D 3.2: Criteria and protocols for restoration of shallow hard bottoms and mesophotic habitats

Marine Ecosystem Restoration in Changing European Seas MERCES

Grant agreement n. 689518

COORDINATOR: UNIVPM

LEAD BENEFICIARY: 13 - CoNISMa

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SUBMISSION DATE: 30/11/2017

DISSEMINATION LEVEL

PU	Public	**X**
CO	CO Confidential, only for members of the consortium (including the Commission Services)	

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1. Introduction

The main goal of Deliverable 3.2. is to provide the rationale and synthetize the main criteria for the elaboration of techniques for the restoration of shallow hard bottoms and mesophotic habitats. Besides general principles supporting the development of the proposed techniques in WP3, the guidelines were also intended to provide step by step indications to guide the application of proposed to techniques for future restoration actions. This document benefits from the results and experiences acquired during the implementation of experiments and actions planned within WP3 (between M1 and M18).

In WP3, as already stressed in previous deliverables, within the shallow hard bottoms and mesophotic habitats we focused on macroalgae and mesophotic coralligenous ones since i) there are increasing evidences of relevant changes and lost of these two habitats across the whole Europe and ii) they are featured by the presence of species of considered critical for the functioning of the coastal marine systems. In addition, these habitats display contrasted characteristics in terms of dominant groups (macroalgal species vs. macroinvertebrate), driving processes (trophic interactions-physical factors vs. competition), dynamics (fast vs. low turnover) and environmental conditions (shallow-light vs. deep-dim light habitats).

Macroalgal forests such as kelps and fucoids are dominant habitat-forming species in rocky intertidal and subtidal habitats around all the Mediterranean and Atlantic/Norway coasts. Macroalgal forests are recognized hot spot of diversity and provide food and habitat to diversified assemblages of understory species and enhance coastal primary productivity. Macroalgal forests can potentially thrive from the intertidal to the circalitoral, then depth can be considered a driver for algal development. Macroalgal forests are featured by different dominant species dwelling at each depth and generally, community structure (i.e. diversity and species richness) increases in complexity, and population and community dynamics (i.e. productivity, turnover and growth rates) slows with depth (e.g. Ballesteros, 1989; 1990; Ballesteros et al., 1998; 2009; Garrabou et al., 2009; Capdevila et al., 2015; 2016). As a response to multiple stressors, including urbanization, eutrophication and increasing sediment loads in coastal areas, these habitats (shallow and deep) are being lost at alarming rates and descriptive and manipulative experiments have demonstrated that these systems may switch towards the dominance of barrens or algal turfs if the canopy is removed or damaged (references in Ling et al., 2015).

Coralligenous outcrops are hard bottoms of biogenic origin that are mainly produced by the accumulation of calcareous encrusting algae growing at low irradiance levels. Coralligenous outcrops harbour approximately 10% of marine Mediterranean species, most of them are long-lived algae and sessile invertebrates, which exhibit low dynamics and belong to various taxonomic groups such as sponges, corals, bryozoans and tunicates (Ballesteros, 2006, Teixidó et al., 2011). This habitat is extended around all the Mediterranean coasts with a bathymetrical distribution ranging from 20 to 120 m depth depending on the local environmental variables, mainly light conditions (Ballesteros, 2006; Martin et al., 2014). Coralligenous assemblages are affected by several pressures such as nutrient enrichment, invasive species, increase of sedimentation, mechanical impacts, mainly from fishing activities, as well as climate

change (Ballesteros, 2006; Balata et al., 2007; Garrabou et al., 2009; Piazzi et al., 2012).

Very few evidence of natural recovery has been reported in macroalgal forests (Scheffer et al., 2001; Perkol-Finkel & Airoldi, 2010; Norderhaug & Christie, 2009), and for mesophotic coralligenous (Linares et al., 2010; 2012; Cupido et al., 2008; 2009) even when the area switches back to predisturbed conditions. Relict populations will finally disappear if effective restoration methods that promote their recovery will not be attempted together with stressor identification, mitigation or abolishment that should be integral part of any restoration plan (Orth et al., 2006).

Under these circumstances, human-induced recover can be of critical importance. Despite the increasing effort towards restoration, results highlight a high heterogeneity of criteria, targets and methods across habitats. By developing more standardized approaches for habitat restoration, synergy can be achieved through cooperation and cost-effective measures. This deliverable aims to fill some knowledge gaps for macroalgae and mesophotic coralligenous species by answering the following questions with concrete information and details: Which species? Where? How to restore? How to measure the success? These guidelines developed by the contribution of all the restoration scientists and practitioners involved in WP3 together with the experience matured during the restoration activities run in WP3 MERCES represent an excellent baseline to benefit future actions which could be also used in other ecological contexts. However, we emphasize that the criteria and protocols here developed cannot be considered conclusive. Additional knowledge will likely be needed to reduce the uncertainty related to restoration activities in the marine environment at present.

During the GA in Crete (June 2017) and following skypes meetings, two writing teams (one for each habitat) were identified, following past and successful experiences (see Deliverable 3.1): Macroalgal (Simonetta Fraschetti, Laura Tamburello, Loredana Papa, Giuseppe Guarnieri, Annalisa Falace CoNISMa, Emma Cebrian and Jana Verdura UdG-CSIC, Bernat Hereu UB, Camila With Fagerli NIVA) and Coralligenous (Joaquim Garrabou CSIC, Cristina Linares UB, Carlo Cerrano UNIVPM and Silivja Kipson PMF Zagreb). The writing teams were supported by the other participants in WP3. Besides a general introduction section, the document is organized in two main parts, one for each habitat.

2. General introduction for restoration actions on macroalgal / coralligenous habitats

2.1. Criteria for selection of target species

Key points

- Identification of habitat-forming species. Criteria should be based on ecological relevance (i.e. associated biodiversity, ecosystem functioning).
- Consideration on easiness of manipulation.
- Knowledge of life-history traits of target species.

A critical step in planning successful restoration actions is the selection of species deserving human intervention. The general criteria in the identification of target species should be based upon their ecologically relevance and status. Due the current

degradation of habitats, restoration actions on habitat forming species and/or on species with relevant ecological roles (foundation species, keystone species) may result in significant changes in the status of the involved species as well as in the structural complexity of the habitats. In fact, a successful restoration of habitat forming species combined with manipulation of other species (e.g. sea-urchins) while providing structurally complex and highly productive habitats, would hopefully support the recovery of the associated assemblages together with the ecosystem functions and services they provide.

Logistic considerations on accessibility or easiness to manipulate the target species should play a secondary role in the prioritization of restoration interventions. Species for which verified manipulation techniques are available should be selected first. If no specific information is available, we recommend, before starting, preliminary assessment at small scale, include removal experiments as well as implantation techniques.

Finally, an in-depth knowledge of ecology and life-history traits of target species is a key requisite for planning efficient restoration actions. This should include information on: i) life cycle (e.g. strategy and timing of reproduction, fertility, growth rate), ii) relevant ecological interactions with extant assemblages (e.g. competition, grazing, predation or susceptibility to pathogens), iii) environmental requirements (i.e. physical-chemical characteristics of the substratum and water column, disturbance regimes), iv) vulnerability to local anthropogenic stressors. All these requirements might vary at different life stages of the target species. As they could represent critical conditions or processes limiting the species survival, strategies to mitigate specific stressors during the restoration actions of target species can be implemented, thus optimizing restoration success. Unfortunately, in most cases, complete information is lacking. Thus, it is important to identify what are the most critical life-history traits, ecological interactions, environmental drivers and anthropogenic stressors affecting the survival of the selected species. Specific research projects should be developed to fill the identified gaps in view to ensure efficient restoration measures.

2.2. Restoration donor sites and specimens

- Selection of donor site - availability of target species and/or recruits.
- Genetic features.
- Assessment of exploitation impact on donor site.

The first criteria for the suitability of potential donor populations for restoration are mainly based upon the large availability of populations (e.g. extension, cover) of target species. Populations to be considered as donors should show a good conservation status implying a mature demographic structure and minor signs of disturbance. This will allow donor populations to rapidly recover from the removal of specimens devoted to restoration actions.

Secondly, donor populations should display levels of genetic variability allowing specimens selected for restoration actions to provide sufficient genetic variation to be able to adapt to environmental changes and avoid inbreeding. While the importance of genetic variability of donor populations has been investigated for seagrasses or saltmarsh plants (Procaccini & Piazzi, 2001; Oudot-Canaff et al., 2013; Evans et al.,

2017), studies for macroalgal and coralligenous species are still lacking. Likewise, bearing in mind that WP3 targeted habitat forming species (e.g. *Cystoseira* spp., *Corallium rubrum*) tend to be genetically differentiated at small spatial scale (i.e. 10 to 100s meters, Ledoux et al., 2010; Buonuomo et al., 2017) since they display very limited dispersal capacity (generally < 10 m), it cannot be discarded that donor populations can be locally adapted (Ledoux et al., 2015). Transplantation to other conditions can result in a reduced adaptive capacity of the restored population. Further investigations on this topic are advocated. However, it would be preferable to follow the precautionary principle of selecting multiple donor populations for restoration actions.

Finally, a crucial gap of knowledge in planning restoration programs is the evaluation of impact on donor populations due to sampling and manipulation. It is expected that thresholds exist for the maximum amount of specimens that could be extracted from donor populations before irreversible damage is caused. Dedicated studies could help identifying critical levels of density preceding the assemblage collapse, which are likely to be specific for each target species (Rindi et al., 2017). Benedetti-Cecchi et al. (2015) document a switch in *Cystoseira* between alternative states (turf vs *Cystoseira* canopy) with a loss of approximately 75% of the canopy biomass. In addition, appropriate sampling campaigns should be programmed to estimate the density and size structure of the donor populations before and during the restoration action, and, by comparing donor populations with unmanipulated populations of the target species, to quantify population size within the study period. Also, restoration techniques can be optimized to minimize the impact on donor population. For example, strategies applying fragments generated by natural disturbances have been suggested for seagrass restoration (Balestri et al., 2011). However, it should be stressed that techniques implying seedling or germling transplantation should be preferred against adult transplant.

