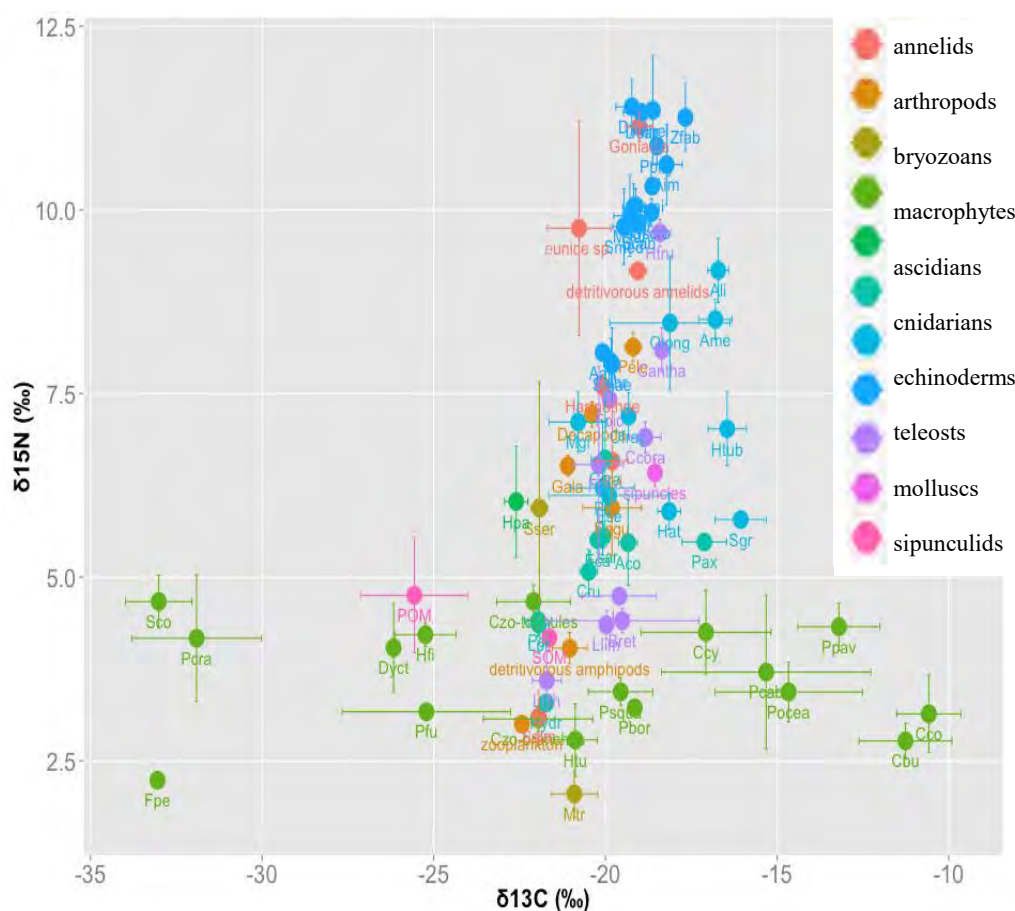


Fig. 1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean $\pm$ standard deviation, ‰) of the taxa and compartments sampled in the coralligenous ecosystem. See Tab. 1 for abbreviations used.

Among the potential sources of OM, macrophytes encompassed all the range of $\delta^{13}\text{C}$ values, from the most ^{13}C -depleted *Flabellia petiolata* to the most ^{13}C -enriched *Codium coralloides*, while their $\delta^{15}\text{N}$ values ranged from 2.2 ± 0.1 ‰ to 4.7 ± 0.2 ‰. *Posidonia oceanica* detritus had a high $\delta^{13}\text{C}$ value (-14.7 ± 2.2 ‰) and an intermediate $\delta^{15}\text{N}$ value (3.4 ± 0.4 ‰), while SOM and POM presented intermediate $\delta^{13}\text{C}$ value (-21.6 ± 0.1 ‰ and -24.5 ± 1.5 ‰, respectively) and $\delta^{15}\text{N}$ value (4.2 ± 0.2 ‰ and 4.8 ± 0.8 ‰, respectively). To determine which sources fuelled the food web, an ascending hierarchical classification clustered the potential OM sources in five groups with similar isotope composition (Fig. 2). Based on their extreme $\delta^{13}\text{C}$ values and the discrepancy between these values and those of the first consumers of the food web, two groups of macrophytes could be excluded as important OM sources in this ecosystem: *Flabellia petiolata*, *Palmophyllum crassum* and *Sphaerococcus coronopifolius* with low $\delta^{13}\text{C}$ values (from -33.1 ‰ to -31.9 ‰) and the two *Codium* species with high $\delta^{13}\text{C}$ values (-11.3 ‰ and -10.6 ‰). Three groups could probably contribute to the coralligenous food web functioning. The first one gathered *Padina pavonica*, *Posidonia oceanica*, *Pseudolithophyllum cabiochae* and *Caulerpa cylindracea*. The second one contained Dictyotales, *Halopteris filicina*, *Pseudochlorodesmis furcellata* and POM. A third group gathered *Peyssonnelia squamata*, *Peyssonnelia bornetii*, *Halimeda tuna*, *Cystoseira zosteroides* and SOM.

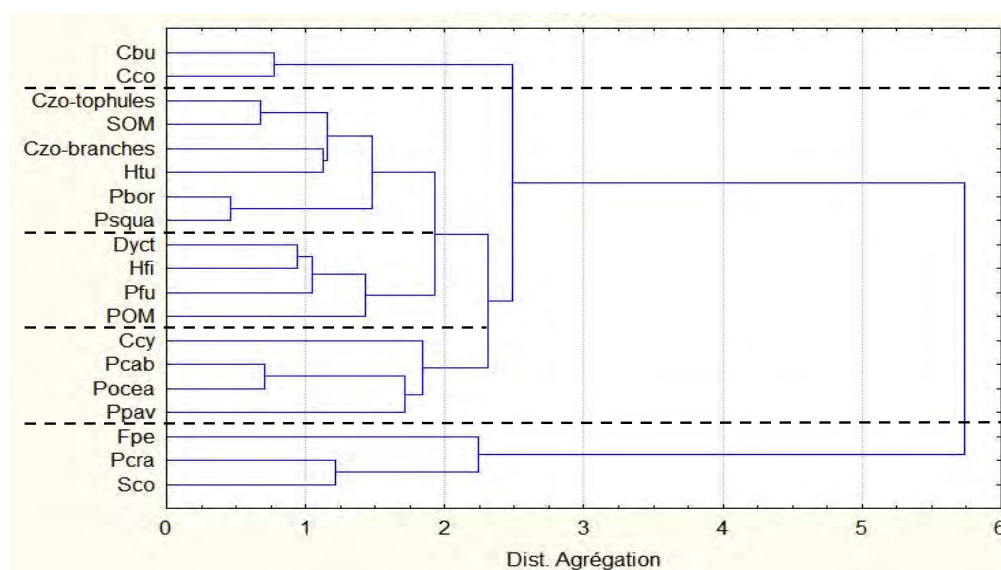


Fig. 2: Hierarchical clustering (minimum jump, Euclidean distance) of OM sources based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. See Tab. 1 for abbreviations used.

Invertebrates, which constituted the largest part of the consumers in the coralligenous ecosystem, presented isotope composition ranging from -22.6 ± 0.3 ‰ to -16.1 ± 0.8 ‰ for $\delta^{13}\text{C}$ values, and between 2.1 ± 0.3 ‰ and 11.1 ± 0.2 ‰ for $\delta^{15}\text{N}$ values. Among them, zooplankton feeders (cnidarians, bryozoans, ascidians, bivalves and the annelid *Salmacina* sp.) presented low mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (4.7 ± 0.4 ‰ and -20.9 ± 0.3 ‰), while carnivorous invertebrates (annelids, actinians, gastropods and echinoderms) presented high mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (8.1 ± 0.4 ‰ and -18.9 ± 0.5 ‰). Herbivores (gastropods and echinoids), detritivores (sipunculids, molluscs, arthropods and echinoderms) and omnivores (annelids, arthropods and echinoderms) had intermediate stable isotope

composition (from 5.8 ± 0.4 ‰ to 8.0 ± 0.6 ‰ for mean $\delta^{15}\text{N}$ values, and from -19.9 ± 1.0 ‰ to -18.9 ± 0.6 ‰ for mean $\delta^{13}\text{C}$ values). The isotope composition of teleost fishes presented narrower ranges of $\delta^{15}\text{N}$ values (from 7.9 ± 0.5 ‰ to 11.4 ± 0.4 ‰) and $\delta^{13}\text{C}$ values (from -20.1 ± 0.1 ‰ to -17.7 ± 0.2 ‰). The zooplankton feeders, *Chromis chromis*, *Spicara maena* and *Anthias anthias*, had low $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (from 7.9 ± 0.3 ‰ to 8.1 ± 0.1 ‰, and from -19.8 ± 0.2 ‰ to -20.1 ± 0.1 ‰, respectively). Four species had the highest $\delta^{15}\text{N}$ values: two macrocarnivorous species, *Zeus faber* and *Muraena helena*, and two mesocarnivores: *Diplodus vulgaris* and *D. sargus* (from 11.3 ± 0.5 ‰ to 11.4 ± 0.4 ‰). The other teleost species, whether meso- (labrids, mullids) or macrocarnivores (scorpaenids, serranids), were characterized by intermediate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Discussion and conclusions

The stable isotope values recorded for coralligenous species, particularly teleost fishes, were close to those obtained by Cresson *et al.* (2014a, b) on nearby artificial reefs in the Bay of Marseille. The range of $\delta^{13}\text{C}$ values measured in this study was much wider for the potential food sources than for consumers, thereby indicating that not all sources were used in the food web. Among the macrophytes, some are well known to be not palatable, such as *Pseudolithophyllum* or *Peysonnelia* spp., which have calcareous thallus or *Pseudochlorodesmis*, which is an encrusting species. The major food sources susceptible to fuel the food web of the coralligenous habitat were some macrophytes, such as the Dictyotales and *Cystoseira zosteroides* consumed by the herbivores, the POM, highly consumed by filter feeders, and the SOM, ingested by the detritivores. The range of $\delta^{13}\text{C}$ values of invertebrates supported the fact that different sources of OM were at the base of this food web. The coralligenous ecosystem included numerous filter-feeding species, which increased the flux of OM from pelagic to benthic habitats. However, a large part of sessile filter-feeding invertebrates (bryozoans, ascidians, cnidarians), while playing a crucial role in the construction and architecture of the coralligenous ecosystem (Ballesteros, 2006), were poorly palatable and hardly consumed by other organisms. This trophic pathway could be considered therefore as a dead end for the coralligenous food web because of a lack of direct transfer of their organic matter up in the food web. The main trophic pathway of OM transfer seemed to be constituted by mobile invertebrates and soft-bodied filter feeders up to carnivorous species. This trophic way fuelled the entire food web up to the highest trophic levels and was the major way of transfer of the organic matter from the lowest to the highest trophic levels. The high range of $\delta^{15}\text{N}$ values between OM sources and carnivorous teleosts indicated that at least three trophic levels of consumers were present. The low $\delta^{15}\text{N}$ values of filter-feeding invertebrates placed them as primary consumers, followed by herbivorous and detritivorous invertebrates. Higher in the food web, one found zooplankton-feeding teleosts, then omnivorous invertebrates and mesocarnivorous teleosts. Carnivorous annelids and the macrocarnivorous teleosts, which prey mainly on fish, were found at the top of this coralligenous food web. After this first attempt of an isotopic characterization of the coralligenous food webs, it would be interesting to analyse their spatial and temporal variations and to compare their functional structure with that observed on other coralligenous ecosystem or on the highly diversified coral reefs (Behringer & Butler, 2006).

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RESTORATION PROTOCOLS FOR THE MEDITERRANEAN CORALLIGENOUS HABITATS

Abstract

The temperate coralligenous bioconcretions harbour approximately 10% of marine Mediterranean species (about 1600 species), including long-lived algae and invertebrates. Enhanced by climate change, several pressures affect coralligenous assemblages, leading to recurrent mass mortalities and dramatic loss of habitat complexity and biodiversity.

The EU-funded project MERCES is developing innovative methodologies to restore macroinvertebrate habitat-forming species from three key taxonomic groups: Cnidaria/Anthozoa, Porifera/Demospongiae and Bryozoa. Restoration protocols combined transplants from donor organisms using different techniques and recruitment-enhancing devices designed for habitat-forming species. Considering the life-history traits, population dynamics and population genetics of the selected species, restoration actions should be mainly based on transplants of small to medium size collected from donor specimens. Bearing in mind that survival of transplants is higher in species with slow growth rates such as those dwelling in the coralligenous than in more dynamic species, transplantation efforts in the coralligenous will require low initial effort but a long period will be required to fully recover habitat complexity, i.e. decades. We identified survival and growth of transplants and recruitment as the most suitable short-term indicators of the success of the restoration actions.

Collaboration with volunteers (divers and diving operators) in several phases of field activities are being explored to cost-effective scaling up the restoration actions and to increase the sense of stewardship in a major users' segment.

Key-words: Porifera, Cnidaria, transplantation, habitat recovery, ecosystem engineer, Mediterranean Sea

Introduction

Bioconstructions are generally considered key habitats in the marine environments owing to the complex architecture they build, offering opportunity of refuge, feeding, reproduction and recruitment for many species. In the Mediterranean Sea, coralligenous habitats represent the most important bioconstructions, mainly built by the accumulation, in hundreds or thousands of years, of dead thalli of encrusting coralline algae growing in dim light conditions. Their current distribution has been assessed in detail only for Italian coasts (Ingrosso *et al.*, 2018) but predictive models offer a good estimation of its abundance in the whole Mediterranean basin (Martin *et al.*, 2014). Being mostly developed along the rocky coastline, several anthropogenic pressures are affecting the integrity of the coralligenous, compromising its resilience especially in the face of increasingly frequent mass mortality events (Rivetti *et al.*, 2017). These are particularly affecting filter feeders like sponges, gorgonians, bivalves and tunicates (Cerrano *et al.*, 2000; Garrabou *et al.*, 2009). The Marine Strategy Framework

Directive (MSFD) requires the restoration of habitat integrity to recover ecosystem functioning and services where damages are recorded. However, this process is being delayed by the lack of sufficient historical baselines and by hurdles in the definition of shared robust methodologies to assess integrity at the regional scale.

Aiming to facilitate the establishment of a marine restoration culture in Europe, the EU-funded project MERCES (www.merces-project.eu) also focuses on coralligenous habitats. New methodologies to restore macroinvertebrate habitat-forming species from three key taxonomic groups as Cnidaria/Anthozoa, Porifera/Demospongiae, and Bryozoa are in the testing phase in Spain, Italy and Croatia, allowing for a strong validation of the different approaches at a regional level.

Material and methods

Work in the coralligenous focuses on colonial species. Restoration protocols combine transplants from donor organisms, using different techniques and recruitment-enhancing devices designed for habitat-forming species. Given the peculiar life-history traits of the chosen taxa (Porifera: *Spongia lamella*, *S. officinalis*, *Petrosia ficiformis*, *Chondrilla nucula*; Cnidaria: *Corallium rubrum*, *Paramuricea clavata*, *Eunicella cavolini*, *E. singularis*), their population dynamics and their genetics, restoration actions are based on small- to medium-size transplants collected from donor specimens.

As a first step, target species and donor populations are selected. The latter shall dwell in areas where environmental characteristics similar to those present where the transplanted population will be located.

Three techniques have been developed for sponges, as their structure can be hard, soft or collagenous. In the first method, fragments from sponges with a hard skeleton are directly glued to the substratum with a two-component epoxy putty (e.g. *P. ficiformis*). In the second method, used for sponges with a soft skeleton, a plastic dowel is inserted into the base of the fragments and then glued to the substratum (e.g. *Spongia* spp.). Dowels can be gently inserted into the fragment after it has been detached from the donor sponge or, alternatively, the dowel is inserted directly into the donor sponge until the sponge tissue overgrows it and a fragment can be cut off. In case of sponges with a collagenous structure, the fragments are laid on a horizontal and clean substratum and kept in place with a grid until natural attachment occurs (which usually requires around 2 weeks – one month) (e.g. *C. nucula*). Since most sponges are very sensitive to manipulation during transplantation, success heavily depends on minimizing sources of stress during these phases.

Five approaches have been tested for gorgonians. Transplants from gorgonian species with a rigid scleraxis or with large sclerites in the coenenchyme can be directly glued to the substratum with the epoxy putty. Indeed, such skeletal features increase the adhesion of the fragments into the putty itself (e.g. *C. rubrum* and *P. clavata*) (Linares et al., 2008; Montero-Serra et al., 2018).

For species displaying thin scleraxis (e.g. *E. cavolini*, *E. singularis*), however, it is recommended to reinforce the basal area of the fragments to ensure a better survival rate. Otherwise, when immersed in the epoxy putty, the coenenchyme will rapidly dissolve with the risk of triggering necrotic processes, leading to the weakening of the organic scleraxis and to the loss of the colony. To such an end, a silicon tube around the basis may be used (e.g. 1-2 cm of airline tubing for aquaria). When the gorgonian fragment is inserted into the plastic tube filled with the epoxy putty, it will be such a tube to be handled and inserted in the epoxy putty placed on the substrate. Another very simple

approach is to transplant Y-shaped branches, so that the putty can host the fragment and cover the bases of branches blocking them at the separation level.

Plastic grids with adult colonies and plastic panels with newly settled colonies have been also adopted for transplant and their effectiveness assessment is in progress.

The spatial arrangements of transplants may include relatively small patches (0.2-1 m in diameter) separated by distances similar to the sizes of the same transplant patches, to create functional reproductive units. The density within the transplant patches may be moderate to high (compared to naturally-occurring ones) in order to enhance the reproductive success and recruitment rates.

Results

Regarding sponges, the most delicate phases are the cutting and the attachment to the substrate, actions that can squeeze the sponge increasing the risk to lose cells. Sponges have not true tissues and cells are poorly bound, so compression can free cells from the fragment. The loss of cells will obviously compromise regeneration, triggering necrotic processes around the wound.

In case of sponge species living in symbiosis with autotrophic organisms, it is also important to consider light exposure when selecting the transplant area.

Tab. 1. Comparison among different methodologies applied to different organisms.

	Sponges			Gorgonians	
	soft skeleton	hard skeleton	collagenous	hard scleraxis	elastic scleraxis
dowel	✓	✓			
Raw		✓		✓	
plastic tube				✓	✓
plastic grid			✓	✓	
plastic panel				✓	
Y shape				✓	✓

We identified survival and growth of transplants as well as reproductive output and recruitment as the most suitable short-term indicators of the success of the restoration actions. The tested methods show different percentage of survival already after 1 month, while values tend to stabilize afterwards. Indeed, the inclination of the substrate and its exposure to currents are key aspects at the very inception of the restoration procedure. As the putty takes around two hours to harden properly, gravity and water movements may negatively affect the stability of transplants, eventually leading to their dislodgment. Based on our data, this is the primary cause of loss in the tests – albeit one of the easiest to avoid.

Discussion

Our results suggest that the restoration action in coralligenous habitats can be promoted by focusing on ecosystem engineer species. Ecosystem engineers have positive effects on other species, facilitating the recovery process of the whole damaged habitats. In this sense, massive and erect sponges contribute to building the architectural complexity of

coralligenous assemblages, play a central role in benthic-pelagic coupling and host a large number of associated species (including those spending the most delicate period of their life cycles sheltered inside the canals of the aquiferous system (Cerrano *et al.*, 2006). Gorgonians generate a wide and complex series of effects within coralligenous assemblages, including reduction of water movement (Scinto *et al.*, 2009) and light penetration (Ponti *et al.*, 2018), sediment resuspension (Valisano *et al.*, 2016), and on the associated fauna (Ponti *et al.*, 2014; 2016; Valisano *et al.*, 2016).

In general, the survival of transplants is relatively high and restoration actions focused on these species require low initial effort, nevertheless it is important to realize that a long period will be required to fully recover habitat complexity, i.e. decades (Montero-Serra *et al.*, 2018). The different percentage of success recorded in our experiments depend both on the specific structural features of the target species and on the chosen transplant technique, which in turn need to match the characteristics both of the selected species and of the sites. Moreover, it is important to check the specific ecological needs because inadequate environmental features (e.g. sites with too much or insufficient light, or poorly exposed to water current) could compromise the survival of the transplants.

Yet, the techniques developed within the MERCES project are not particularly complex and, if adequately taught by trained professionals (researchers, technicians), can offer the opportunity to involve volunteers in field activities. Collaboration with volunteers (divers and diving operators) in the various phases of field work is a cost-effective approach to scale up the restoration actions, and to increase the sense of stewardship in a major users' segment.

Acknowledgements

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COMMUNITY ECOLOGY OF CORALLIGENOUS ASSEMBLAGES USING A METABARCODING APPROACH

Abstract

Coralligenous habitats are bioconstructed, emblematic habitats of the Mediterranean Sea, which display a remarkably complex tridimensional structure and are considered as one of the most important biodiversity hotspots of the Mediterranean Sea. In order to assess the specific diversity of these habitats we sampled small surfaces (10 cm²) of these habitats using a suction sampler in different environmental conditions on 19 sites in the Marseilles area (France). These samples were crushed and total DNA was extracted. A small portion of the COI gene was amplified using universal primers targeting metazoans. A total of 3029 Molecular Operational Taxonomic Units (mOTUs) were found among all samples. Most of the mOTUs belong to three taxonomic groups: Rhodophyta, Arthropoda and Annelida but many other phyla were detected. Environmental factors such as depth, slope and locality influence the community composition of coralligenous habitats. This first assessment of the specific diversity of these habitats using a metabarcoding approach confirms the status of biodiversity hotspot of these habitats and underlines the importance of abiotic variables to structure the community. Methodological developments can be used to design new monitoring protocols of these habitats.

Key-words: coralligenous, metabarcoding, community ecology, COI

Introduction

Coralligenous habitats are bioconstructed, emblematic habitats of the Mediterranean Sea, which present a remarkably complex tridimensional structure resulting of the permanent dynamics between bioconstruction and bioerosion. The main builders of these habitats are coralline red algae but other marine invertebrates such as bryozoans directly contribute to the frameworks of the habitats as they build their own calcareous skeletons. This highly complex framework represents a habitat for around 1600 species (Ballesteros 2006) and is thus considered as one of the most important biodiversity hotspots of the Mediterranean Sea. It provides many ecosystem services such as: (i) recreational (coralligenous habitats are the favorite spot of divers in the Mediterranean Sea), (ii) food provision (several species of high commercial values live or feed on these habitats), (iii) jewelry, as it is the habitat where the red coral is harvested. These habitats are threatened by human activities, in particular fishing activities and global change. Mechanical degradation due to anchoring, divers' fins or nets of fisheries are responsible for a direct destructing of the habitats. Many organisms of these habitats are affected by global warming especially gorgonians, which undergo mass mortality events correlated with positive thermal anomalies of the seawater. Combination of acidification and warming has been shown to have a deleterious impact on calcareous organisms of the habitats such as calcareous red algae or bryozoans. The increasing amount of organic matter due to human activities, invasive species and overfishing are also impacting the composition of

coralligenous communities. Moreover, as coralligenous habitats are found quite deep in the sea, they are poorly studied and our lack of knowledge is probably the major constraint to the setup of efficient protection strategies. Until now the assessment of species richness and monitoring of coralligenous habitats has been mainly conducted using conventional approaches especially direct assessment by scuba diving or based on photographic surveys. These methods can be inefficient in detecting particular taxonomic groups or very small organisms in particular in the very complex framework of coralligenous habitats. Moreover, they rely on a very good taxonomic expertise, which make them difficult to apply for monitoring purposes (Weeler *et al.*, 2004). Metabarcoding is a fast, powerful, potentially cost effective molecular approach to study species diversity (Dejean *et al.*, 2012), and has not yet been used to study coralligenous habitats.

Materials and methods

240 samples from 19 localities of the bay of Marseilles were collected by scuba diving using a suction sampler and a hammer to extract a 10 cm² of coralligenous habitats. Depth of sampling and topographic data such as slope and exposition were recorded during sampling. Samples were collected at 20, 30 and 40 meters depth, on vertical walls as well as flat bottoms. A site is defined by its location, depth, slope and exposition. On each site 4 samples placed at a 1 meter radius circle were collected. The samples were weighted, examined and a quick counting of the different easily recognizable taxa (Figure 2) was done before crushing them with a blender. DNA extractions, PCR amplification of a 313 bp fragment of the COI and of the 28S gene were conducted. Sequencing was completed on an Illumina Miseq sequencer (in a 2x250 bp Paired End run). After bioinformatic processing and Molecular Operational Taxonomic Units (mOTUs) clustering with the SWARM algorithm (Mahé *et al.*, 2014; 2015), a mOTU abundance table was obtained and filtered according to sequence quality, length, chimera detection, taxonomic affiliation and comparison of abundances with negative control abundances (for each mOTU). Community ecology analyses (e.g. PERMANOVA) were realized with the statistical software PRIMER (Anderson *et al.*, 2008; Clarke *et al.*, 2014; 2015) using the Sorensen distance.

Results

We found a total of 3029 mOTUs in 59 sampling sites on Marseilles area (Tab. 1). Among those mOTUs 13,4 % very unidentified, 63 % were assigned to animal organisms and 23 % to algae. Most mOTUS belonged to three taxonomic groups: Arthropoda (19,5%), Rhodophyta (18,7%), Annelida (14,5%). Other taxonomic groups such as Bryozoans, Cnidarians, Mollusca and Porifera represented a fair proportion of the mOTUS. The total number of mOTUs on each site varied from 151 to 678 (Fig. 1). Environmental factors influenced the community composition of our samples (Tab. 2): the locality, the depth and the slope had a significant effect on assemblage composition.

Tab 1: Number of mOTUs assigned to the different taxonomic groups

Taxonomic group	Number of mOTUs
Annelida	438
Arthropoda	591
Bryozoa	171
Chloroplastida	3
Chordata	32
Cnidaria	172
Echinodermata	75
Fungi	14
Mollusca	150
Other animals	52
Other eukaryotes	6
Porifera	233
Rhodophyta	567
Stramenopiles	120
Unidentified	405
All	3029

Tab. 2: PERMANOVA table for the model including the environmental factors: locality, slope, depth and exposition.

