D 10.6: Restoration of marine ecosystems: a manual for users

Marine Ecosystem Restoration in Changing European Seas
MERCES

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1. Introduction

Global change is transforming European seas more than other regional seas, exacerbating by the deleterious anthropogenic impacts. Direct and indirect human pressures on marine ecosystems are expected to further increase in the next few decades, leading to a serious loss of marine habitats, their biodiversity and to the impairment of ecosystem functioning. It is now widely recognized that restoration actions are needed to halt further decline. Restoration is a key action of the Aichi Biodiversity Targets and in the UN Sustainable Development Goal 14 “conserve and sustainably use the oceans, seas and marine resources for sustainable development” in particular of target 14.2 “by 2020, sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, including by strengthening their resilience, and take action for their restoration, to achieve healthy and productive oceans”. The Decade dedicated by UN to “Ecosystem Restoration” and the UN Decade on “Ocean Science for Sustainable Development” that will both start in 2021 represent a unique combination and opportunity for the follow up of the MERCES results. These global UN initiatives offer a great challenge to restore marine ecosystems within European seas and beyond, and will help and support transition of our societies to a sustainable future. The experience and results achieved in the framework of the MERCES project could represent an important contribution to maximize future initiatives of restoration in marine ecosystems. MERCES project can thus provide a potentially huge contribution to the perspective of the European Green Deal, the European Commission's blueprint and roadmap to make Europe the first climate neutral continent by 2050, with a sustainable economy that leaves no one behind.

1.1 Restoration and EU initiatives

On 1 March 2019, under Resolution 73/284, the United Nations General Assembly proclaimed 2021-2030 to be the United Nations Decade on Ecosystem Restoration (hereafter referred to as the UN Decade), with the primary aim being to prevent, halt and reverse the degradation of ecosystems worldwide. All initiatives within the UN Decade will consequently have a dual focus on protecting as well as restoring ecosystems. The local social, economic and ecological context of each initiative will determine the appropriate balance of conservation and restoration in a particular landscape. Integrated land-use planning, undertaken in a rights-based manner, where all stakeholders are informed of the full range of benefits to be gained through conservation, restoration and sustainable use of natural resources in their local ecosystems, assists in achieving this balance. United Nations (UN) Sustainable Development Goals (SDGs) for 2030 call for the restoration of

marine and coastal ecosystems (Goal 14), as well as forests and other ecosystems that have been degraded (Goal 15). The UN Environmental Programme (UNEP), Food and Agriculture Organization (FAO), Global Landscapes Forum (GLF), and International Union for Conservation of Nature (IUCN), among others, are expected to lead implementation and knowledge exchange programs for the Decade on Ecosystem Restoration.

The Convention on Biological Diversity (CBD) has a target of restoring 15% of degraded ecosystems by 2020 to mitigate the impacts of climate change and to combat desertification (Aichi Biodiversity Target 15), and views ecological restoration as key to delivering essential ecosystem services (Aichi Biodiversity Target 14). The CBD has adopted a Short-Term Action Plan on Ecosystem Restoration (CBD 2016), and restoration is expected to play an even larger role as the current biodiversity targets expire and are revised for the post-2020 biodiversity framework. The CBD (2018) also encourages Parties to further strengthen their efforts "... to identify regions, ecosystems and components of biodiversity that are or will become vulnerable to climate change ... to promote ecosystem restoration and sustainable management post-restoration."

The EU Biodiversity Strategy for 2030 is an ambitious strategy that delivers on the EU and Member State commitments as parties to the UN Convention on Biological Diversity. The strategy aims to ensure that ecosystems are healthy, resilient to climate change, rich in biodiversity and deliver the range of services essential to the prosperity and well-being of citizens. Key topics addressed are: protected areas, **restoration of ecosystems**, habitat and species status, urban green spaces, biodiversity to benefit climate and people, new biodiversity governance framework enabling transformative change, and supporting biodiversity through EU external policies. The targets address the main drivers of biodiversity loss and aim to reduce key pressures on nature and ecosystem services in the EU. The Strategy further outlines the ambition to strengthen the biodiversity proofing framework for EU programmes and financing instruments and unlock at least €20 billion a year for spending on nature via e.g. a dedicated natural capital and circular-economy initiative under Invest EU, the European Green Deal Investment Plan, the EU budget dedicated to climate action, and the mobilisation of further public and private funding at national and EU level. Nature-based Solutions are highlighted as a key instrument for climate adaptation and mitigation and for greening cities. The ambition is high, but also necessary given that the previous EU Biodiversity Strategy to 2020 failed on many accounts (EFH, 2019, Langhout, 2019).

The Strategy contains specific commitments and actions to be delivered by 2030, including:

- Establishing a larger EU-wide network of protected areas on land and at sea, building upon existing Natura 2000 areas, with strict protection for areas of very high biodiversity and climate value.
- An EU Nature Restoration Plan - a series of concrete commitments and actions to restore degraded ecosystems across the EU by 2030, and manage them sustainably, addressing the key drivers of biodiversity loss.
- A set of measures to enable the necessary transformative change: setting in motion a new, strengthened governance framework to ensure better implementation and track progress, improving knowledge, financing and investments and better respecting nature in public and business decision-making.
- Measures to tackle the global biodiversity challenge, demonstrating that the EU is ready to lead by example towards the successful adoption of an ambitious global biodiversity framework under the Convention on Biological Diversity.

The Biodiversity Strategy for 2030 outlines an EU Nature Restoration Plan to restore damaged ecosystems and ensure their sustainable management. Europe's biodiversity will be on the path to recovery by 2030 for the benefit of people, the planet, the climate and our economy, in line with the 2030 Agenda for Sustainable Development and with the objectives of the Paris Agreement on Climate Change. The awareness that protecting the nature will not be enough to bring nature back into our lives makes priority nature restoration to reverse biodiversity loss. The experience gathered during the MERCES project could represent an important contribution to the new EU Nature Restoration Plan making Europe a worldwide leader of this ambitious task. The plan will help improve the health of existing and new protected areas, and bring diverse and resilient nature back to all landscapes and ecosystems. This means reducing pressures on habitats and species, and ensuring all use of ecosystems is sustainable. It also means supporting the recovery of nature, limiting soil sealing and urban sprawl, and tackling pollution and invasive alien species. The plan will create jobs, reconcile economic activities with nature growth and help ensure the long-term productivity and value of our natural capital.

2. Restoration of marine habitats: protocols tested during the MERCES project

MERCES is the first project in which restoration actions are carried out in different marine habitats and target species along the EU seas, including the deep sea. This multi-habitat and multi-regional approach allows to test and setup the best protocols for restoring different marine ecosystems and to analyze different aspects and implications of the ecological restoration in different EU seas. MERCES project has explored the potential of restoration actions in shallow soft and hard bottoms (including mesophotic) and deep-sea habitats at pan-European scale, from Norway to Turkey. MERCES is giving a special attention on the most fragile and vulnerable habitats, including seagrass meadows, algal and kelp forests, coralligenous outcrops, cold-water corals, canyons, seamounts and fjords in 25 different pilot areas. Twenty-five protocols (including species translocation and transplanting, seedling and grazer removal, artificial and biodegradable substrates) have been tested to increase restoration efficiency and to identify the criteria for the selection of target species and habitats. Pilot restoration actions have been successfully carried out on seagrasses (*Zostera marina*, *Z. noltii*, *Cymodocea nodosa, Posidonia oceanica*), coupled with bivalves to activate ecological facilitations processes (e.g., *Mytilus edulis*, *Pinna nobilis*, *Macoma balthica*). Coral, gorgonians and sponge species (e.g., *Chondrilla nucula*, *Aplysina aerophoba*, *Spongia officinalis*, *Corallium rubrum*, *Paramuricea clavata*, *Eunicella singularis*, *E. cavolini*) have been used to restore hard bottoms. Finally, a challenge of the MERCES project has been the setup of restoration protocols for deep-sea habitats, including soft and hard bottoms and species of cold-water corals (e.g., *Callogorgia verticillata*, *Paracalyptrophora josephinae*, *Viminella flagellum*, *Lophelia pertusa (Desmophyllum pertusum)*). Here we report the protocols tested in the shallow soft bottom habitats (section 2.1), shallow hard bottom (section 2.2) and mesophotic habitats (section 2.3) and finally the deep-sea habitats (section 2.4), including passive restoration approach. The success of fieldwork activities is highly dependent upon the environmental conditions, the presence of anthropogenic and the protocols tested. The experience acquired by MERCES project provides evidence that the feasibility of restoration activities in marine shallow habitats can be partly compromised by extreme/episodic events (e.g., storms, heat waves). Independently by the methodologies utilized for marine ecological restoration, the presence of extreme climate-driven events immediately after the restoration actions can compromise the survival of target species. The success of most of the pilot restoration actions and the failure, are now lessons learn which allowed the identification of the best solutions to make successful future restoration actions at large spatial scale.