2.3. Selection of restoration site

- Historical presence of target species. Availability of data from scientific literature, grey literature.
- Knowledge of stressors/causes of disappearance of target species and evaluation of actual mitigation/removal of anthropogenic stressors.
- Assessment of extant assemblage and identification of species, which could potentially influence the success of restoration (e.g. characterization of herbivore assemblage, biodisturbance, presence of invasive species).

Careful selection of restoration sites is an important step, and several conceptual models to optimize site selection have been developed (e.g., Calumpong & Fonseca, 2001; Campbell, 2002; Short et al., 2002). However, site selection requires additional insights into habitat requirements and characteristics of eligible habitats at local scale. In particular, an historical presence of the target species should be documented for an eligible restoration site. Although this is apparently trivial, availability of scientific records are generally scarce. Analyzing grey literature or local ecological knowledge approaches (e.g. photo and video reporter, fishermen, scientific researchers) may help filling this gap of knowledge.

In addition, a rational restoration planning requires identifying which factors caused and maintained the loss of the target species in a putative restoration site. It would likely be unsuccessful to attempt restoration actions in locations where stressors have not been mitigated or abolished. Although we apparently state the obvious, an in-depth, causal relationship between stressors and species loss is still lacking for macroalgal and coralligenous habitats targeted in WP3. Therefore, it is still a priority to develop studies aiming to identify the effects of anthropogenic stressors acting separately or in combination, their impact on different life-stages of target species and tolerance ranges to different stressors.

Beyond examining abiotic environmental conditions, a careful assessment of extant assemblages at the eligible restoration site is essential to identify species that could potentially limit the success of restoration. In general, the disappearance of habitat-forming species results in a less complex and productive assemblages, dominated by opportunistic or stress-tolerant taxa. The establishment of new feedback mechanisms in degraded assemblages contributes to their self-sustainment and prevents the natural recovery of habitats. Even when habitat-forming species are reintroduced, ecological interactions with extant species may play an influential role in determining the success of restoration action.

Finally, all restoration steps must be planned carefully before implementation. Protocols must be user-friendly, but volunteers and other potential stakeholder should never take initiatives without the appropriate scientific supervision of experts.

2.4. Restoration protocols (Techniques) tested within MERCES for macroalgal and coralligenous

3.1. Protocol M1. Adults transplanting in the fringe: *Cystoseira amentacea*

3.2. Protocol M2. *Cystoseira amentacea* adult transplanting

3.3. Protocol M3. Germling transplanting of *Cystoseira amentacea*

3.4. Protocol M4. Ex-situ seddling of *Cystoseira* species (e.g *C. barbata* and *C. crinita*)

3.5 Protocol M5. Transplant of adult kelp to restore a kelp forest patch on an urchin grazed barren ground: *L. hyperborea* and *S. latissima*

4.1. Protocol C1. Transplant of adult arborescent macroinvertebrates species

4.2. Protocol C2 Adult sponge transplants

4.3. Protocol C3. Macroinvertebrate recruitment enhancement techniques

3. Protocols for macroalgae and kelps

3.1. Protocol M1. Adults transplanting in the fringe: *Cystoseira amentacea*

The transplant of adult specimens of *Cystoseira* has already been tested (Falace et al. 2006; Sales et al., 2011), although never on a large spatial and temporal scale. For this scope, it is necessary to select one or possibly mutiple donor locations, characterized by assemblages dominated by dense canopy of the target species, and suitable restoration locations, represented by shores with sparse individuals or where the target species desappeared. Donor and recipient locations can be at a range of distances (from few kilometers apart to large distances). Within each location, the intervention will focus on multiple sites (approximately few 10s meters long and at a distance of 100s of meters apart from each other). In each recipient site, an appropriate number (at least 10) of 30 x 30 cm plots should be identified for transplant.
One crucial condition required to select a suitable restoration site is the historical presence of the target species and the effective mitigation of the stressors previously responsible of the disappearance of the target species.

3.2. Protocol M2. *Cystoseira amentacea* adult transplanting

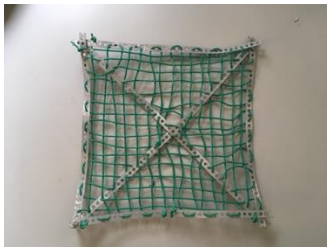
Materials

Cystoseira amentacea thalli, epoxy putty and plastic gloves, hammer and chisel, aluminium frames with PVC strings, screws, bolts, washers, underwater drill, fridges and ice blocks, metal fences.

Protocol/Methodology

Step 1.
Prepare the necessary material:

a) build aluminium frames. These are structures made by a 30 x 30 cm aluminum frame with PVC strings, which will ensure and facilitate the attachment of *C. amentacea* thalli.



b) screws, bolts, washers and underwater drill are necessary to fix aluminium frames on the substratum.

c) hammer and chisel, to remove *C. amentacea* thalli and to clean the surface at the recipient site.

d) epoxy putty and protective plastic gloves, to fix transplants on the substratum. Small quantities are needed to do the job.

e) fridges and ice blocks, to transport *C. amentacea* thalli from donor to the recipient site.

f) build metal fences. These are rectangular, parallelepiped structures (30 x 30 x 40-50cm), made by metal mesh and plastic tighteners.

Step 2.

In the donor sites, identify and mark with epoxy putty 30 x 30 cm plots in the middle of canopy beds. These will represent the reference conditions to evaluate transplant efficiency. Also, some plots will allow to tease apart the intrinsic impact of

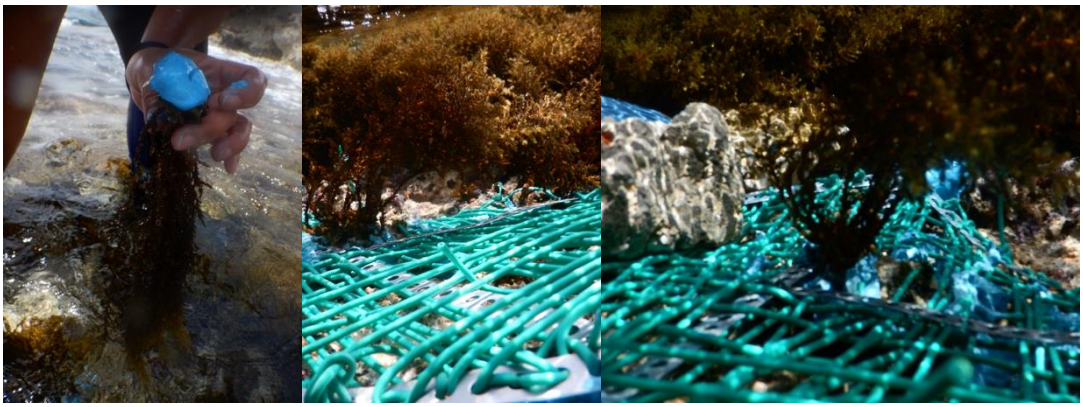
transplantation technique from the effects of local environmental conditions on the survival of transplanted specimens.

Step 3.
In restoration sites, identify and mark with epoxy putty 30 x 30 cm plots at an appropriate depth. Plots should be cleaned to bare rock with hammer and chisel. Using an underwater drill, aluminium frames need to be anchored to the substratum in recipient experimental units.

Step 4.
Before removing adults from the donor sites, it is necessary to evaluate the appropriate number of clumps necessary to reproduce, at the recipient sites and for the expected recipient units, a cover of *C. amentacea* similar to that observed in healthy assemblages. Approximately 13 clumps of *C. amentacea*, during its maximum vegetative period, are sufficient to cover a 30 x 30 cm surface.

In the donor locations, clumps of *C. amentacea* are removed with hammer and chisel, paying attention not to damage their basis. All removed individuals should be stored in cool conditions into fridges for transport to the recipient site.

Step 5.
Within the same day, clumps of *C. amentacea* should be glued to the substratum with portions of epoxy putty on the bases, fixing them below the PVS strings. Frames will facilitate the attachment phase.



Step 6.
To separate the intrinsic impact of transplantation technique from the effects of environmental conditions on the survival of transplanted specimens, transplantations are needed within and between donor sites. Thus, at least in one site for each donor location, characterized by healthy macroalgal canopy, it is necessary to clean additional quadrats. Specimens of *C. amentacea* are dislocated and relocated in the same position, to evaluate the impact of manual removal and handling; other specimens are translocated from one site to the other within the same location and further specimens are cross-transplanted between sites of different donor locations. A

comparable number of marked plots in each donor site will not be manipulated and will serve as controls.

Step 7.
All aluminium frames in donor and resipient locations can be removed after the hardening of the epoxy putty used to fix transplanted thalli of *C. amentacea.*

3.3. Protocol M3. Germling transplanting of *Cystoseira amentacea*

In the project MERCES, this technique has been used for *Cystoseira amentacea*. However, it can be adopted also for all species belonging to the genus of *Cystoseira*. Ex-situ seeding seems to be a feasible management option, providing a large number of healthy individuals to be re-introduced in the environment without impacting the natural populations (Falace et al., 2006; Sales et al., 2015; Verdura et al., 2015; Falace et al., in press).
To realize a restoration action on large spatial scales it is necessary to select multiple donor and recipient locations, eventually at a distance of few kilometers apart. Within each location, the intervention will focus on multiple sites (approximately few 10s meters long and at a distance of 100s of meters apart from each other). In each site an appropriate number of 30 x 30 cm plots should be selected.