	Df	SS	MS	Pseudo F	P(perm)	Unique Perms
Locality	17	55292	3252	1,52	0,0001	9503
Slope	1	4033	4033	1,89	0,0015	9858
Depth	2	7146	3572	1,67	0,0011	9867
Exposition	2	4848	2424	1,13	0,1991	9828
Res	29	61972	2137			
Total	51	1.42E05				

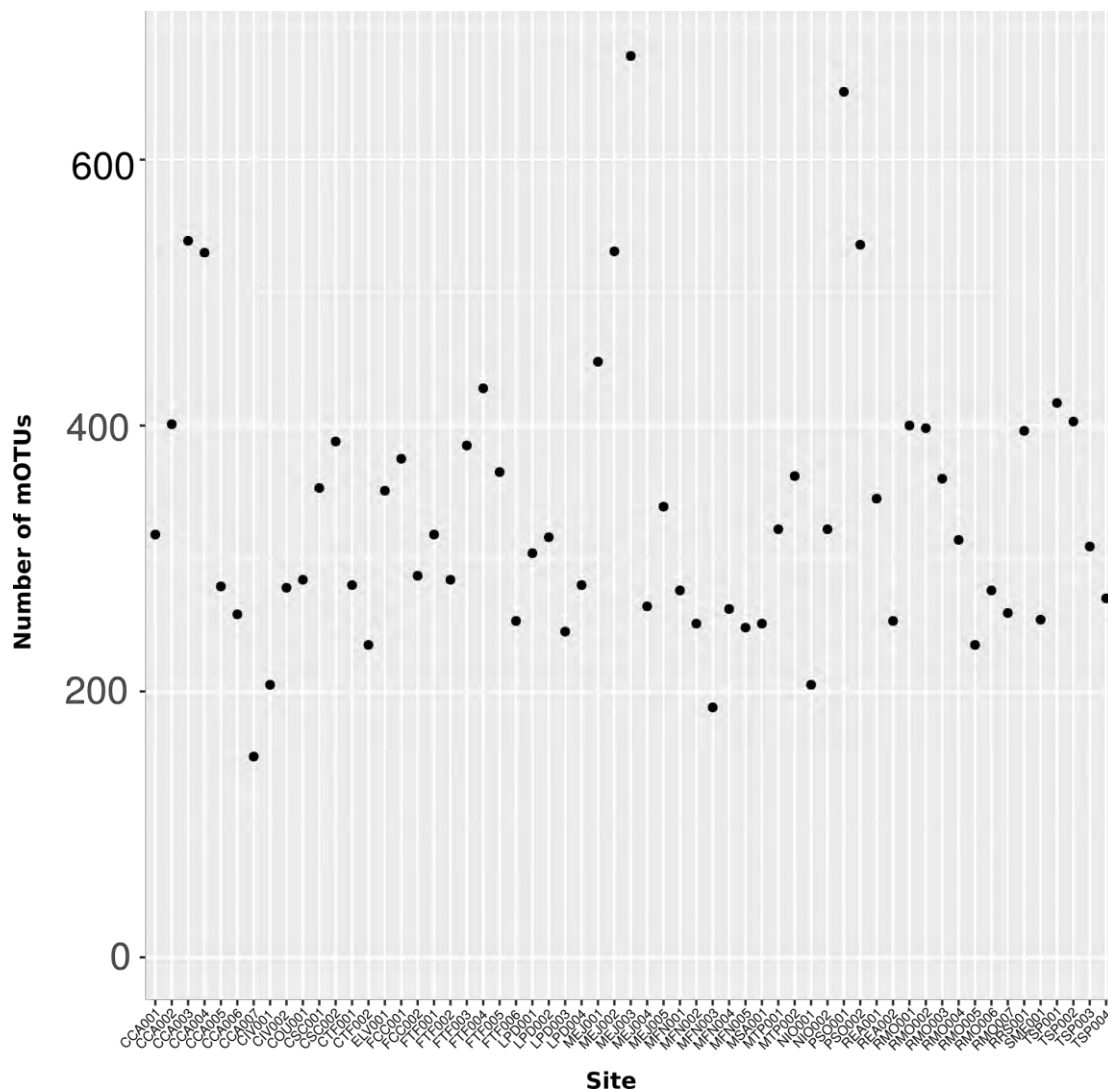


Fig. 1: Number of mOTUs in each of the 59 sampling sites from the Marseille Area (France)

Discussion and conclusions

The high number of mOTUs found in this study strengthens the status of biodiversity hotspot of coralligenous habitats and suggests that an important part of the species occurring in coralligenous outcrops are not described yet.

The strong influence of environmental variables on the assemblage composition points the importance of selective mechanisms in shaping the specific biodiversity structure of these habitats. Light, temperature and sediment deposition were considered (prior to our study) the main environmental factors influencing the composition of coralligenous assemblages (Balata *et al.*, 2005; Ballesteros, 2006). In our study, depth and slope influence the quantity and quality of light received by surface area, and slope also influences sediment deposition. The effect of the locality factor can be due to a combination of factors as localities differ from each other in their distance from the coast, their protection status or the importance organic matter input from the continent.

This type of approach could be implemented to monitor these habitats because of its power of detection. In fact, this approach allows the detection of cryptic and hidden species in the complex framework of coralligenous habitats and is less time-consuming and costly than traditional morphological identification (Aylagas *et al.*, 2018). The

increased resolution obtained by using this approach allowed us to detect the effect of environmental factors such as depth that was not detected in a study using a photographic approach in the same area (Doxa *et al.*, 2016). Improvements of the first protocol, completion of sequences databases and the constant decrease of sequencing costs make this approach a very promising method to monitor coralligenous habitats.

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A MASS MORTALITY EVENT OF THE SPONGE *CLIONA VIRIDIS* IN THE CORALLIGENOUS OUTCROPS IN THE CÔTE AGATHOISE MPA (GULF OF LION, NORTHWESTERN MEDITERRANEAN)

Abstract

*In 2014, a mass mortality event (MME) affecting the sponge *Cliona viridis* was observed in the Côte agathoise Marine Protected Area in coralligenous outcrops dwelling at 20 m depth.*

*Previous to the necrosis, the specimens displayed a discoloration process, from green to pale yellow. Then, as necrosis progressed with the appearance of a bluish bacterial veil the internal sponge structures were exposed. At the last stage, the sponge has completely disappeared and erosion canals were visible. To monitor coralligenous outcrops, semi-permanent transects were setup in 2010 in 3 sites within the MPA. The transects were monitored using photoquadrats. Four Surveys were carried between 2010 and 2017. In November 2014, a mass mortality in *Cliona viridis* populations was observed in one site. The mortality (partial or total necrosis) affected 90% of surveyed specimens. In November 2017, the affected population showed only a partial recovery of specimens. The causes of the mortality are unknown. Unfortunately, neither necrotic tissue samples to conduct microbial analysis were available, nor the water samples to analyze potential sources of pollution. However, the mass mortality was concomitant with the occurrence of a positive thermal anomaly (at least 2 weeks > 21°C, at 20 m depth). As in other events observed in the North-Western Mediterranean, temperature conditions can be considered as a triggering factor of the observed mortality.*

Key-words: Coralligenous, Mass mortality, *Cliona viridis*, thermal anomalies.

Introduction

Abundant in the infralittoral and circalittoral area of the western Mediterranean (Rosell & Uriz 1991), and considered a bioindicator species (Carballo *et al.*, 1996, Bary *et al.*, 2016), *Cliona viridis* is one of the most abundant sponge species in the coralligenous habitat (Uriz *et al.*, 1992). Sponges as sessile benthic organisms are exposed to environmental conditions and therefore are good candidates to evaluate the effects related to stressors such as pollution, sedimentation and temperature extrem events.

In the NW Mediterranean, the mass mortality events of 1999 and 2003 (Cerrano *et al.*, 1999 Garrabou *et al.*, 2003) affected a wide variety of fixed organisms (anthozoa, sponges, bivalves and ascidians). Other mortality events specifically affecting sponges have also been reported in the Mediterranean (e.g. Di Camillo & Cerrano, 2015).

Since 2010, a coralligenous benthic communities monitoring has been implemented in the Côte Agathoise Marine Protected Area (CAMPA) (Blouet *et al.*, 2011, 2012, 2013). The main goal of this monitoring program is to track changes affecting coralligenous benthic sessile organisms and, if necessary, to implement management and recovery measures to maintain the good conservation status of the habitat.

During the 2014 surveys an unprecedented massive mortality of *Cliona viridis* specimens was observed. The purpose of this paper is to provide a first report of the *Cliona viridis* mortality phenomenon. Through the temporal comparison (2010 to 2017) of the

photoquadrats, we were able to provide quantitative data on the incidence of the mortality and recovery of the affected population. Finally, some insights on potential factors behind the observed mortality were addressed.

Materials and methods

To monitor the benthic communities of the CAMPA coralligenous, series of underwater photographs were carried out along two semi-permanent transects (11-15 meters in length) setup around 20 m depth in 3 sites within the Côte Agathoise Marine Protected Area (NW Mediterranean) (CAMPA) (Fig 1). The transects were sampled in 2010, 2013, 2014 and 2017. To carry out the sampling, a full frame Nikon™ D3X camera with a Nikon 18mm f/2.8 AF-D lens and electronic strobes (mounted on a photoquadrat framer) were used to photograph 0.25 m² quadrats. For each sampling period a total of more 30 photographs were obtained (7–9 m² in surface) in each site.

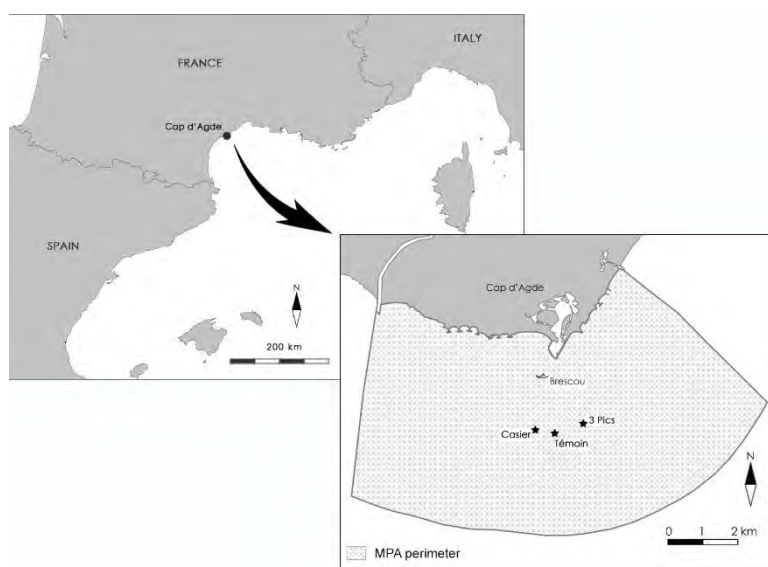


Fig.1 : Map displaying the study area and the protected surface of the Côte Agathoise Marine Protected Area (NW Mediterranean).

The processed images were analyzed using Seascap software to estimate the recovery of each individual. This semi-automatic underwater image analysis software has proven to be particularly effective in quantifying the recovery of benthic organisms by providing precise surfaces (Teixido *et al.*, 2011).

The photoquadrats analysis has been carried out for the years 2010, 2013, 2014 and 2017. Like in the Cebrian *et al.* (2011) study, specimens displaying signs of necrosis were considered as “affected” and those showing 100% of necrosis were considered as “dead” individuals.

Temperature was recorded *in situ* by HOBO v2 autonomous temperature data loggers setup at the study sites. The temperature was recorded at 1 h intervals providing high resolution temporal data series. The temperature data loggers were fixed along a rope with a data logger every 10 meters between -30 m and -10 m depth and deployed and recovered by divers during the monitoring surveys on annual basis (for more information see wee.t-mednet.org).

Results

The mortality of *Cliona viridis* was observed on a platform coralligenous area developing at 20 m depth at the site Temoin (Fig 1). In the other sites no control diving could be done in 2014.

The photographic monitoring allowed to follow the *Cliona viridis* specimens degeneration process (Fig. 2). Previous to the necrosis the specimens displayed a discoloration process, from green to pale yellow (Fig 2 A-B). Then, as necrosis progressed with the appearance of a bluish bacterial veil, the internal sponge structures were exposed (Fig. 2 C). At the last stage, the sponge completely disappeared and erosion canals were visible (Fig. 2 D).

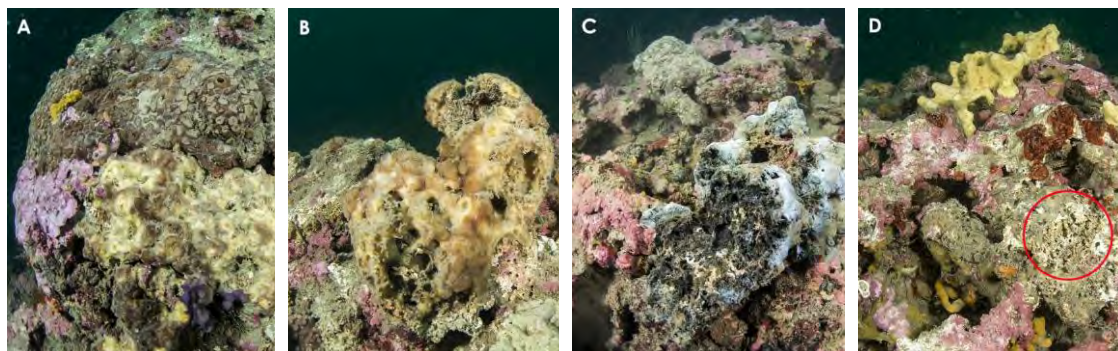


Fig.2 : *Cliona viridis* photographic mortality proces observed in November 2014 in Côte Agathoise Marine Protected Area (NW Mediterranean).

In November 2014, 71.4% of surveyed *Cliona viridis* specimens were dead individuals and 19% specimens display signs of necrosis. Data analysis shows that the percentage of healthy *Cliona viridis* specimens per quadrat decreased from 100% for the years 2010 and 2013, to 9.5% for the year 2014 (Fig. 3).

In 2017, the percentage of dead individuals increased by more than 2.5%, a consequence of the evolution of necrosis in 2014. The number of healthy individuals also increased by more than 13.5%. This result reflects the high level of recruitment or high retention in the site.

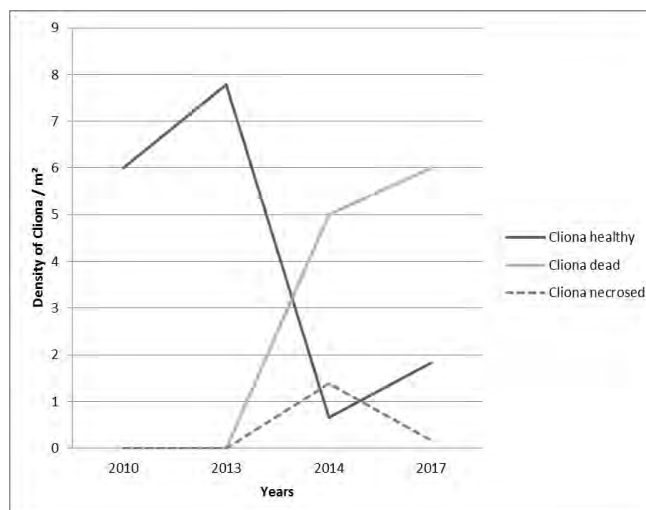


Fig. 3: Density of healthy, necrosed and dead *Cliona viridis* specimens per m² between 2010 and 2017 at the Témoin site within the in Côte Agathoise Marine Protected Area (NW Mediterranean).

In 2017, coralligenous benthic communities have been monitored in 2 nearby sites ("Casier" and "3 Pics"), respectively located at 800 m and 1 km from the Témoin study site. On these 2 sites, the number of dead and affected specimens found in 2017 indicated that the 2014 mortality event was indeed affecting the entire coralligenous habitat within the marine protected area (Fig. 4).

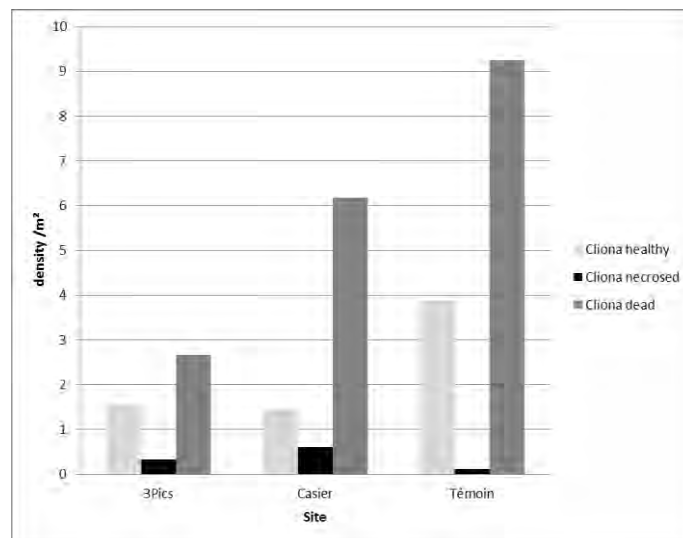


Fig. 4: Density of healthy, necrosed and dead *Cliona viridis* specimens per m² in 2017 at the “3Pics”, “Casier” and Témoin sites within the in Côte Agathoise Marine Protected Area (NW Mediterranean).

The temperature monitoring of the MPA marine waters, near the study site (using HOBO v2 temperature data loggers) showed a temperature increase in the supra-thermocline above the 21 °C threshold, for 14 days in September 2014. Since 2010, this thermal anomaly is unique (Fig. 5).

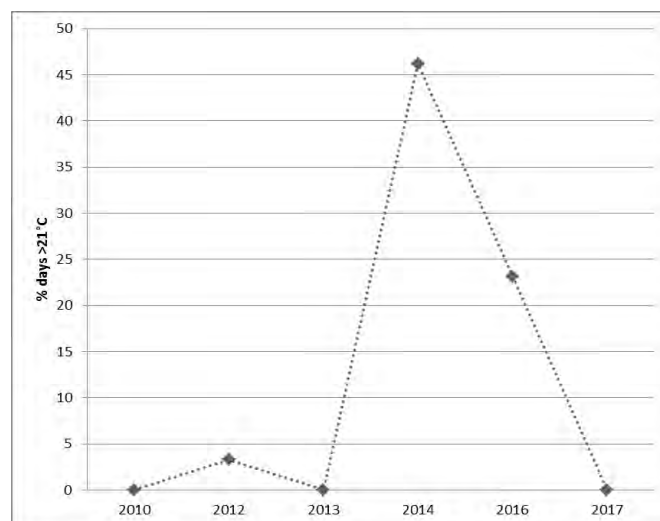


Fig. 5: Percentage of days >21°C in Septembre over the 2010-2018 period at 20 m depth within Côte Agathoise Marine Protected Area (NW Mediterranean).

This abnormal 21 °C threshold overrun, at 20 m depth, during several weeks, may have been a stress factor for *Cliona viridis*.

Discussion and conclusion :

Global warming has been clearly identified as a cause of thermal stress affecting a wide range of organisms, both directly and indirectly resulting in massive mortality of organisms (Jones *et al.*, 2004, Hughes *et al.*, 2018). In the Mediterranean Sea, large scale massive mortality events have affected more than 30 species from 5 different phyla

(Garrahou *et al.*, 2009). Focusing in sponges, mass mortalities affected different Mediterranean species such as *Ircinia variabilis* (Stabili *et al.*, 2012; Di Camillo *et al.*, 2012), *Sarcotragus fasciculatus* (Cebrian *et al.*, 2011; Riesgo *et al.*, 2016), *Chondrosia reniformis* (Di Camillo & Cerrano, 2015), *Sarcotragus spinosulus* (Di Camillo *et al.*, 2012), *Spongia officinalis* (Gaino & Pronzato, 1989; Gaino *et al.*, 1992; Di Camillo *et al.*, 2012;), *Hippospongia spp.* (Gaino *et al.*, 1992). In most of these events, anomalous high temperature conditions were reported prior of each event.

The mass mortality event affecting reported in this study affected exclusively the *Cliona viridis* and was the first time reported in the Mediterranean. Nevertheless, previous evidences indicate recent bleaching episodes of specific Clionid species in the Florida Keys (Caribbean) which were directly related to positive thermal anomaly (Hill *et al.*, 2016). In contrast, Marlow *et al.*, (2018) reported a *Cliona aff. viridis* bleaching event in Indonesia, coinciding with a 0.8 °C drop in water temperature.

In the absence of samples from affected *Cliona* individuals, ultra-structural analysis could not be conducted to determine if the rate of symbionts had decreased, which could have partly explained the tissues depigmentation and degeneration reported. The fact that the mortality episode only affected *Cliona viridis*, suggests that its specific symbionts may have played a central role in the phenomenon.

Although the factors triggering this mortality event on *Cliona viridis* could not be clearly identified, heat stress conditions are the most likely factor. During the summer of 2014, the high number of windless days and the low occurrence and intensity of NW sector winds resulted in a thermal anomaly. Similar conditions were also observed during a previous mass mortality event recorded in 1999 affecting the Gulf of Marseille (France) (Romero *et al.*, 2000).

This first report on mass mortality *Cliona viridis* species in the NW Mediterranean enlarge our understanding of the global warming phenomenon and the changes it might cause within the marine ecosystems

To face warming impacts within the marine protected area, the CAMPA management plan is implementing a marine citizen science initiative with local dive centers to support the observation of early signs of necrosis of species and the assessment of mortality events. These observations will be precious in order to plan more specific surveys and to provide a more comprehensive view on the impact of these mortalities. In the current context of warming, these mortalities are expected to affect recurrently shallow Mediterranean bottoms and gaining information on these impacts is key to address management and conservation measures.

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DEMOGRAPHY AND DISTURBANCE LEVELS OF THE CORAL *ASTROIDES CALYULARIS* (PALLAS, 1766) IN THE TUNISIAN MARINE PROTECTED AREA OF ZEMBRA (CENTRAL MEDITERRANEAN)

Abstract

Mediterranean coral species are increasingly threatened by human activities, particularly global warming. In fact, Astroides calyularis populations are the focus of conservation efforts (listed in the Bern and Barcelona Conventions and the Convention on International Trade in Endangered Species of Wild Flora and Fauna). Demographic studies on coral species are able to provide information on the conservation status of populations. Astroides calyularis is one of the coral contributing to build coralligenous outcrops. Data regarding the demography and the state of conservation of this species is poorly studied in Tunisian coasts. In order to fill this gap scuba diving surveys were carried out between 2015 and 2016 in two sites of the MPA of Zembra. A total of 1109 colonies were surveyed randomly using photo-quadrats. Density of colonies, number of polyps and extent of injury per colony were assessed. For both sites, the upper distribution limit was at 10 - 15 m depth. Mean population density per m² was 369.03 ± 10.7 SD while the mean number of polyps per colony was 18.9 ± 21.9 SD in Capo Grosso and 20.2 ± 24.0 SD in Lamparo site. The size structure of populations was dominated by recruits and juvenile colonies (from 1 to 10 polyps). Colonies affected by injuries was very low, less than 1% of all surveyed colonies in both populations. Most colonies showed signs of partial mortality due to recent necrosis of polyps. No total mortality was observed. The results obtained in this work provide a robust baseline to evaluate future changes of populations in the MPA of Zembra and to compare the conservation status at Mediterranean scale.

Key-words: Coralligenous, Scleractinia, Climate change, Necrosis, Mediterranean Sea.

Introduction

Several studies have been carried out on the demography of Mediterranean anthozoans species contributing to set the conservation status of these species (Linares *et al.*, 2008). The Mediterranean Sea is considered as a hotspot of marine biodiversity threatened by multiple anthropogenic disturbances such as pollution, overfishing and invasive species (Coll *et al.*, 2010, Templado 2014, Cramer *et al.*, 2018). Climate change has exacerbated the degradation of ecosystems and biodiversity, and its impacts have mainly affected sessile benthic species (Garrabou *et al.*, 2009, Cramer *et al.*, 2018).

Regression of the biogeography of the populations of the anthozoan *Astroides calyularis* in the Western Mediterranean (Zibrowius 1995) and its cantonnement in the south-central part (Zibrowius, 1995) and in the north-east of the Adriatic (Goffredo *et al.*, 2010) has led to its inclusion in the list of species protected by the Bern and Barcelona Conventions and the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES). Due the scarcity of population ecology studies on *A. calyularis*, especially in the southern coast of the Mediterranean, it is difficult to assess the

conservation status of this species. Therefore, this study was designed to build robust baselines able to inform on the conservation status of the populations in Tunisian waters.

Materials and methods

Scuba diving surveys were carried out between 2015 and 2016 in two sites of the Marine Protected Area of Zembra (Tab. 1). Colonies density, number of polyps per colony, proportion of injured surface and type of injury were chosen as the main population descriptors. Measurements were taken using 25×25 cm² photoquadrats haphazardly placed within the *Astroides calycularis* populations. Density was determined based on the number of colonies present within 25×25 cm² quadrats, averaged and recalculated for 1 m² surface. For each colony found, the number of polyps was counted. Two descriptors were used to assess the impact of potential disturbances: Extent of injury per colony and type of injury. Extent of injury was estimated as the proportion of the colony's total surface with denuded polyps and/or that was overgrown by other organisms. Based on the presence/ absence of epibionts, three types of injury were identified; Type A: denuded polyps, indicating a recent tissue necrosis; Type B: colony overgrowth by pioneer species (e.g. hydrozoans); Type C: presence of multiples species and/or slow growing epibionts species indicating an old injury. Overall, a total of 1109 colonies were assessed. Colonies with less than 10% of injured surface were considered as healthy, colonies with injuries >10% of total surface were classified as affected, whereas 100% of injury corresponded to dead colonies. The number of the polyps in each colony were grouped into four size-classes: 1–10 (recruits and juveniles), 11–20 (small-sized colonies), 21–50 (medium sized colonies) and >50 cm (large colonies). To assess the population structure, different descriptors were used: mean size, skewness (g1) and kurtosis (g2). Coefficients of g1 and g2 were considered significant if the ratio to their standard error was >2.

Tab. 1. Geographical position and characteristics of the studied sites in Tunisia.

Locality	Site	GPS Coordinates	Depth range	Habitat	Quadrats number
Zembra	Lamparo	37°08'441"N 010°47'736"E	5 – 15 m	Wall	22
	Capo grosso	37°08'200"N 010°48'160"E	0 – 15 m	Wall	26

Results

In Zembra, the mean density is 369 colonies·m⁻² with a minimum density of 160 colonies per m². Results suggest that no significant variability in density exists among sites ($p < 0.05$).

The mean ($\pm$ SD) number of polyps per colony is 18.91 $\pm$ 21.85 for Capo Grosso and 20.17 $\pm$ 23.98 for the Lamparo site. Polyp number of *Astroides calycularis* populations appears to be relatively symmetrical for the populations at the two studied sites. Kurtosis is positive for all sites suggesting a predominance of one or the other of the two smaller size classes (1-10 and 11-20 polyps/colony). The small sized colonies between 1 and 10 polyps were the most abundant in both sites indicating the dominance of recruits and juveniles (49.5% and 46.47% in the Capo Grosso site and Lamparo, respectively). The maximum size was of 156 and 196 for the two respective sites and a minimum of 1 polyp per colony (Tab. 2).