2.1 Shallow soft bottom habitats

2.1.1 Protocol: Seagrass-bivalve co-restoration using *Zostera marina* and *Mytilus spp*

1. Rationale

In this study, our aim was to determine whether co-restoration of eelgrass and blue mussels could benefit one or both species. Previous studies have shown that these species can facilitate each other, though few have attempted co-restoration. Mussels can facilitate eelgrass through their feeding activities, reducing water turbidity through filtration and increasing nutrient availability in sediment through biodeposition of pseudo-faeces, and by reducing hydrodynamic stress on eelgrass. Similarity, eelgrass can also facilitate mussel survival by providing shelter from predators and hydrodynamics. Prior to launching this field experiment, we ran a preliminary aquarium experiment in which we found that mussels promoted eelgrass growth by fertilizing sediment porewater.

2. Objectives

- To test if planting eelgrass and blue mussels together can increase the restoration success (survival and growth) of either or both species;
- To test if site exposure can moderate this interaction.

3. Target species and habitats

Species: *Zostera marina, Mytilus edulis/trossulus*

Locations: Eelgrass sandy sites in Estonia, Finland, Norway.

Criteria for site selection

Sites need to be subtidal, sandy (sandy-muddy) habitats i.e. areas where blue mussels and eelgrass occur naturally**.**

4. Material:

- Plastic grid nets mesh size 25×25 mm
- Eelgrass
- Blue mussels
- Cable ties
- Metal hooks
- Temperature and light loggers

5. Description of the protocol and activity

We selected six subtidal (2-4 m depth) sandy sites: one exposed and one sheltered site in each country. In each site, we planted 30 plots including 6 × eelgrass alone, 6 × mussels alone, and 6 × eelgrass and mussels together, along with control plots.

Step 1. The eelgrass was collected from a nearby donor site, then 16 shoots and rhizomes were attached to a plastic grid with cable ties.

Step 2. The grid was then buried several centimeters under the sediment and kept in place with 2-3 metal pins.

Step 3. The mussels (1 litre) were then placed on top of the plot.

Step 4. We checked eelgrass shoot length and growth, as well as mussel condition index after 3 months, and then monitored eelgrass shoot density and mussel percent cover after 3 months (one growing season), 12 months (one winter season), and 15 months (a second growing season).

Results & Monitoring: Adding mussels did increase eelgrass growth, but this did not translate to higher shoot length or shoot density in the field. Eelgrass also had no effect on mussel condition index or percent cover. In all exposed sites and one of the sheltered sites, the mussels disappeared from the plots within the first three months. The eelgrass survived in most plots after one growing season, but had disappeared from most plots after the winter season. However, eelgrass survived in several plots in the sheltered sites, and these showed high increase in shoot density after the second growing season.

6. Observations and Recommendations

- Mussels can indeed promote eelgrass growth in aquaria and field plots, especially in more sheltered locations, but this does not translate to higher shoot density in the field.
- Eelgrass seems to have no effect on mussel cover or condition index.
- Exposure was a determining factor in eelgrass survival, all plots in exposed sites disappeared.

7. Challenges and barriers

Eelgrass had difficulty surviving in some sites, due to several site-specific factors: drift algal mats in Finland, sediment burial in Estonia, and erosion in Norway. Exposure seemed to be the determining factor affecting eelgrass survival and restoration success. It was impossible to determine whether mussels could facilitate eelgrass growth and survival, as most of them were washed away from the plots, thus providing mussel substrate is a crucial aspect of ensuring mussel survival in co-restoration efforts.

2.1.2 Protocol: Seagrass-bivalve co-restoration using *Zostera marina, Mytilus spp* and BESE (Biodegradable Ecosystem Engineering Structures)

1. Rationale

The results of the first field experiments (Protocol 2.1.1) showed that exposure and hydrodynamics were a limiting factor in eelgrass and mussel survival in small restoration plots. The stabilizing effect of these two ecosystem engineers was not enough to overcome strong hydrodynamics, and thus here we added Biodegradable Ecosystem Engineering Structures (BESEs; Temmink et al. 2020) to stabilize the sediment for seagrass and provide substrate for mussels. Biodegradable structures (i.e. artificial ecosystem engineers), made of hessian bags (Kidder et al. 2015) or biodegradable polymers (Temmink et al. 2020), for supporting seagrass shoots holds promise for increasing seagrass restoration success, but has never been trialed in conjunction with biogenic ecosystem engineers.

2. Objectives

- To test if biodegradable structures (BESEs) can provide substrate for blue mussels in co-restoration efforts with eelgrass;
- To assess if BESEs facilitate eelgrass blue mussels survival.

3. Target species and habitats

Species: *Zostera marina; Mytilus edulis/trossulus*

Locations: Sandy eelgrass sites in Denmark, Estonia, Finland, Norway.

Criteria for site selection:

Sites need to be subtidal, sandy (sandy-muddy) habitats i.e. areas where blue mussels and eelgrass occur naturally.

4. Material

- BESE units 30 × 30 cm 10 cm thick
- Eelgrass
- Blue mussels
- Biodegradable rope or string
- Metal hooks
- Temp and light loggers
- Aquaria or plastic tanks

5. Description of the protocol and activity

Subtidal sandy sites, one site per country except for two sites in Norway (Fig. 1). At each site we set up 32 plots: 16 with BESEs and 16 on sand. Within these we had four plot treatments: control with organisms, eelgrass alone, mussels alone, eelgrass and mussels together. We measured eelgrass shoot density and mussel cover after 3 months (one growing season), 12 months (one winter season), and 15 months (a second growing season).

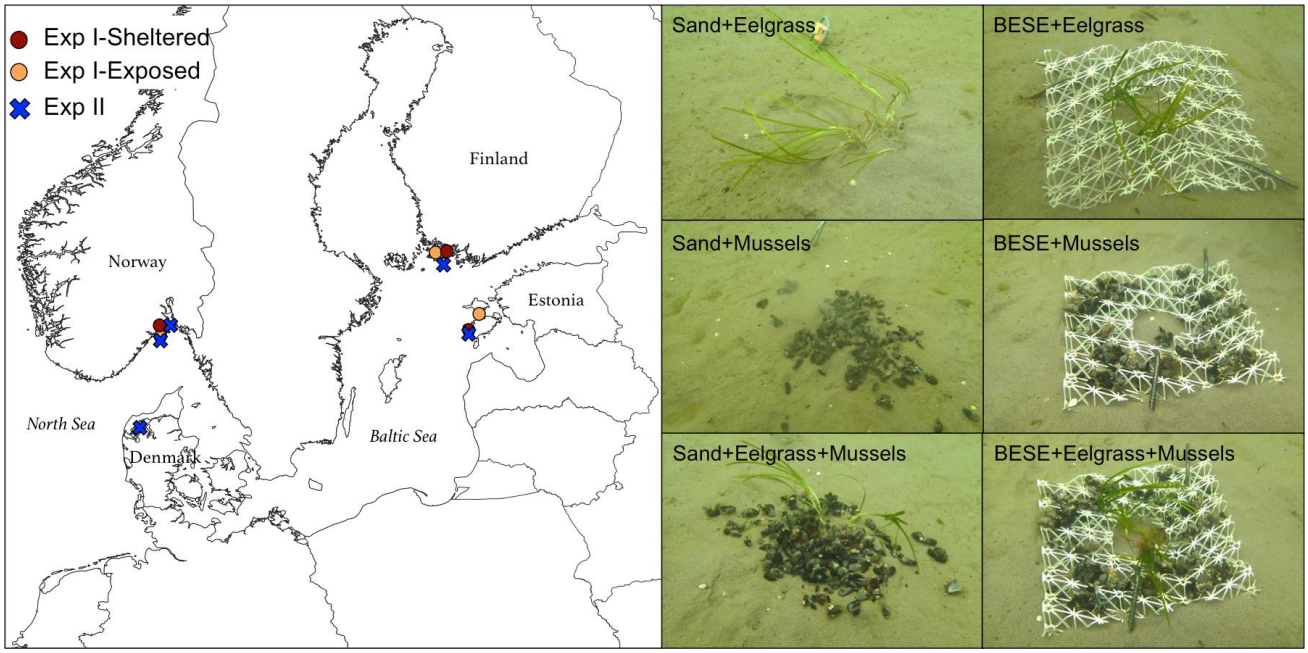


Fig. 1 Experimental sites for both eelgrass-mussel co-restoration experiments. Sites used in the first experiment are shown in red (sheltered) and yellow (exposed) circles, and sites in the second experiment with BESEs are shown in blue. Note that two sites (one in Norway and one in Estonia) were used in both experiments. Also shown are pictures of the experimental plots from the second experiment in the Finnish sites. In the left column are sand plots, and in the right column are BESE plots. (Photo credit: Karine Gagnon).