Materials

Cystoseira amentacea fertile thalli, enclosure cages and cages with openings (made by metal mesh and metal wire), hammer and chisel, screws, bolts, washers, underwater drill, epoxy putty and gloves, scissors, aluminium foil, seawater-wetted towels, fridges and ice blocks, clay dishes, Stosch's enriched seawater (VSE), sea-water filters, autoclave, air pumps, aquaria, brush.

Protocol/Methodology

Step 1.
Several donor populations, characterized by dense *C. amentacea* cover, should be identified and monitored in order to detect the reproductive time of the year, when receptacles of the target species become available.

Step 2.

In the meantime, material and facilities at the recipient sites can be prepared.

Necessary material:

a) build double-mesh metal cages (to be used in those areas where herbivory has been found a relevant driver). These are 20 x 20 cm structures made with metal mesh and wire, which will protect *C. amentacea* from grazers. To estimate the efficacy of cages in reducing grazer impact and to assess an eventual artifact due to the presence of the cage, a certain number of cages have 3 x 4 cm openings on each side, in order to allow the access of herbivores.

b) screws, bolts, washers and underwater drill are necessary to fix cages on the substratum.

c) epoxy putty and protective plastic gloves, to seal cages to the substratum and to fix germling clays on the substratum.

d) hammer and chisel, to clean the surface where cages and germling clays will be fixed at the recipient site.

e) fridges and ice blocks, to transport mature apexes from donor sites to the laboratory and germling clays from the laboratory to the recipient site.

f) scissors to collect mature apexes.

g) aluminium foil and seawater-wetted towels to pack mature apexes for transport from donor sites to the laboratory.

h) lab: clay dishes, Stosch's enriched seawater (VSE), sea-water filters, autoclave, air pumps, aquaria, brush.

Step 3.
Prepare facilities at the recipient sites.

In each site, an appropriate number of 30 x 30 cm plots should be marked with epoxy putty and cleaned to bare rock with hammer and chisel. Plots should be provided with double metal mesh cages, which will protect germlings from grazing. Cages can be fixed to the substratum by screwing them with an underwater drill and sealing them with epoxy putty.

Also, to ensure juveniles protection from hydrodynamic disturbance and reduce desiccation stress, adult specimens of *C. amentacea* can be transplanted in recipient plots from healthy populations. This require arranging anchoring facilities (aluminium frames with PVC) into metal cages.

Step 4.
When *C. amentacea* fronds exhibite mature receptacles at donor sites, apexes need to be collected for fertilization and cultivation of germlings in the aquarium. Personal observations reports that from 200 fertile receptacles (mature apexes) are required to generate 400 adults, which are the number necessary to restore several square meters of rocky shore. 3-4 cm apexes can be cut with scissors. During harvesting, almost 3 fertile apexes should be collected from each individual, in order to ensure a minimum degree of genetic variability and to avoid compromising the reproductive capability of exploited individuals.

Step 5.
In the laboratory, apexes need to be checked for the presence of mature receptacles and packed in aluminum foil. During transportation to the nursery facility apexes wrapped with seawater-wetted towels should be kept in cool, humid and dark conditions. Transport should be completed within 48 hours from collection.


Step 6.

In the meantime, nursery facilities are appropriately set up. Temperature and photoperiod should be selected to reflect typical seasonal conditions in the donor site. Light irradiance (LED lamps) should be set at 100-125 µmol photons m−2s−1. The medium used for the culture should be Stosch's enriched seawater (VSE). The seawater has to be filtered and autoclaved prior to VSE addition. Aquaria filled with culture medium will be renewed every 3 days to minimize possible limiting effect of nutrients depletion and continuously aerated by air pumps.

Step 7.

Arriving at nursery facilites, fertile apices have to be gently cleaned with a brush and rinsed with sterile seawater, in order to remove the adhering biofouling and detritus on their surface. Then they are placed in the aquaria. 3 apices (randomly chosen among the total available) with mature receptacles are placed on each clay tile (ca. 4 cm diameter) to guarantee a wide coverage of settled germling. After 2-hour gametes are released and visible on substrata and the receptacles should be removed.

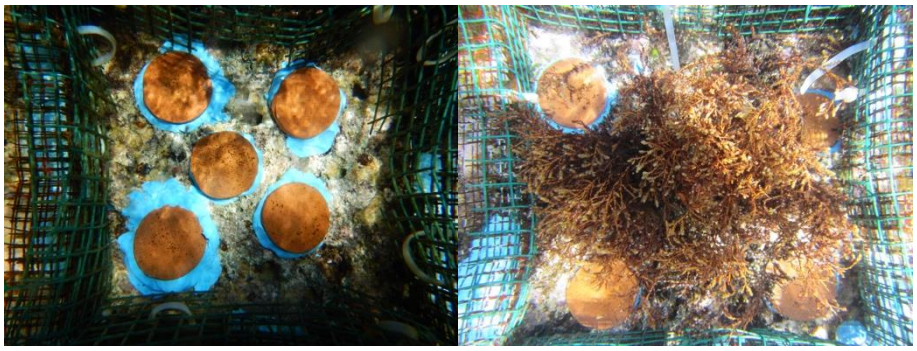
Cultured germlings could grow on small substrates (clay plates) at least for 4 weeks, after which they can be transported to the field to be attached.

Step 8.

During germling culturing, adult thalli of *C. amentacea* can be transplanted at recipient sites, according to the "*Cystoseira amentacea* adult transplanting protocol".

Step 9.

The transport of germlings from laboratory to the field should be carried out in cool and dark conditions. Once at destination, the attachment of clay dishes should take place rapidly, to avoid thermal stress of germlings. In each cage, five clay plates with germlings are fixed to the substratum with epoxy putty.



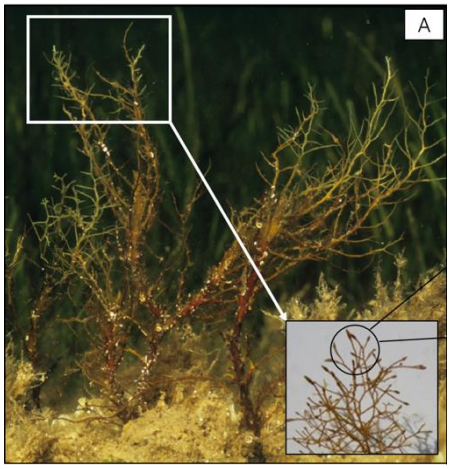
3.4. Protocol M4. *Ex-situ* seddling of *Cystoseira* species *(e.g C. barbata* and *C. crinita)*

Materials

Cystoseira sp. fertile thalli, hammer and chisel, mesh, tieds, scissors, cooler, zip lock plastic bags.

Step 1.

Collect fertile apical branches from the donor population 5 cm long with a scissors.



See the fertile apical branches from adult population.

Step 2.

Transport of the fertile branches should be done without water inside a plastic zip-lock bag and cold/fresh conditions.

Step 3.

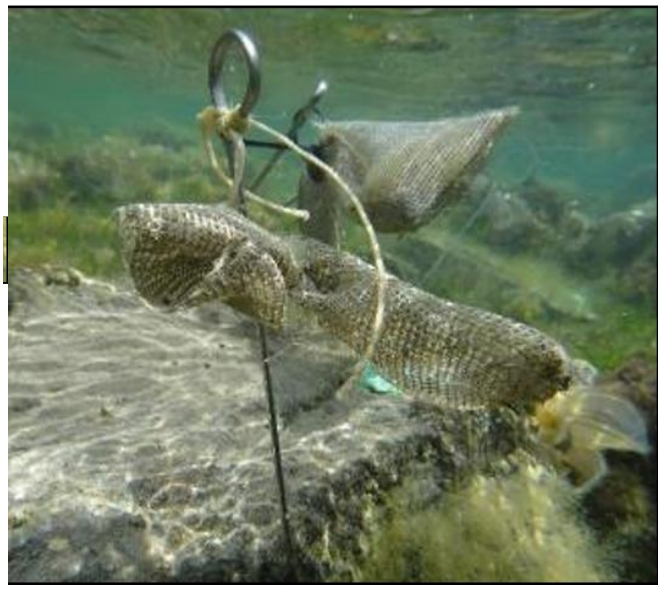
Fertile branches should be placed in CDAPs (see below the Container-Dispersor of Algal Propagules).

CDPA (Container-dispersor of algal propagulae)

Step 4.
The receiving area should be divided in several sites (from 25 - 30m) and 200m apart each other.

Step 5.
For each receiving site eight CDAPs containing fertile branches should be placed interspaced and separated by few meters (at least 2 meters apart, depending on the species).