Tab. 2. Population size structure of *Astroides calycularis* populations at Zembra National Park (Tunisia)

Locality	Site	Number of colonies	Density mean/m ²	SD	Number of polyps per colony					
					Mean	SD	Max	Min	Skewness	Kurtosis
Zembra	Capo Grosso	612	376.6	106.3	18.9	21.8	156	1	23.3097	36.491
	Lamparo	497	361.5	106.2	20.2	24.0	196	1	26.0304	55.291

In the Zembra National Park, 0.3% of the 612 and 0.4% of the 497 colonies observed were affected in Capo Grosso and Lamparo respectively. All studied populations have an average lesion extent of less than 10%. The majority of affected colonies have type A injuries (recent necrosis) (Tab. 3).

Tab. 3. Degree of injury of *Astroides calycularis* populations at Zembra National Park (Tunisia)

Locality	Site	Depth (m)	N	Extent of colony injury (%)		Proportion of uninjured, injured and dead colonies			Proportion of Colonies per type of injury		
				Mean	SD	< 10%	≥10% - ≤99%	100 %	A	B	C
Zembra	Capo Grosso	5-10	612	0.3	3.1	98.4	1.60	0	1.96	0.16	0
	Lamparo	5-10	497	0.4	4.5	99.2	0.8	0	0.6	0.4	0

Discussion and conclusions

Astroides calycularis is poorly studied in the Mediterranean Sea. Most studies have focused on polyp biometrics and reproduction (Goffredo *et al.*, 2010). Similarly, Casado-Amezua *et al.*, 2012 and 2013, focused first on the genetic population structure in the Alboran Sea populations and then on the morphological study of the species at two Mediterranean sites. Associated macrofauna as well as geographical distribution and % recovery in Alboran Sea were analysed by Terron Sigler *et al.* (2015). The average densities of the species observed in this study are about 360 colonies per m². These values are similar to those found in the Alboran Sea with a recovery rate of up to 75% (Terron-Sigler, 2015). There is a dominance of recruits and juveniles (a number of polyps between 1 and 10 per colony). This could be explained by a high recruitment rate. The high frequency of small colonies could be attributed to sedimentation in studied sites. In fact, Zembra is located in the Gulf of Tunis, whose sedimentation is under the influence of the important inputs of Wadis. Coarse sediments remain confined to the Gulf while the finer can reach Zembra with existing traffic in this area, influenced by external currents (Azouz 1973). Indeed, according to Nugues and Roberts (2003), this parameter is considered as one of the main stressors for coral reef formations by affecting coral development and diversity, colony size and recruitment (Babcock & Smith, 2000). This factor can also influence population size structures by acting on their maintenance and recovery potentials. Large colonies that are less vulnerable may be reduced in size by partial colony mortality due to high sedimentation (Goffredo & Chadwick-Furman, 2003). Climate change has been identified as a major threat to corals

that are unable to adapt their physiology to environmental disturbances. The current global warming may have severe impacts in local populations of multiple macroinvertebrate species belonging to different groups such as cnidarians, sponges and mollusks (Garrahou *et al.*, 2009). The thermal stress can also have important effects on the reproduction output of affected species as was demonstrated for the red gorgonian *Paramuricea clavata* affected by mass mortality (Linares *et al.*, 2008). Indeed, Cerrano *et al.* (2000), Rodolfo-Metalpa *et al.* (2000) reported necrosis and bleaching of scleractinian corals including *Cladocora caespitosa* and *Balanophyllia europaea*. However, studies of the effects of water warming on the species *Astroides calycularis* are missing. A factor of considerable importance must be taken into account is that of the effect of ocean acidification, which will probably affect considerably scleractinian corals (Veron *et al.*, 2009). Indeed, acidification is an unfavorable factor for the growth of these species by reducing the rate of calcification. Experimental studies assessing the role of different environmental parameters (temperature, pH, water flow) must be taken into account in future studies to better understand how expected environmental changes will affect the *Astroides calycularis* and the habitats that it constitutes. The results obtained in this work provide a robust baseline to evaluate future changes of *A. calycularis* populations in the MPA Zembra and to compare the conservation status at a Mediterranean scale. The patterns perceived offer insights as to how biotic, abiotic, and anthropogenic factors may affect the structure and dynamics of populations for this species, and consequently impact their aptitude to environmental changes. The implementation of similar methods to survey diverse species would provide reliable information which would allow develop effective management measures for the conservation of coralligenous assemblages, a key habitat in the Mediterranean basin.

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ACTIVE RESTORATION ACROSS MARINE COASTAL HABITATS: A FOCUS ON THE MEDITERRANEAN SEA

Abstract

Active restoration is considered a profitable strategy to return ecosystems to their pre-disturbance state, in a reasonable time frame. However, ecological restoration of marine ecosystems is still in its infancy if compared to the terrestrial context. A review of 498 studies published in the last 25 years was carried out within the MERCES H2020 EU project to understand the effects of marine restoration actions across coastal habitats at global scale. Here, an overview focused on the Mediterranean Sea was carried out to identify both contextual and methodological determinants of restoration success in the basin. Results show that restoration efforts across habitats are increasing, especially in seagrasses and saltmarshes, but never approached at ecosystem level. Targets, methods, response variables and standards are still very heterogeneous. Short project duration (one-two years), small restoration areas (< 1 ha), lack of controls and knowledge of baselines are still a limit for deriving generalities. Finally, restorations rarely consider future challenges linked to global change, thus impairing long-term success stories. Marine restoration is a promising approach to counteract habitat loss in coastal areas. However, restoration science needs more robust approaches leading to the development of best practices (e.g. protocols, monitoring of the effects, reasons for failure) to be applied at spatial and temporal scales so as to answer to present and future disturbance regimes.

Key-words: Restoration ecology, marine coastal habitats, review, conservation policies.

Introduction

Despite crucial advances in environmental legislation, the Mediterranean Sea is considered as ‘under siege’ since exposed to intense and increasing pressures from a variety of human activities. In this framework, success stories of recovery are mostly linked to the presence of Marine Protected Areas (Lotze et al., 2011; Franchetti et al., in press) and mitigation strategies on the effects of human activities are probably not enough to reverse present trajectories of changes.

Active restoration (defined as management actions and techniques that aim to enhance recovery) is considered one of the most profitable strategies to assist the recovery of damaged, degraded or destroyed ecosystems in a reasonable time frame (Wilson, 1992; Dobson et al., 1997; Suding, 2011; Hobbs, 2004), with the Convention on Biological Diversity and European member states, agreeing upon targets to restore at least 15% of degraded ecosystems by 2020 (Aichi Target 15; CBD, 2014; Target 2, EU, 2011).

Whilst there is a clear impetus to restore degraded areas, the success of restoration actions depends upon the potential to effectively implement them. It represents a challenge with a number of practical, political, economic and societal questions remaining unanswered (Aronson & Alexander, 2013; Ockendon et al., 2018). This is particularly true in marine ecosystems, where restoration is still in its relative infancy compared to terrestrial systems (Blignaut et al., 2013), in part due to the intrinsic difficulty and elevated costs of working in the marine environment (Bayraktarov et al., 2016). As a result, several gaps still remain to improve the efficiency and success rates of restoration (e.g., Elliott et al., 2007; Suding, 2011; Duarte et al., 2015). Here, capitalizing on a global review carried out by the project MERCES (Marine Ecosystems Restoration in Changing European Seas, <http://www.merces-project.eu>) consortium (Papadopoulou et al., 2018), we provide a preliminary overview of the restoration efforts carried out in the Mediterranean Sea. The aim of the study is to provide specific recommendations aimed at setting restoration priorities improving the strategies addressing the biodiversity changes urgent conservation issues faced by marine ecosystems in the basin, as well as in other parts of the world.

Materials and methods

A systematic literature review was used to identify restoration actions in coastal (i.e. strictly marine and semi-terrestrial) systems at global scale. Totally 31 terms indicating habitats/ecosystems, in combination with the terms Restor* OR Rehab*, has been used in our search. From a total of 4066 search results, 498 articles were considered eligible since strictly related to active restoration interventions (according to Elliott et al., 2007). From the total eligible articles, 36 have been carried out in Mediterranean Sea (Fig. 1). From each record a suite of information was extracted (see Tab. 1). The success of the restoration action was inferred from the study and classified as failure, partial success or success. Furthermore, in order to provide more contextual information, we downloaded the public version of the World Database on Protected Areas (<http://www.protectedplanet.net/>). ArcGIS was employed to explore the possible overlap of the location where the restoration action was carried out with the presence of any protection measure (e.g. sanctuaries, parks, marine reserves, etc.). A similar approach was used to determine a gradient of human impact (i.e. cumulative impact score, according to Halpern et al., 2008) across the restoration actions.

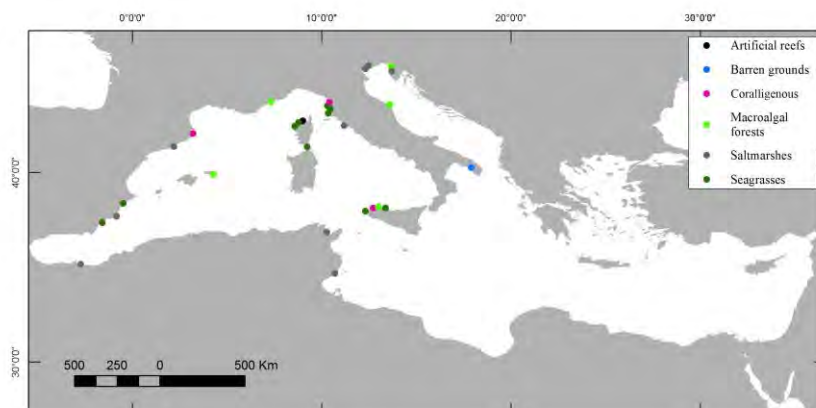


Fig. 1: geographical distribution of the studies on active restoration carried out across habitats in the Mediterranean Sea.

Tab. 1: summary of the studies of active restoration carried out in the Mediterranean Sea (extracted from the global analysis carried out by the MERCES consortium [Papadopoulou *et al.*, 2018]).

Contextual information			Methodology information						Restoration outcome (d)	Reference
Habitat	Protection (a)	Impact (b)	Type of restoration action (c)	Technique	Scale	Duration	Target species /assemblage	Response variables		
Artificial reefs	Y	L	Habitat enhancement	Artificial reefs or structures	NA	2-4 years	Assemblage	Community structure	NA	Schuhmacher & Schillak (1994)
Barren grounds	Y	M	Rehabilitation	Biological stressor removal, Physical/Hydrological setting	100's-1000's m ²	6-12 months	Assemblage	Community structure	S	Guarnieri <i>et al.</i> (2014)
Coralligenous	Y	M	Habitat enhancement	Transplanting	10-100's ha	2-4 years	<i>P. clavata</i>	Survival Growth	S	Linares <i>et al.</i> (2008)
	Y	MH	Habitat enhancement	Artificial reefs or structures	< 1 m ²	6-12 months	<i>C. rubrum</i>	Recruitment	PS	Benedetti <i>et al.</i> (2011)
	N	L	Re-introduction	Transplanting	< 1 m ²	6-12 months	<i>A. calycularis</i>	Survival	S	Musco <i>et al.</i> (2017)
Macroalgal forests	Y	M	Restoration	Transplanting	NA	6-12 months	<i>C. barbata</i>	Growth	S	Falace <i>et al.</i> (2006)
	Y	MH	Re-introduction	Transplanting	10's-100's m ²	1-6 months	<i>C. amentacea</i>	Survival	S	Susini <i>et al.</i> (2007)
	N	L	Creation	Artificial reefs or structures	100's-1000's m ²	10-16 years	<i>Cystoseira</i> spp. and <i>Sargassum</i> spp.	Biodiversity	S	Vega Fernández <i>et al.</i> (2009)
	Y	M	Restoration	Planting, Physical/Hydrological setting	1-10's ha	6-12 months	<i>C. barbata</i>	Size	F	Perkol-Finkel & Airoldi (2010)
	Y	M	Re-establishment	Transplanting	NA	6-12 months	<i>C. barbata</i>	Survival	PS	Sales <i>et al.</i> (2010)
	Y	M	Restoration	Planting, Physical/Hydrological setting	100's-1000's m ²	6-12 months	<i>C. barbata</i>	Survival	S	Perkol-Finkel <i>et al.</i> (2012)
	Y	M	Restoration	Planting, Physical/Hydrological setting	100's-1000's m ²	6-12 months	<i>C. barbata</i>	Survival	S	Perkol-Finkel <i>et al.</i> (2012)
Saltmarshes	N	M	Remediation	Biological stressor removal, Physical/Hydrological setting	1-10's ha	1-2 years	Assemblage	Community structure Distribution	PS	Lardicci <i>et al.</i> (2001)
	N	M	Remediation	Biological stressor removal, Physical/Hydrological setting	1-10's ha	6-12 months	Assemblage	Environmental variables Distribution Other	F	Lenzi <i>et al.</i> (2003)
	Y	M	Remediation	Physical/Hydrological setting	NA	6-12 months	Phytoplankton	Community structure	S	Kobbi-Rebai <i>et al.</i> (2012)
	N	M	Habitat enhancement	Physical/Hydrological setting	10-100's ha	2-4 years	<i>P. australis</i> , <i>S. maritimus</i> , <i>S. litoralis</i>	Biomass Environmental variables	PS	Calvo-Cubero <i>et al.</i> (2013)
	N	MH	Habitat enhancement	Physical/Hydrological setting	10-100's ha	16-30 years	Assemblage	Cover	NA	Scarton <i>et al.</i> (2013)a
	N	MH	Habitat enhancement	Physical/Hydrological setting	10-100's ha	16-30 years	Assemblage	Composition	NA	Scarton <i>et al.</i> (2013)b
	N	M	Restoration	Planting	100's-1000's m ²	1-6 months	<i>S. frutescens</i>	Environmental variables	F	Gonzales-Alcaraz <i>et al.</i> (2013)
	N	M	Creation	Physical/Hydrological setting	NA	10-16 years	Assemblage	Community structure	NA	Nordstroem <i>et al.</i> (2015)
	Y	M	Re-creation	Physical/Hydrological setting	1-10's ha	4-7 years	Assemblage	Community structure	S	Ivajišić <i>et al.</i> (2016)
	N	MH	Restoration	Physical/Hydrological setting	10-100's ha	10-16 years	None	Environmental variables	F	Abidi <i>et al.</i> (2018)
Seagrasses	Y	M	Re-establishment	Transplanting	10's meters	6-12 months	<i>P. oceanica</i>	Survival	PS	Meinesz <i>et al.</i> (1992)
	Y	L	Restoration	Transplanting	100's-1000's m ²	6-12 months	<i>P. oceanica</i>	Survival Growth	PS	Molenaar <i>et al.</i> (1993)
	Y	M	Habitat enhancement	Transplanting	NA	2-4 years	<i>P. oceanica</i>	Survival	S	Balestri <i>et al.</i> (1998)
	Y	M	Restoration	Transplanting	100's-1000's m ²	2-4 years	<i>P. oceanica</i>	Survival	S	Piazzi <i>et al.</i> (1998)
	Y	M	Restoration	Transplanting	kilometers	2-4 years	<i>P. oceanica</i>	Survival	S	Procaccini & Piazzi (2001)
	Y	M	Re-establishment	Transplanting	NA	1-6 months	<i>P. oceanica</i>	Survival	S	Vangeluwe <i>et al.</i> (2004)
	N	M	Creation	Transplanting	10's-100's m ²	2-4 years	<i>P. oceanica</i>	Density	F	Sánchez-Lizaso <i>et al.</i> (2009)
	Y	M	Habitat enhancement	Transplanting	NA	2-4 years	<i>P. oceanica</i>	Survival Growth Recruitment	S	Balestri <i>et al.</i> (2011)
	Y	MH	Creation	Nurseries, Transplanting	NA	6-12 months	<i>C. nodosa</i>	Survival Growth	S	Balestri & Lardicci (2012)
	N	L	Restoration	Transplanting	10's-100's m ²	6-12 months	<i>P. oceanica</i>	Survival Growth	F	Dominguez <i>et al.</i> (2012)
	Y	M	Restoration	Nurseries, Transplanting	100's meters	1-6 months	<i>P. oceanica</i>	Density	F	Alagna <i>et al.</i> (2013)
	N	M	Restoration	Transplanting	10's-100's m ²	2-4 years	<i>P. oceanica</i>	Survival	NA	Terrados <i>et al.</i> (2013)
	Y	M	Habitat enhancement	Nurseries, Transplanting	NA	1-2 years	<i>C. nodosa</i>	Biomass	F	Balestri & Lardicci (2014)
	N	M	Restoration	Transplanting	10's meters	4-7 years	<i>P. oceanica</i>	Recruitment	S	Pirrotta <i>et al.</i> (2015)

(a) GIS based elaboration derived from WDPA (www.protectedplanet.net). Y=yes, N=no

(b) GIS based elaboration derived from Halpern *et al.* (2008). L=Low, M=Medium, MH=Medium high impact

(c) Based on the classification of human mediated restoration actions provided by Elliott *et al.* (2007)

(d) Inferred from the study and classified as failure (F), partial success (PS) or success (S)

Results

Approximately 7% of studies reviewed at global scale have been carried out in the Mediterranean Sea. Most of the studies have been carried out in the western area of the basin (Fig. 1). The most targeted habitat of restoration is represented by seagrasses (in total 14 records, mostly focused on *Posidonia oceanica*) followed by saltmarshes (Fig. 1, Tab. 1). For macroalgal forests, 6 records have been found, mostly related to *Cystoseira* species. Three studies focused on species typical of coralligenous (i.e. *Astroides calycularis*, *Corallium rubrum*, *Paramuricea clavata*). About 64% of studies (i.e. 23) documented success of the restoration action (also partial), and most of them have been carried out in areas featured by some form of protection and by medium/medium high levels of impact. Most of the studies (i.e. about 56%) cover a maximum time span of one year. Long-time series have been found exclusively for saltmarsh habitat. This is also the habitat in which most of interventions (generally involving a modification of physical/hydrological settings) covered the largest spatial scales (i.e. from 1 to 100's of hectares). By contrast, a huge amount of studies carried out in other habitats (approximately 80%) show small scale transplanting of target species. In these studies, the response variable mostly assessed is the survival of transplanted units (15 studies out of 25).

Discussion and conclusions

The number of records obtained from the literature review demonstrates a wide adoption of active restoration practices to support the recovery of disturbed ecosystems at global scale. As far as the Mediterranean, these practices are still uncommon and mostly concentrated in the western part of the basin. In spite of the substantial increase in restoration efforts across habitats, to date studies have been principally focused on seagrasses (i.e. *P. oceanica*) and saltmarshes habitats. The application of active restoration techniques to macroalgal forests or coralligenous shows a significant delay compared to the others habitats and, in many cases, we are still in the explorative phase. Independently of the habitat targeted by restoration, more than the 50% of the studies documented the total or partial success of restoration outcomes. However, even considering the same methodological approach, the restoration success strongly depends on a combination of key factors, including the species or habitat being restored, the method(s) used as well as contextual factors such as the location, the conservation status and the duration of the project as suggested in the literature (Keenleyside et al., 2012; Perring et al., 2015; Chang et al., 2016; Darwiche-Criado et al., 2017; Montero et al., 2018). For instance, several studies have been carried out in the Mediterranean on *P. oceanica* transplants, showing a gradient of success up to complete failure. Moreover, also in presence of successful results, defining the success rate on a short time frame can be not reliable. Restoration of *P. oceanica* meadows, as well as for other habitats, requires long time periods (decades) and better knowledge on a number of issues potentially affecting the outcomes of the restoration action (see van Katwijk et al. [2016] for a review). Generally, the measure of success across studies is heterogeneous and sometimes also vaguely reported, with an absence of baselines or reference sites for direct comparisons. Where criteria for success were stated explicitly, they typically related to simple structural metrics (mainly survival) of a target species. With the exception of transitional systems, most restoration studies were found to cover relatively small areas (< 1 ha). In addition, most restoration studies in the database showed a limited duration (i.e. no more than 1-2 years) corresponding to the lifetime of development projects,

research grants, or academic theses. These are the main reasons leading to general criticism of restoration practices in the marine environment. Ten years ago, a study reported that restoration research on coral reefs had been focused on the development of techniques rather than on assessing the application of established methodologies in large-scale restoration projects (Zimmer, 2006). Unfortunately, the present review indicates that still little is changed and many studies are still experimental, covering small scale and very limited durations. Whilst small-scale restoration actions can be informative in testing a technique or theory, their success should be evaluated at multiple spatial scales to match the scale of disturbance leading to habitat degradation. Our study suggests that, especially for the Mediterranean Sea, the added benefit of active restoration must be interpreted cautiously. In the basin, active restoration should continue to be encouraged where specific contingencies are present, together with effective measures of passive recovery still representing a critical option to counteract the effects of human threats on coastal environments.

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BLEACHING OF CRUSTOSE CORALLINE ALGAE IN THE MEDITERRANEAN SEA

Abstract

Here we report the incidence of coralline algae bleaching in the western Mediterranean Sea. In October 2015, after a summer characterized by positive thermal anomalies, we observed a spread of bleaching and mortality affecting several crustose coralline algae (CCA) species from 0 to more than 30 m depth at several sites in the NW Mediterranean Sea. We have observed this phenomenon every post-summer period from 2015 until 2017. Syndromes of CCA bleaching were characterized by a white discoloration affecting the living algal tissue. In some cases, especially at shallow depths, CCA had locally disappeared resulting in bare rock areas, with some evidence that sea urchins grazing effects may have exacerbated this process. The extent of this mortality in the Montgrí, Illes Medes i Baix Ter and Cap de Creus Natural Parks showed a heterogeneous bleaching pattern, affecting from 15% to 70% of the CCA surface, depending on the species and depth.

Although the causes and mechanisms of CCA bleaching are still poorly understood, diseases in coralline algae have been related with elevated seawater temperatures, and their effects may be amplified by future ocean warming and acidification. Further research is needed to confirm the nature of such mortality events, but these observations suggest that CCA bleaching may be a widespread and previously overlooked phenomenon in temperate seas, and especially in the Mediterranean Sea. Because coralline algae play key structural and ecological roles in temperate seas, the emergence of these diseases may have dramatic consequences on the structure and functioning of marine ecosystems.

Key-words: Coralline algae, bleaching, diseases, climate change, stressors

Introduction

Crustose (non-geniculate) coralline algae (CCA) occur worldwide from polar to tropical regions, occupying the entire depth range inhabited by photosynthetic organisms, from upper-intertidal regions to depths as great as 312 m (Friedlander et al., 2014), and are important components of ecosystems in tropical and temperate reefs (Littler and Littler, 2013). CCA also play important ecological roles including substrate consolidation, and providing food for grazers, space and cues for invertebrate larval settlement, and habitats for a high diversity of associated organisms (Nelson, 2009).

This is especially true in the Mediterranean Sea, where CCA create and maintain habitats used by other organisms at different bathymetric ranges. At the mediolittoral zone, the red calcified alga *Lithophyllum byssoides* create large bioconstructions known as *L. byssoides* rims (Laborel, 1987; Verlaque, 2010). In the infralittoral zone, several CCA species establish the primary substrate for the development of complex algal communities (Verlaque, 1987). In the circalittoral zone, CCA build-up coralligenous outcrops, a coralline algal reef that thrives exclusively in Mediterranean deep waters (20–120 m depth) (Ballesteros, 2006).

Evidences of CCA diseases were unknown until 1993, when the Coralline Lethal Orange Disease (CLOD) was first discovered in Aitutaki Atoll, Cook Islands (Littler and Littler, 1995). Recently, a white CLOD-like pathogen has become even more abundant worldwide (Littler and Littler, 2013), together with the discover of new types of diseases, such as the Coralline Fungal disease (CFD) (Littler and Littler, 1998), the Coralline White Band Syndrome (CWBS) (Goreau *et al.*, 1998), Coralline Cyanophyte Disease (CCD), Coralline White Patch Disease (CWPD) (Vargas-Ángel, 2010), and an unknown species of target-shaped coralline pathogen first reported from the Great Astrolabe Reef on 2007 (Littler *et al.*, 2007). It was not until 2015 that CCA bleaching was also observed in a non-tropical sea, the Mediterranean Sea (Hereu and Kersting, 2016), highlighting that this phenomenon is not restricted to tropical areas.

The aim of the present study was to describe the CCA bleaching phenomena detected in 2016 in the Catalan coast (NW Mediterranean), identify the affected species, and quantify the extent of this phenomena at different benthic communities along a depth gradient.

Material and Methods

This study was performed in the Montgrí, les Illes Medes i el Baix Ter Natural Park, and the Cap de Creus Natural Park (NW Mediterranean) during 2016 and 2017, under the framework of the Catalan Marine Natural Parks monitoring program.

To quantify the extent of this phenomenon, in the *Lithophyllum byssoides* rim community, 16 sites were selected throughout both Marine Natural Parks, under different protection levels. At each study site three areas were selected, and at each area we took 20 pictures of 25x25 cm² quadrats distributed haphazardly along the mid-level of the rim. The sampling was performed in October 2017, as it is the recommended period to study this community (Verlaque, 2010). Pictures were analyzed calculating the percent cover of four different categories: living, bleached, covered by epiphytes and broken *L. byssoides* portions.

For the sublittoral and circalittoral communities, a study was performed in October 2016 in the Montgrí, Illes Medes and Baix Ter Natural Park, where 5 sites were selected and three different depths: at 5 and 15 meters on algal-dominated communities, and at 25 meters on coralligenous outcrops. At each site and depth, we took 20 pictures of 50x50 cm² quadrats distributed haphazardly on the bottom. Pictures were analyzed distinguishing the cover and the living and bleached surface of the most common and conspicuous species.

Results

We observed a spread of bleaching affecting several species of crustose coralline algae from 0 to more than 30 m depth along the Catalan Natural Parks.

For *Lithophyllum byssoides*, bleaching appeared in patches distributed homogeneously along the rim, showing a defined concentric white band following the perimeter, with the inner part also white discolored (Figure 1A). *Neogoniolithon brassica-florida* showed also the same pattern, but forming big patches from 10 cm to 1m in diameter, above/below the *L. byssoides* rim, and distributed heterogeneously along the coast (Figure 1.B). For *Lithophyllum incrustans*, the mortality showed the same pattern, sometimes triggered by the appearance of star-shaped white spots, from where whitening was extended (Figure 1.C).

For *Mesophyllum alternans*, we observed brown areas that darkens to gray or green tones, giving a necrotic aspect of the tissue (Figure 1.D). In other cases, we observed a thin

brown band followed by a white decolored area (Figure 1.E), or other typology of bleaching showing concentric spots without any transition between white and colored areas. *Neogoniolithon mamillosum* generally showed the emergence of bleaching from the formation of white spots that were spreading along the surface of the algae. However, in some cases, we observed that the expansion of mortality also occurred in the form of white bands (Figure 1.F). In the case of *Lithophyllum stictaeforme* (Figure 1.G) we observed a similar pattern.