6. Observations and Recommendations

- The mussels survived much better on the BESEs than on bare sand, and in some site, the BESEs also attracted new mussel recruits.
- After 3 months, eelgrass shoot density was higher in BESE plots than sand plots, but there were no significant differences between plots with or without mussels.
- After 12 and 15 months, BESE plots without mussels showed much higher eelgrass shoot density; most eelgrass in all other treatments died.

- The BESEs are effective at retaining mussels and at attracting new mussel recruits. The BESEs also increased eelgrass survival over the winter season.

7. Challenges and barriers

Due to a heat wave across northern Europe in 2018, there was high eelgrass mortality across all treatments and high temperatures might have caused blue mussel die off too. Mussels did not facilitate eelgrass, however BESEs were very effective in assuring both eelgrass and mussel survival (though not when planted together).

2.1.3 Protocol: Seagrass-bivalve co-restoration using *Macoma balthica* and *Zostera marina*

1. Rationale

The tellinid clam *Macoma balthica* is an important component of the macrofauna community in the North Sea and Baltic Sea with strong impacts on nutrient fluxes between the sediment-water interface. Throughout the Baltic Sea, *M. balthica* is the dominant infaunal bivalve, often associated with vegetated habitats such as eelgrass (*Zostera marina*) meadows. To date only nine studies have addressed the interaction between *Z. marina* and *M. balthica*, and these studies show mixed effects. The effect of tellinid bivalves and specifically *M. balthica* on seagrasses has not been tested in a manipulative experimental way to date, despite their natural co-occurrence. The method below is published in Meysick et al. (2020)

2. Objectives

- Test if planting eelgrass and infaunal clams (*M. balthica)* together increase the restoration success (survival and growth) of either or both species.

3. Target species and habitats

Species: *Zostera marina; Macoma balthica*

Location: Finland, Fårö, Archipelago Sea.

Criteria for site selection:

Sites need to be subtidal, sandy (sandy-muddy) habitats i.e. areas where clams and eelgrass co-occur naturally. Sediments with high porewater nutrient concentrations should be avoided (see below).

4. Material

- Plastic grid nets mesh size 30×30 mm
- Eelgrass
- Macoma bivalves
- Metal hooks
- Temp and light loggers

5. Description of the protocol and activity

Step 1. We planted 60 plots consisting of 16 eelgrass shoots each, attached to a 25 × 25cm plastic grid.

Step 2.The grid was buried several centimeters under the sediment and kept in place with 2-3 metal pins.

Step 3. We then added 10 densities (0-2800 ind. m^{-2}) of adult clams (>8mm) to the plots. Three replicates of each treatment were recollected after 75 days (n=30) and again after 14 months (n=30).

6. Observations and Recommendations

- All plots, independent of clam density, survived the 14 months and increased in biomass and size over time.
- Infauna samples indicated that most clams stayed in place during the first 2 months.
- Shoot, root and rhizome biomass were highest at high clam densities in combination with low ammonium concentrations and *vice versa*.
- Conclusions: The effect of infaunal clams on eelgrass seems to be context-dependent, potentially due to increased nutrient release from the sediment. The best effect of bivalves was in lower porewater nutrient conditions.

7. Challenges and barriers

High clam densities combined with high ammonium concentrations however, resulted in an inhibition of biomass production. Since porewater nutrients were not sampled after 14 months, we could not test whether this effect was still apparent over time. The condition index of clams was significantly lower in plots and in the adjacent eelgrass meadow, compared to bare sand, potentially through reduced food availability.

2.1.4 Protocol: Intertidal seagrass restoration using eelgrass seed transplantation (I)

1. Rationale

Previous attempts at intertidal seagrass restoration in the Dutch Wadden Sea (e.g. the BuDS-method; Pickerell et al. 2005) had overall poor results, therefore the new seeding method “Dispenser Injection Seeding” (DIS) was developed. With the DIS-method we aimed to reduce seed losses by storing seeds overwinter and injecting the seeds directly into the sediment come spring.

2. Objectives

- Investigate the viability of the DIS-method, as well as how plot size (20 vs. 200 m^2) and seed density (2 vs. 20 seeds/injection) affect restoration success of *Zostera marina* in the intertidal zone.

3. Target species and habitats

Species: *Zostera marina.*

Location: The Netherlands, Uithuizen, North of the Dutch Groningen coast.

Criteria for site selection

Low hydro- and sediment-dynamics;

Suitable elevation, 0 - +15 cm NAP;

Presence or historic presence of seagrass;

Suitable sediment.

4. Material

- *Z. marina* seeds
- Mudflat mud
- Overwinter seed storage
- Caulking guns
- Sealant tubes
- Nozzles for sealant tubes
- 1 m^2 grids for seeding

5. Description of the protocol and activity

Intertidal mudflat.

Step 1. *Zostera marina* seeds were collected in late summer from a substantial intertidal seagrass meadow in Schleswig-Holstein, Germany. In the Netherlands the seeds were separated from other plant material and organic debris. Once separated the seeds were treated with a low concentration of copper-sulfate (0.2 ppm) to combat a prevalent mold infection. Afterwards the seeds were stored in a cold and dark climate chamber over winter.

Step 2. In March, before seeding the seeds were soaked in freshwater for 24h, with the goal to kickstart a stress reaction that initiates the germination process. After soaking the seeds were mixed with mudflat-sediment and the mixture was pushed into 300 ml dispenser tubes.

Step 3. In the field the seed-mud mixture was injected directly into the sediment with sealant-/caulking guns. Before seeding, the caulking guns were calibrated to inject the desired amount of seeds/mud each injection.

6. Observations and Recommendations

- Overall, this first DIS-experiment was a success and provided us valuable insight on the potential of this method.

7. Challenges and barriers

Only a few plants emerged the next summer, so our ultimate goal of establishing a self-sustaining seagrass population was not achieved. Seed losses were still high.

2.1.5 Protocol: Intertidal seagrass restoration using eelgrass seed transplantation (II)

1. Rationale

The first field attempts with the DIS-method proved promising, but restored plant densities still remained relatively low and seed losses high. Therefore, the method needed further optimization.

2. Objectives

- Optimize the DIS-method by investigating how seeding depth, injection density and seed density affects restoration success of *Z. marina*.

3. Target species and habitats

Species: *Zostera marina.*

Location: (1) Intertidal sandflat Northeast of the Dutch Wadden Sea island Griend;
(2) intertidal mudflat at Uithuizen, North of the Dutch Groningen coast.

Criteria for site selection:

Low hydro- and sediment-dynamics.

Suitable elevation ~0 NAP

Presence or historic presence of seagrass

Suitable sediment

4. Material

- *Z. marina* seeds
- Mudflat mud
- Overwinter seed storage
- Caulking guns
- Sealant tubes
- Nozzles for sealant tubes
- 1 m^2 grids for seeding

5. Description of the protocol and activity

We investigated how three seeding variables affected restoration success, crossing injection density (100 vs. 25 injects/m^2), seeding depth (4 vs. 2 cm) and seed density (20

vs. 2 seeds/inject) (Fig. 1 a-e). We seeded six 4-m² replicates of the eight treatments at a sandy and a muddy site (48 plots/site).

In a 2nd experiment, we tested what seed density yields the highest plant numbers and the lowest seed loss. We tested five seed densities in the mesocosm (2, 4, 6, 8 & 10 seeds/inject) and seven densities in the field (2, 4, 6, 8, 10, 16 & 20 seeds/inject).