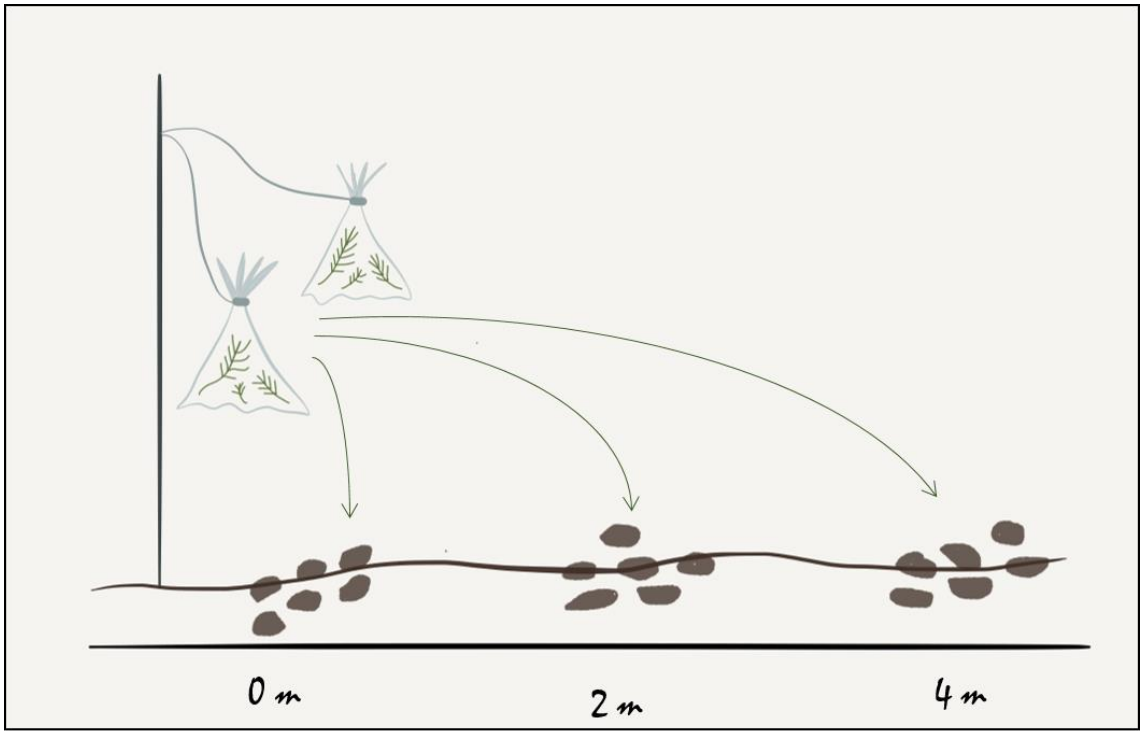
Step 6.
Each CDAP should be tied in a pick and directly fixed to the substratum using a hammer.



Detail of the CDPAs fixed in situ with the free substrate provided to promote *Cystoseira* recruitment.

Step 7.

Provide free substrate close to the CDPAs: flat stones with similar surface (aprox. 0.04 m^2) deprived of any meio- and macrobenthic organism should be placed close to the CDAPs to promote settlement of *Cystoseira*.



Scheme of the in situ seedling technique, with the CDPAs providing zygotes to the free substrate available.

Critical points

Generally, most active restoration actions in macroalgae cover a temporal interval of few months (from 6 to 12). Very few studies cover longer time scales. This can be extremely limiting as to assess recovery of ecosystem functioning and the outcome of

restoration (success or failure) the period of observation is extremely critical. In addition, most active interventions have been carried out at a spatial scale lower than few meters which is extremely unrealistic to match the scale of human disturbance. It has been demonstrated that restoration scale and feasibility are positively correlated in seagrass meadows (Katwijk et al., 2016), due to mechanisms that are likely relevant also for macroalgal forests. First, introduction of target species over larger extensions could spread the mortality risks due to stochastic effects of natural variability. Secondly, settlement of more specimens would provide a critical mass for stress amelioration by the starting founders, thus enhancing self-sustaining feedbacks that, in turn, would increase further population growth. However, further studies are required to identify the minimum spatial extension of intervention over which these mechanisms may become relevant and beneficial in macroalgal forests.

Since the reproductive capability of a species depends by several environmental conditions, zygotes/germlings availability could be extremely compromised. As demonstrated by Marion et al. (2010) seed production in donor beds can vary dramatically from year to year. Therefore, it is crucial to operate as far as possible during the short reproductive season of the selected species to collect an appropriate number of mature apexes. Their availability represents an intrisic limit of the restoration technique which cannot be repeated until the following reproductive period of the target species.

Furthermore, the transports between the laboratory and the field could pose risk to all life cycle steps of macroalgae. It is essential to ensure that the transport is carried out in dark and cool condition to minimize mortality. As conditions and duration of germlings transport represent a critical bottleneck for their survival, proximity of nursery structures to restoration sites can be critical. Likewise, in adult transplant experiments the proximity between donor and recipient sites may determine the feasibility of restoration intervention.

Finally, a further drawback to consider is the chance to lose an indefinite number of attached tiles in as occurred in our study in one of the most exposed site.

3.5 Protocol M5. Transplant of adult kelp to restore a kelp forest patch on an urchin grazed barren ground: *L. hyperborea* and *S. latissima*

Kelp is considered a foundation species that provides habitat and resources for numerous invertebrate and fish species (Christie et al., 2009). The transplant of two kelp species such as *Laminaria hyperborea* and *Saccharina latissima* can be tested on barren grounds overgrazed by sea urchins. As a result of warming sea temperatures due to climate change, in many areas the density of the cold-water urchin *Strongylocentrotus droebachiensis* has substantially declined during recent years (Fagerli et al. 2013). However, despite reduced grazing pressure, the overgrazed kelp forest has not recovered. Low recruitment success of kelps, due to either the low supply of kelp propagules or removal of seedlings by remaining urchins, may explain lack of kelp recovery. Transplant should be carried out at 5-7 m depth on a barren ground with low densities of sea urchins and moderate exposure. The site selected for restoration actions should be located within an area where the target kelp species earlier were naturally occurring. The sea depth selected for kelp transplantation should be similar to depths where naturally occurring kelp at the donor populations is densely distributed. Important physical properties of the restoration site should be evaluated prior to kelp transplantation. Key features that should be considered include:

- availability of rocky substrate for kelp attachment;
- hydrographic conditions (e.g. wave exposure) to ensure high water movement;
- sedimentation rate (low sediment loads are preferable);
- densities of sea urchins (low densities are preferable).

Collection procedure for kelp at the donor site

Materials

L. hyperborea kelp, tow-camera, knife, wet towels, tags, industrial chains, cable ties, polyethylene ropes

Protocol/Methodology

A tow-camera operated from a small boat can be used to identify suitable donor populations according to kelp density and biological condition of the kelp. It is preferable to perform collections and transplantation during early spring when the kelp fronds are healthy and clean as they tend to get grown with epiphytes during summer.

Step 1.

To collect kelp, a knife should be gently pressed under the kelp holdfast and slightly pushed from side to side until the entire plant can be detached from the substratum.

Step 2.

During boat transport to the transplant site the kelp should be kept moistened with sea water to prevent the kelp tissue from drying out and to increase the likelihood of survival of the associated flora and fauna. A simple method to keep kelp moist is to cover it with wet towels and regularly splash it with sea water from a bucket.

Immediately after arrival at the restoration site the collected kelp should be submerged in sea water until reattachment.

Step 3.
To increase the chance of restoration success and survival of transplanted kelp, the density of sea urchins in vicinity to the transplanted kelp should be reduced by manual removal. As an example, in MERCES approximately 500 sea urchins were removed when the kelp was deployed. The removal was repeated after four months during monitoring of the transplanted kelp.

Transplant of adult kelp: *L. hyperborea*

Step 1.
60 m^2 kelp forest patch can be created by transplanting 130 adult *L. hyperborea* to the selected barren site. To evaluate the transplantation technique, a sub-set of kelps collected from the donor populations has to be processed and transplanted back into the donor sites (using the identical procedure) to serve as procedural controls. To account for natural growth and mortality in the donor populations, 20 undisturbed individuals of *L. hyperborea* at the donor sites have to be tagged and measured.

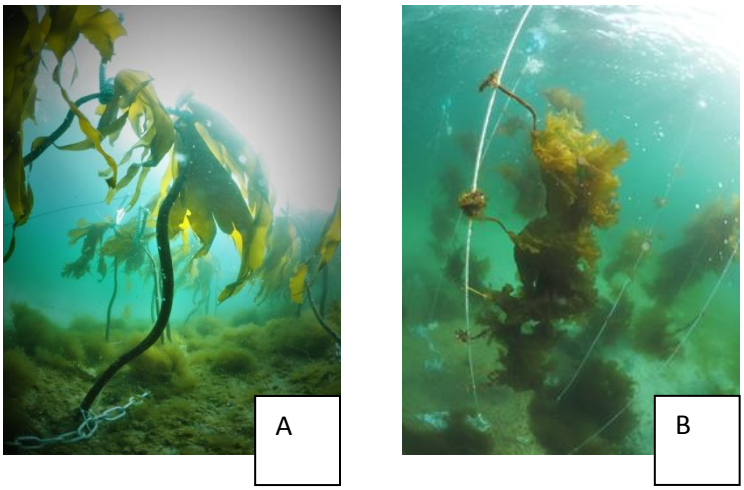
Step 2.
During transplantation, kelps should be attached to heavy weight that will remain relatively stable on the seafloor despite of wave action. In MERCES, *L. hyperborea* kelps were attached to heavy 5 m long industrial chains. Each individual kelp has to be attached to the chain by cable ties with a 50 cm maximum distance between each kelp. One cable tie is loosely fastened around the kelp stipe just above the holdfast, while two cable ties are threaded through the holdfast and attached to the industrial chain. The chain is stretched in a line along the sea floor and positioned so that it provided some support and stability for the attached kelp. A small float is attached to the upper part of the kelp stipe, just below the frond, to ensure the kelp remained upright.

Kelps has to be measured and tagged in order to monitor growth and survival.

Transplant of adult kelp: *S. latissima*
Based on differences in morphology and growth forms, different transplant set-ups has to be applied for *S. latissima. S. latissima*, which has a short and flexible stipe and a bulky lamina that rests on the sea floor, is more susceptible to herbivory compared to *L. hyperborea,* which has a longer and more rigid stipe. *S. latissima* has to be mounted on vertical ropes and suspended in the water column. In MERCES, a total of 42 kelps divided among 7 ropes were deployed at the transplant site. Two ropes were deployed as procedural controls at the donor site for evaluation of the transplant method.