In some cases, the mortality of the tissue starts with brown spots, but more often it directly starts with the appearance of white spots and bands which are extended through the algal surface, usually in the form of a continuous band or in a circular shape. Once the tissue is dead, it can be covered by algal epiphytes, which provide a greenish color, and finally the tissue is lost, leaving the bare substrate, a process that can be enhanced by sea urchins grazing (Figure 1.H). The extension of the injuries varied from several centimeters to circles about 1 m in diameter.

In the *L. byssoides* rim, we observed a bleaching of coralline crustose algae in all studied sites, with an average coverage of bleached surface of 32.2% in the Cap de Creus Natural Park and 49.4% in the the Mongrí, les Illes Medes i el Baix Ter Natural Park.

In the subtidal communities, a general pattern can be observed both in the distribution of crustose coralline algae species, and also in the affectation and extension of bleaching. *M. alternans* was the most abundant species at the depths sampled, with the other CCA species being much less prolific. The mortality rates oscillated between 10% and 70% on average in all species, without significant differences among them. On the other hand, the highest mortality rates were observed at the shallowest depths, with a mean mortality of 50.1%, 37.1% and 38.2% at 5, 15 and 25 m depth, respectively.

Discussion and conclusions

The CCA bleaching phenomena described in this study is a new threat not only for the species affected but also for the habitats they form. Unlike the bleaching phenomena described for corals, we observed that the CCA bleaching causes in all the cases the mortality of the affected tissue.

For several species we have found a great similarity to the disease described as White Band Syndrome (CWBS), with a characteristic circular, expanding white band that leaves non-pigmented dead algal tissue behind. A different pattern of bleaching corresponding to the described Coralline White Patch Disease (CWPD) affected the same species.

We observed crustose coralline algae bleaching in both Catalan Natural Parks, from 0 to more than 30 meters depth. Moreover, further observations were reported along several locations along the Mediterranean Sea, such as Columbretes islands (Spain) (Hereu and Kersting, 2016, Hereu pers. obs), Banyuls (France) (M. Verlaque, pers. comm.), Marseille (France) (M. Verlaque, S. Sartoretto, pers. comm.), Port-Cros (M. Verlaque, pers. comm.) (Hereu pers. obs.), Nice (France) (Mangialajo, pers. comm.), Sicily (Italy) (Caronni *et al.*, 2017) and Lastovo Nature Park and Mljet National Park (Croatia) (Petar Kružić, pers. comm.). These observations confirm that CCA bleaching events are not an isolated, but a widespread phenomenon.

Although several CCA diseases have been documented in tropical seas, almost nothing is known about their etiology, spatiotemporal dynamics and relationships with extrinsic environmental drivers (Vargas-Angel, 2010). In tropical seas, diseases of coralline algae have been linked to elevated seawater temperatures (Vargas-Angel, 2010; Williams *et al.*, 2014), which may also have interactive effects with other environmental stressors, such

as ocean acidification (Williams *et al.*, 2014). In the NW Mediterranean Sea, the CCA bleaching can be a natural phenomenon, especially in the *L. byssoides* rims due to the seasonal desiccation and thermal stress (Verlaque, 2010). Nevertheless, observations of massive bleaching of CCA in sublittoral communities were made for the first time after a summer period characterized by positive thermal anomalies (Hereu and Kersting, 2016), suggesting a relationship between seawater temperature and bleaching. Similarly, the relationship between temperature and bleaching is also denoted by the higher mortality percentages occurring at the shallowest depths.

Our observations indicate that this is a general phenomenon in the Mediterranean, probably related to the increase of seawater temperature as a consequence of climate change, and comparable to the mass mortalities occurred with other benthic organisms such as gorgonians, corals, or sponges (eg Garrabou *et al.*, 2009).

Although the CCA have a certain ability to recover after mortality events by growing, recurrent bleaching episodes as the described in this study may limit their recovery. Moreover, although individual algae could grow again and recover their coverage, this individual growth may not be enough to maintain or restore the biogenic structures that they form, such as the *L. byssoides* rims or the coralligenous outcrops.

It is worth to mention that this perturbation can be enhanced by others stressors. For example, trampling in the *L. byssoides* rims, or sea urchins overgrazing, can erode faster the CCA surface of the algal communities. These interactive effects may ultimately destroy the three-dimensional structure of the algal communities, leaving bare rock, changing the composition and structure of the whole community, as well as its ability to be restored.

Further research is needed to confirm the nature of this pattern, but these observations suggest that coralline bleaching may be a new widespread and probably overlooked phenomenon in the Mediterranean Sea and other temperate regions worldwide. Nevertheless, the relationship of CCA diseases with increasing seawater temperature and ocean acidification, support the hypothesis that the recurrence of these diseases are increasing as a consequence of climate change.

Despite their ubiquity and their key structural and ecological role, many aspects of the biology and ecology of CCA remain poorly understood, especially regarding the causes and distribution pattern of diseases, which may have dramatic consequences on the structure and functioning of marine ecosystems

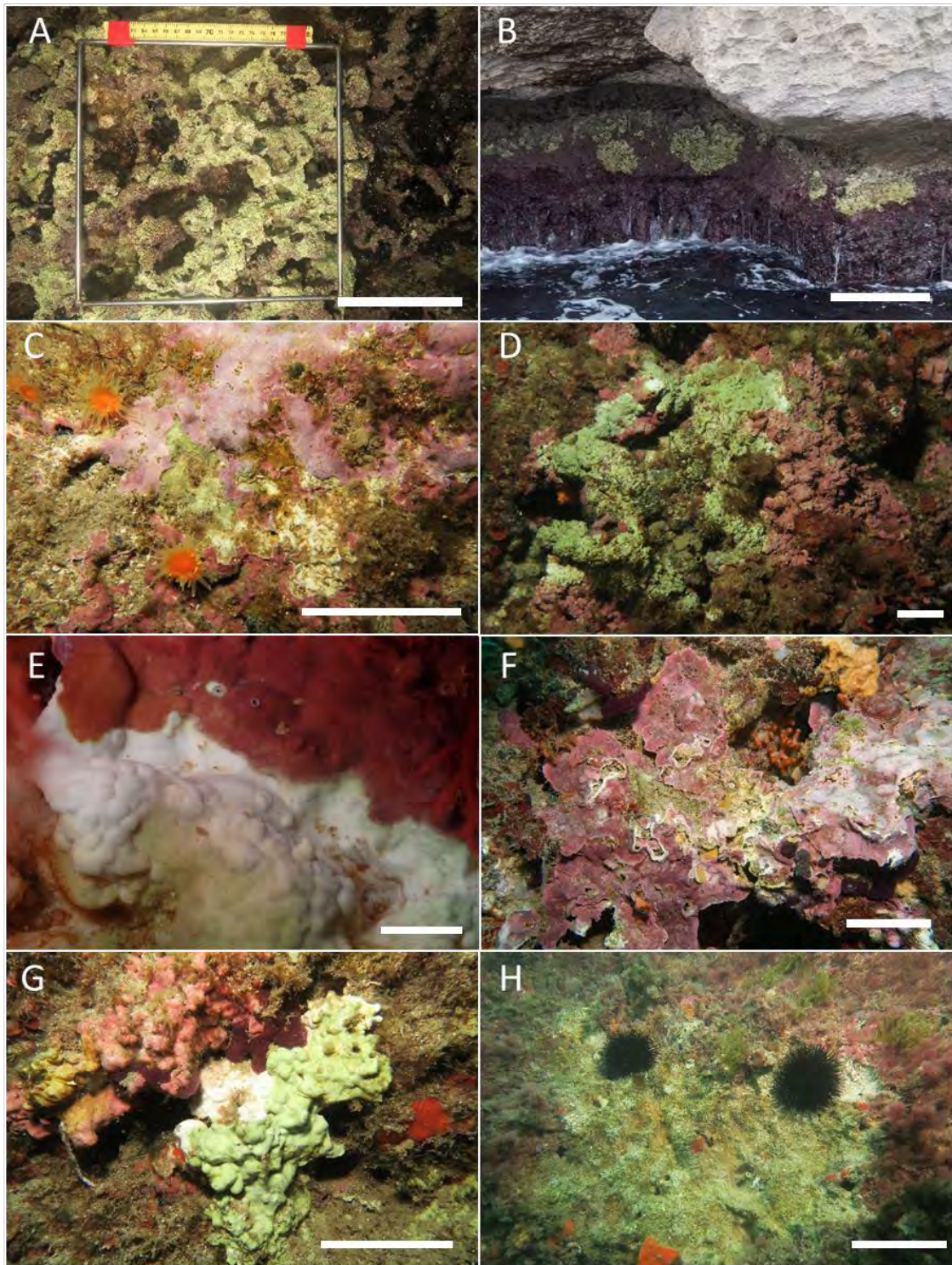


Fig. 1- Bleaching of several Coralline Crustose Algae species observed in the Catalan Natural Parks (NW Mediterranean): **A)** *Lithophyllum byssoides*, **B)** *Neogoniolithon brassica-florida*, **C)** *Lithophyllum incrustans*, **D)** *Mesophyllum alternans*, **E)** *Mesophyllum alternans* detail, **F)** *Lithophyllum stictaeforme*, **G)** *Neogoniolithon mamillosum*, **H)** bare rock after bleaching and dead of *M. alternans* and the grazing effect of sea urchins. Scale bars represent 10 cm in all figures, except in B and E, where they represent 100 and 1 cm, respectively.

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CAN FACILITATION PROCESSES ENHANCE THE EFFECTIVENESS OF RESTORATION ACTIONS IN THE CORALLIGENOUS HABITAT?

Abstract

*Coralligenous outcrops are slow-dynamic habitats exhibiting high structural complexity. If these valuable habitats are degraded, relatively long time is required to recover their structure and full functionality. In order to reduce recovery times, restoration actions can focus on target habitat-forming species. Facilitation processes (i.e. positive species interactions), rarely considered to date, may be further explored to enhance the effectiveness of coralligenous restoration initiatives. Here we test if erect habitat-forming species, putatively modifying light penetration and/or water movement, can affect the survival and growth of co-occurring encrusting and massive ones. For that purpose, we designed an experiment using the red gorgonian *Paramuricea clavata* and bryozoans, important coralligenous structural animals, as model organisms. The experiment, replicated in Spain, Italy and Croatia, is based on the comparison between series of 0.25 m² experimental plots. Experimental treatments include addition of bryozoan recruitment-enhancing devices (plastic grids) with and without gorgonian colonies and controls (empty and artefact ones) for a total of 4 replicates per treatment. The hypothesis is that the erect layer (15 *P. clavata* fragments up to 20 cm in maximal height per experimental plot) could facilitate the settlement of bryozoan colonies onto grids within the experimental plots and enhance their survival and growth. The experiment was initiated in May-July 2017 within the EU Horizon 2020 project MERCES (Marine Ecosystem Restoration in Changing European Seas, <http://www.merces-project.eu/>) and it is still ongoing. In this contribution we present and discuss our preliminary experimental findings.*

Key-words: coralligenous assemblages, Mediterranean Sea, restoration, facilitation, gorgonians

Introduction

Coralligenous outcrops are vulnerable habitats subjected to cumulative threats such as global warming, invasive species, habitat destruction and overfishing (Ballesteros, 2006). In general, biological and ecological processes in these habitats are slow (e.g. Teixidó *et al.*, 2011), hence if degraded, it can take long before structured populations and communities can be restored. Identification of species able to facilitate such processes and thus reduce recovery time is an important step to enhance the effectiveness of coralligenous restoration approaches.

Gorgonians are key structural species in the coralligenous habitat (Ballesteros, 2006). Their forests can modify light penetration, sediment deposition and micro-scale water movement. Moreover, they can affect the early-stage recruitment of sessile epibenthic assemblages, promote bioconstruction processes in their understorey (Ponti *et al.*, 2014; 2018 and references therein) and offer protection from mechanical damage to smaller fragile species, such as erect bryozoans (Garrabou *et al.*, 1998).

Here we test if such erect habitat-forming species can facilitate the survival and growth of co-occurring encrusting and massive ones. We report our preliminary findings on recruitment of early-stage sessile assemblages, with special focus on bryozoans, important coralligenous animal builders.

Materials and methods

Experimental set-up. We carried out a manipulative field experiment using the red gorgonian *Paramuricea clavata* (Risso, 1826) and bryozoans as model organisms. The experiment was replicated in Spain (Medes Islands Marine Protected Area (MPA; 42°2'N, 3°13'E)), Italy (Portofino Promontory MPA; 40°48'N, 14°9'E) and Croatia (Cape Sokol, Krk Island, Natura 2000 site; 44°58'N, 14°49'E), thus encompassing 3 regions: the Northwestern Mediterranean, the Ligurian and the Adriatic Seas. These sites host natural *P. clavata* populations and the experiment was set in the species upper bathymetric range: at 18 m depth in Medes Islands and 35 m depth in Portofino and Krk Island.

Following previous successful trials, which indicated the suitability of plastic grids as a substrate for bryozoan recruitment and growth (see Fig. 1), we used them in experimental treatments with and without gorgonian colonies (15 *P. clavata* fragments up to 20 cm in maximal height per experimental plot). Rubber ribbons (5 cm wide) were used to fix gorgonian transplants in the experiment (*P. clavata* forest treatment; PC) and additionally two types of controls were set: empty (plastic grid only; C) and artefact one (plastic grid + rubber ribbons; AC). There were 4 replicates per treatment and the size of each replicate plot was 0.25 m². The simulated density of gorgonians was comparable to the maximum values observed in natural populations (Linares *et al.*, 2008). The experiment was initiated in May-July 2017 within the EU Horizon 2020 project MERCES and it is still ongoing.

Sampling and data analysis. Photosampling was carried out seasonally, using the 25 x 25 cm quadrat to facilitate taxa identification. Images were analysed with PHOTOQUAD software (Trygonis & Sini, 2012), by superimposing a grid of 400 equal-sized squares. Organisms were identified to the lowest possible taxonomic level and the percentage cover of each taxon/category was calculated from the ratio of respective taxa cell counts and cumulative count of the experimentally set artificial substrate.

A Bray-Curtis similarity matrix was constructed on square root-transformed percent cover data and non-metric multidimensional scaling (MDS) ordination was performed to visualize patterns of community similarities. Differences in early-stage sessile assemblage structures inside and outside (including both empty and artefact control) of gorgonian forest (Tr: fixed factor with 3 levels) and among sites (Si: fixed factor with 3 levels in fall and 2 levels in spring) were assessed by permutational multivariate analysis of variance (PERMANOVA) for each season: fall (October 2017) and spring (April 2018). Values of $P < 0.05$ were considered significant. When less than 9999 unique values in the permutation distribution were available, asymptotical Monte Carlo P-values were used instead of permutational P-values. Significant interactions among main factors were investigated by post-hoc pair-wise tests. Univariate tests were run on untransformed data using the Euclidean distance. SIMPER was used to assess the percentage contribution of each taxon to the average dissimilarity among sites and the first three taxa with the highest % contribution were reported. Statistical analyses were performed using PRIMER 6 with PERMANOVA+ add-on package (Clarke & Gorley, 2006 and references for respective procedures therein; Anderson *et al.*, 2008).



Fig. 1: Illustration of recruitment, survival and growth of bryozoan *Pentapora fascialis* over a period of 1.5 years on plastic meshes set within Medes Islands MPA.

Results

In total, 40 different taxa/categories recruited on the available artificial substrate within the first 10 months of experiment's deployment: 21 in Medes, 20 in Portofino and 14 on Krk Island. MDS clearly showed three distinct clusters in fall 2017 (Fig. 2a) and two in spring 2018 (Fig. 2b), corresponding to sites which differed significantly in their early-stage sessile assemblages (Tab.1).

Average dissimilarity between Krk and Medes Islands was 53.4% and crustose coralline algae (26.2%), algal turf (12.4%) and *Peyssonnelia* spp. (12%) contributed the most to the observed dissimilarities, being more abundant in Medes (SIMPER two-way analysis). Similarly, average dissimilarity between Portofino and Medes MPAs was 53.3% but in this case animal turf contributed the most (16.4%), being more abundant in Portofino whereas green algae (9.6%) and crustose coralline algae (9.3%) were more abundant in Medes. Lastly, average dissimilarity between Krk and Portofino was 61.9% and the main contributors to the observed dissimilarity were animal turf (21.9%) and crustose coralline algae (16.8%), being more abundant in Portofino, whereas serpulids were (surprisingly) only recorded on Krk Island (11.3%). The similar pattern between these two sites persisted in spring, with their average dissimilarity of 57.7% and serpulids (30.3%) and encrusting orange bryozoans (12.5%) as main contributors to observed dissimilarity, being more abundant on Krk Island, while animal turf (28.8%) continued to dominate in Portofino (SIMPER two-way analysis).

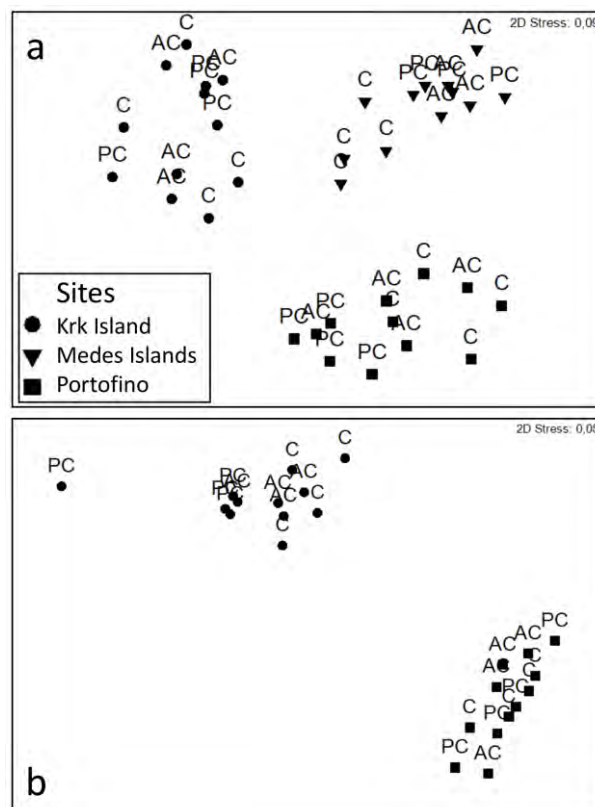


Fig. 2: Non-metric multidimensional scaling (MDS) plots comparing early-stage sessile assemblages inside and outside *Paramuricea clavata* forest among 3 sites belonging to different Mediterranean regions in two seasons: a) fall (October 2017) and b) spring (April 2018), based on Bray-Curtis dissimilarities of square root transformed percent cover data. Experimental treatments include: C = control, AC = Artefact control and PC = *Paramuricea clavata* forest.

Furthermore, the effect of *P. clavata* forest on early-stage sessile assemblages depended on the site (Tab.1). No difference among experimental treatments was observed in Portofino (Ligurian Sea), regardless of season. On the other hand, in spring 2018 on Krk Island (Adriatic Sea) difference between *P. clavata* forest treatment and control was significant (pair-wise test, $P=0.013$) while it was borderline in respect to the artefact control (pair-wise test, $P=0.05$). Abundance of both serpulid polychaetes and *Peyssonnelia* spp. differed among sites and treatments (PERMANOVA, $P=0.0075$ and $P=0.0411$, respectively), being significantly lower inside *P. clavata* forest on Krk Island (pair-wise test; PC vs. C, $P=0.0143$; PC vs. AC, $P=0.0143$ for serpulids and PC vs. C, $P=0.0089$; PC vs. AC, $P=0.037$ for *Peyssonnelia* spp.). The abundance of crustose coralline algae also differed among sites and treatments (PERMANOVA, $P=0.0221$) being significantly higher outside the forest, though only in the (empty) control treatment (pair-wise test; PC vs. C, $P=0.0205$; AC vs. C, $P=0.045$). The recruitment of encrusting orange bryozoans notably increased after winter period and reached significantly higher abundance (% cover; PERMANOVA, $P=0.001$) in April 2018 on Krk Island (up to $12.02\% \pm 3.29$ SE) than in Portofino (up to $1.18\% \pm 0.34$ SE). However, *P. clavata* forest had no effect on the bryozoan recruitment on both sites (PERMANOVA, $P=0.59$). The partial loss of experimentally set substrate in Medes Islands precluded the analysis of recruitment after winter on that site.

Tab. 1: Summary of PERMANOVA tests on the effects of *Paramuricea clavata* forest on the recruitment of sessile assemblages in different sites and different seasons (based on Bray-Curtis dissimilarities of square root transformed percent cover data).

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
a) Fall 2017						
Si	2	28883	14442	38.45	0.0001	9935
Tr	2	858.36	429.18	1.14	0.3314	9903
Si x Tr	4	3456.6	864.14	2.30	0.0009	9906
Res	27	10142	375.63			
Total	35	43340				
b) Spring 2018						
Si	1	18016	18016	82.58	0.0001	9925
Tr	2	1276.7	638.34	2.93	0.0035	9941
Si x Tr	2	913.26	456.63	2.09	0.0242	9928
Res	18	3927	218.17			
Total	23	24133				

Discussion and conclusions

The *P. clavata* forest effects on the early-stage sessile assemblages within coralligenous habitat depended on the site and differed among taxa. Within the analysed period of 10 months significant differences in taxa abundance were evident only for serpulid polychaetes and *Peyssonnelia* spp. on Krk Island Natura 2000 site. Their abundance was lower inside the *P. clavata* forest - an effect that was already recorded for serpulid polychaetes elsewhere (e.g. in Tavolara, Tyrrhenian Sea; Ponti *et al.* 2014).

No differences were observed in bryozoan abundance inside and outside of the gorgonian forest, contrary to Ponti *et al.* (2014) reporting *P. clavata* facilitation of encrusting bryozoans at both investigated sites, including our site Portofino but using a different substrate (PVC panels). Nevertheless, the pulse of bryozoan recruitment was recorded after winter in this study and plastic grids have proven to be a suitable settlement substrate, at least on Krk Island (Adriatic Sea). Besides potential differences in larval supply and/or other local factors, lower bryozoan recruitment success observed in this study may be the consequence of unavailable substrata, already occupied by competitive animal turf that continuously dominated the sessile assemblage, putatively preventing the bryozoan settlement in spring.

The newly recruited assemblages differed significantly among sites. Such an outcome is in concordance with the study by Ponti *et al.* (2014), that revealed significant spatial variation of recruitment patterns between Portofino (Ligurian sea) and Tavolara (Tyrrhenian Sea), suggesting variability in larval supply, sedimentation, chemico-physical parameters and hydrodynamism as causes of observed site differences.

As the experiment presented herein continues, it remains to be seen whether the observed patterns will persist.

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ASSESSING THE SENSITIVITY OF MARINE HABITATS TO ANTHROPOGENIC PRESSURES: A KEY TOOL IN EVALUATING RISKS TO MEDITERRANEAN BENTHIC HABITATS

Abstract

Standardised sensitivity data on marine habitats is an essential support in the management of human activities at regional, national and international scales. We developed a methodology for assessing the sensitivity of marine habitats to human pressures, as well as associated guidance on how to use the resulting sensitivity assessments. The sensitivity of 41 French Mediterranean benthic habitats to 12 physical pressures was assessed as part of the first stage of this project; other physical, chemical and biological pressures will be defined and assessed shortly. Results will feed into risk assessments allowing the identification of conservation priorities and the development of spatial planning strategies. By maximising methodological consistency in the generation of sensitivity data with other Member States, and sharing the outputs of this project internationally, we hope to facilitate collective use of sensitivity information under the Habitats Directive, the Marine Strategy Framework Directive, the Marine Spatial Planning Directive and the Regional Sea Conventions (such as Barcelona Convention).

Key-words: benthic habitats, sensitivity, pressures, impacts, human activities

Introduction

Understanding benthic habitats' sensitivity to anthropogenic pressures is fundamental to the effective management of the marine environment and sustainable economic activities. Sensitivity assessments are required:

- To identify pressures that might impede the achievement of good environmental/conservation status for habitats;
- To assess habitats' vulnerability or risk of being impacted by human activities;
- To identify and prioritise monitoring programmes and appropriate management measures that are consistent at a local, national and regional scales.

Different approaches have been used to assess sensitivity in the marine environment for the past 40 years (Gundlach & Hayes, 1978; Hiscock *et al.*, 1999 and 2006; Clarke Murray *et al.*, 2014; Montefalcone *et al.*, 2017). This project aimed to develop a scientific methodology to assess the sensitivity of French benthic habitats to anthropogenic pressures, to produce standardised results at the national level and to be consistent (insofar as possible) with other equivalent European methodologies, in order to support risk/vulnerability assessments at a national and international scale.

The terminology, habitat and pressure units, methodological framework and guidance on how to use the resulting assessments are available as a technical report (La Rivière *et al.*, 2016).

This methodology aims to be (i) pragmatic (ii) applicable to all benthic habitats and relevant human pressures (iii) consistent (insofar as possible) with other equivalent European methodologies, iv) able to produce standardised results at a national level, v) adaptable to both site-scale and regional scale marine management [under the Habitats Directive (HD, 92/43/EEC), the Marine Strategy Framework Directive (MSFD, 2008/56/CE), the Marine Spatial Planning Directive (MSPD, 2014/89/EU), Regional Sea Conventions, etc.], and (vi) based on best available knowledge.

Sensitivity assessments are pressure-based, and pressures can be either physical, chemical or biological. A single activity can generate one or more pressures and the same pressure can result from one or more activities (Fig. 1).

Assessments of French Mediterranean benthic habitats' sensitivity to twelve physical pressures were undertaken, presenting resistance, resilience and sensitivity scores, as well as associated confidence indices and evidence descriptions (La Rivière *et al.*, 2018). The sensitivity of a benthic habitat is defined as the combination of resistance and resilience; resistance being the ability of a habitat to tolerate a pressure without significantly changing its biotic or abiotic characteristics, and resilience as the time a habitat needs to recover from the effect of a pressure, once that pressure has been alleviated.

The project outputs aim to serve as a key tool in assessing the risks posed by human activities to benthic habitats and improving the effectiveness of management.