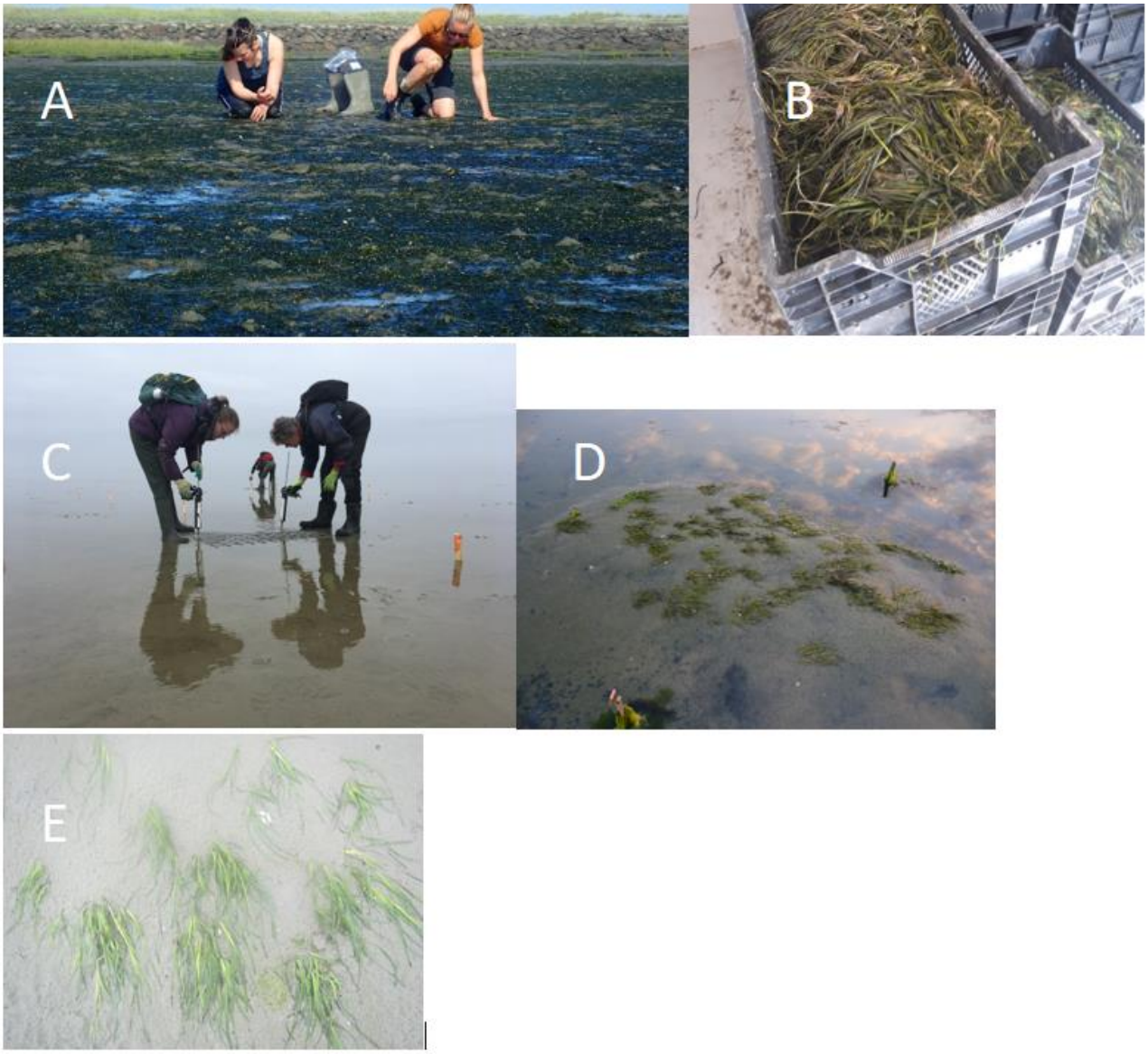


Fig. 1 a. *Z. marina* seed collection at German donor site. b. Harvested annual *Z. marina* plants with ripe seeds. c. Seeding with the DIS-method, Griend March 2018. d. Experimental plot at Griend, August 2018. e. Closeup of restored *Z. marina* plants (Photo credit: Laura Govers: A, C & D; Max Gräfnings: B & E).

6. Observations and Recommendations

- The first experiment was successful at the sandy site, near the island of Griend. The best treatment (100 injections/m², 2 seeds/injection, 4 cm depth) resulted in high plant densities (>10 plants/m²), with low seed loss (~94%) compared to previous experiments (99%), yielding up to 10-fold higher plant densities.
- In the second experiment, we found that the lowest seed densities (2 and 4 seeds/inject) performed best in the mesocosm as these densities produced similar plant densities compared to high-density treatments.

  Conclusions: We conclude that the DIS-method is viable for large-scale restoration in stable sediments. However, in contrast to earlier findings, we found that sediment trapping by high-density intertidal eelgrass beds enhances low-tide water drainage, increasing the populations' vulnerability to desiccation. Mesocosm experiments highlight that high-density seeding yields lower net germination, presumably due to intraspecific competition. We conclude that when focusing on single-species restoration, seeding should be done at relatively low densities at sites that remain moist during low tide.

7. Challenges and barriers

Low-tide drainage caused by sediment trapping combined with mid-summer heat waves decimated high-density plots. Nevertheless, we estimate that overall, over 10,000 adult plants emerged from our seeds at this site; approximately 1/3 of size of the largest current eelgrass population in the Dutch Wadden Sea. At the muddy tidal flat near Uithuizen, high seedling densities emerged in May. However, the majority of the plants washed away during June, which was most likely caused by the PVC-poles marking the experimental plots that caused heavy scouring here. We did not find any clear results in the field in the seed density experiment, as experimental plots were overrun by large aggregates of cockles, which dislocated/burrowed the majority of the seagrass seedlings.

2.1.6 Protocol: Eelgrass *Zostera marina* transplantation with rope method

1. Rationale

Eelgrass *Z. marina* has been historically reported in the north-eastern region of the Gulf of Riga, the north-eastern Baltic Sea but due to eutrophication and extreme storm events in the 1980s these communities have decreased significantly throughout the region. The restoration strategy involved transplanting *Z. marina* shoots from the donor area to sites where eelgrass was known to have previously existed.

2. Objectives

- To develop a new restoration technique to restore seagrass *Z. marina* in those sites where eelgrass was known to have previously existed.

3. Target species and habitats

Species: Eelgrass *Zostera marina.*

Location: Estonian coastal waters, north-eastern Baltic Sea.

Criteria for site selection

suitable substrate (e.g. sediment organic content);

suitable environmental conditions (e.g. light, nutrients, salinity);

the donor site was selected because its communities providing a good stock and its environmental conditions are similar to that of those found in the transplanting site.

4. Material

- Rope
- Eelgrass
- Cable ties
- Metal hooks
- Temp and light loggers

5. Description of the protocol and activity

Step 1. The 10 shoots containing long rhizomes were attached to a 1 m rope using cable ties and the ropes are buried under the sediment.

Step 2. The ropes were held in place by driving attached metal pins into the sediment at a depth of 3.0 m. A density of 50 shoots per $1m^2$ was achieved using this method. The first

attempt of transplanting experiment was performed in five replicates and three replicates used for the second attempt.

6. Observations and Recommendations

- Despite seagrass being lost from most ropes over the first winter, some ropes were found to have seagrass expansion after the second growing season. So, even if most transplanted shoots do not survive, only a small number are required to survive in order to establish new seagrass meadow.

7. Challenges and barriers

The main reason for failure was likely due to drifting algal mats smothering the seagrass shoots.

2.1.7 Protocol: Seagrass restoration using Biodegradable EcoSystem Engineering elements (BESE)

1. Rationale

Biodegradable EcoSystem Engineering elements (BESE, producer: Bureau Waardenburg, The Netherlands) consist of 91 × 45.5 × 2 cm sheets that can be combined to form three-dimensional establishment structures. The modular units are designed to temporarily mitigate harsh environmental conditions to allow establishment of transplants, seeds or larvae of ecosystem engineering species. Once matured, these organisms should form biogenic structures that sufficiently improve the organism's own environment to allow it to thrive, after which the structures will naturally biodegrade.

2. Objectives

- To test if BESE can enhance establishment and restoration yields of seagrass transplants in exposed environments where mature meadows facilitate and maintain themselves by attenuating hydrodynamic energy and stabilizing sediments.

3. Target species and habitats

Species: *Zostera marina, Thalassia testudinum, Posidonia oceanica*

Locations: Seagrass habitats in Finland, Bonaire (Caribbean Netherlands), Sweden, USA and Croatia.

Criteria for site selection

To test BESE, sub-tidal exposed and sheltered sites with unvegetated sediment bottom in an area with historical / recent records of a study seagrass species, were selected. Moreover, additional criterion for site selection was a good water transparency, as BESE cannot lower or solve turbidity issues that may adversely impact seagrass restoration efforts.