Step 1.
During transplantation *S. latissima* kelps should be mounted to a 10-12 mm diameter polyethylene rope with twisted strands. Individual kelps should be fixed to the rope by threading the holdfast through the strands. In MERCES, six kelps were transplanted to each 4 m long rope and spaced approximately 40 cm apart. Ropes should be anchored to a heavy weight on the sea floor; industrial chains should be used. A float should be attached to the unanchored end of the rope to ensure a vertical position in the water column. Alternative cultivation and transplantation techniques are already developed

for *S. latissima* for commercial purpose and can be found in literature (see e.g. Forbord et al., 2012; Sandersen et al., 2012; Peteiro et al., 2013).



Transplant set-up for A) *L. hyperborea* and B) *S. latissima* kelps

Monitoring and maintenance

Transplanted kelp should be monitored systematically (minimum every 6-8 months) for survival and optionally for growth. To increase the chance for transplantation success, transplanted kelps and the floats should be checked and cleaned for algal overgrowth. Sea urchins should be removed from the vicinity of the transplanted kelp to reduce the grazing pressure on the transplanted kelp.

If successful, these transplanted kelps should reduce sea urchin densities naturally through physical abrasion and by lowering grazing intensity and natural urchin recruitment. Healthy kelps produce a large supply of spores, and the reduced water flow within artificial canopies should increase the retention of these propagules, increasing natural settlement and recruitment of kelps in nearby reefs.

4. Protocols for coralligenous

The life-history traits typically displayed by coralligenous species (slow growth rates, low recruitment rates and high mortality rates of recruits and juvenile colonies) point to the use of transplantation techniques, rather than recruitment-enhancing techniques, as the most appropriate and effective for habitat forming species in the coralligenous such species as gorgonians, sponges and some bryozoans. However, for some species such as the bryozoan *Pentapora fascialis* and probably other similar species, whose skeleton is very fragile for manipulation and obtaining fragments, recruitment enhancement techniques can be useful alternatives for restoring their populations. In this guidelines we provide two restoration protocols based on adult transplants and one in recruitment enhancement.

4.1. Protocol C1. Transplant of adult arborescent macroinvertebrates species

Material

Plastic bags and scissors, coolers, ice-packs, two-component epoxy putty, plastic gloves, knife, slate and pencil, underwater camera (e.g. GoPro)

Methodology

Step 1.

Prepare the material needed:

a) scissors to cut the transplants

b) zip-lock bags to store transplants and prepared epoxy putty

c) epoxy putty to fix transplants (e.g. Ivegor, Veneziani Subcoat S)

d) gloves to protect hands while mixing the epoxy putty

e) knife or a metal brush to clear the surface at the point of transplant attachment underwater

f) slate and pencil to draw the location and position of the transplants and to anotate their presence, their health status and size during subsequent surveys

g) alternatively, an underwater camera to film the area and build a photogrammetric reconstruction of the site

Material required for Protocol C1

Step 2.

Underwater, use scissors to collect 5-10 cm long apical fragments of mature, healthy donor specimen of selected species. As a reference, in gorgonians a colony is considered healthy when less than 10% of its surface presents necrosis and/or epibiosis. In the case of the red coral *Corallium rubrum* or the bryozoan *Myriapora truncata*, the fragments from colonies are broken by hands from colonies collected by illegal fishermen (in the case of red coral) or in both cases from colonies collected from the bottom.

Once back to surface and on board, the plastic bags should be placed in coolers for transportation to the restoration location. Use coolers with ice-packs if necessary to keep the temperature between 16 and 21ºC, or in any case limit the thermal-shock during the maintenance of the samples

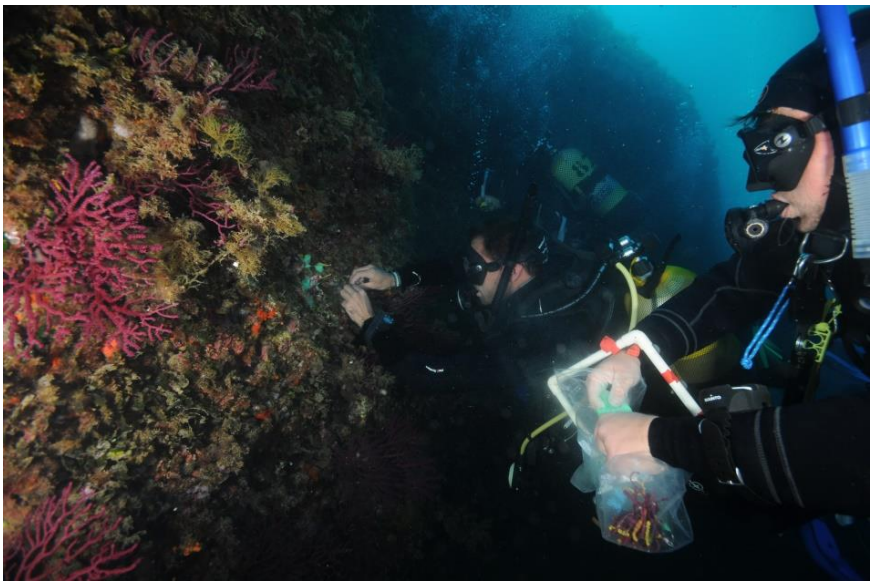
Step 3.

On board/land, put the plastic gloves on and prepare the epoxy putty by mixing equal parts of two components, following manufacturer's instructions. Should the resin tend to harden too quickly before the transplantation work has finished, a lower proportion of hardener might be used. However, less than 30-35% hardener component would usually translate in insufficient hardening when deployed with transplants. Store it in the wet zip-lock bag that you will take underwater.The epoxy putty will serve as a glue to attach transplants to substrata.

Step 4.

Again underwater, use a knife or metal brush to clear the surface where you plan to attach transplants and thus ensure better adherence to substrata. Ideally look for small natural holes and crevices and fix the base of the transplants with portions of prepared epoxy putty. Adjust your technique according to the species involved – e.g. gorgonians with thin scleraxis may firstly require placement of a fragment into a silicone tube filled with the epoxy putty and then fixation of the tube with the additional epoxy putty to the substrate (see Specific treatments section below). Attach fragments in small patches (0.2 - 1 m in diameter), separated by distances similar to the sizes of the transplant patches.

In other words, to set the spatialy arrangement transplants use small pvc quadrats (e.g. 20 x 20 cm). Within each quadrat place 6-8 transplants (which corresponds to natural density 50 colonies/m^2). Once you finish, move the quadrat 20-25 cm apart and repeat the operation (see Density of restoration patches and spatial arrangement section).



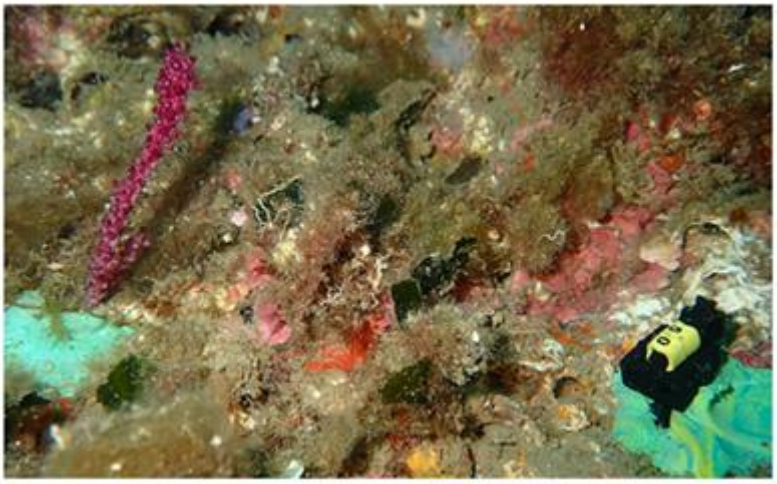
Step 5.

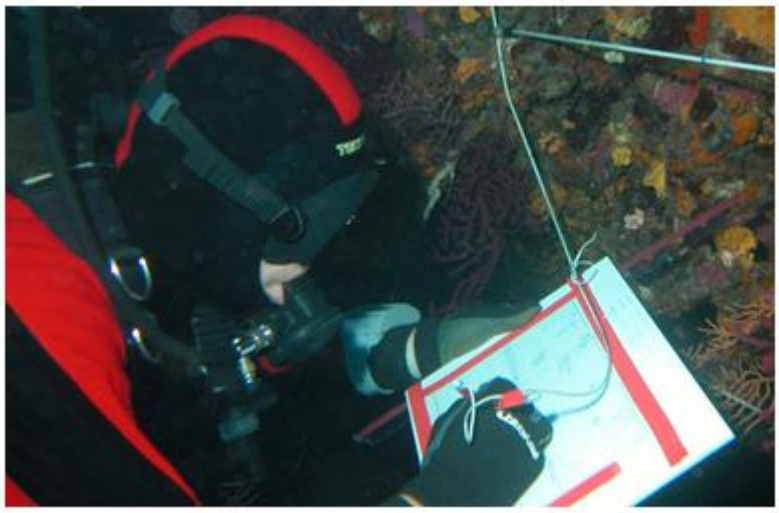
Ensure that epoxy putty and the transplant within are firmly attached to the substrate. After a while, transplants and/or other benthic organisms will overgrow the epoxy putty, blending it with the environment.

Although the use of epoxy at the first glance could seem to be toxic or aggressive from a visual point of view, gorgonians are able to overgrow the epoxy, covering the entire surface within one year hindering the recognition of transplanted colonies.