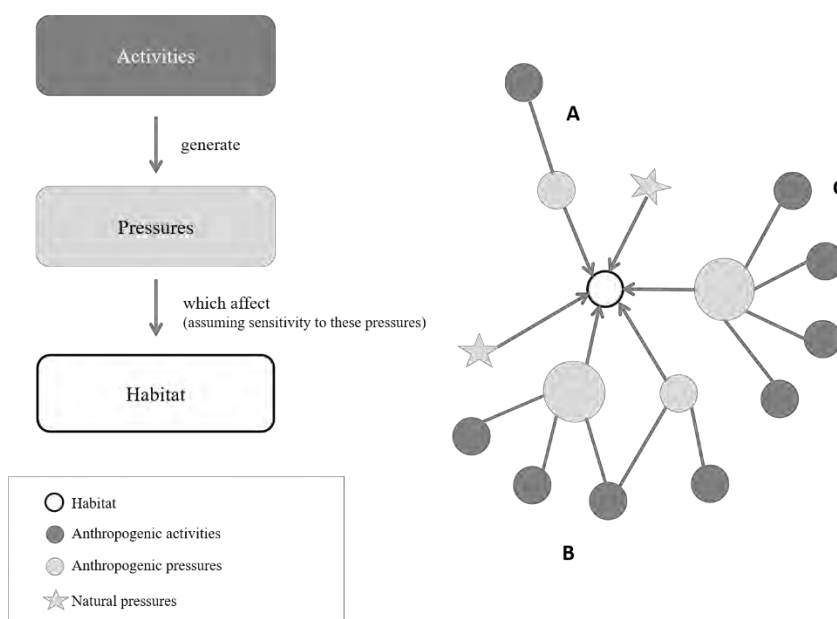


Fig. 1: Conceptual relationship between different sources of pressures affecting a habitat in three different scenarios (A, B and C) with their relative (co-occurring) intensity indicated by the size of the circles (adapted from Knights *et al.*, 2013; Clarke Murray *et al.*, 2014).

Materials and methods

Existing approaches for assessing marine habitats' sensitivity from other countries were reviewed. It was decided that the assessment of benthic habitat sensitivity would be based principally on expert judgement drawing on available scientific literature wherever possible, following recommendations from McBride *et al.* (2012) and Barnard & Boyes (2013). French benthic scientists from both the Mediterranean and Atlantic/English Channel/North Sea were asked to contribute to assessments.

Habitat sensitivity was assessed at the “biocenosis” level (which takes into account biotic and abiotic characteristics; see Dauvin *et al.*, 2008), in accordance with the French Mediterranean benthic habitat classification (Michez *et al.*, 2014). Assessments were based on a range of criteria including characteristic, structuring and/or engineer species’ life history traits, substratum type, hydrodynamic conditions and bathymetric range.

As a first step, 12 physical pressures were defined and assessed. Pressures were defined according to their ecological and technical relevance. Assessments could only be made against single pressures and “one-off” pressure events; the cumulative effects of pressures were not assessed within the scope of this project. The spatial extent of a pressure was assumed to allow for habitat recovery via recolonization from remaining habitat edges or from adjacent areas.

Assessing sensitivity involved the following steps for each pressure and each habitat:

- Identifying the key biotic and abiotic elements affecting habitat sensitivity;
- Assessing the habitat’s resistance to the pressure;
- Assessing the habitat’s resilience to the pressure;
- Combining resistance and resilience scores to generate an overall sensitivity score.

Four resistance categories (None, Low, Medium, High) and five resilience categories (None, Low, Medium, High, Very high) were defined to reflect habitats’ differences in terms of ecological responses to pressures and in relation to management timescales, noting that resilience assumes that the pressure has been alleviated or reduced to a magnitude that no longer causes an impact (i.e., allowing recovery of the habitat). A final semi-quantitative sensitivity assessment for each habitat was derived from the resistance and resilience scores (5 categories of sensitivity: Very Low, Low, Medium, High, Very High; Fig. 2).

Resilience Resistance	None > 25 yr	Low 10-25 yr	Medium 2-10 yr	High 1-2 yr	Very High < 1 yr
None	Very High	High	High	Medium	Low
Low	High	High	Medium	Medium	Low
Medium	High	Medium	Medium	Low	Low
High	Medium	Medium	Low	Low	Very Low

Fig. 2: Sensitivity scale defined by the combination of resistance and resilience scores

A confidence index was assigned to each assessment (resistance, resilience, sensitivity) as an indication of the quality of supporting evidence. Wherever possible, assessments were based on empirical data demonstrating the resistance and/or resilience of benthic habitats. Confidence scores were derived from the combination of three aspects for each resistance and resilience assessments:

- the quality of information sources: expert judgement, peer-reviewed papers, grey literature, etc.
- the applicability of evidence: the same habitat/area/pressure is assessed
- the degree of congruence between evidence sources and quantity of evidence available.

The evidence base and justification for the assessments are recorded in the final assessment matrix.

Results

The methodological report is available online, along with the assessment matrices (INPN, 2018). Data for French Mediterranean habitats will be published in two stages: firstly for the physical pressures (La Rivière *et al.*, 2018), and secondly for all other pressures (other physical, chemical and biological pressures) at the end of 2019. Data derived from the sensitivity assessments are presented in a sensitivity database.

For each habitat unit (n=41), a sensitivity assessment matrix shows the scores of resilience and resistance along with the derived sensitivity score (and their associated confidence indices) for each of the assessed pressures, as well as the evidence base and type (Fig. 3).

IV.3.1 Coralligenous communities

7 associations and 5 facies

[Relationship with other classifications](#)

Category	Pressure	Resistance	CI resistance	Resilience	CI resilience	Sensitivity	CI sensitivity	Evidence base	Evidence type
Physical disturbance or damage (temporary and/or reversible change)	Trampling	M	L	M	L	M	L	Resistance is assessed as Medium if compression only affects erected species and doesn't alter the biogenic substratum and the sheltered fauna. Some erected species are flexible, but vertical compression can break some individuals. NB: if compression reaches the bioconstructed substratum, cavities will be obstructed and their sheltered communities will be destroyed. Resilience is then estimated at over 25 years.	Expert judgment
	Surface abrasion	M	H	N	H	H	H	Surface abrasion doesn't alter the coralligenous formation nor the sheltered fauna, but will eliminate all erected species that are characteristic of the specific associations or facies. Erected individuals that will be injured without being removed will rapidly be colonised by epibiotic organisms and will break under their weight. Global resistance of the habitat is assessed as Medium because the substratum isn't affected. As the affected species are long-lived, the time needed to recover is estimated at several decades. <u>This pressure is likely to induce changes in the facies/associated without changing the biocenosis itself.</u> NB: All facies and associations of this biocenosis don't have the same resistance and resilience characteristics to this pressure. <u>Associations IV.3.1.a, b, c and Facies IV.3.1.h, i, j, k: resistance None; resilience None – Sensitivity Very High. Associations IV.3.1.d, e, f, g, and Facies IV.3.1.l : resistance Medium ; resilience Medium - Sensitivity Medium</u>	Directly relevant peer-reviewed literature (see references in La Rivière <i>et al.</i> , 2018)

Fig. 3: Extract of a sensitivity assessment matrix for coralligenous communities (Mediterranean habitat IV.3.1) to two physical pressures (Trampling and Surface abrasion) (from La Rivière *et al.*, 2018).

CI = Confidence index, H = High, L = Low, N = None, M = Medium, VH = Very High.

Discussion and conclusions

The French ecological sensitivity project provided a methodological framework and sensitivity assessments for 41 Mediterranean benthic habitats to 12 physical pressures.

Certain limitations have to be taken into account when using the sensitivity assessment results, several of which are related to methodological assumptions (La Rivière *et al.*, 2016). Sensitivity assessments are not absolute: scores are dependent on how pressures were defined (by their magnitude). Thus, if an activity generates a pressure below the magnitude described in the pressure definition, this would not necessarily result in an absence of impact. Despite cumulative effects (of same or various co-occurring pressures) being commonplace in the marine environment, it was not possible to consider all scenarios in our generic sensitivity assessments, as the ways individual pressures interact are largely unknown (Halpern *et al.*, 2008). The duration and frequency of pressures should also be considered in the development of appropriate management measures.

Nevertheless, the standardised habitat sensitivity data provided by this project constitute an essential support in the management of human activities in the marine environment at a regional, national and international scales. The resulting assessments will (i) allow the identification of pressures impeding the conservation of marine habitats, (ii) feed into vulnerability/risk assessments such as “fishing risk analyses” within marine Natura 2000 sites across Europe and (iii) help to define priorities for MPA management or spatial planning strategies. These actions are essential to delivering the objectives set out under European Directives, including the HD, the MSFD, and MSPD, as well as those of the Regional Sea Conventions such as the Barcelona Convention. Thus, this project is a good example of how the scientific community’s expert advice contributes to the implementation of environmental policies.

Lastly, as most of the assessments were based on expert judgement, this project highlighted significant knowledge gaps regarding the response of benthic habitats to pressures. This type of exercise also raises the question of the relevance of numerous units in habitats classifications when only a few are well known and described.

In the interest of European compatibility and biogeographical-scale management, links with similar initiatives underway in neighbouring countries are being pursued. By ensuring methodological consistency in the generation of sensitivity data with other Member States, we hope to facilitate sharing and collective use of sensitivity information under the HD, MSFD, MSPD and Regional Sea Conventions.

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ENHANCING THE EFFECTIVENESS OF RESTORATION ACTIONS IN CORALLIGENOUS HABITATS: INSIGHTS FROM A TRANSREGIONAL THERMOTOLERANCE EXPERIMENT

Abstract

Understanding the response of organisms facing climate change is critical to buffer the on-going biodiversity crisis. The Mediterranean coralligenous habitats are among the richest but also most threatened habitats of the Mediterranean. Indeed, coralligenous habitats, dominated by long-lived and structural species such as octocorals or sponges, have been impacted by different marine heat waves causing large-scale mortality events. Considering the slow dynamics of many impacted species, mortality events question the future of coralligenous habitats and restoration actions appeared as one of the most relevant tools to buffer biodiversity losses.

Effective restoration actions should rely on an understanding of the interactions among the organisms to be restored and their local environment in order to allow the identification of relevant donor populations to restore vulnerable and degraded populations.

*The main aim of the present study was to characterize the interactions between *Paramuricea clavata*, a habitat-forming octocoral severely but differentially impacted by mortality events, and its thermal environment. We carried out a common garden experiment in aquaria focusing on the response to thermal stress of 12 populations from five different regions (Catalunya, Corsica, Northern Italy, Croatia and Southern Portugal) within the North Western Mediterranean, the Adriatic and the Atlantic. The objectives of this study were: 1) to further the acquisition of basic information about the thermotolerance features of *P. clavata*; 2) to evaluate the role of biological processes in the differential responses to thermal stress by conducting genomic analyses; 3) to characterize the micro-eukaryotic and prokaryotic communities associated to the targeted populations. Here, we will present the first results of this study. We will discuss their implications for restoration actions of the coralligenous habitats dominated by *P. clavata* in the context of on-going climate change.*

Key-words: climate change, mass mortality events, common garden experiment, local adaptation, microbiome.

Introduction

How biodiversity will respond to environmental variations driven by climate change is a critical issue for ecologists and managers. While mounting evidences suggest that extreme climatic events are already severely impacting worldwide biodiversity (Hughes et al. 2018), our ability to understand how eco-evolutionary processes shape the differential responses of organisms and populations facing environmental changes is key to buffer the current biodiversity crisis. In this context, the study of population-by-environment interactions (PEI),

and particularly, the study of local adaptation, received increasing attention from theoretical and empirical perspectives. While deciphering PEIs remain challenging, recent advances in sequencing technologies combined to experimental approaches, such as common garden experiments are promising tools to understand the impact of the shift in the regime of selection on biodiversity (de Villemereuil et al. 2016).

Climate change, and warming in particular, is one of the major concerns for Mediterranean biodiversity. While this sea represents less than 1% of the world ocean, it harbors 4 -18 % of worldwide marine biodiversity. Moreover, this hotspot of marine diversity was identified as a hotspot of climate change. In the last decades recurrent marine heat waves, “*periods of extreme warm sea surface temperature that persist from day to month and can extend up to thousands of kilometers*” (Frölicher & Laufkötter 2018), were reported in the North Western Mediterranean Basin (Garrahou et al. 2009). These heat waves were related to large scale mass mortality events (MME) impacting thousands of kilometers of coastal ecosystems. Coralligenous communities, which are one of the most diverse Mediterranean communities (Ballesteros 2006), were profoundly impacted by MMEs. Indeed, tens of different invertebrate species from these communities, including sponges, octocorals and bryozoans, suffered from different levels of tissue necrosis driving the individual to death in many cases (Garrahou et al. 2009). Due to their slow population dynamics, many of these species seem particularly vulnerable to the recurrence of the MMEs, which unambiguously question the future of coralligenous communities.

Interestingly, intensive field surveys conducted during the MMEs demonstrated that these events are characterized by their differential impacts at all the biological levels considered: among individuals, populations and species and even within colonies for colonial species such as octocorals. It has been proposed that individuals or populations within species may show different levels of tolerance to thermal stress. Nevertheless, the underlying eco-evolutionary processes explaining those differential responses remain to be fully characterized.

Here, we focus on *Paramuricea clavata*, a structural octocoral species from the coralligenous, which was severely impacted during past MMEs (Garrahou et al. 2009). Our main objective was to shed new light on the eco-evolutionary processes and environmental factors involved in the differential responses to thermal stress observed in the field. More particularly, we combined a trans-regional common garden experiment with whole genome sequencing and metagenomics analyses to evaluate the role of eco-evolutionary processes (with a focus on local adaptation, genetic drift, acclimatization) and environmental factors (with a focus on the microbiome) on the differential responses of individuals and populations to thermal stress. The results should guide the identification of potential resistant populations and/or colonies to be used in restoration actions.

Materials and methods

Biological model. The red gorgonian, *Paramuricea clavata*, is a habitat-forming octocoral with a key role in the structure and functioning of coralligenous habitat (Ballesteros 2006). Accordingly, *P. clavata* is of major interest for conservation and management purposes. This is a long-lived and gonochoric species that reproduces annually and shows late sexual maturity (13 years of age) with a short larval phase (Linares et al. 2008). *P. clavata* populations are characterized by a patchy distribution mainly in the Western Mediterranean and in the Adriatic. During the MMEs, populations of *P. clavata* were differentially impacted by MMEs. For instance, following the 2003

MME, populations from the Provence coast (France) showed percentage of affected colonies ranging from 2 to 80% (Garrahou et al. 2009).

Experimental set-up. We carried out a common garden experiment in which individuals from different origins were submitted to a common thermal stress in controlled conditions and their responses were assessed during the time of the experiment. We sampled 30 colonies coming from twelve populations located in five different regions (Southern Portugal, Catalonia, Corsica, Northern Italy and Croatia) separated by tens of meters to hundreds of kilometers within the North and West Mediterranean and the Atlantic Ocean and inhabiting contrasting temperature regimes at the regional and local scales (Fig. 1a).

The experiment involved two aquarium sets: Control and Treatment. In the Control set, seawater temperature was maintained at 16-18°C during the whole experiment (Fig. 1b). In the Treatment set, the heat stress consisted of a stepwise temperature augmentation from 18°C to 25°C during 2-3 days. Once the 25°C temperature (i.e. critical temperature for this species; Crisci et al. 2017) was reached, the condition was kept constant for 25 days.

Response variable. The response variable was the level of tissue necrosis per individual (Fig. 1c), which was visually monitored daily in the Treatment and Control tanks. From these observations, the percentage of affected colonies (i.e., the proportion of colonies displaying tissue necrosis > 0%) and mean necrosis values per day were computed to assess the response to thermal stress.

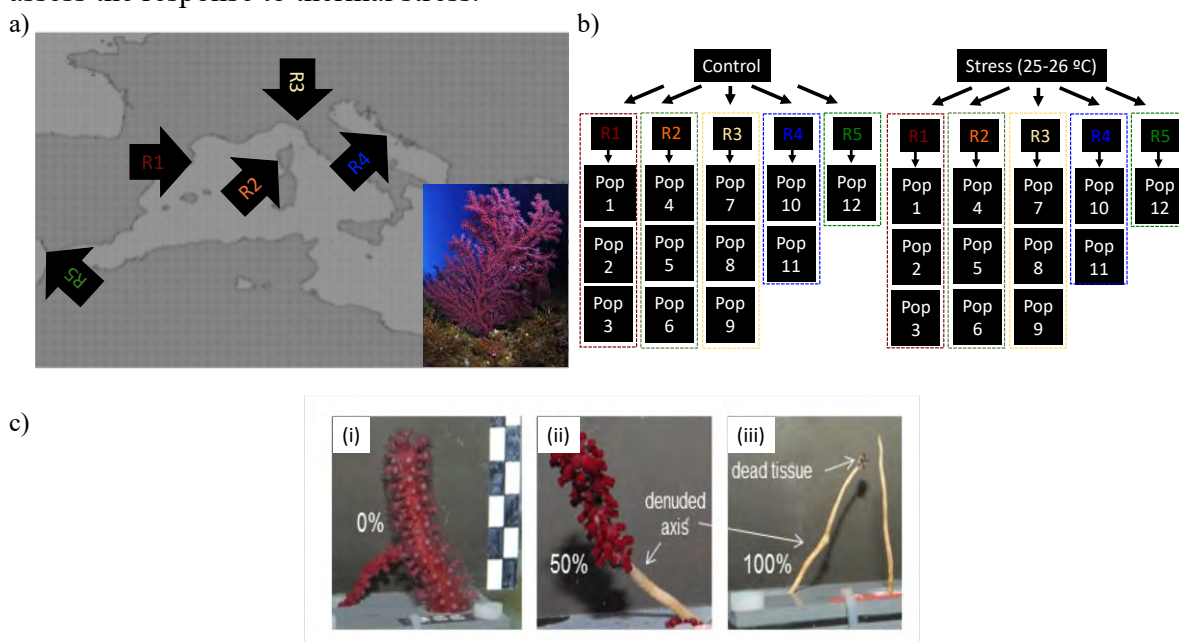


Fig .1: a) Twelve populations coming from five different regions (R1: Catalunya; R2: Corsica; R3: Northern Italy; R4: Croatia and R5: Portugal) were sampled for the common garden experiment; inset shows a *Paramuricea clavata* colony; b) experimental set-up showing the two treatments (Control at 18°C vs. Treatment at 25°C); c) The level of tissue necrosis monitored daily during the experiment: (i) no necrosis, (ii) 50% of necrosis, (iii) 100% of necrosis was monitored daily during the experiment.

Whole genome sequencing analyses of *P. clavata*. In order to look for putative genetic factor(s) and to estimate the relative impact of eco-evolutionary processes involved in the differential response to thermal stress, we conducted a whole genome sequencing analysis. As a preliminary step, we generated the whole genome sequence of one individual of *P. clavata* coming from the Catalan coast. This *de novo* whole genome

assembly was done following a hybrid strategy combining the data obtained from Illumina and Oxford Nanopore Technologies sequencing. RNA-seq from three different individuals coming from three different populations and submitted to control and treatment conditions are ongoing to annotate the genome. This strategy should allow covering a large amount of the genes expressed during thermal stress. In parallel, we used the data on the response variable to identify five resistant and five sensitive colonies per population (120 individuals in total), which will be re-sequenced at lower coverage (10x). These re-sequencing data will be used to look for genetic variant and putative locus involved in the differential responses to thermal stress.

Microbiome analyses of *P. clavata*. In parallel, we conducted microbiome analyses. These analyses aimed to: i) characterize the temporal dynamic of microbiome diversity in colonies of *P. clavata* submitted to thermal stress; ii) to look for microbiome differences among sensitive and resistant individuals. The microbiome of the individuals used in the experiment will be sequenced using metagenomic approach. Amplicon sequencing was performed by the Integrated Microbiome Resource facility at the Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University. Two distinct sets of amplicon analyses were conducted: one focused on associated eukaryote and one focused on procaryotes. In both cases, sequencing was performed on an Illumina MiSeq using a 300 bp paired-end read design.

Results

Experiment and response variable.

First signs of necrosis appeared at least in some colonies for all populations after only one-week exposure at 25 °C. At the end of the experiment, 88 % of the total number of colonies showed 100% tissue necrosis (dead colonies) while only 5.6% (mainly from Portugal and one population from Italy: Lighthouse) remained without necrosis. Despite the general patterns of sensitivity, significant differences in the survival probability were found among populations ($p < 0.001$). The most resistant population was found in Portugal, with 43% of colonies affected and an average of 30.3% of mean tissue necrosis after 3 weeks exposure at 25 °C. The second most resistant population, a population from Italy displayed 54.7% of mean tissue necrosis and 80 % of affected colonies over the same period. All other populations showed more than 80% of mean tissue necrosis after 3 weeks exposure at 25 °C. The most sensitive population was Palazzu from Corsica, which was affected after only 6 days and reached more than 80% of mean tissue necrosis after only 15 days, followed by a complete death of all its colonies in less than 3 weeks. From these results, we selected, in each population, five resistant individuals identified as the last five individuals reaching 50% of tissue necrosis and five sensitive colonies identified as the first 5 individuals reaching 50% of tissue necrosis (Fig. 2) to be re-sequenced.

Whole genome sequencing analyses of *P. clavata*.

While the genome-size estimated by Genomescope (Vurture et al. 2017) is close to 750 Mb, the resulting genome assembly of *P. clavata* is 760.25 Mb long. The contiguity is relatively low (contig N50 14.32 and scaffold N50 15.33 Kb). The gene completeness of this assembly was estimated with BUSCO v3.0.2 (Simão et al. 2015) identifying 76.2% of complete genes, 8.8% of fragmented genes and 15% of missing genes. From this assembly, the number of heterozygous single nucleotide variants (SNV) was estimated to more than 2×10^6 corresponding to a heterozygous SNV rate 3.8×10^{-3} SNVs/bp (i.e. we expect to find almost 4 SNPs per Kb).

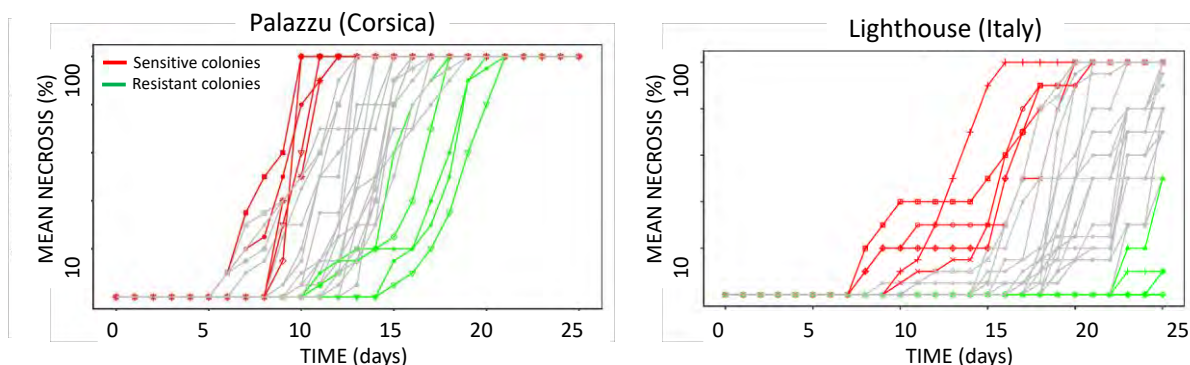


Fig. 2: Percentage of tissue necrosis during the experiment for two populations showing contrasted responses (Palazzu from Corsica vs. Lighthouse from Northern Italy). In those two populations, sensitive and resistant colonies are shown in red and green, respectively.

Microbiome analyses of *P. clavata*.

Based on our preliminary data, there is a difference in the prokaryotic communities among populations and among sensitive and resistant individuals to temperature increases. In all the individuals the bacterial communities are dominated by gammaproteobacteria and the coral associated communities are clearly different from the surrounding water bacteria as well as the bacteria associated to their food source.

Moreover, we have observed a shift in the microeukaryotic communities when increasing the temperature. When the temperature was 18° C the community was dominated by ciliates and changed to apicomplexans when we increased it to 25° C. As it happens with the prokaryotic community, the microeukaryotes associated to the corals are clearly different from those in the surrounding water and the provided food.

Discussion and conclusion

Field observations following mass mortality events suggest that the capacity of the habitat-forming octocoral, *Paramuricea clavata*, to deal with thermal stress may vary among individuals and populations. The results of the trans-regional common garden experiment conducted here confirmed previous field surveys. Indeed, the level of tissue necrosis varies significantly among individuals and populations. Interestingly, the population from Portugal and one population from Italy seem only marginally affected by the thermal stress.

Previous studies demonstrated that different factors may be involved in the differential responses to thermal stress in *P. clavata*. For instance, the physiological status of the colony was acknowledged as a central factor in the differential responses. Indeed, the impact of thermal stress was stronger in starved colonies compared to well-fed colonies (Coma et al. 2009). Then, the sexual maturity (adults vs. juveniles) and the sex (male vs. female) of the colonies play a significant role in the differential responses observed within population. Juveniles are more resistant compared to adults while female's reproduction is more impacted than male reproduction (Arizmendi-Meija et al. 2015). Focusing on eco-evolutionary processes, genetic drift has been suggested as a main driver of the differential responses, hampering potentially adaptation to local environmental conditions (Crisci et al. 2017). Nevertheless, some limitations of the approaches used in this study preclude definitive conclusions on the respective role of selected and neutral eco-evolutionary processes on the differential responses to thermal stress.

The high number of SNVs identified in the present study and their genotyping and comparison between resistant and vulnerable individuals from the experiment should allow

the identification of genetic regions involved in the responses to thermal stress. In the meantime, we expect to improve our understanding of the processes driving the differential responses and to deepen our knowledge on the PEIs in this species.

Regarding the spatial patterns of the microbiome of *P. clavata*, previous studies suggested a lack of geographic structure in the bacterial community inhabiting *P. clavata* (La Rivière et al. 2013). The metagenomic approach developed here refined those results. While this study is still on-going, it seems that prokaryotic communities are different among the different populations used in the experiment. Interestingly, our results also suggested variations of the microbiome community with the duration of the thermal stress.

Understanding the eco-evolutionary processes and environmental factors, including the microbiome, involved in the differential responses of organisms to climate change is a critical issue for conservation biologists. The increasing frequency and intensity of marine heat waves may severely jeopardize sessile species characterized by slow population dynamics such as habitat-forming octocorals from the coralligenous. Indeed, it is unlikely that recolonization and resilience capacities of the impacted populations are sufficient to counteract the recurrent and negative impacts of mortality events. In this context, restoration actions are a promising tool. Our study should refine our understanding of the processes and factors underlying the resistance to thermal stress of colonies and populations of *P. clavata*. Accordingly, we should be able to improve the selection of source populations of *P. clavata* to enhance the effectiveness of restoration actions. Considering the central ecological role of *P. clavata* in the coralligenous, restoration actions will benefit the associated biodiversity.