4. Material

- BESE units
- L-shaped metal rebars to anchor BESE
- Hammer
- Shovel and/or a knife (collecting seagrass/digging hole)
- Seagrass shoots
- U-shaped metal pins

- Small cable ties (for tying rhizomes to u-shaped pins)
- Temperature loggers
- Light loggers
- Snorkeling/diving equipment (depending on a depth)

5. Description of the protocol and activity

Seagrass ramets were transplanted in 91 × 91 × 6 cm BESE-modules that were either placed above- or belowground, and compared with transplants in unmanipulated controls. Seagrass rhizomes were anchored by U-shaped metal pins directly or they were tied by cable ties onto the u-shaped pins, whereas BESE units were anchored by ca 50 cm long L-shaped metal rebars (6 per unit). Each of the three treatments was block-wise replicated four times per site.

Step 1. Transporting BESE to a chosen restoration site.

Fig. 1 (Photo credit: S. Kipson)

Step 2. Setting up of BESE underwater. Note the pre-cut hole in the middle of BESE to place a seagrass transplant.

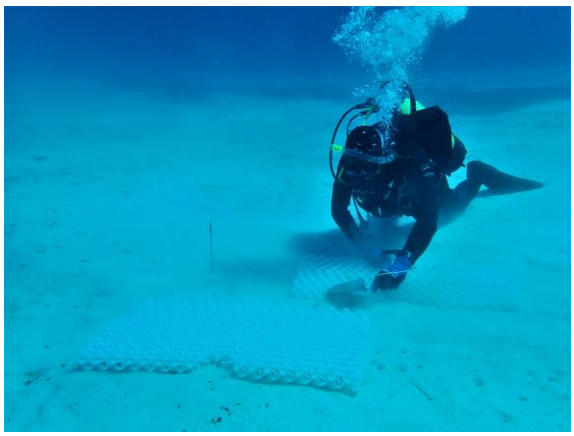


Fig. 2 (Photo credit: S. Kipson)

Step 3. Using a U-shaped pin to anchor seagrass ramet.

Fig. 3 (Photo credit: D. Petricioli)

Step 4. Further attaching seagrass ramets with cable ties onto the u-shaped pins (if needed)

Fig. 4 (Photo credit: M. Belosevic)

Step 5. Anchoring BESE with L-shaped metal rebars.

Fig. 5 (Photo credit: D. Petricioli)

Step 6. Final setup: aboveground BESE with seagrass ramets (example of *Posidonia oceanica* in Croatia).

Fig. 6 (Photo credit: M. Belosevic)

6. Observations and Recommendations

- BESE can be used to enhance seagrass transplant establishment and restoration success at sites where key population-level traits generating self-facilitation (in our case anchoring and sediment stabilization by mature root mats) can be mimicked.

7. Challenges and barriers

BESES significantly enhanced establishment and growth in exposed environments, while having a non-significant negative effect in sheltered areas. Aboveground BESE positively affected *Thalassia* yields in Bonaire, but had neutral (Finland, USA) to negative effects on *Zostera* yields. In Croatia, almost half of BESE modules with seagrass ramets were lost during strong winter storms, with belowground structures being more resistant. Observed losses of BESE resulted from the breakage of the structures and not due to their inadequate anchoring, implying the existence of a threshold in hydrodynamics beyond which the application of BESE modules may be severely compromised.

2.1.8 Protocol: Seagrass restoration using biodegradable materials

1. Rationale

Seagrass meadows cover about 0.1-0.2% of the global ocean (Duarte 2002) and their spatial distribution at global scale has reduced of ca. 30% in the last century (Waycott et al. 2009). Seagrass habitats are highly productive (Duarte 2002) and play a key-ecological role in the provisioning of ecosystems' goods and services (Nellemann et al. 2009; Barbier et al. 2011; Mtwana Nordlund et al. 2016). Thus, seagrass meadows are included in the list of priority habitats to protect and conserve as reported in the EU directives (e.g. Council Directive 92/43/EEC 1992). Natural recovery of altered ecosystems rarely occurs (Lotze et al. 2011) and current conservation initiatives have proven insufficient to stop and/or reduce the negative effect of anthropogenic impacts (Lindegren et al. 2018). Considering the important ecological role of seagrasses, the recovery of degraded meadows is a priority in the field of ecological restoration (Paling et al. 2009) and is considered an effective strategy to supplement current conservation and management actions for these ecosystems (Perring et al. 2015). The experiment reported is fully explained in Da Ros et al. (2021 in press)

2. Objectives

- Test the efficacy of a new seagrass transplantation technique based on the use of biodegradable bags and jars by monitoring shoot density and leaf biomass of *Cymodocea nodosa*

3. Target species and habitats:

Species: *Cymodocea nodosa.*

Location: Gabicce Mare North – Western Adriatic Sea, Italy.

Criteria for site selection

high recovery potential and no evident anthropogenic impacts,

similar physical characteristics (sediment type, depth, temperature, exposure, salinity, and nutrients) to the donor site;

similar biological characteristics (presence of grazers feeding on eelgrass or preventing algal blooms, bioturbators, facilitating species) to the donor site.

4. Materials

- Diving equipment
- Stainless-steel corer (diameter: 10cm; length: 25cm) and corks

- Hammer
- Biodegradable bags
- Biodegradable jars
- Wooden stakes and kitchen twine
- Plastic boxes for transportation
- U-shaped stainless-steel rods

5. Description of the protocol and activity

After a preliminary survey scientific operators identify the suitable donor seagrass meadows and the adjacent transplanting area. A team of four divers is necessary to safely complete all steps of the protocol.

Step 1. The operator inserts a stainless-steel corer in the seagrass in order to collect the whole clod with rhizome and leaves avoiding their damage. Leaves are wrapped on themselves, inside the corer (Fig. 1).

Fig. 1 (Photo credit: F. Torsani)

Step 2. The use of a hammer can facilitate the insertion of the corer in the sediment. To collect the clod is necessary to insert at least 2/3 of the corer height in the sediment (Fig. 2).

Fig. 2 (Photo credit: F. Torsani)

Step 3. The upper opening of the corer is closed with a cork. This facilitates the maintenance of the clod with rhizomes and leaves inside the corer. This is very important for the next step when the corer is removed (Fig. 3).

Fig. 3 (Photo credit: F. Torsani)

Step 4. The operator gently removes the corer with the clod from the sediment (Fig. 4).

Fig. 4 (Photo credit: F. Torsani)

Step 5. The operator put the corer closed in the upper opening inside a biodegradable bag included in a biodegradable jar. These biodegradable materials are prepared by the second operator who receive all stuffs (Fig. 5).

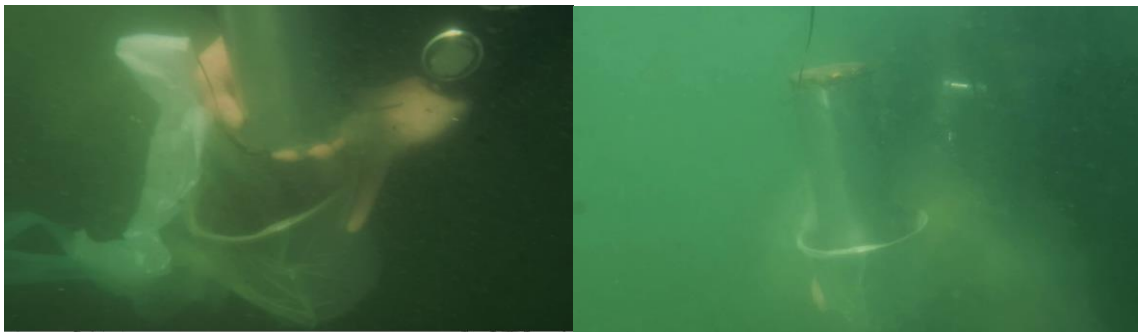


Fig. 5 (Photo credit: F. Torsani)

Step 6. The operator emerges with attention and facilitate the removal of the water from the biodegradable jar maintaining the vertical position of the corer (Fig. 6).

Fig. 6 (Photo credit: F. Torsani)

Step 7. The cork is removed to release the clod with rhizome and leaves in the biodegradable bag and jar (Fig. 7).

Fig. 7 (Photo credit: F. Torsani)

Step 8. Several clods are collected and stored in plastic boxes with seawater to avoid any drying to the seagrasses removed from the donor meadows (Fig. 8).

Fig. 8 (Photo credit: F. Torsani)

Step 9. Contextually to the seagrass collection for transplanting, other two diving operators prepare the receiving sites, delimiting 3 plots (1m × 1m) using wooden stakes and kitchen twine (Fig. 9). These stuffs are biodegradable and easy to find. The plots that host

transplanted seagrass are prepared digging holes using manual corers. Each hole received one jar with the clod. The number of jars is depending on the density of the donor meadows. Each jar is inserted in one of the prepared holes and anchored with a U-shaped stainless-steel rod to increase the stability of the stuff and avoid the removal in case of high-energy conditions.