Step 6.

Using the same technique as for transplants attachment, place permanent marks (e.g. screw with plastic tags) to facilitate the mapping of transplants and the subsequent monitoring. Using the slate and pencil, now you can annotate the position and draw a map of your permanent marks and transplants. The maps will be used for the monitoring of the restoration actions (see Monitoring restoration section) and may also include information on the size of transplants.





To sum up:

Density of restoration patches and spatial arrangement

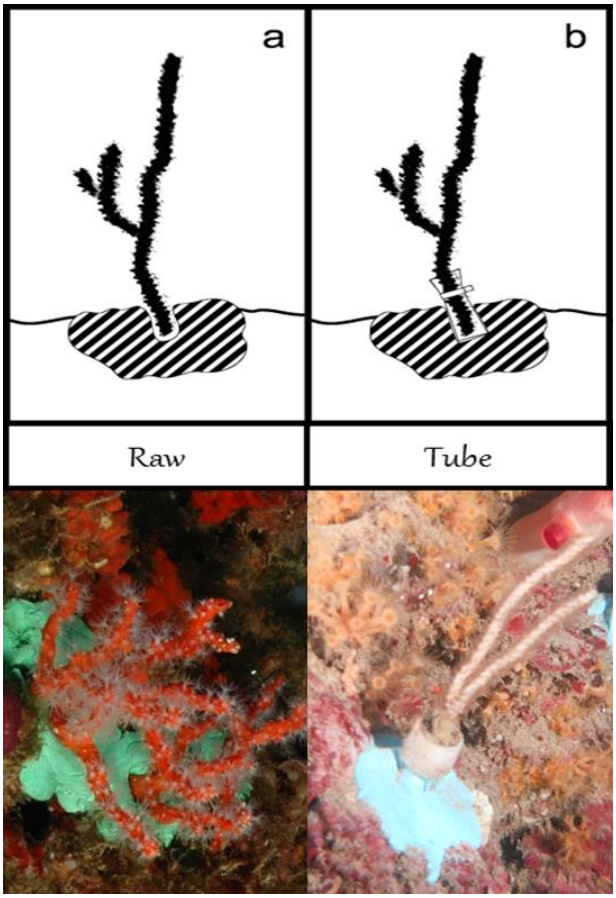
The spatial arrangements of transplants may include relatively small patches (0.2-1 m in diameter) separated by distances similar to the sizes of the transplant patches. The density within the transplant patches may correspond moderate-high population densities (up to 50 colonies or more per m^2). This will fit with the natural densities and while is expected to enhance the reproductive success and potentially increase the recruitment in the space inter-transplant-patches. Overall this kind of arrangement should enhance the resilience of restored populations firstly by the growth of the transplants and secondly by enhancing the reproduction success of the populations.

Specific treatments for species

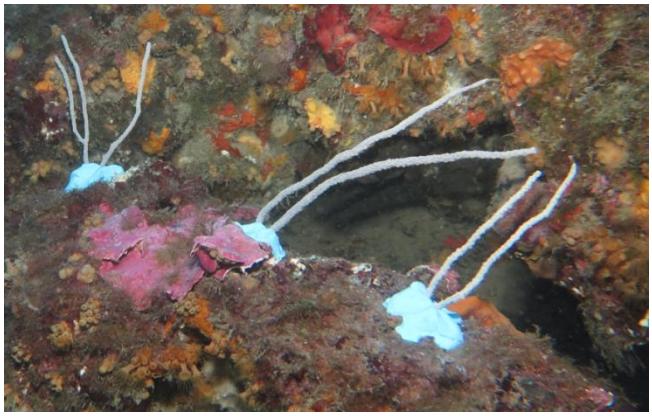
The technique that will be adopted for transplants has to take into account the skeletal structure of the species.

For species with a rigid scleraxis or displaying large sclerites in the coenechyme the putty can be used directly for the transplants since such skeletal features increase the adhesion of the fragments into the putty itself. Succesful tests have been carried out for *Corallium rubrum and Paramuricea clavata.*

For species displaying thin scleraxis however it is recommended to reinforce the basal area of the fragments to ensure a better survival rate, indeed, when immersed in the epoxy putty, the coenenchyme will rapidly dissolve with the risk to trigger necrotic processes, the weakening of the organic scleraxis and a consequent loss of the colony. For instance the utilization of a silicon tube around the basis may be used (e.g. 1-2 cm of airline tubing for aquaria).Tests have been run with *Eunicella singularis*, and *E. cavolini* and it could be applied to other species such as *Leptogorgia sarmentosa*, The introduction of the gorgonian fragment into a plastic tube filled with the epoxy putty will permit to handle directly the plastic tube and insert it in the epoxy putty placed on the substrate.



Alternatively, when the use of a tube or other material is not feasible we recommend using fragments obtained by cutting the tips just below the branching node i.e. with a V shape. In this way the basal branching immersed in the putty will securely anchor the transplant.



The methods herein described for the transplantation of adult fragments of macroinvertebrate species with arborescent forms have been mainly tested in gorgonian species. However, they can be also applied to bryozoans. For species such as *Myriapora truncata*, the raw technique performs very well thanks to the rigid skeleton of the species. In other species, such as *Pentapora fascialis*, this technique does not work well given the fragility of the skeleton when it is manipulated. In this case, the fragment shall be first glued to a base, which is then glued to the substrate with the putty, to avoid the direct manipulation of its fragile skeleton (note: this is an approach similar to the one used for sponge species).

Period to implement restoration protocol

We recommend performing the restoration action between April and September to avoid the winter months with high frequency and intensity of storms, which can substantially increase the loss of transplanted colonies. Within this period, April and May just before the reproductive period of gorgonians (main target species of this technique) could be the best months to perform the restoration activity in order to firstly enhance possibility for the best weather conditions during following months and secondly, to allow larvae from transplanted colonies to settle in the new area.

Monitoring the success of transplantation

Survival and growth of transplants and recruitment would be the most suitable indicators of the success of the restoration actions. The survival of transplanted colonies should be monitored one month after the restoration action to evaluate the efficiency of the restoration technique applied (number of transplants in place) and approximately every six months or once per year afterwards to evaluate the survival and growth of transplants.

Critical points: Pros and cons

Pros:

The technique proposed is simple and can be easily performed not only by scientists, but also by volunteers and managers of MPAs working together under the supervision of experts.
The technique can be applied using fragments obtained from donor colonies that would otherwise die. For instance, these could be fragments from surviving detached colonies found at the bottom of walls, entangled in fishing nets (both in the bottom or recovered by fishermen). Likewise for red coral this approach has been proven to restore populations with colonies seized from illegal fishing activities. This will diminish the pressure over the donor populations.

As clonal organism, small fragments display similar reproductive output as the donor colonies.

Technique failure can cause an important loss of transplanted colonies mainly due to either a break in the epoxy/substratum attachment or the loss of the dowel due to poor installation. However, after an initial period of attachment failure, well-attached transplants had survival rates similar to those of natural colonies. The contrast between the losses due to attachment and the survival of well-attached transplants shows two different phases. In the first phase (first days-month), the mortality due to attachment failure is higher, but in the second phase the survival of transplants is similar to that exhibited by natural colonies.

High effort during the first days of the installation of transplants on the substratum should reduce the loss of transplants and probably allow use of either of the different techniques with good results.

Cons:

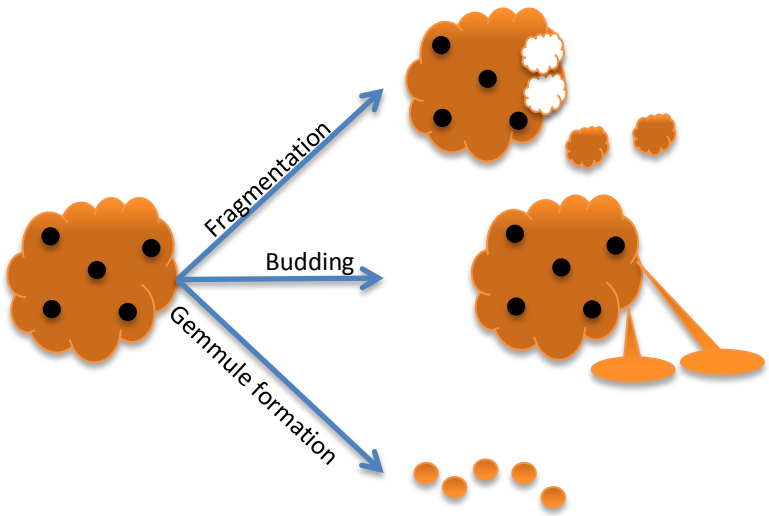
The use of small size of transplants. Bearing in mind the low growth rates (e.g. less than 1 cm in height per year for red gorgonian *Paramuricea clavata*), significant contribution to the structural complexity of the community cannot be expected before at least 10 years after the restoration action has commenced.

The transplantation of the whole colonies although possible, but bears more risks (in terms of the loss of the colonies) given their higher resistance to water flow. Preliminary experiments to test the effect of size on the survival of transplants, by comparing two different sizes of transplants: small (3–10 cm) and large (10–20 cm) indicated that large transplants always displayed higher failure of attachment regardless of the technique chosen. This was due to the higher resistance of large transplants to water flow, which easily opened a hole at the base of the transplant before the putty solidified. As a consequence, we recommend using small transplants, collected from apical branches, since they showed better attachment success.

4.2. Protocol C2. Adult sponge transplants

Sponge fragments or whole specimens?

Fragmentation is one of the strategies for asexual reproduction displayed by marine modular organisms and is expressed in Porifera (sponges) through different paths. This strategy has been extensively leveraged upon to develop propagation and transplantation techniques for sponges, mostly in the frame of aquaculture approaches. Building from these experiences, fragmentation represents an opportunity for restoration actions with sponges.