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ADVANCES IN THE CONSERVATION OF RED CORAL POPULATIONS: LONG-TERM DEMOGRAPHIC DATA, MANAGEMENT TOOLS AND RESTORATION ACTIVITIES

Abstract

*Coralligenous assemblages face cumulative impacts from multiple stressors acting both at local and global scales. The red coral *Corallium rubrum*, one of the most emblematic coralligenous species, is threatened by historical overharvesting and, since the last decades, by the effects of global warming. Nine red coral populations have been annually monitored during several years in different Mediterranean localities. Long-term data revealed a high vulnerability to multiple human-driven stressors with strong effects of local management and ocean warming. In addition, a regional survey along the Catalan coast performed last year within the two largest and oldest MPAs in the area showed that the conservation status of the red coral population is far from what we expect within protected areas. The goal of this presentation is to understand the main mechanisms driving the persistence and the structural simplification of red coral populations after being harvested during millennia, and to assess the current effectiveness of different conservation tools such as the fishing ban proposed recently by the Catalan Government, the role of marine protected areas (MPAs) and their interaction with other stressors, and the use of active restoration for illegally harvested colonies. The final aim is to discuss the conservation status and different management actions at the Mediterranean scale in order to ensure a solid scientific consensus on the conservation of fully functional *C. rubrum* populations.*

Key-words: Coralligenous, Habitat-forming species, Overfishing, Climate change, Marine Protected Areas

Introduction

Coralligenous assemblages are affected by several disturbances such as destructive fishing practices, ghost fishing, anchoring, recreational diving, mucilaginous algal aggregates and algal invasions (Ballesteros, 2006). Additionally, mass mortalities related to marine heatwaves have been reported across NW Mediterranean populations (Cerrano *et al.*, 2001; Garrabou *et al.*, 2009).

Among all coralligenous species, the red coral *Corallium rubrum*, is one of the most emblematic species given its important cultural and ecological role in the Mediterranean Sea. Due to historical overexploitation and still widespread unsustainable fishing practices, most red coral shallow populations are depleted and can be considered functionally impaired or even ecologically extinct (Bruckner, 2009; Garrabou *et al.* 2017a). Marine Protected Areas (MPAs) are one of the main conservation tools to recover red coral populations, although only after several decades of proper enforcement MPAs can recover size-structure of red coral populations (Linares *et al.*, 2010; 2012; Bavestrello *et al.*, 2015). Nevertheless, MPAs may not be enough to ensure their persistence under

climate change. Due to the risks associated with overexploitation and climate change, this species has recently been listed as an endangered species in the IUCN Mediterranean Red List of Threatened Species (Otero *et al.*, 2017). In this context, a recent new legislation approved by the Government of Catalonia in 2018 based on scientific data and advice will totally ban red coral extractions for a period of ten years (Garrahou *et al.*, 2017b).

In order to assess the effectiveness of different conservation strategies, demography is a powerful tool that can also help to understand the evolution of life histories. However, it is particularly challenging for long-lived species due to a general scarcity of long-term and fine-scale demographic data. During the last two decades, several studies focused on the study of the demography of red coral populations in NW Mediterranean Sea (Garrahou & Harmelin 2002; Tsounis *et al.*, 2006; Rossi *et al.*, 2008; Linares *et al.* 2010; 2012; Priori *et al.*, 2013; Bramanti *et al.*, 2014). However, most of these studies were restricted to short-term and local spatial scales hindering our understanding of the processes driving the dynamics of this threatened species and its conservation needs.

In this study, we will specifically provide information about the long-term monitoring of red coral populations at different Mediterranean locations as well as about the current conservation status of red coral populations in the Catalan coast. Additionally, we will discuss the current effectiveness of different conservation tools such as the fishing ban proposed recently by the Catalan Government, the role of marine protected areas (MPAs) and the use of active restoration for illegally harvested colonies. We also aim to discuss the conservation status of this species and propose different management actions at the Mediterranean scale in order to ensure a solid scientific consensus on the conservation of fully functional *C. rubrum* populations.

Material and methods

Regarding to the long-term monitoring of red coral populations, nine populations placed at different locations in the NW Mediterranean Sea at relatively shallow depths (15 to 25 m) were monitored during periods ranging from 3 to 13 years. Among the studied coral populations, six were located within MPAs and three were non-protected and subjected to several fishing events during the monitoring. One of the protected populations, located in the Réserve Naturelle de Scandola (Corsica, France), has been recurrently affected by positive thermal anomalies since 2003, which have triggered coral mass mortality events (Garrahou *et al.*, 2009). A total of 1144 coral colonies were individually monitored using photographic techniques ($n=30$ photoquadrats of 20 x 20 cm at each population) over periods ranging from 3 to 10 years between 2003 and 2011. Based on these photographic series, we estimated temporal trends in density and recruitment as well as size-dependent annual survival, fecundity and growth rates.

Regarding to the current conservation status of red coral populations in the northern Catalan coast during 2017 we obtained demographic information for 21 red coral populations inside and outside MPAs. From each of these populations, we quantified the density and size of colonies as well as the proportion of large coral colonies (>100 mm) as a proxy for population structural complexity and conservation status (Linares *et al.*, 2010; Montero-Serra *et al.*, 2018a). Given that the total ban of red coral extractions approved by the Government of Catalonia for the following ten years (2018-2028) was based in previously published data in scientific papers and reports, information on the current conservation status of red coral populations as we obtained in this study was totally needed and will be crucial to be used as a baseline at the beginning of the fishing ban.

Results

The long-term monitoring of red coral populations located in different regions of the NW Mediterranean Sea revealed that *C. rubrum* displayed slow dynamics over the nine studied populations along the NW Mediterranean, displaying a high stability in adult density in all populations throughout the study (Fig. 1). Recruit densities were very limited and very stable over the study period within most of the cases being zero or one recruit per quadrat (Fig. 1).

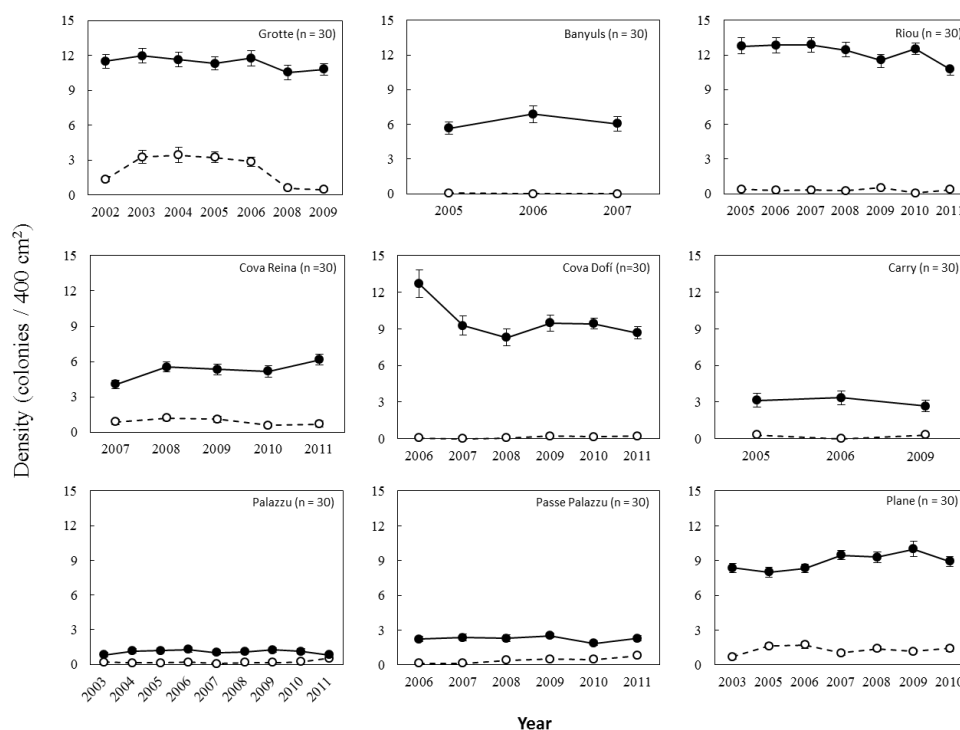


Fig. 1: Long-term population trends at nine red coral populations in the NW Mediterranean. Black dots indicate adult and white dots recruit densities

The study performed in 2017 in order to assess the current conservation status of red coral populations in the Northern Catalan coast showed contrasting differences between both studied Natural Parks, where Montgrí, Illes Medes and Baix Ter Natural Park showed larger mean height colonies in most of the locations than those observed in Cap de Creus Natural Park (Fig. 2). However, all of them were far from a desirable conservation status in terms of colony size and biomass. Only three populations (Encalladora, Vaca Nord and Paieta) out of the 21 studied showed some colonies larger than 100 mm but only representing less than 3% of the total colonies.

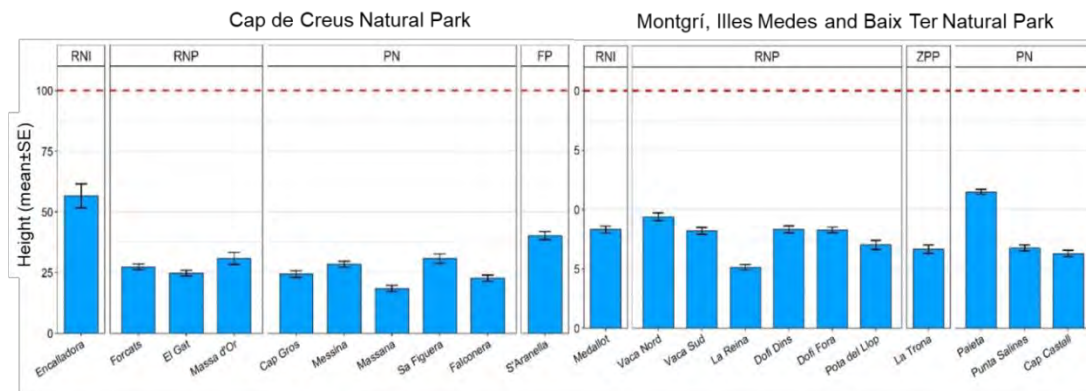


Fig. 2: Mean height in mm of red coral colonies in the 21 populations studied in 2017 in the Cap de Creus Natural Park and Montgrí, Illes Medes and Baix Ter Natural Park in the Catalan coast. RNI: No-Take area where all activities are forbidden except research activities. RNP: Natural Reserve where all fishing activities are forbidden. ZPP: buffer zone where only some artisanal fisheries can be performed. PN: Natural Park where most of the activities are allowed included the red coral extraction in Cap de Creus. FP: Outside the Natural Park. Note that the red coral extraction is not allowed in any location inside the Montgrí, Illes Medes and Baix Ter.

Discussion and conclusions

The long-term monitoring showed a much more homogenous pattern of low recruitment at larger temporal and spatial scales, than showed in previous studies (Bramanti *et al.*, 2005; Santangelo *et al.*, 2012). Recruitment limitation can seriously hinder the ability of populations to recover after intense perturbations. For species with limited recruitment, adult survival becomes of paramount importance to ensure their persistence. We found extremely high survival for *C. rubrum* with estimated mortality even lower than found in previous experimental studies (Garrabou & Harmelin, 2002).

The regional survey along the Catalan coast performed in 2017 after the declaration of the total red coral fishing ban in Catalan waters showed that the current conservation status of red coral population is far from what we expect even within protected areas. These findings corroborate the scientific recommendation about the ban of red coral extractions from 2018 to 2028 approved by the Government of Catalonia, which was based in previously published data in scientific papers and reports (Garrabou *et al.*, 2017b).

Although MPAs can enhance the recovery of exploited populations in terms of biomass and size-structure (Linares *et al.*, 2010; Bavestrello *et al.*, 2015), they are often far from the expected goals given the existence of other threats that can affect red coral populations within MPAs such as poaching and diving activities and the effects of climate change. However, given the low recruitment rates and limited dispersal of gorgonian larvae (Ledoux *et al.*, 2010), it is unlikely that protected populations act as a source to exploited populations ensuring their long-term persistence. Therefore, we should not just consider improving the actual network of MPAs but also moving towards a more restrictive legal framework for red coral populations. Beyond the establishment of annual quotas and minimum harvesting sizes, the ban on using dredges as well as the recent recommendation on total ban on harvesting of *C. rubrum* populations under 50 m depth (Cau *et al.*, 2013), we should discuss the conservation status and more restrictive management actions at the Mediterranean scale, like total bans of red coral fishery, in order to ensure the conservation of fully functional *C. rubrum* populations.

Finally, the results obtained to date regarding to the restoration activities performed in this species (Montero-Serra *et al.*, 2018b) confirm the potential success of these actions and strongly support the feasibility of these techniques, at least at local spatial scales and specially in case of poaching, which is expected to increase as a result of the total ban on red coral harvesting.

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EFFECTIVE SFM-BASED METHODS SUPPORTING CORALLIGENOUS BENTHIC COMMUNITY ASSESSMENTS AND MONITORING

Abstract

Animal forests enhance Mediterranean coralligenous habitat biodiversity and functioning. Their monitoring is paramount to assess the conservation status of the habitat and to plan restoration actions for addressing the Good Environmental Status as required by the EU legislation. Scuba diving investigations allow fine data collection up to -70 m but are limited in small surfaces sampled as a result of the short available time. Moreover, human observations in this stressful condition are likely to be affected by errors. Structure from Motion (SfM) photogrammetry is an on-growing technology supporting rapid and accurate indirect data collection through optical scanning over organisms and habitats, thus empowering scuba diving sampling performances. A tools-set of SfM based methods using scaled point clouds, 3D models and quasi-orthorectified projected images were developed to support the study of the coralligenous benthic communities. Population structure, morphometrics, biomass and secondary production of sponges and gorgonians were calculated using real case of studies and assessed with ground truth datasets. The performances of different cameras (GoPro Hero4 and Sony NEX7) in data collection were tested. The accuracy in population density estimation were always above 70% for all the considered species. The precise measurements of the organisms morphometries supported the estimation of the average growth rate of the mapped population. These findings highlight the potential use of this technique for environmental accounting studies (emerging approaches) required by the European Marine Biodiversity Strategy Directive whilst contributing to the quantitative estimation of the organic carbon turned out by the species.

Key-words: scientific diving, natural capital, marine protected areas, remote sensing, climate change.

Introduction

In the Mediterranean Sea, animal forests are well represented by several octocoral species such as the gorgonian *Paramuricea clavata* (Risso, 1826), the red coral *Corallium rubrum* (Linnaeus, 1758) and by large sponges such as *Sarcotragus foetidus* (Linnaeus, 1753). These species form dense patches or sparse populations along outcrops, cliffs, overhanging marine caves entrance and biogenic substrates from -15 m to -200 m depth (Ballesteros, 2006). *P. clavata* is of special interest in coralligenous habitats because its canopy reduces the range of environmental variability, which favour a rich associated biota (Valisano et al., 2016). *C. rubrum* is protected under the Annex II of the SPA/BD Protocol of the Barcelona Convention (Parry et al. 2010) because overexploited for centuries (Garrabou et al., 2017). *S. foetidus* is a cosmopolitan demosponge included in the RAC/SPA list of the endangered or threatened species (Parry et al., 2010) with a rich associated zoobenthic community living the complex porous network of the connective tissue (Cinar et al., 2002).

Mass mortalities events affecting sponges, as well as octocorals, have been recorded since 1998 as a result of climate change and human pressure on benthic assemblages (Linares et al., 2008). These dramatic events may cause local extinctions and trigger shifts of the benthic communities leading functional changes in habitat (Cerrano & Bavestrello, 2008, 2009). Therefore, monitoring changes in the population structure over time is mandatory to quantify the magnitude of environmental disturbances, to assess the recovery capacity of the populations and to inform managers in calibrating conservation strategies for reaching the habitats Good Environmental Status (Directive 2008/56/EC). However, sampling methodologies are often not standardized (Pergent, 2011). Advances in scuba diving techniques allows scientist to operate at remarkable depth (up to -70 m) minimizing the negative effect of Nitrogen gas narcosis (Cerrano et al., 2010) but, underwater working conditions can be stressful even in shallower areas with consequences on the reliability of *in-situ* observations. Photos and videos sampling are not affected by human bias but are limited to standard surfaces usually defined a priori (i.e. photos surface 0.5 m² and video transects among 10 m) replicated across small areas. Moreover, destructive samplings, needed for estimating organisms' morphometrics (i.e. biomass), are rarely implemented in monitoring programs to avoid sampling impacts on the communities (Mistri & Ceccherelli, 1994). Consequently, our knowledge on the composition of benthic communities is spatially fragmented and lack in resolution.

In recent years the uptake of new underwater remote sensing technologies has supported researchers and surveyors with the collection of *in-situ* observations. Structure from Motion (SfM) photogrammetry support rapid and accurate data collection through optical scanning over organisms and habitats (Figueira et al., 2015; Ferrari et al., 2017; Palma et al., 2017). SfM surveys are implemented collecting multi-view cameras and by processing image datasets with commercial (i.e. Agisoft Photoscan) or open-source software (Coro et al., 2018) for generating organisms or seascapes 3D models, orthomosaics and Digital Terrain Models from dense point clouds.

Here we present a tools-set of SfM based methods developed and tested with different cameras, to support the study of the coralligenous benthic communities. Scaled point clouds, 3D models and quasi-orthorectified projected images were used to estimate organisms' morphometrics (volume, maximal height, maximal width, planar surface, biomass) and density. Information on Carbon and Energy trapped in the gorgonian living tissues were also estimated supporting energetic based calculation of the natural capital of coralligenous habitat.

Material and method

SfM underwater surveys were performed in 2017 and 2018 at Punta del Faro (Portofino Marine Protected Area, Italy) by scuba divers who mapped representative areas larger than 50 m² where the selected species were present. Surveys were scaled with Ground Control Points (GCPs) and image datasets were processed using Photoscan software (Agisoft LLC., St. Petersburg, Russia) to estimate the relative camera poses and generate dense clouds.

S. foetidus individuals were counted and mapped singularly. From the scaled 3D model of each sponge, has been calculated the volume interpolating fitting a plane to the base of the sponge. The weight of collected dried sponge samples were measured and the volume was estimated both in laboratory and from 3D models to calculate a volume to biomass conversion factor through linear regression. Finally, the biomass of the entire population was estimated.

SfM surveys were performed in January 2017 after 10 months on a *P. clavata* population using at the same time a GoPro Hero 4 Black edition (GP) recording time-lapse images at maximal frame dimension and a Sony NEX7 Alpha (SN) recording video at maximal frame dimension. Performances of the two cameras in estimating density, maximal height, maximal width, fans' planar surface, generated from point clouds analysis were assessed with in-situ ground truth sampling using traditional sampling methods (standard surface quadrats replicates, photos with dimensioned background). The metric 3D canopy was calculated interpolating the gorgonians point cloud with best fitting planes dimensioned 5 cm x 5 cm expressing a more accurate estimation of the filtering surface of the gorgonian population.

The point clouds of selected colonies were interpolated with best fitting planes using Meshlab software (Cicogni et al., 2008) and the best image among those used to generate the point cloud was projected on the plane generating the quasi-orthorectified images. The procedure was replicated with the datasets obtained with the two sampling events. The length of apical branches per colony was measured to estimate the gorgonians' linear growth.

The coenenchymal surface of a set of collected and dried colonies was measured in the lab and by calculating the mesh surface from high-resolution 3D models generated through SfM to estimate the accuracy of the photogrammetric procedure. The factors to convert coenenchymal surface to biomass, organic Carbon and Energy content of living tissues (Mistri & Ceccherelli, 1994; Brey et al., 1988) were applied to the 3D model mesh surfaces using virtual samples dimensioned 5 cm x 5 cm randomly positioned over the 3D gorgonian models. These metrics were then estimated over the whole study area (52 m²) and to the full extent of the coralligenous habitat along the MPA of Portofino (Punta del Faro, Italy) (86,300 m²) converting the 3D canopy metric value. Details on the methodology applied have been published in Palma et al. (2018) and in Pavoni et al. (2018). Red coral colonies were segmented over a point cloud in Meshlab software (Cignoni et al., 2008) by classifying the dataset per colour and by defining the minimal distance to the interpolated background mesh obtained interpolating the substrate point cloud. Then a clustering analysis was performed with the remaining *C. rubrum* point cloud using the Connected Component plugin in Cloudcompare software (Girardeau-Montaut, 2014). The accuracy of the measurement was estimated comparing the results obtained from SfM procedure with the value obtained through images visual count of the colonies.

Results

The distribution of *S. foetidus* counted 19 individuals over an area of about 200 m². The conversion factor (volume to biomass) was estimated as 0.1082 gr/cm³ (+5.55~SD) on 13 samples with different sizes and from different specimens. The linear regression analysis showed a strong correlation between the sponge samples dry weight and their volumes R²=0.82. The average volume per sponge is 5000 cm³ (±2300 SD) with a total dry weight biomass of 8516 g estimated for the whole population.

A SfM survey was performed on January 2017 and replicated after 10 months to map gorgonians population over 52 m² using a GoPro Hero 4 (GP) and a Sony Nex7 Alpha (SN). The GP camera showed lower departures from the gorgonians abundance ground truth values overestimating the colonies density less than 1.7 colonies m⁻², whereas the SN camera presented larger departures, <6.4 colonies m⁻². Linear regression between SfM and ground truth shows R²= 0.72 with GP and R²=0.82 with

SN for maximal height. The $R^2=0.90$ and $R^2=0.79$ were calculated respectively for the GP and the SN, through linear regression between planar surface estimated with SfM method and digital image analysis as ground truth.

The gorgonian linear growth estimated over 30 apical branches from four selected colonies within the mapped population, results as 1.5 cm (± 3.0 SD) with a maximal growth value of 6.8 cm and a maximal negative growth of -5.9 cm.

The linear regression analysis between coenenchymal lab measurements and SfM 3D models surface calculation showed a strong correlation $R^2=0.99$. The mean coenenchymal surface calculated over 56 virtual sampling quadrats dimensioned 5 cm $\times$ 5 cm positioned on the collected colonies 3D models, was 23.24 cm² (± 0.06 SD), corresponding to 0.109 g (± 0.0750 SD) of coenenchymal biomass per surface unit. The 3D canopy surface converted in coenenchymal surface over the study site was 4.74 m² (GP) and 4.61 m² (SN) corresponding to 223 g and 217 g for the GP and the SN. In the study area, the amount of Carbon was estimated as 24.5 g (GP) and 23.8 g (SN) and the Energy amounted to 922 KJ (GP) - 898 KJ (SN). For the entire MPA, 40.7 Kg (GP) - 39.65 Kg (SN) of Carbon and 1.53 GJ (GP) - 1.49 GJ (SN) of Energy were calculated.

C. rubrum survey took 8 minutes of data recording to cover an area of 55 m² where three different populations were surveyed. The experimentation was performed on three population covering a surface of 2 m² each. Dense point cloud generated from the selected areas ranged between 3.5MM points and 1.7MM points. The average segmented and cleaning point cloud counted for 13k points (± 5.6 SD) and the point cloud clusters generated corresponding to the red coral colonies were 104 (± 25 SD). The calculation performed over standard surfaces of 0.5 m $\times$ 0.5 m each population, counted a total of 34 points cloud clusters against 39 colonies, 25 points clusters against 29 colonies, and 15 points clusters against 12 colonies of *C. rubrum* counted through visual image examination.

Discussion and conclusion

The SfM based methods developed are accurate and provided relevant information on the benthic selected species distribution and biology. The estimation of biomass, growth, Carbon, and Energy tissue contents from SfM outcomes is novel and provide new perspective for effective applications of the technology for long-term monitoring as well as for rapid habitat characterizations.

SfM based method for the biomass calculation of the large sponge *S. foetidus* support the total census of the species (19 sponges) over 200 m² which is a relevant information can't be obtained with traditional sampling methods. Moreover, the calculation of the factor to convert volume to dry weight allowed to estimate the biomass contribution of the species in the habitat composition by estimating the sponges' volume through their 3D models geometry. Dense populations of *C. rubrum* and *P. clavata* which are unlikely used for generating 3D modeling have been accurately mapped by processing the point clouds obtained from SfM surveys.

The estimation of the *C. rubrum* colonies abundance performed through point cloud segmentation based on colour criteria, showed small departure to the colonies count performed by analyzing the images. The planar growth shape of *P. clavata* supported the calculation the best fit planes obtained interpolating the colonies point clouds. The fan planes obtained supported fine calculation of the colonies morphometrics and abundance across the mapped population. GP and SN cameras showed high to moderate accuracy with ground-truth data for *P. clavata* abundance, maximal height, maximal width and

planar surface of the colonies, highlighting the flexibility of the technology which can be successfully implemented using sensors with different quality and recording videos as well as photos. The replication of the SfM survey over time on the same gorgonian population supported the estimation of the colonies average linear growth calculated as the average value of 1.5 cm (± 3.0 SD) in 10 months which is similar to the values reported in literature for the species (1.8 cm/yr to 2.7 cm/yr) (Mitri & Ceccherelli, 1994).

The new metric 3D canopy provides accurate approximation of the gorgonian population surface by interpolating the species point cloud with a mosaic facets of standard maximal dimension (5 cm $\times$ 5 cm). The metric provides complementary information to those traditionally used in gorgonian population structure studies (i.e. abundance, density, maximal height, maximal width, fan surface) and supported the estimation of the coenenchymal surface and the related metrics (biomass, carbon, energy tissues contents) avoiding destructive sampling. The carbon and Energy estimation suggest that the SfM framework can be a suitable tool for estimating the indirect contribution of *P. clavata* to local and regional carbon budgets.

These findings demonstrate that SfM based methods in data processing can provide relevant information on coralligenous habitat key species. The presented point clouds data processing methods supported the estimation of relevant metrics for natural capital estimation in Marine Protected Areas and represents a tool-box for coralligenous benthic assemblage characterization and monitoring for managers and practitioners.

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GHOST MED: ASSESSMENT OF THE IMPACT OF LOST FISHING GEAR IN THE FRENCH MEDITERRANEAN SEA

Abstract

Lost and abandoned fishing gear affects all marine habitats, both coastal and offshore habitats such as bathyal habitats, including canyons. Not only professional fishing nets, longlines and fishing traps are concerned, but also recreational fishing tackle (fishing lines, lead weights, hooks, etc.). This lost gear causes various forms of negative impact including ghost fishing, that has been extensively studied for several decades. However, the lack of information on the quantification of lost gear and the assessment of impacts other than ghost fishing is critical worldwide. The aim of the Ghost Med program is (i) to quantify the lost fishing gear in the Mediterranean and characterise the habitats concerned, (ii) to address federate underwater observers and citizen science to better collect data on fishing gear loss events, (iii) to assess their impact on species and habitats, and (iv) to propose tools to managers in order to prioritize, or not, their removal. The protocol is based on an assessment of the environmental and seascape impact of the lost gear as well as the technical risks involved in its removal. Each of these parameters was measured using several criteria, such as the number of mobile species trapped in the gear, the number of fixed species damaged or uprooted and the alteration of the habitat relief. All these criteria are weighted by a score which will then be used to calculate the lost gear Removal Aid Index (RAI). This simple-to-use protocol should help Marine Area managers to determine whether gear removal should be considered. This protocol is currently used as a basis for the assessment and removal of fishing nets in France, and could be extended to all Mediterranean coasts.