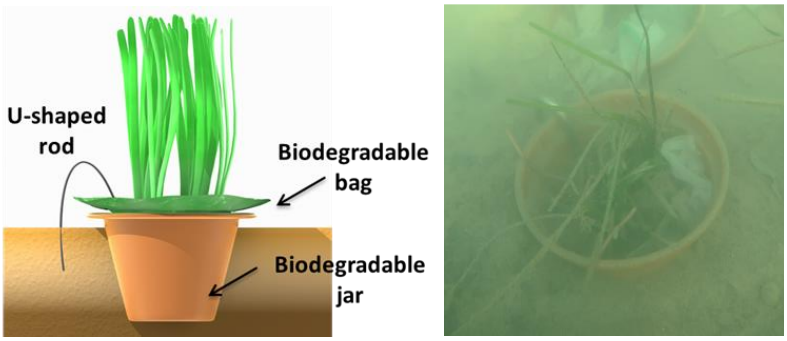


Fig. 9 (Photo credit: F. Torsani)

Step 10. The boards of the biodegradable bags that come out of the jar are removed using scissors (Fig. 10).

Fig. 10 (Photo credit: F Torsani)

Step 11. The activities can require several days depending on the area (square meters) covered by transplanting and the number of divers employed to transplant seagrass. The monitoring of the efficacy of the transplanting should cover one year, preferentially on monthly basis. Fig. 11 shows the transplanting seagrass after one year.

Fig. 11 (Photo credit: F. Torsani)

6. Observations and Recommendations

- Preliminary survey is important to assess the shoot density of the donor seagrass meadows in order to select a conservative shoot density to transplant limiting the potential impact of shoots removal from the donor site.
- The life cycle of the selected species (fast vs slow growth) could favour the success of the seagrass transplanting.
- The seagrass transplanting should be carried out immediately after the removal from the donor seagrass to limit the operative stress to the plants.
- The selection of the best suitable site for transplanting is a priority: an area close to a natural park, as far as possible from the crowded beaches (especially in Summer), limits the anthropogenic impact which could potentially compromise the conservation of the transplanted seagrasses.
- Spring has been identified as the best suitable period to conduct successfully seagrass transplantation in temperate ecosystems. This period favours the settlement, maintenance and the vegetative growth of the underground rhizome of the transplanted seagrass. Good environmental conditions immediately after the transplanting favour the settlement and conservation of the transplanting seagrass.
- The use of biodegradable bag and jar maintains and stabilizes the consistency of the clod with rhizomes and leaves. Besides, the lack of high-energy events along the shoreline immediately after the transplanting favours the expansion of the roots and the settlement of the transplanted plants in the bare sediments.
- One year monitoring is the minimum temporal interval to verify the efficacy of the translating approach to cover the temporal variability.
- Experimental plots have to be replicated and included donor and transplanted seagrass to assess the efficacy of the restoration activities.

7. Challenges and barriers

The physical stress due to high-energy storms that generally occur during the Winter can compromise the settlement of the transplanted seagrass and slow down their potential recovery, since waves and currents could dislodge poorly anchored plants that did not have enough time to establish and expand their roots. Our results show that this restoration technique was successful, enabling the seagrass survival (approximately 30%) even in high-energy conditions occurring in winter. Transplanted *C. nodosa* requires a certain time scale to anchor their roots to the new substrate, suggesting that the transplanted seagrasses can show a delay in reaching density and biomass comparable to

those reported in donor seagrass meadows. The scaling-up of the transplanting requires a team of divers with a good experience on restoration activities and good environmental conditions for a period enough to complete the activities and allow the stabilization of the transplanted seagrass. The effects of seagrass transplanting should be evaluated using a multi-levels approach in which not only seagrass density or biomass are evaluated by also the sedimentary trophic status; ecosystem functioning and the abundance and diversity of associated fauna. The sediments hosting the transplanted seagrasses showed an increase of trophic availability and of rates of organic matter cycling. The coastal area are generally characterized by an important touristic activity during the summer and this can be an issue for the conservation of the transplanting plots. A dedicated campaign to raise awareness for tour operators and tourists can support the preservation and maintenance of the restoration activities.

2.1.9 Protocol: Seagrass-bivalve co-restoration: *Pinna nobilis* and *Cymodocea nodosa*

1. Rationale

Seagrass meadows are unique, productive, and highly diverse ecosystems, which provide habitat and food for organisms (Hemminga and Duarte 2000). Seagrass meadows can provide shelter from water flow caused by wind waves or tides and increase the particle supply to associated filter feeders. The fan mussel *Pinna nobilis* (Linnaeus, 1758) is endemic to the Mediterranean Sea, where it typically occurs in association with *Posidonia oceanica* meadows, and is long-lived, achieving life spans in excess of 20 years (Galinou-Mitsoudi et al. 2006; Richardson et al. 1999). Fan mussels live partially buried upright in the sand, anchored by their numerous byssus filaments to the rhizomes and shoots of *P. oceanica*. Currently, *P. nobilis* has become a threatened and vulnerable species and is legally protected under Annex II of the Barcelona Convention (SPA/BD Protocol 1995), Annex IV of the EU Habitats Directive (EU Habitats Directive 2007), and the Spanish Catalogue of Threatened Species (Category: Vulnerable, Royal Decree 139/2011). The population numbers of the bivalve *P. nobilis* are currently in decline (Centoducati et al. 2007) due to both an increase in anthropogenic impacts on coastal areas resulting from increased human population growth and incidental damages by trawling and anchoring and collection by divers (Katsanevakis 2007; Richardson et al. 2004). Recent mass mortality events of the pen shell *Pinna nobilis* are reported over hundreds of kilometers of the western Mediterranean coast of Spain, except for Catalonia and along the Tyrrhenian coast of Italy (Carella et al. 2019; Vázquez-Luis et al 2017; Darriba et al., 2017). This makes priority to find best practices for bivalve transplanting and restoration.

2. Objectives

- Test the efficacy of a protocol for the translocation of specimens of *P. nobilis* from a donor to receiving area in order to test interspecific interactions between mussels and seagrass and their potential effects on the seagrass restoration.

3. Target species and habitats

Species: *Pinna nobilis; Cymodocea nodosa.*

Location: Gabicce Mare, North – Western Adriatic Sea, Italy.

Criteria for site selection

high recovery potential and no evident anthropogenic impacts,

similar physical characteristics (sediment type, depth, temperature, exposure, salinity, and nutrients) to the donor site,

similar biological characteristics (presence of grazers feeding on eelgrass or preventing algal blooms, bioturbators, facilitating species) to the donor site.

4. Material

- Diving equipment
- Plastic boxes for transportation of mussels
- Cloths soaked with seawater for the maintenance of pen-shells during transportation
- Stainless-steel corer and its cork
- Hammer
- Pebbles
- U-shaped stainless-steel rods
- scissors

5. Description of the protocol and activity

Before any translocation of *P. nobilis*, it is necessary to request and obtain authorization from the competent national authority. After a preliminary survey scientific operators identify the suitable plots in which bivalves can be transplanted. A team of at least four divers is necessary to safely complete all activities.

Step 1. Specimens of *P. nobilis* are identified by scientific diving operators in the donor area. The number of specimens collected from the donor to the translocation area should be select to minimize any negative effects on the specimens of the donor site (Fig. 1).

Fig. 1 (Photo credit: Marco Lo Martire)

Step 2. The operators gently remove the pen shells from the donor area paying attention to avoid any damage to the byssus and shells. Each bivalve is marked and measured following the guidelines of Bottari et al. (2017). During the immediate transfer to the receiving site, specimens are stored in wet conditions using refrigerated boxes (*in situ* temperature).

Step 3. Before the pen-shells collection for transplanting, two diving operators prepare the receiving sites, delimiting plots (1m × 1m) using wooden stakes and kitchen twine. These stuffs are biodegradable and easy to find. The receiving areas can include experimental plots with seagrass and transplanted seagrass. Seagrass transplanting has to be performed a week before the transplanting of bivalves. The protocol for seagrass transplanting is described above (protocol 2.1.8).

Fig. 2 (Photo credit: Marco Lo Martire)

In the centre of each plot, a housing for the transplanting bivalve is prepared in the sediment using a stainless-steel corer. The dimension of the hole should be suitable for the size of *P. nobilis* and should contain pebbles. The presence of pebbles can facilitate the byssus attachment (Fig. 2).