Two techniques have been used to test the attachment efficiency of sponge fragments obtained for restoration purposes, both using a two-component epoxy putty as glue. In the first method (raw), sponge fragments are directly glued to the substratum using the putty. In the second method, a plastic dowel is inserted into the base of the fragments

and then glued to the substrate using the epoxy. 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 the dowel and a fragment can be cut off.

Since most sponges are very sensitive to manipulation during transplantation, success heavily depends on minimizing sources of stress during these phases. Key steps can be listed taking into account whether the sponge has a solid/hard structure or a soft one.

Material

Zip-log bags and cutter, coolers, ice-packs, two-component epoxy putty, plastic gloves, knife or brush, slate and pencil, underwater camera (e.g. GoPro)

Methodology

Step 1.

Prepare the material needed:

a) cutter with the possibility to change the blade underwater to cut the transplants

b) blades for the cutter

c) zip-lock bags to store transplants

c) epoxy putty to fix transplants (e.g. Ivegor, Veneziani Subcoat S)

d) gloves to protect hands while mixing the epoxy putty

e) knife or a brush to clear the surface from sediments at the point of transplant attachment underwater

f) slate and pencil to draw the location and position of the transplants and to annotate their presence, their health status and size during subsequent surveys

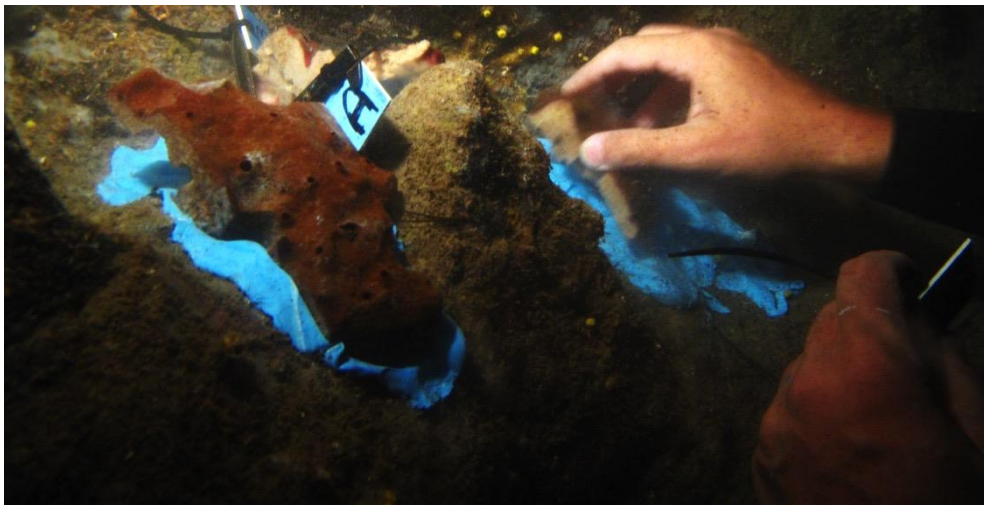
g) as an alternative, an underwater camera to film the area and build a photogrammetric reconstruction of the site

Step 2. Collection and maintenance or arrangement before transplantation

Depending on the sponge species involved, choose to apply appropriate transplantation technique:

Technique 1

Raw technique, gluing transplants directly to the putty. This technique is feasible for species with a hard skeleton and an evident basal portion such as *Petrosia ficiformis*.



Cutting of transplants should be done with a sharp blade, in order to minimize torsion and stretching of fragments during detachment.

Be sure that at least one side of the fragment was totally covered by the exopinacoderm (skin) otherwise the sponge will never cicatrize its cut surfaces.

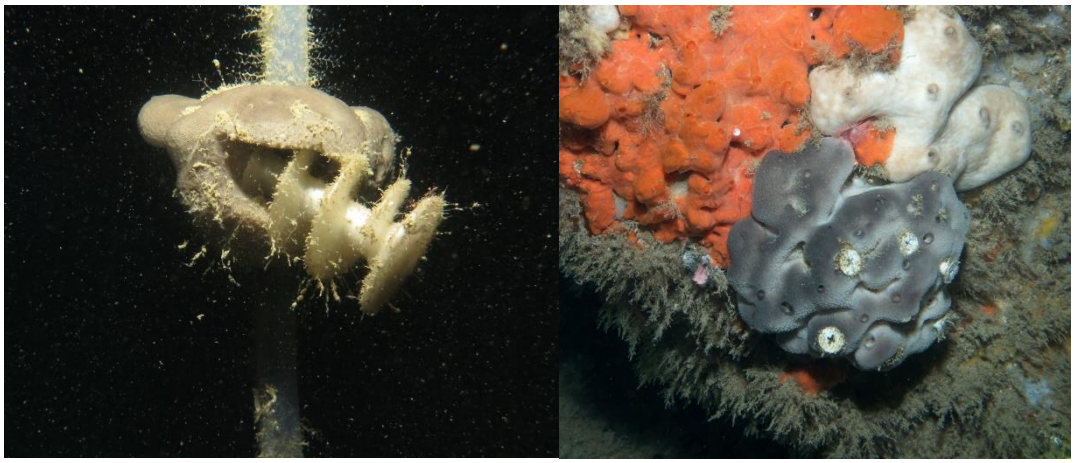
Underwater, use a cutter to collect portions of sponge with a minimum volume of 100 ml from mature, healthy donor specimen of selected species. As a reference in sponges specimen is considered healthy when it displays less than 10% of necrosis on its surface.

Once on board, the plastic bags should be placed in coolers for transportation to the restoration location. Use coolers with ice-pack if necessary to keep the temperature close to the one present in the collection site or colder.

Technique 2

In case of sponges with a soft skeleton (e.g. *Spongia* spp., *Sarcotragus* spp., *Ircinia* spp.) it is very important to avoid squeezing of the samples. For this reason it is fundamental to arrange the transplants with a dowel, either inserting it directly in the donor sponge, before cutting off the fragment, or inserting it into the already detached sponge fragment. The choice shall be made in order to minimize the stress imposed on the fragments, and depends on several factors, including the shape and condition of the donor sponge, the time allowed for underwater work, the temperature at the time of work and so on.
.

Inserting dowels into fragments (left picture) or directly into the donor sponge (right picture). These are two approaches that can limit the manipulation of the fragments and increase their survival. Here is shown one example with a dowel placed into a fragment of *Spongia lamella*.and with several dowels placed into *Spongia officinalis* still *in situ*.



Handling of transplants should be always done carefully, without squeezing them (the production of milky water means loss of cells fundamental for regeneration) and, in any case, keeping manipulation to the minimum.

Exposure to air should always been avoided. Abrupt changes in temperature, also over short period of time, can negatively affect the transplants and/or may cause reactions such as the expulsion of eggs/sperms in mature sponges.

Step 3. Specimens handling and transportation to the transplantation site

Once on board, the plastic bags with the fragments (with or without dowels inserted in) should be placed in coolers for transportation to the restoration location. Use coolers with ice-pack if necessary to keep the temperature similar to the one in the sea during collection.

On board/land, put the plastic gloves on and prepare the epoxy putty by mixing equal parts of two components, following manufacturer's instructions. The epoxy putty will serve as a glue to attach transplants to substrata.

Step 4. Transplantation

Again underwater, bringing the fragments to transplant, use a brush to remove sediments from the surface where you plan to attach transplants and thus ensure better adherence to the substrate. Ideally look for small natural holes and crevices and fix the base of the transplants with portions of prepared epoxy putty. Adjust your technique according to the species involved depending on the consistency of the skeleton.

To set the spatial arrangement of transplants it could help to know the local currents and It is important to keep a minimum distance between fragments of 30-40 cm, to avoid re-inhaling the expelled water from the adjacent sponges.

In case of sponge species living in symbiosis with autotrophic organisms it is important to select places adequately exposed to light. In case of other species it is important to check their 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.

Step 5. Checking transplantation

Ensure that the epoxy putty and the transplants inside it are firmly attached to the substrate. After a while, transplants and/or other benthic organisms will overgrow the epoxy putty, blending it with the environment.

Applying the same technique used for attachment of transplants, you can place permanent marks (e.g. screw with plastic tags) to facilitate the mapping of transplants and the subsequent monitoring. Using the slate and pencil, now you can annotate the position and draw a map of your permanent marks and transplants. The maps will be used for monitoring of the restoration actions (see Monitoring restoration section). The maps may include information on the size of transplants. You can also use an underwater camera to film the area and apply photogrammetric techniques to record the disposition of the transplants and allow a detailed monitoring.

In the next figure a donor-specimen of *Spongia lamella* is shown before manipulation, just after the cutting of the portion to be transplanted (Nov. 16) and one year later (Nov. 17) to document the survival and the complete recovery of the mother-sponge.

Furthermore, an example of the evolution of a transplant of the hard skeleton sponge *Petrosia ficiformis* is shown below:

May 2017

July 2017

August 2017

September 2017

Lastly, the transplant of the soft skeleton sponge *Spongia lamella* is shown in different phases, using the technique 2: cicatrization of the sponge tissue surrounding the dowel (January 2017), attachment of the dowel into the putty, keeping the sponge close to the substrate (May 2017) and complete recovery of the sponge and its adhesion to the substrate (September 2017).

January 2017

May 2017

September 2017

Period to implement restoration protocol

Early-winter months should be generally avoided, as they are characterised by high frequency of storms. This can substantially increase the loss of transplanted specimens. The period from March to June occur just before the main reproductive period of many western Mediterranean sponges. These months could therefore be the best for transplanting sponges as likely good weather conditions are likely ahead, and

larvae from transplanted colonies might already develop and settle in the new area. However, depending on the climatic condition, summer could represent a very critical period for many filter feeders in the Mediterranean Sea and, in case of thermal anomalies, transplantation efforts in this season should be avoided.