Key-words: Ghost Med Program, lost fishing gear, ghost fishing, Mediterranean, coralligenous.

Introduction

More than 8 million metric tons (t) of waste are discharged into the global oceans each year. The marine garbage consists mainly of land origin waste, while the main source of marine litter is the merchant marine fleet. The amount of lost and discarded nets has only been sketchily estimated, but Macfadyen *et al.* (2009) estimated that they represented around 10% of the marine waste. In the Mediterranean Sea, the impact of lost fishing gear (LFG) is of importance because of the high intensity of artisanal fishing activities off the coast and the offshore industrial fishing effort, involving the use of various devices: gillnets, trammel nets, trawls, longlines, traps, etc. In the coastal zone, recreational fishing activities also contribute in a significant way to the LFG in the form of fishing lines, lead, lure, etc. Fishing gear lost in the Mediterranean Sea has been estimated at between 2637 and 3342 tons per year (Golik, 1997); nevertheless, the overall assessment remains uncertain, and often only concerns small geographical areas. Professional fishing activities are regulated by very strict guidelines. Most fishermen follow the rules because of the cost of the fishing gear; in addition, they have an interest in adopting environmentally friendly practices in order to ensure sustainable resources. The loss of fishing gear is mostly accidental, for example due to rough weather conditions, lack of familiarity with the marine areas, user conflicts or gear drifting with the current.

The LFG has various forms of impact on the marine environment. Firstly, the best-documented is ghost fishing, which generates the unintentional and unnecessary trapping of mobile fauna. The more recent the LFG the more the ghost fishing is abundant (Ayaz *et al.*, 2006). The impact decreases over time, but could last over considerable periods if the net remains deployed in the water column. Moreover, it acts with a cascade effect because the trapped organisms attract other predators or scavengers, which are in turn trapped, and so on. LFG also damages the benthic habitat and the species attached to the substrate and alters the functioning of the habitat. The risk of chemical pollution linked to the presence of LFG is based in the introduction of synthetic materials into the trophic chain of ecosystems. Finally, they present a significant danger to the users of the sea, namely, navigators, swimmers and divers. On the other hand, the colonization on old LFG submerged for a long time can constitute new complex structures, sometimes with heritage value species. This aspect must be considered for the overall assessment of the impact of LFG because their removal may be more harmful for the habitat than beneficial.

In the Mediterranean Sea, information on LFG is sparse and incomplete. The aim of this study is: (i) to quantify the LFG in the French Mediterranean and characterise the habitats concerned; (ii) to address federate underwater observers and citizen science to better collect data on fishing gear loss events; (iii) to assess their impact on species and habitats, and (iv) to propose tools to managers to prioritize, or not, the removal of lost gear. The aim of the Ghost Med program is to coordinate the available information, initially with regard to French waters, with a view towards extending the investigation to the Mediterranean as a whole.

Materials and methods

Since 2015, a database has been constituted to gather all the data available on LFG in the French Mediterranean Sea. This database collects observations from three sources: (i) scientific, (2) managers and (3) citizen science. The scientific observations come from scientific campaigns usually devoted to naturalist knowledge such as Natura 2000 site descriptions, Medseacan and Corseacan missions for the exploration of Mediterranean canyons (Fourt & Goujard, 2012), and seabed mapping. The managers observations are provided by *in situ* observations of field-based staff. The citizen observations are provided by an online data recovery form (<https://www.mio.univ-amu.fr/ghostmed/en/form/>) that enables members of the public to declare the observation of LFG. The form provides for the entry of at least the following information: type of fishing gear, depth, concerned marine habitat, geographical position, date of observation and identity of the observer. A 'Comments' box is provided to allow for any additional details. To federate underwater observers and citizen science, social networks, the development of a website, participation in conferences, trade fairs and press releases were used.

Environmental, seascape and technical criteria are used to assess the impact of LFG on the marine environment. Each criterion is quantified by a set of relevant parameters. Each parameter is assessed by a semi-quantitative or a qualitative scale, and a score is assigned. The sum of the scores gives the assessment of the criterion. Note that the scores can be negative because some impacts may have a positive effect, e.g. the colonization of the fishing gear.

Parameters for environmental impact (EI) (Tab. 1).

- The **colonization** of the fishing gear is assessed within four colonization stages: (0) without epibiosis; (1) by filamentous algae; (2) by macroalgae and hydrozoa; and (3) by encrusting epibiosis (bryozoa, macroalgae, annelida, etc.). The more developed the colonization is, the less the removal of the gear would be appropriate.

- The **trapped mobile fauna** is quantified by the number of individuals trapped in the LFG on the basis of a semi-quantitative scale.
- The **removed fixed species** concerns all the benthic species fixed to the substrate that have been torn off by the action of the fishing gear. It is estimated by the number of individuals removed in the LFG.
- The **damaged fixed species** are species that undergo necrosis or breakage due to contact with the LFG. This is assessed by the number of individuals damaged by the LFG.
- The **presence of outstanding species** concerns the species that have colonized the LFG. Outstanding species are species presenting heritage value, such as protection status and rarity, and/or commercial value.
- The **obstructed cavities** are quantified by the number of cavities that are no longer accessible for mobile fauna.
- The **abrasion of the substrate** is the observation or not of a friction effect of the LFG on the substrate which would then damage the colonization.
- The **habitat creation** is when the LFG sustains an ecological role for the marine fauna such as nursery, hideout or pantry.

Tab. 1: Parameters for assessing the environmental impact (EI) of lost fishing gear.

Parameter	Assessment	Score
Colonization	Stage 0	0
	Stage 1	-2
	Stage 2	-4
	Stage 3	-6
Trapped mobile fauna	0	0
	1 to 5 individuals	2
	> 5 individuals	5
Removed fixed species	0	0
	1 to 10 individuals	1
	> 10 individuals	2
Damaged fixed species	0	0
	1 to 10 individuals	1
	> 10 individuals	2
Presence of outstanding species	Yes	0
	No	1
Obstructed cavities	0	0
	1 to 10 cavities	1
	> 10 cavities	2
Abrasion of the substrate	No	0
	Yes	1
Habitat creation	Yes	-2
	No	2
Total		-8 to 15

Parameters for seascape impact (SI) (Tab. 2). The impact is assessed according to five criteria, with ratings between -2 and 5. The sum of the scores of these criteria provide a basis for estimating the seascape impact of the LFG. The total of the scores range from 15 (very negative) to -2 (positive effect on the seascape). The "seascape alteration" criterion is based on the observation of a visual alteration of the seascape. For example, a large net laid flat on a seabed will profoundly alter the seascape, while on its own, a fishing line placed across a coralligenous habitat will not.

- The **distance of visibility** is the distance at which the LFG is visible.
- The **extent of impact** corresponds to the surface concerned by the LFG (usually the surface area occupied by the gear on the bottom).
- The **seascape alteration** is the recognition or not that there is an alteration of the seascape.
- The **qualifying adjective** used to qualify the alteration of the seascape could be neutral, positive or negative.
- The **relief** created by the LFG may or not alter the natural relief of the site. For example, if the gear is lying on a rocky scree, it tends to detract from the relief, whereas if it is deployed in the water column, it enhances the relief.

Tab. 2: Parameters for assessing the seascape impact (SI) of lost fishing gear.

Parameter	Assessment	Score
Distance of visibility	< 1 m	1
	From 1 m to 5 m	2
	> 5 m	3
Extent of impact	< 5 m ²	1
	From 5 m ² to 20 m ²	3
	> 20 m ²	5
Seascape alteration	No	0
	Yes	3
Qualifying adjective	Neutral	0
	Negative	2
	Positive	-2
Relief	No alteration	0
	Diminution of the relief	2
	Enhancement of the relief	-2
Total		-2 to 15

Parameters for technical risk (TR) (Tab. 3). The technical risk is considered with regard to the diver's intervention or the technical equipment required for the removal of the LFG. The technical risk is composed of two criteria that alone can tip the decision on whether to remove the fishing gear.

- The **depth** of the LFG.
- Attachment of the LFG **to the bottom** is a criterion that modulates the time spent by divers on the bottom and the use or not of specific tools.

Tab. 3: Parameters for assessing the technical risk (TR) of removing the lost fishing gear.

Parameter	Assessment	Score
Depth	≤ 20 m	1
	20 to 50 m	2
	> 50 m	3
Attachment to the bottom	Relatively easy	0
	Difficult and time-consuming	2
Total		1 to 5

Removal Aid Index (RAI). The aim of this index is to assist managers in their decision-making for the removal of nets. The calculation is based on the use of the environmental impact (EI), seascape impact (SI) and the technical risk (TR):

$$RAI = \frac{EI + SI}{TR}$$

The RAI ranges from -10 to 30. The higher the value, the more it will be advisable to remove the LFG. The technical risk is placed in the denominator because the higher the risk, the less the removal is a priority. This means that fishing gear with a maximum environmental and landscape impact of 30, but with a maximum technical risk of 5, will never be given priority for removal. To interpret the RAI, we use 3 classes of decision:

$20 < \text{RAI} \leq 30$: the removal of the LFG is strongly recommended, priority 3;

$10 < \text{RAI} \leq 20$: the removal of the LFG is recommended, priority 2;

$0 < \text{RAI} \leq 10$: the removal of the LFG is not a priority, priority 1;

$-10 < \text{RAI} < 0$: The removal of the LFG is not recommended, priority 0.

Results

The database includes 1256 observations of LFG (121 manager observations, 111 citizen observations and 1024 scientific observations; Tab. 4). The number of scientific observations was high until 2015, because we began to collect scientific data from scientific campaigns such as the campaigns in the Mediterranean canyons Medseacan and Corseacan (900 observations), and we reviewed all available studies on habitat description and mapping (e.g. Houard *et al.*, 2012; Charbonnel *et al.*, 2013). The Ghost Med team currently includes 12 scientists, 7 managers and 70 citizen observers.

Tab. 4: Total number of observations collected in the database. Natura 2000: habitat studies of Natura 2000 sites conducted by coastal oceanography and marine environment offices; GIS Posidonie: observations collected in different GIS Posidonie studies; CG06: French local council of the Alpes-Maritimes, Ghost Med: present program on LFG.

Date	Total number of observations	Main sources	Number of citizen observations
2005	3	Natura 2000	1
2006	6	Natura 2000	1
2007	12	Natura 2000	1
2008	59	Natura 2000	1
2009	494	Medseacan	0
2010	440	Corseacan	5
2011	20	Natura 2000	2
2012	5	Natura 2000	3
2013	44	GIS Posidonie, CG06	2
2014	32	Ghost Med	20
2015	35	Ghost Med, FFESSM	13
2016	25	Ghost Med	16
2017	18	Ghost Med	7
2018	63	Ghost Med	39

Among the data, some relate to Mediterranean canyons and others to coastal habitats (356 observations). The main coastal habitat concerned for the database is the coralligenous habitat (Tab. 5). The damage to coralligenous habitats is much greater than for infralittoral rocky reefs. Cnidarians and bryozoans can be broken or injured by contact with nets. Among the 1256 listed LFG, 50 have been removed.

The use of the RAI in several cases led to contrasting results, depending on the situations. This index has the advantage of being objective and based on parameters that can be quickly assessed *in situ*. It is a tool proposed to managers in order to prioritize, or not, the removal of lost gear. Of the 10 cases investigated *in situ*, we obtained values ranging from -5 to 30. The weakest values concerned old lost nets that were completely colonized and/or on soft substrates, and so not obstructing cavities. The highest RAI value was

obtained for fishing gear lost on a shallow bottom and not attached to the rock; many species were trapped in the net and it had uprooted and damaged several species fixed on the bottom; it also obstructed numerous cavities and its movements abraded the substrate; this gear was not colonized and did not create any habitat; it therefore had a very strong negative environmental impact. From a seascape point of view, this LFG was extensive, visible and strongly altered the seascape. This net has a maximum RAI value (30), and its removal was therefore highly recommended because of its severe environmental and seascape impact and because the technical risk is limited. Even if the environmental impact of the deeper LFG, especially in the marine canyons, is often significant, their removal is not a priority, mainly because of the technical risk related to the depth.

Tab. 5: Number of observations of LFG according to habitats and the type of fishing gear.

Coastal habitat	Number of observations	Type of fishing gear	Number of LFG
Canyons	900	Fishing nets	393
Coralligenous	202	Longlines	315
Macroalgae-dominated infralittoral rocky reefs	79	Fishing tackle (lead, lure, fishing hook, etc.)	248
<i>Posidonia oceanica</i> meadow	28	Undetermined	230
Artificial reefs	18	Fishing lines	58
Wrecks	15	Traps	4
Sandy bottoms	9	Ropes	4
Coastal detrital bottoms	5	Trawl nets	4

Discussion and conclusions

The contribution of the citizen science is essential to have new reports of sightings when working on extensive geographical areas; however, this concerns mainly the sites most popular among scuba divers. This is why it is essential to collect information from environmental managers and scientific campaigns. In this context, collaboration with fishers is important for receiving early warning when they lose fishing gear. The coralligenous habitat is one of the coastal habitats most affected by LFG, and is very sensitive to their mechanical impact. The erect species are the most sensitive to the abrasion due to fishing nets and to their removal, due to the pressure exerted. The Ghost Med program enhances efforts to address and mitigate the incidence and impact of all kinds of lost or abandoned gear, to support decision-making for their removal and to adopt mechanisms to monitor and reduce discards. It could now be extended to cover the whole of the Mediterranean Sea.

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POSTERS

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CAP NEGRO-CAP SERRAT (TUNISIA, MEDITERRANEAN): A MARINE AREA WITH ENVIRONMENTAL VALUES OF NATIONAL AND REGIONAL INTEREST

Abstract

The marine habitats of the future Marine and Coastal Protected Area (MCPA) of Cap Negro-Cap Serrat were investigated during 2015 -2016 within the framework of the MedKeyHabitats project to elaborate maps of the main assemblages and bottom types between 0 and 50 m in order to provide the APAL with a tool to better manage the site. The habitats and species distribution were defined based on a geophysical survey and biological studies. A monitoring network of the coralligenous biocenoses was also set-up in the study area to evaluate and track their conservation status and detect changes associated to human pressures and natural processes. The main marine key habitats of conservation interest present in the area are Posidonia oceanica meadows and Coralligenous biocenosis hosting two main facies: Eunicella singularis - Flabellia petiolata and Eunicella cavolini - Leptogorgia sarmentosa. About 37% (17.89 km²) of the total surface area (47.79 km²) surveyed were covered by coralligenous biocenosis including coralligenous platforms, mosaic of coralligenous on rocky bottoms and bio-concretion assemblages. All priority habitats cover an area of almost 30 km². A total of 18 benthic species that are protected or considered as key species of priority habitats were found in the site. The exceptional extension of the Coralligenous Platforms is one of the main particularities of this area, which is characterized by strong currents that seem to play a key role in conditioning the environment: High presence of gorgonians; ripple marks on detritic bottoms; erosion at the base of the coralligenous platforms.

Key-words: Cartography, Monitoring network, Coralligenous platforms, priority habitats, Tunisia.

Introduction

The candidate Marine and Coastal Protected Area (MCPA) of Cap Negro-Cap Serrat is located in the North-West coast of Tunisia and ranges from the shoreline to the isobath of -100 m. This study is part of the "MedKeyHabitats" project, it aims at improving the knowledge about the distribution of key marine habitats and on seabed types in this area between 0 and -50 m.

Materials and methods

In 2015 and 2016, a geophysical survey, using a Side Scan Sonar (EdgeTech 4125) and a single beam echo-sounder (Odom Echotrack) was undertaken. It was supported by a biological survey (ground truthing) including an underwater video survey along transects (Mini ROV, towed camera and GoPro), macrobenthos sampling with Van Veen grab, scuba diving surveys and fish visual census. A monitoring network of the coralligenous assemblages was set-up according to Garrabou *et al.* (2014).

Results

The main marine key habitats of conservation interest present in the area are *Posidonia oceanica* meadows and Coralligenous biocenosis hosting mainly facies with *Eunicella singularis* and *Flabellia petiolata*; facies with *Eunicella cavolini* and *Leptogorgia sarmentosa*. About 37 % (17.89 km²) of the total surface area prospected (47.79 km²) are covered by coralligenous biocenosis including coralligenous platforms, mosaic of coralligenous on rocky bottoms and bio-concretion assemblages (Fig. 1). The priority habitats cover a surface area of almost 30 km². A total of 18 benthic species that are protected or considered as key species of priority habitats were found: six species listed in the Appendix II of the SPA / BD Protocol, eight species listed in the Appendix II of CITES; three key species of priority habitats and three species included in the Annex III of the SPA/ BD Protocol. Two species, considered very rare in the whole Mediterranean, have been observed for the first time along the North African coast: *Felimare gasconi* (Gastropoda, Nudibranchia) and *Cervera atlantica* (Cnidaria, Stolonifera). The cryptogenic coral *Oculina patagonica* (Cnidaria, Scleractinia), whose expansion is attributed to global warming, has been observed in three stations. The Sarato's goby *Gobius fallax*, found only in Central East Atlantic, North Mediterranean and Black Sea, was found in the area, which constitutes a first record of the species in the Southern Mediterranean.

The exceptional extension of Coralligenous Platforms is one of the main particularities of this area, which is characterized by strong currents that seem to play a key role in shaping the seabed and the environmental conditions.

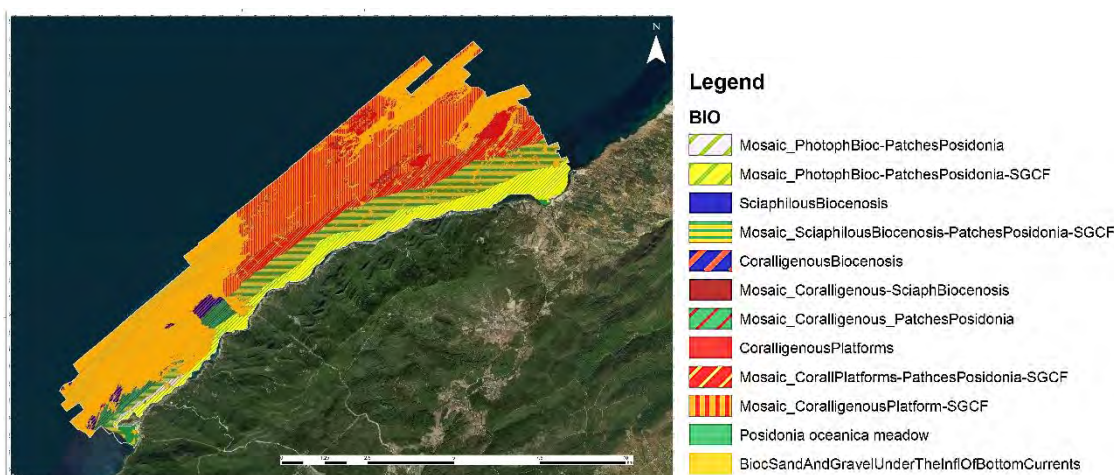


Fig. 1: The biocenotic map of the Cap Negro-Cap Serrat

Conclusion

The present study has widely contributed to fill the gap in the knowledge of the distribution of the most important benthic biocenoses in the Cap Negro-Cap Serrat MCPA. The techniques used have proven effective tools for habitat mapping and useful support for the implementation of protection and management measures in the area.

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SIZE DISTRIBUTION, DENSITY AND DISTURBANCE LEVEL ON THE GORGONIAN *EUNICELLA SINGULARIS* (ESPER, 1791) (CNIDARIA, OCTOCORALLIA) AROUND PALOMA ISLAND, ALGERIA (SOUTHWESTERN MEDITERRANEAN)

Abstract

Gorgonians (Cnidaria, Octocorallia) are emblematic species of sublittoral communities in the Mediterranean Sea. This taxonomic group play an important ecological role in structuring the habitats, especially in the highly diverse Mediterranean coralligenous outcrops. In the Western Mediterranean Sea Eunicella singularis is found at high densities on sublittoral rocky bottoms in shallow waters and on coralligenous formations in deeper sublittoral waters. In this study, we assess the size distribution, density and disturbance level of E. singularis. During late summer 2017, a population of E. singularis dwelling between 15 and 25 m depth was surveyed by SCUBA diving around Paloma Island (western of Algerian coast). Density ranged between 44.5 and 117 gorgonians per m² and mean colony height was 16.2 cm. The size distributions indicated the prevalence of the smaller height classes (<20 cm). The mean extent of injury was 23.1%. The size structure indicates a high level of recruitment, as well as low levels of impact suggesting a current favorable conservation status. This study provides a first baseline for the monitoring of Algerian coralligenous habitats through the survey of gorgonians populations.

Key-words: Demography, disturbance level, Gorgonian, Paloma Island.

Introduction

In the Western Mediterranean Sea, *Eunicella. singularis* is a common species on Mediterranean sublittoral rocky bottoms in shallow waters (Carpine and Grasshoff 1975). Gorgonians have been affected by mass mortality events and other disturbances during the last two decades in several locations in the Mediterranean Sea (Garrabou et al. 2009). This study provides the first analysis on the demographic characteristics and disturbance levels of *E. singularis* in the Algerian coast. The study quantifies density, size structure and injuries of the populations of *E. singularis* at Paloma Island in Western Algerian coast.

Materials and methods

Paloma Island lies in the Western Coast of Algeria (35°46'14.44"N, 0°54'3.47"W) in the Southwestern of the Mediterranean basin. Two bathymetric levels dominated by coralligenous habitat were selected (15 and 25 m) to carry out a quantitative demographic study and disturbance impact level. Six stations separated 500 m around Paloma Island were surveyed. At each site and depth range, 24 quadrats (50 x 50 cm) were placed randomly within the *E. singularis* populations by scuba divers between October and December 2017. The density, colony height (cm), injury extent (% of injury extent) and type of injury were chosen as the main descriptors.

Results

A total of 6 stations were prospected and 917 colonies of *Eunicella singularis* examined. Density of *E. singularis* ranged between 44.5 and 117.0 gorgonians per m², with a mean density of 76.42 ± 28.97 cm (mean $\pm$ SD) per m². The density did not exhibit significant differences between both depths (t-test $t = -0.509$, $p = 0.613$). Mean colony height was 16.2 ± 7.6 cm (mean $\pm$ SD), while the maximum recorded height was 42 cm. The populations dwelling in deeper waters displayed significantly larger mean colony height than shallower populations (t-test, $t = 6.68$, $p < 0.001$). The size distributions of population of *Eunicella singularis* at Paloma Island is unimodal and exhibited a significant positively skewed indicating the prevalence of the smaller height classes in this population (< 20 cm). The colonies affected by total or partial mortality, (i.e. showing extent of injury > 10 % of denuded axes or axis with epibiosis) was on average 23.1 ± 5.83 % (mean $\pm$ SD) and did not exhibit significant differences between both depths (t-test, $t = 0.165$; $P = 0.869$). Moreover, 0.3 ± 0.8 % (mean $\pm$ SD) of the colonies affected were dead colonies, while the majority of the remaining colonies had less than 25% of the injured surface. Considering only the affected colonies, 41.4 ± 18.1 % (mean $\pm$ SD) of colonies showed an old epibiosis stage (type C), characterized by colonized algae, sponges, bryozoans and other calcareous organisms. Denuded axis (type A) and new epibiosis stage (type B) included overgrowth by pioneering species, filamentous algae and hydrozoans, showing respectively means of 23.9 ± 16.3 % and 35.0 ± 18.2 %.

Conclusions

The mean height and density of the *E. singularis* populations of Paloma Island are higher compared to other areas of the western Mediterranean that are close to our study area (Linares et al., 2008). The population was dominated by the first (0–10 cm) and second (10–20 cm) size classes. The larger proportion of small colonies observed in *E. singularis* suggests high recruitment rates (Linares et al. 2008), which is characteristic of a suitable habitat, thus indicating expanding populations (Grigg, 1975). The relatively low proportion of recently injured colonies suggests a low level of stress and a good conservation state of these populations.

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SHAPE ANALYSIS OF THE MEDITERRANEAN RED CORAL *CORALLIUM RUBRUM* (LINNAEUS, 1758): COLONIES HARVESTED FROM DIFFERENT HABITATS OF THE NORTH OF TUNISIAN COMMERCIAL BANK

Abstract

*While taxonomic studies focus on species, conservation focuses on populations, which are the main conservation unit. Conservation actions, therefore, should focus on the identification and management of local populations. The aim of this study is to use a mathematical description (elliptical Fourier analysis) of the shape of *Corallium rubrum* colonies in order to discriminate their populations in the northern coast of Tunisia. The shape analysis using the elliptic Fourier descriptors had shown that the colonies coming from different populations do not have significantly different shapes. Nevertheless, this structuring has been observed for populations of different depth strata in the Bizerte zone (Wilks $\lambda = 0$, $P < 0.0001$) so the depth can be a criterion for population morphological differentiation. A precise characterization of the shape, combined with demographic structure and genetic analysis, could give as useful tool for stock identification and help in the development of a management plan of this precious commercial species.*

Key-words: shape analysis, red coral *Corallium rubrum*, Tunisia, Habitats

Introduction

Corallium rubrum is currently considered as a threatened species, and has been included in several international conventions for the conservation of marine biodiversity. The objective of the present work is the discrimination of the red coral populations based in mathematical description (elliptical Fourier analysis) of the colonies' shape (Kuhl & Giardina 1982; Lestrel 1997; Stransky & MacLellan 2005)

Materials and methods

From April 2010 to September 2011, Tunisian coral fishermen collected 769 colonies of *C. rubrum* by SCUBA diving at a depth comprised between 55 and 114 m in 4 areas (Bizerte, Esquerquis, La Galite and Tabarka). The shape of each colony was assessed with elliptic Fourier analysis by using 83 harmonics (332 variables).

Results

The shape analysis using the elliptic Fourier descriptors had shown that the populations of red coral do not have a significant difference according to the shape of the colonies (Fig. 1).

Nevertheless, this structuring has been observed for populations of different depth strata in the Bizerte zone (Wilks $\lambda = 0$, $P < 0.0001$) (Fig. 2).