Step 4. Each bivalve is gentle insert in the hole with pebbles. A stainless-steel U-shaped rod can be used to anchorage the specimen to the seafloor (Fig. 3).

Fig. 3 (Photo credit: Marco Lo Martire)

Experimental plots have to be replicated and included donor and transplanted seagrass to assess the efficacy of the *P. nobilis* in the restoration.

6. Observations and Recommendations

- Preliminary survey is important to identify the best suitable site to perform both *P. nobilis* and/or seagrass transplanting and also to limit any negative effects on species involved.
- Be sure to use densities of *P. nobilis* and seagrass from donor sites that do not affect the conservation of the donor species in terms of density and biomass.
- *P. nobilis* transplanting should be carried out immediately after the removal from the donor site to limit the operative stress to the bivalve. During the transfer from the donor to the receiving site is important to keep the conditions of storage similar to the environmental ones.
- The selection of the best suitable site for transplanting is a priority: an area close to a natural park, as far as possible from the crowded beaches (especially in Summer), limits the anthropogenic impact which could potentially compromise the conservation of the transplanted species.
- Spring and Summer is the suitable periods to perform transplanting according to the life cycle (reproductive periods) of *P. nobilis* and seagrass. This period favours the settlement and conservation of species. Good environmental conditions immediately after the transplanting favour the settlement and maintenance of the transplanting species.
- The presence of high density seagrass favour the stabilization and conservation of the *P. nobilis* specimens.

- The seagrass offers an efficient repair to the *P. nobilis* to the high-energetic hydrodynamic conditions that can occur during the winter season.
- The use of a hole and pebbles is efficacy in the maintenance of the *P. nobilis* byssus.
- The presence of *P. nobilis* may increase the availability of food sources for benthic fauna associated with seagrasses meadows.
- One year monitoring is the minimum temporal interval to verify the efficacy of the translating approach to cover the temporal variability.
- Experimental plots have to be replicated and included donor and transplanted seagrass to assess the efficacy of the restoration activities.

7. Challenges and barriers

The physical stress due to high-energy storms that generally occur during the Winter can compromise the settlement of the transplanted specimens and slow down their potential recovery, since waves and currents could dislodge poorly anchored plants and bivalves that did not have enough time to establish and expand their roots and byssus, respectively. The effects of seagrass and *P. nobilis* transplanting should be evaluated using a multi-levels approach in which not only seagrass density or biomass and *P. nobilis* survival are evaluated by also the sedimentary trophic status; ecosystem functioning and the abundance and diversity of associated fauna. The coastal area are generally characterized by an important touristic activity during the summer and this can be an issue for the conservation of the transplanting plots. A dedicated campaign to raise awareness for tour operators and tourists can support the preservation and maintenance of the transplanted species. The setup of efficient protocols and guidelines for *P. nobilis* transplanting is priority on the light of the recent mass mortality events reported over hundreds of kilometers of the western Mediterranean coast.

2.1.10 Protocol: Seagrass-bivalve co-restoration using *Pinna nobilis* and *Cymodocea nodosa*

1. Rationale

Both the noble pen shell *Pinna nobilis* (Linnaeus, 1758) and a seagrass *Cymodocea nodosa* (Ucria) Ascherson 1870 are endangered and strictly protected Mediterranean habitat formers. Despite of numerous examples of bivalve-seagrass facilitation, for these two species such interactions were not previously explored.

2. Objectives

- To test if transplantation of *P. nobilis* into existing seagrass meadows can increase the growth/survival of either or both species.

3. Target species and habitats

Species: *Pinna nobilis; Cymodocea nodosa.*

Location: Javorike Bay, Brijuni MPA, North Adriatic Sea, Croatia.

Criteria for site selection:

The nearby marine protected area (Brijuni MPA) was selected as a host site because it ensured protection of pen shells from direct adverse impacts such as anchoring and illegal extraction. The selected bay already harbour a sparse *P. nobilis* population within a well developed *Cymodocea nodosa* meadow at a shallower depth.

4. Materials (the same as used in the protocol for *Pinna nobilis* translocation from a disturbed site)

- Trowels
- Plastic boxes sub-divided by a rope
- Metal rods
- Tanks (preferably with a constant supply of fresh seawater) or at least with air pumps
- Boat (preferably supplied with a water pump)/other mean of transportation, depending on a distance between donor and a host location
- Diving equipment

5. Description of the protocol and activity

Step 1. Noble pen shells were collected from a donor site to be transplanted into the $1m^2$ plots at 12m depth with and without seagrass (on unvegetated sandy bottom).

Step 2. During transplantation, pen shells were carefully dug out using trowels and planted at host sites without provision of any additional anchoring substrate (burying approx. 1/3 of the shell-the anterior part, as ocurring naturally for this semi-infaunal bivalve). Plots with pen shells were assigned either to low (1 ind m^2) or high density (5 ind m^2) treatment whereas controls contained no pen shells. There were 5 replicates per each treatment (25 plots in total).

Step 3. Bivalve survival and growth were monitored, as well as seagrass growth after 1 and 2 years post-transplantation.

Fig. 1. High density (5 ind m^2) of *Pinna nobilis* treatment in *Cymodocea nodosa* bed (Photo credit: D Petricioli)

Fig. 2. High density (5 ind m^2) of *Pinna nobilis* treatment on bare sand (Photo credit: D Petricioli)

Fig. 3. Marked *Cymodocea nodosa* shoots; measuring seagrass growth by a pin-holed method (Photo credit: D. Petricioli)

6. Observations and Recommendations

- Pen shell survival on bare sediment was high 5 months post-transplantation but was compromised by an autumn storm. In such an exposed site, transplanting pen shells within seagrass meadow substantially increased their survival.
- Growth of seagrass *C. nodosa* was enhanced by high-density pen shell treatment (5 ind m^2) and in general, total nitrogen levels were higher (although not significantly) in the sediment of plots with pen shells 1 year post-transplatation.
- This is the first study to show mutual facilitation of the noble pen shell *P. nobilis* and a seagrass. Transplanting *P. nobilis* within seagrass meadow enhances its survival in exposed areas, given that transplantation is (ideally) carried out during early summer, thus providing enough time for pen shells to regenerate byssus and anchor well, prior to winter storms. Furthermore, transplanting pen shells in high density (e.g. 5 ind m^2) may enhance *C. nodosa* growth through a putative fertilization effect, but the overall effect may be context dependant i.e. influenced by environmental conditions such as light intensity and hydrodynamism.

7. Challenges and barriers

Unlike for majority of bivalves, considerable time (i.e. several months) is needed for a byssus of the noble pen shell *Pinna nobilis* to fully regenerate, and hence for a pen shell to anchor well. Therefore, transplanting pen shells shortly before the likely incidence of heavy storms at exposed sites should be avoided. *Cymodocea nodosa* beds, through sediment stabilization and provision of additional substrate for pen shell's anchoring in the form of intermingled matrix of rhizomes and roots, may significantly enhance pen shell survival in

conditions of elevated hydrodynamism, but again, initial transplantation needs to take place during a calm season. The effect of the noble pen shell on *Cymodocea nodosa* may be context dependent. Given the current disease alert for the noble pen shell (March 2020), all activities including its translocation should be ceased until any doubt can be excluded that targeted populations and individuals are affected. At the moment, greater knowledge on the factors involved in disease outbreaks is urgently needed in order to properly plan future conservation and restoration actions involving this critically endangered bivalve.

2.1.11 Protocol: *Pinna nobilis* translocation using cages

1. Rationale

Pinna nobilis L., 1758, the Mediterranean endemic fan mussel mainly habitats seagrass meadows and is a good indicator for marine ecosystem changes. Its presence is known to increases oxygen levels and potentially affect the health of seagrass for the positive while seagrass may have some benefits over *Pinna sp.* by utilizing increased organic compounds.

2. Objectives

- Test if a protective cage can help *Pinna nobilis* to establish after translocation?

3. Target species and habitats:

Species: Noble pen shell *Pinna nobilis.*

Location: Gökova Bay, Turkey, Eastern Mediterranean.

Criteria for site selection

Gökova Bay is a marine region in the southeastern Aegean Sea with a narrow entrance into the land. It is considered ecoregion as a biodiversity hotspot in the Mediterranean Sea which highlights the importance of the Gökova Bay MPA by means of being core breeding and resting site for rare and threatened species. It is also the MPA in Turkey with first NTZs, which also provided a unique opportunity to experimentalize various active restoration methods in an area that is strictly restricted to human activities in order to understand efficiency of restoration actions.