Monitoring the success of transplantation

The survival of transplanted sponges should be monitored ideally the day after transplantation (to check for any procedural issue), about every week or ten days during the first month and then on a monthly basis during the first six months. After this initial period, survival and other processes (e.g. growth and/or reproduction) may be checked approximately every six months or once per year, unless there is evidence of or concern for acute stressors (such as mass mortalities, heat waves etc.) that warrant emergency checks. In addition to survival rates, the reproductive potential of colonies provides crucial information to assess the viability of the action in a long-term. Reproduction from samples collected and fixed in 4% formaldehyde just before the period of spawning for NW Mediterranean sponges, could be assessed once per year after the transplantation.

Critical points: pro and cons

Transplantation of long-lived species can be deemed successful upon demonstration of survival and persistence *in situ* over several years. However, this is not sufficient to label the endeavour as “restoration”, which can be only considered successful when the population starts to reproduce and recruit.

In case of sponges, we do not know what the optimal sex-ratio shall be therefore it is important to have a high number of transplants, collected from several sponges, to maximise the chance of avoiding unbalanced sex-ratios.

Pros:

The technique can be applied using fragments obtained from donor colonies that can easily regenerate and grow to the original size. As clonal organism, small fragments display similar reproductive output as the donor colonies.

If the putty and the dowels are correctly fixed to the substratum and if the sponge tissue is firmly attached to the dowel, transplants had survival rates similar to those of natural colonies. The assessment of the transplants during the first months will clarify if the process

Cons:

Even if the technique proposed is simple and can be easily performed not only by scientists, but also by volunteers and managers of MPAs working together, the supervision by researchers is required to adequately select the species, because their correct identification in the field could be difficult. Moreover it is important to know details on the biology and on the natural history of the target species to correctly select the transplantation area.

The density and the structure of the sponge skeleton strongly affects the final result and a short training is required to explain to the volunteers how to manipulate the samples.

If the coverage of the exopinacoderm on the fragments is not sufficient, cicatrisation will not occur.

4.3. Protocol C3. Macroinvertebrate recruitment enhancement techniques

Adult transplants or recruitment enhancement techniques

As commented for Protocol of transplants of adult erect macroinvertebrate species, in some species developing fragile skeletons such as the bryozoan *Pentapora fascialis* manipulation and obtaining skeleton fragments may be challenging. For these species, recruitment enhancement techniques are useful alternatives for restoring their populations.
Tests on the bryozoan *Pentapora fascialis* have been carried out. This species usually settle in erect substrates such as skeletons or damaged tissues of gorgonians, hence this technique could be particularly very effective in this case.

Materials

Plastic screws, plastic ties, plastic mesh, two-component epoxy putty, plastic gloves, knife, slate and pencil

Protocol/Methodology

Step 1.

Prepare the material needed:

a) plastic mesh where the recruits of *Pentapora fascialis* will settle

b) plastic ties to attach the mesh to the plastic screws

c) plastic screws that will be attached to the rock using the epoxy putty

d) epoxy putty to fix plastic screws that will serve as anchors for plastic mesh (e.g. Ivegor, Veneziani Subcoat S)

e) gloves to protect hands while mixing the epoxy putty

f) knife or a metal brush to clear the surface at the point of attachment of a plastic screw underwater

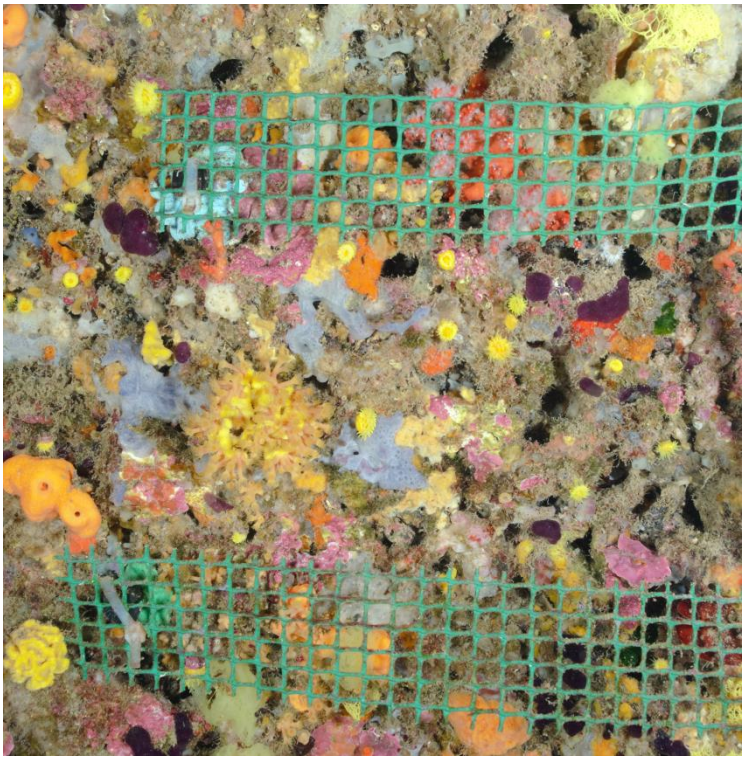
g) slate and pencil to draw the location and position of the transplants and to anotate their presence, health status and size during posterior surveys

Step 2

On board/land, put the plastic gloves on and prepare the epoxy putty by mixing equal parts of two components, following manufacturer's instructions. Store it in the wet zip-lock bag that you will take underwater. The epoxy putty will serve as a glue to attach the screw to substrata. These screws will serve as anchors to attach the mesh.

Step 3

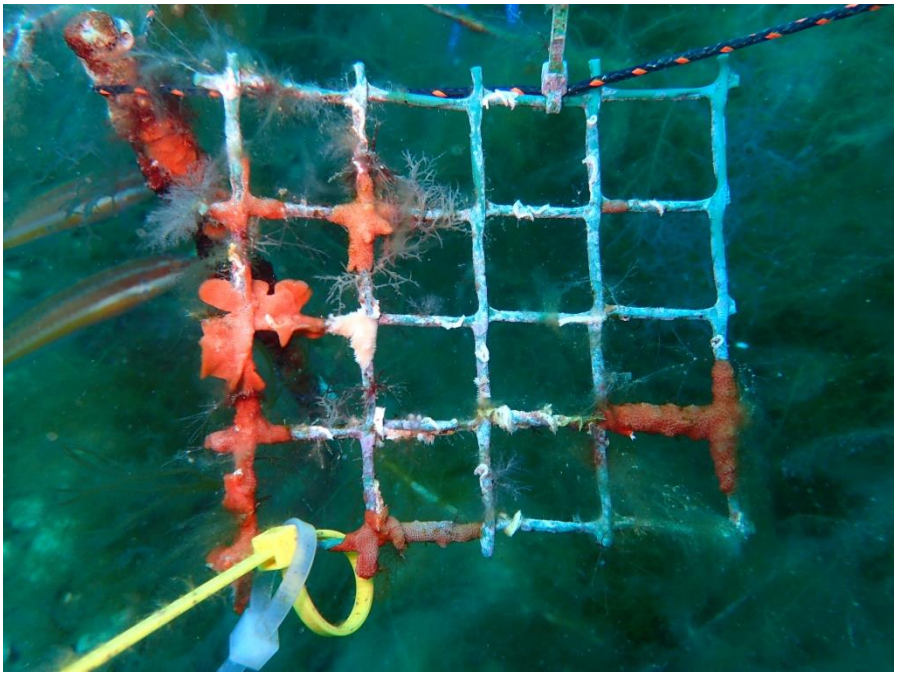
Again underwater, use a knife to clear the surface where you plan to attach the mesh with the screw and thus ensure better adherence to substrata. Ideally look for small natural holes and crevices and fix plastic screws with portions of prepared epoxy putty. When the epoxy putty hardens (approx. after 24 h) you can fix the plastic mesh to the screws with plastic ties.



Step 4

Using the slate and pencil, now you can annotate the position and draw a map of the different surfaces that you installed. In the following visits you can also annotate their

presence, the health status and their size. Be patient until new bryozoan colonies are installed on the mesh.



Step 5.

Once the colonies reach a significant size, we can cut the meshes with scissors and install them in the area where we have detected a significant decline or complete loss of this species. Once the meshes are installed in the new site, we must individually identify the colonies in order to assess their survival and growth.

Period to implement restoration protocol

We recommend performing the restoration action between September and April to avoid the maximum development of algae and their growth over the meshes which can inhibit the settlement of new colonies. Moreover, the development of dense and thick formations of filamentous algae covering large extensions of littoral (such as the ones

occurring during the last two years, caused by seasonal proliferation of several species such as *Acinetospora crinita*) may inhibit the settlement of bryozoans and even cause the mortality of new recruits.

Monitoring the success

Recruitment and growth rates would be the most suitable indicators of the success of this restoration action.The recruitment in the installed meshes should be monitored monthly or at least every two months between the first six months and the first year; after that survival can be noted approximately every six months or once per year. Growth rates can also be assessed to investigate the time needed to achieve their structural role.

Critical points

The applicability of this technique is currently being investigated. Although we have scientific evidences of the success of recruitment on this plastic meshes and the relatively fast growth of the recently settled bryozoans, in this moment we cannot indicate the best period to ensure the maximum recruitment rates on the meshes.

The last step involving the installation of the bryozoan with the mesh in other areas has not been proven to date, since we are currently assessing the success of the first part of the technique as recruitment enhancers.

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