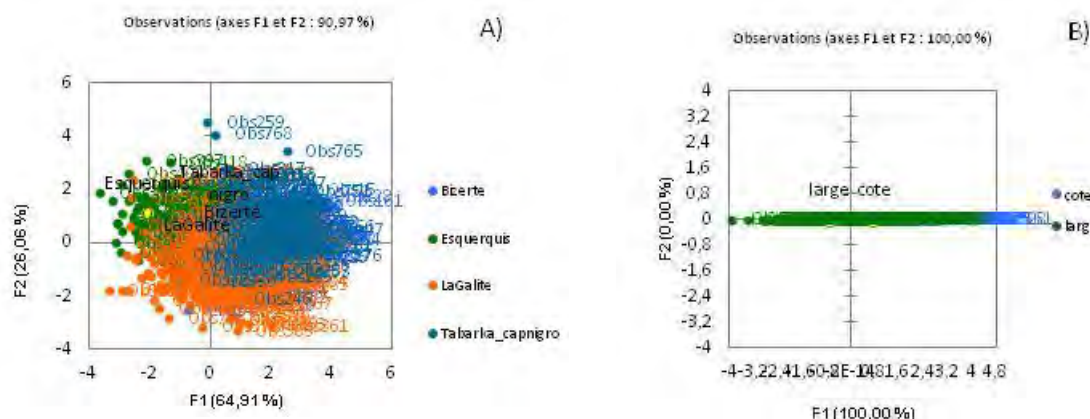


Fig. 1: Discriminant analysis (AFD) for A) the 4 areas (Bizerte, Esquerquis, La Galite and Tabarka-Cap Negro) B) for coastal and offshore

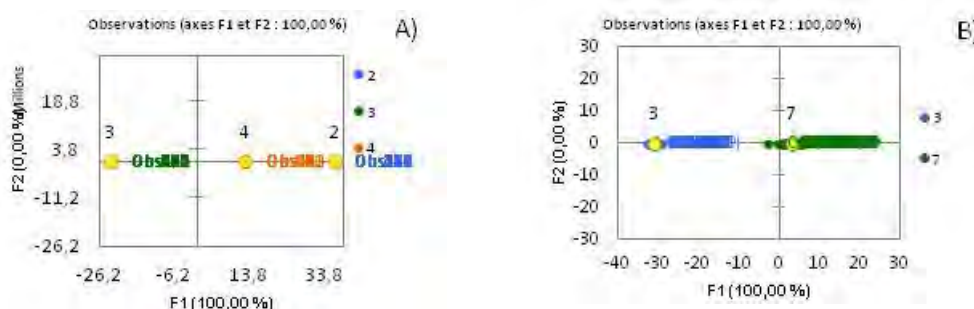


Fig. 2: Discriminant analysis (AFD) for A) Bizerte B) LaGalite with; strate 2 [50 ; 60]; strate 3 [60 ; 70]; strate 4 [70 ; 80]; strate 5 [80 ; 90]; strate 6 [90 ; 100] et strate 7 >100 m

Discussion and conclusions

Red coral populations can have different shapes regarding to depth. A precise characterization of the shape, combined with demographic structure and genetic analysis, could give as useful tool for stock identification and help in the development of a management plan of this precious commercial species.

Acknowledgments

This study was carried out in the framework of a cooperation agreement between stakeholders in the red coral fisheries: researchers, administration, and fishermen (UTAP, divers, traders, and ship-owners). A special thanks to all of them. We declare no conflicts of interest.

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PRESENCE OF TWO *PARAMURICEA MACROSPINA* DENSE POPULATIONS IN THE TYRRHENIAN SEA (MEDITERRANEAN SEA)

Abstract

Paramuricea macrospina (Koch, 1882) is a small endemic gorgonian of the Mediterranean Sea, dwelling on rocky bottoms and maërl beds between 40–2000 m depth. Although recent investigations have highlighted a wider distribution and abundance of this species, few data are available on its presence, ecology, biology and population structure. In fact, it is classified as data deficient in the IUCN red list. Moreover, in most of the localities where it has been recorded there is evidence of damage by fishing activities, which probably causes population decline. A multi-year Remoted Operated Vehicle (ROV) investigation, allowed the identification of two new sites characterized by large *P. macrospina* populations around Italy (Tuscany Archipelago and Nord-East Sicily). Density, habitat preference and ecological observations for this species are described, adding new knowledge on deep Mediterranean gorgonian species.

Key-words: Deep gorgonians; *Paramuricea macrospina*; Mediterranean Sea; ROV-imaging; Fishing impact.

Introduction

Paramuricea macrospina (Koch, 1882) is an endemic gorgonian of the Mediterranean Sea, dwelling on rocky bottoms and maërl beds between 40–2000 m depth (Hofrichter, 2004). *P. macrospina* grows in small colonies reaching the height of around 10-20 cm with thin and simple branching patterns (Carpine & Grasshoff, 1975). The colony color is very variable and different chromatic morphotypes have been described (Grinyó *et al.*, 2018). The presence of sparse colonies of this species is frequently recorded in many Mediterranean areas but very few studies are focusing on this species (Grinyó *et al.*, 2016). The present study aims to describe two dense populations of *P. macrospina* found along Italian coast at Panarea Island (Aeolian Archipelago) and Giglio Island (Tuscany Archipelago), providing information about the habitat and associated species.

Materials and methods

Data was collected during two ROV surveys onboard the R/V *Astrea* (ISPRA) carried out in January 2012 along Giglio Island (Tuscany Archipelago, Italy) and in 2017 around Aeolian archipelago (North East Sicily). HD video was analyzed and *P. macrospina* density (col. m⁻²) was estimated. Surface area was calculated using two laser pointers as reference for the track width and total track length was measured with the USBL System. Preliminary information about habitat and morphology of this species was also recorded.

Results

In the southern part of Giglio Island, *P. macrospina* forms a dense population (0.7 col m⁻², total area 600 m²), in rocky habitat along pinnacles in the depth range of 70-100 m. In this area the main associated species are *Eunicella cavolini* and the basket star

Astrospartus mediterraneus. In the same area the seafloor is characterized in the shallower part by coralligenous and by *Anthipatella subpinnata* forests in the deeper ones. *P. macrospina* in Panarea Island forms a dense population between 65 and 72 m depth, with a density, estimated within a total area of 454 m², of 1.2 colonies m⁻². In this area, *P. macrospina* is settled on coralligenous and rocky seafloor with evident leaks of fluids of volcanic origin. In absence of these leaks, the rocky seafloor is covered by a thick layer of algae and hydroids, while the most abundant specimens belong to Porifera, Bryozoans, and Tunicates. In both areas, no necrosis or damaged colonies were observed. *Filograna implexa* is the most frequent epibiont but sometimes bryozoans grow attached to the main axis. Dominant chromatic morphotype at Giglio Island is the light purple form while in Panarea Island it is the yellow form.

Discussion and conclusions

The knowledge on *P. macrospina* and its ecology is really scarce. For this reason, the species is classified as DD (Data Deficient) by the Mediterranean IUCN Red List of Anthozoans (Otero *et al.*, 2017). *P. macrospina* is a species with a wide distribution, but very little information is available on the presence of its populations. Ecological information on the *P. macrospina* populations is limited to the Balearic Islands. In the Menorca Channel, the density at the same depth range (60-100 m) is higher compared to the ones observed in this study, and the two chromatic morphotypes (yellow and light purple) of the continental shelf grow on maërl beds and not on rocky substrate (Grinyó *et al.*, 2016, 2018). The two investigated areas show similar density values but different chromatic morphotypes, and both are associated to hard bottoms and coralligenous assemblages. In the new Italian sites signs of high anthropogenic impacts (lost fishing gears) were recorded, but there was not evident damage (entanglement and partial or total necrosis) on *P. macrospina* colonies. However, being this species of small size and fragile consistency, it cannot be excluded that the impact of fishing gear could cause its complete removal.

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DEEP POPULATION OF GIANT RED GORGONIAN (*PARAMURICEA CLAVATA*): A NATURAL HERITAGE TO KNOW AND PROTECT

Abstract

*Along the "Côte Bleue" (West of Marseille, France), a remarkable population of the red gorgonian *P. clavata* was discovered on a large rocky bank between 50 m and 60 m depth. High densities (20-25 colonies m⁻²) and giant colonies up to human size (height 1.5 m - 1.8 m) characterise this population. The origin of the gigantism is currently unknown. Two non-exclusive hypotheses were formulated to explain the gigantism: (i) an adaptation to specific local environmental conditions, such as, for example, trophic richness, constant water temperature, favourable hydrodynamism; (ii) the existence of deep populations, genetically separated from those inhabiting shallower areas. Unfortunately, a strong (and increasing) fishing pressure was observed in the area as well. Conservation measures should be considered to preserve this natural heritage.*

Key-words: *Paramuricea clavata*, environmental adaptation, gigantism, phenotype, Climate Change

Introduction

The red gorgonian *Paramuricea clavata* is a key species structuring rocky bottoms in the Mediterranean Sea. This long-lived species is particularly threatened by human activities (e.g. fishing, anchoring, SCUBA diving) and by mass mortalities linked to environmental climate change. In the Natura 2000 site of the "Côte Bleue", between 50 and 60 m depth, a large rocky bank named 'Katchoffs' shelters remarkable assemblages of gorgonians, dominated by *P. clavata* (Astruch et al., 2011). High density and giant colonies characterise this *P. clavata* population. Recent explorations contributed to the assessment of (i) the extent of these populations and their characteristics (size and density of colonies) and (ii) human impacts. The origin of this gigantism and conservation issues are discussed.

Material and methods

The 'Katchoffs' bank is located 2-3 NM offshore the "Côte Bleue" (West of Marseille, France) and it covers about 2300 ha. Three zones (namely Western, Central and Eastern) were investigated in summer 2016 and 2017, between 50 m and 60 m depth. The area is characterized by the intrusion of the Rhône river plume and by recurrent up-wellings (Frayse, 2014). Investigations were carried out by SCUBA diving and remote operated vehicle (ROV) operated from the surface along transects (5 dives + 3 camera explorations). Divers measured *in situ* the height and density of all gorgonian species found in 3 quadrats of 1 m² surface per transect.

Results

Paramuricea clavata dominates all the investigated areas, mixed with other gorgonians (*Eunicella cavolini*, *E. singularis*, *E. verrucosa*, *Leptogorgia sarmentosa* and *Corallium rubrum*). Giant colonies are mainly located in the Eastern rocky bank, between 50 and 55 m depth.

The tallest colonies measured 1.50 m to 1.80 m (Fig. 1) and may attain an age of up to 100 years (Mistri & Ceccherelli, 1994). Density was about 20-25 colonies m⁻². Fishing lines were entangled in gorgonians and many broken giant colonies were observed. In the Central zone, the density of *P. clavata* was similar, but colonies were smaller (1 to 1.5 m). In this zone the presence of an abandoned trawling net was observed. Finally, at the Western zone showed sparse small colonies of *P. clavata* on the rocky bank, and the presence of *L. sarmentosa* and big colonies of *Eunicella verrucosa* > 60 cm) on the sandy bottom.



Fig. 1. Illustration of the corresponding authors with a broken giant *Paramuricea clavata* found at 'Katchoffs' bank Côte Bleue Nature 2000 site (Marseilles, France)

Discussion

Paramuricea clavata populations of the 'Katchoffs' bank showed a rare case of gigantism. Two non-exclusive hypotheses were formulated to explain this phenomenon: (i) an adaptation to local environmental conditions (e.g. organic matter supply, constant water temperature, favourable hydrodynamism), and (ii) the existence of deep populations genetically separated from those inhabiting shallower areas. Genetic approaches are intended to be used in the future to investigate the origin of the gigantism. Abandoned fishing gears, as well as an estimated daily attendance of 209 recreational fishing boats (50/day on average) (year 2015; Charbonnel et al., 2017), prove a strong pressure from both professional and recreational activities over this bank. The conservation of this unique natural heritage is threatened by human activities, thus to manage its protection should be strongly advised. For this purpose, forthcoming investigations will provide precise mapping and a demographic analysis of the population of giant *P. clavata*. In parallel, consultations with the stakeholders are imperative in order to manage fishing activities and minimize their impact.

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TEMPORARY PATTERNS OF THE CORALLIGENOUS HABITAT OF THE MARINE AREA OF JBEL MOUSSA (SOUTHWEST MEDITERRANEAN)

Abstract

*The Strait of Gibraltar is considered as a vulnerable biodiversity 'hot spot'. In the marine area of Jbel Moussa, between Belyounech Bay and Tanger-Med harbour, an important shallow population of the red coral (*Corallium rubrum*) was reported in 2014. In 2015, 9 monitoring stations were placed within 3 sites to characterize the coralligenous and the colonization by exotic/invasive macroalgae species *Rugulopteryx okamurae* in this locality. Demographic results and the shallow location of these rich coralligenous communities indicate the effective protection (military in this case) for the conservation of endangered red coral populations. Nonetheless, during the 2015-2017 monitoring period, a regression in the conservation status of the coralligenous habitats highlights the urgent need to promote administrative initiatives and actions to implement protection measures of these rich habitats and endangered populations of the red coral.*

Key-words: Biological Indicators; Endangered Cnidarians; Invasive Macroalgae; Benthic Community; Species Monitoring

Introduction

In 2014, during a sampling program performed within the framework of the MedKeyHabitats Project, led by the UNEP/MAP-SPA/RAC, in the marine area of Jbel Moussa (Morocco), an important shallow population of the red coral (*Corallium rubrum*) was reported. This area had been designated a Site of Ecological and Biological Interest by the Moroccan Authorities (PDAPM 1996) and proposed as a future Specially Protected Area of Mediterranean Importance (PNUE-PAM-CAR/ASP 2016).

Materials and methods

In September 2015, three monitoring stations, each composed by four 1 x 1 m fixed quadrats on vertical rocky surfaces at 20 metres depth, were placed within three different sites of the marine area of Jbel Moussa (Fig. 1, up). Sites were sampled in September of the years 2015 and 2017. Sessile bioindicator species and exotic macroalgae coverage data was obtained from stations using photographic methodology (see García-Gómez 2015). A PERMANOVA was carried out on a resemblance matrix based on Bray-Curtis similarities testing factor Year (levels: 2015 and 2017) using Primer-E +PERMANOVA.

Results

Belyounech site was not included in the analyses because this station was not found and had to be re-installed in 2017. For Oued El Marsa and Perejil, sessile coralligenous community varied among years ($G1 = 1$, Pseudo- $F_{1,12} = 3.42$, $p < 0.05$ – see Fig. 1, down).

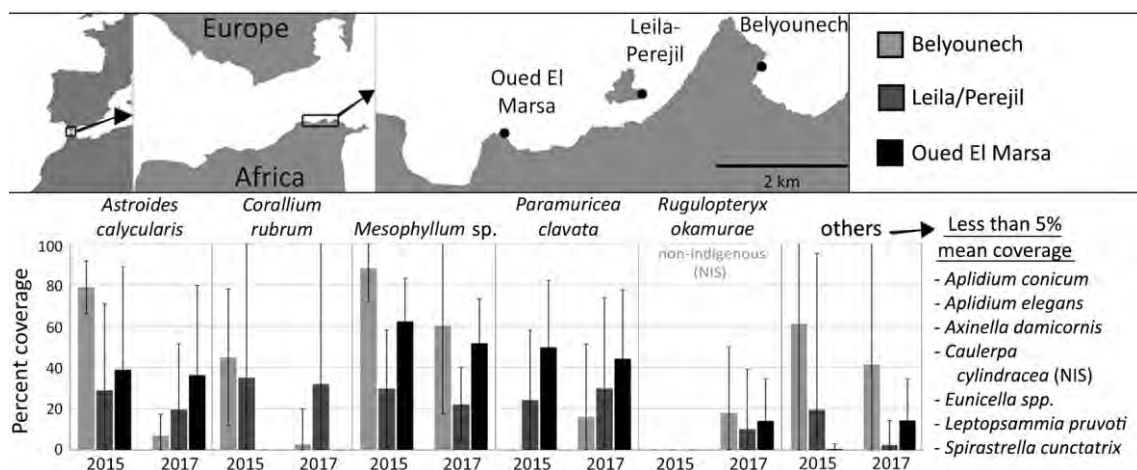


Fig. 1: Up: Belyounech (light grey), Leila/Perejil (grey) and Oued El Marsa (black) sites in the marine area of Jbel Moussa (Strait of Gibraltar). Down: Mean and Standard Deviation of bio-indicator and NIS species coverage in each site for years 2015 and 2017.

Discussion and conclusions

The coralligenous is one of the most diverse benthic habitats of the Mediterranean Sea (Boudouresque 2004). Temporal changes in the coralligenous in the monitored sites in the marine area of Jbel Musa occurred only in two years affecting most bioindicators used. These changes were concomitant with the massive growth of Non Indigenous Species (NIS) *Rugulopteryx okamurae* (García-Gómez *et al.* 2018). Accordingly, the area of Jbel Moussa should be further monitored and protected due the actual regression of this habitat in the Mediterranean Sea (Boudouresque 2004).

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TRANSPLANTATION OF YELLOW GORGONIAN FRAGMENTS FROM A HIGHLY DISTURBED AREA

Abstract

Recently, a significant decline of benthic suspension feeders occurred in the north eastern Sea of Marmara (Prince Islands), following excessive sedimentation of anthropogenic origin. The most important source of sedimentation was the construction and land filling operations held at Yassiada Island where a population of the yellow gorgonian, Eunicella cavolini, was significantly affected with a mortality rate of 88 %. In 2019, similar construction project is known to begin at the neighbouring island, Sivriada, where the yellow gorgonian population was affected with a mortality rate of 37 %. The relatively lower mortality is due to the location of the colonies on walls, e.g. vertical substrate. However higher mortality can be expected in 2019 since the operations will be held much closer to the colonies. In order to preserve the yellow gorgonian population in the area, a transplantation project was initiated. Coral fragments from 90 colonies were transplanted to an uninhabited island (Balıkçı) 13 km SSE, in order to test the feasibility of the transplantation technique in the area. 10 cm fragments that were cut from colonies in Sivriada were put in jars with surrounding seawater and kept in coolers until Balıkçı Island. Colonies were then fixed with two-component epoxy on a vertical wall where unaffected but scarce yellow gorgonian colonies were already present. After 13 months, most transplanted fragments were still alive and showed high growth in length. Higher numbers of colonies are planned to be transplanted before the construction starts in Sivriada.

Key-words: Gorgonians, *Eunicella cavolini*, transplantation, Sea of Marmara, disturbance

Introduction

Octocoral assemblages of soft corals and gorgonians are present in Prince Islands region, north eastern Sea of Marmara, despite high anthropogenic pressures (Topçu & Öztürk 2015), but were severely affected by a mortality event in 2015, following excessive sedimentation of anthropogenic source (Topçu *et al.* in press). The most important source of sedimentation was the construction and land filling operations held at Yassiada Island but another source was the dumping of polluted dredged sediments during the rehabilitation of Kurbağalıdere stream. Most octocorals died directly by clogging of feeding apparatus due to sedimentation, but also due to infections by *Vibrio* spp. and fungi as they became vulnerable to exploitation by opportunistic microorganisms (Topçu *et al.* in press). Dense assemblages of yellow gorgonians in Yassiada were almost entirely lost, while those present at the neighbouring island, Sivriada were less affected due their location on walls. However, similar construction works will start at Sivriada in 2019 and higher mortalities can be expected. Transplantation of gorgonians has been previously suggested as a successful restoration method (Linares *et al.* 2008). Here, we aim to test the feasibility of gorgonian fragment transplantation in the region for further broad-scale restoration purpose.

Materials and methods

Sampling took place at Sivriada (Fig.1) by scuba diving, between 22-30 m depth, in September-October 2017. Fragments of approximately 10 cm in length were cut from gorgonian colonies and placed in jars filled with seawater. Jars were kept in coolers until Balıkçı Island. Fragments were then fixed with two-component epoxy on a vertical wall where 3 transects of 1 m x 1m were previously placed (30 fragments/transect). Transplanted colonies were numbered, mapped and their exact length was measured. Transects were monitored every 2 months for a general check and growth of colonies was measured one year after transplantation date.

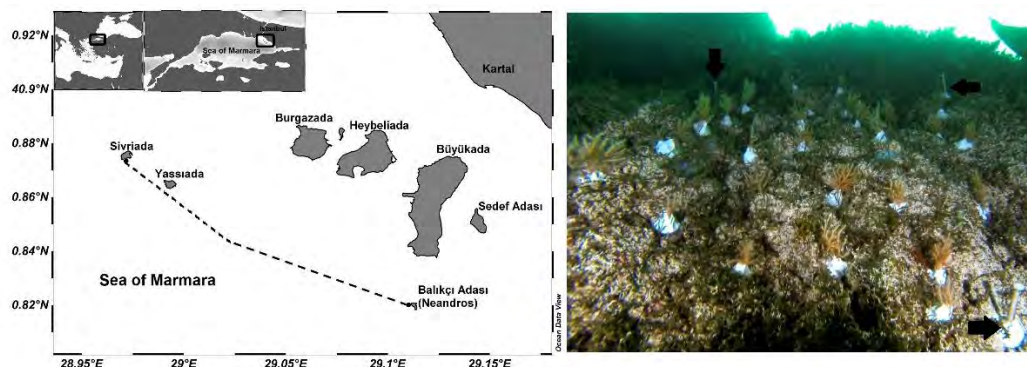


Fig. 1. Study area and transplants. Corners of the transect are shown with black arrows.

Results

Six fragments were found dead one year after transplantation, presenting a survival rate of 93 %. Annual growth in size of colonies was fast, with a mean of 2.4 cm year^{-1} ($\pm 0.97 \text{ cm}$; $N=90$).

Discussion and Conclusions

Transplanted fragments showed high survival and the technique seems to be useful for further broad scale restoration. More fragments will be transplanted from Sivriada where mortality rates will also be monitored during constructions. The Sea of Marmara seems to support well the growth of transplants with year-round $14-15^{\circ}\text{C}$ temperature and mesotrophic to eutrophic waters. However, fragments are threatened by fishing nets.

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SOME PECULIARITIES OF THE HEXACORALLIA ASSEMBLAGES ALONG THE MONTENEGRIN COAST

Abstract

In order to facilitate the creation of future MPAs, about 36 km² of seabottom were surveyed in the Montenegrin coast. This communication aims to provide a preliminary description of specific Hexacorallia assemblages found in Montenegro and discuss possible further actions to improve their knowledge. The main Hexacorallia assemblages found were: 1) Cladocora caespitosa, living colonies and huge dead colonies, along with large-sized sponges and cnidarians. 2) Savalia savaglia facies with high density of colonies dwelling in shallow water (12-25 m) within Boka Kotorska Bay, a unique assemblage at Mediterranean level. Savalia savaglia colonizes the bottom in two small spots of a specific habitat characterized by "vrulja" (submarine spring of fresh water). 3) Extraordinary large madreporaria colonies of Phyllangia americana mouchezii and Polycyathus muelleriae in the vertical walls of a semi-obscure cave, associated with three other madreporaria species and sponges. These peculiarities of the Hexacorallia assemblages along the Montenegrin coast are considered key habitats that are very rare and vulnerable, but they are also providing an important role for the ecosystem functions and biodiversity in general. Because of their rarity and their key role these assemblages should be further investigated, monitored and protected.

Key-words: Adriatic Sea, MPA, Hexacorallia, priority habitats, Montenegro.

Introduction

Unfortunately, Montenegro is one of the very few countries in the Mediterranean where Marine Protected Areas (MPAs) have not yet been established. International projects MedMPAnet (RAC/SPA, UNEP/MAP, 2013) and MedKeyHabitats (RAC/SPA, UNEP/MAP, 2016) were carried out in three areas along the Montenegrin coast in order to facilitate the creation of future MPAs: Inner part of the Boka Kotorska Bay (26.5 km²), Platamuni area (8.4 km²) and Cape Ratac (0.94 km²). In these three areas, mapping of marine habitats and creation of species preliminary check-lists were carried out by performing geophysical and biological surveys. Some peculiarities rise from these studies, especially about Hexacorallia assemblages and this communication aims to provide a preliminary description of these specific coral assemblages and discuss possible further actions in order to improve the knowledge on these key habitats.

Materials and methods

A total of 20 scuba diving surveys were performed (in Boka Kotorska Bay and Krekavica cave) and data about the species were recorded along 100 m transects by biologists and documented by professional photographers with high resolution images.

Results and discussion

In the inner part of the Boka Kotorska Bay two specific Hexacorallia assemblages stand out: One is formed by *Cladocora caespitosa* and the second by *Savalia savaglia*. Large

areas with living and dead colonies of *C. caespitosa*, were known from previous studies (Stjepčević & Parenzan 1980), but now they are precisely mapped. It is possible that *C. caespitosa* had its climax phase in the area hundreds or thousands of years ago and that currently it is in a recession phase, probably due to environmental changes (e.g. increase in temperature and in water turbidity). The *S. savaglia* facies in the habitat “vrulja” (submarine spring of freshwater) on two micro-locations, Dražin vrt and Sopot. Because of the specific ecological niche, this species creates very dense populations (average 2 colonies per m²) at 12-25 m depth and some colonies are even at 8 m depth, representing a unique assemblage at the Mediterranean level. The estimation of 300 existing colonies sums up in number to almost half of the whole Mediterranean known populations (Giusti et al. 2015, Trainito & Baldacconi, 2016). Furthermore, *C. caespitosa* and *S. savaglia* in the Boka Kotorska Bay are accompanied by large-sized sponges (*Axinella cannabina*) and cnidarians (*Leptogorgia sarmentosa* and *Parazoanthus axinellae*).

In the area of Platamuni large colonies (from few polyps up to 250 cm in diameter) of *Phyllangia americana mouchezii* and *Polycyathus muelleriae* are present in the walls of a large semi-obscure Krekavica cave. These colonies are associated with three other Madreporaria species (*Madracis pharensis*, *Leptopsammia pruvoti*, *Paracyathus pulchellus*) and several different sponges.

Having in mind rarity and vulnerability of these key habitats and their important role for the ecosystem functions, in the Krekavica cave a monitoring was set up as a baseline for further studies. Since anthropogenic impacts are very high in Boka Kotorska bay, similar monitoring should be implemented. Furthermore, in order to better understand the possible evolution of benthic communities in Boka Kotorska Bay in-depth study and datation of living and dead colonies should be carried out. Overall further studies would be required to support effective protection and conservation measures of these unique assemblages in the Montenegrin coast.

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RECOMMENDATIONS OF THE 3rd MEDITERRANEAN SYMPOSIUM ON THE CONSERVATION
OF CORALLIGENOUS & OTHER CALCAREOUS BIO-CONCRETIONS

1. Basic information on the geographic and bathymetric (present and past) distribution on coralligenous and other calcareous bio-concretions in the Mediterranean is still missing in most Mediterranean areas.
2. Coralligenous are being impacted by different sources of disturbances related to the warming, fishing activities and invasive species. More research effort is required to better understand the potential biological and environmental causes and their synergies
3. Extensive work needs to be done in terms of monitoring to assess the conservation status of coralligenous and other calcareous bio-concretion. New technological developments such as Structure from Motion photogrammetry can support the cost-effective characterization of coralligenous habitats and their key species
4. Protocols for ecological restoration actions for key species coralligenous species are available to support restoration of natural populations and ecological functions.
5. Citizen science can be an effective approach to conservation of coralligenous that has a great role in collecting data and reporting the impact of pressures on these formations as well as participating in restoration actions.
6. Collaboration schemes, including tools ranging from standardized protocols to information systems and participatory approaches, should be adopted to support the assessment of conservation status of coralligenous and other calcareous bioconcretions and propose effective management and conservation plans across the Mediterranean.

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