4. Materials

- Scuba equipment
- Bag to protect *P. nobilis* with its surrounding sediment during transportation
- Hammers
- PVC pipe cages 1 m x 1 m x 0.5 m cages covered with 1x1 cm plastic mesh
- Metal hooks to anchor cages
- Brush for monthly cleaning of the cages
- Shovel

5. Description of the protocol and activity

Step 1. *P. nobilis* translocation was done by collecting small individuals from the vicinity and digging out with 50 cm radius and 50-60 cm deep sediment to protect the byssus as much as possible.

Step 2. All individuals were then transferred by covering attached sediment with plastic bag and carried underwater.

Step 3. They were placed and covered with their original sediment, and no support was used. Cages (1 × 1 × 0.5 m) were used to cover the individuals (Fig.1).

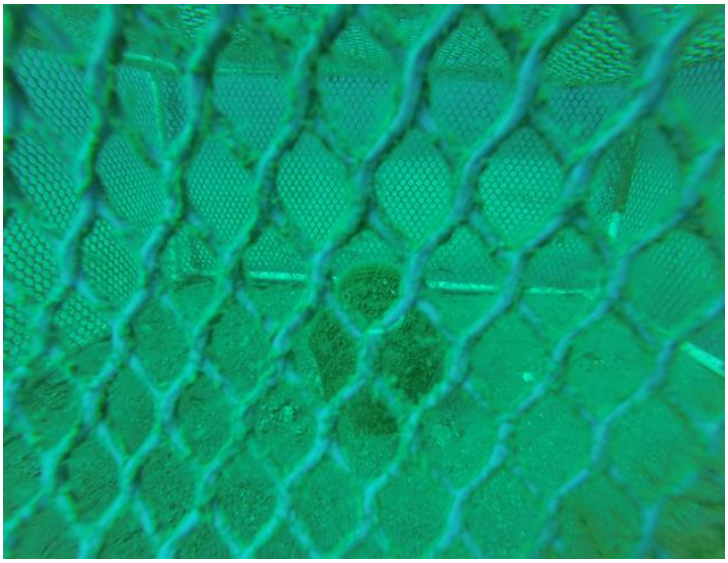


Fig. 1. Translocated *Pinna nobilis* under cage protection

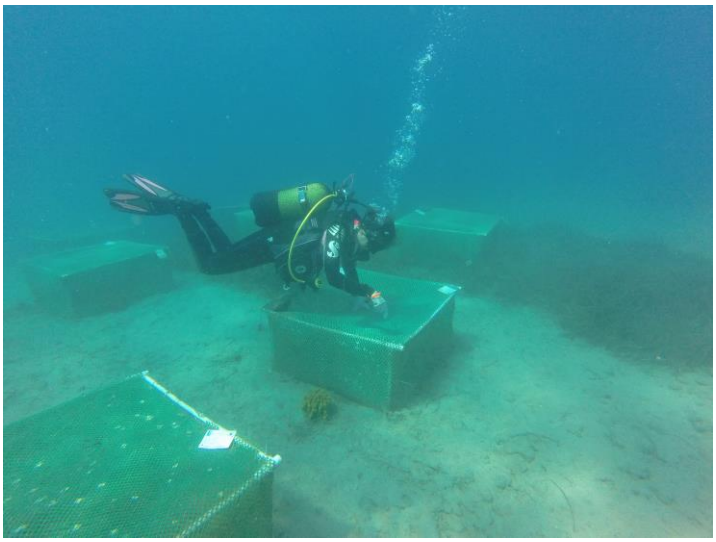


Fig. 2. Periodic cleaning of the cages against organisms or sediment accumulation

6. Observations and Recommendations

- Transplanted *P. nobilis* individuals were alive and healthy after the winter and spring periods. Some new individuals were observed in spring on both cage covered and uncovered plots and few on the frame of the cages.
- It was observed that cages help *P. nobilis* to anchor after translocations and promote recruitment of new individuals, but a solid conclusion cannot be made due to disease outbreak.

7. Challenges and barriers

In July 2018, due to parasite infection all individuals were either looking unhealthy (slowly closing their shell) or even dead. The infection wiped out a large portion of the Eastern Mediterranean *P. nobilis* population (Fig. 3).

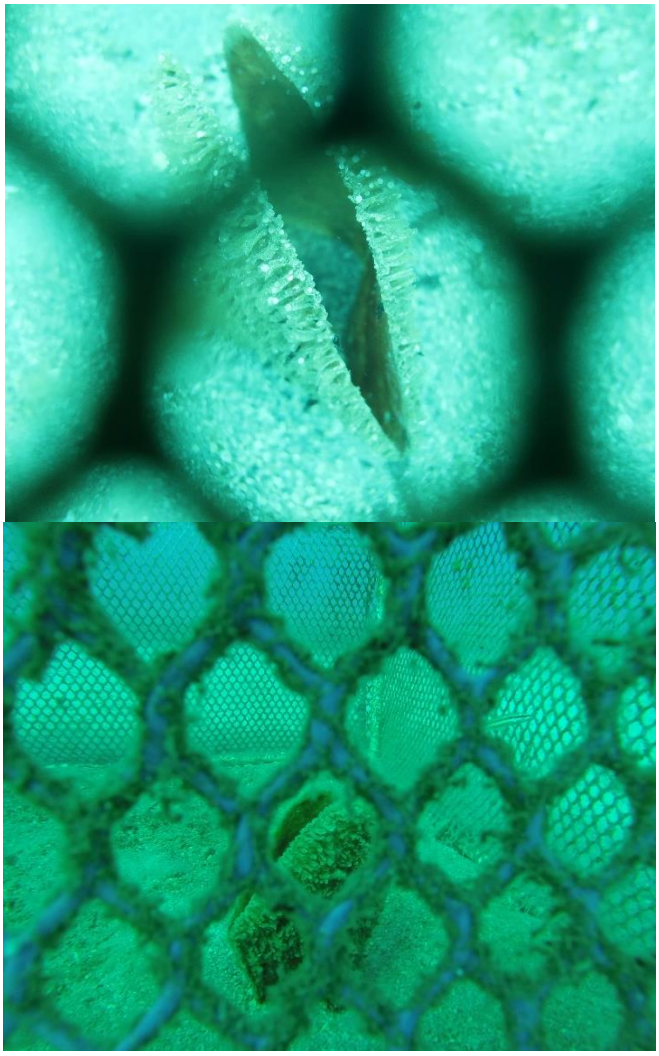


Fig 3. Photos of dead *Pinna nobilis*.

2.1.12 Protocol: *Posidonia oceanica* restoration using cages to prevent herbivory

1. Rationale

The Neptune grass, *Posidonia oceanica* (L.) Delile, is ecologically important, endemic, and abundant seagrass, which provide major ecological functions and services besides hosting very high biodiversity in the Mediterranean Sea. Eastern Mediterranean, Levantine Sea being the front line for the effects of warming of seawater and lessepsian species in addition to anthropogenic factors such as anchoring have decreased Neptune grasses shoot density and health of the meadows.

2. Objectives

- Test if cages help seagrass transplantation success against grazing effects.

3. Target species and habitats:

Species: Neptune grass *Posidonia oceanica*

Location: Gökova Bay, Turkey, Eastern Mediterranean.

Criteria for site selection:

Gökova Bay is a marine region in the south-eastern Aegean Sea with a narrow entrance into the land. It is considered ecoregion as a biodiversity hotspot in the Mediterranean Sea which highlights the importance of the Gökova Bay MPA by means of being core breeding and resting site for rare and threatened species. It is also the MPA in Turkey with first NTZs, which also provided a unique opportunity to test various active restoration methods in an area that is strictly restricted to human activities in order to understand efficiency of restoration actions.

4. Materials

- Scuba equipment
- Hammers
- PVC pipe cages 1 m × 1 m × 0.5 m cages covered with 1 × 1 cm plastic mesh
- Metal hooks to anchor cages
- Brush for monthly cleaning of the cages
- Shovel
- Steel bars
- Zip tie

5. Description of the protocol and activity

Two controls (bare sediment and seagrass) and four experimental treatments were considered, with three replicates (1 × 1 × 0.5 m cages) each placed between 8-11 m depth. Treatments were bare sediment, bare sediment + transplanted *P. oceanica*, and already existing *P. oceanica*.

Step 1. Transplantation was conducted by removing plants with their rhizomes using a shovel.

Step 2. Transplants were chosen from the same depth as the experimental plot and were placed to the plots by digging a hole and covering the rhizomes with the removed sand. To secure transplants, 70 cm long steel rods were pushed into the sediment and shoots were attached using cable ties.

Fig. 1. Transplanted *Posidonia oceanica* under cage

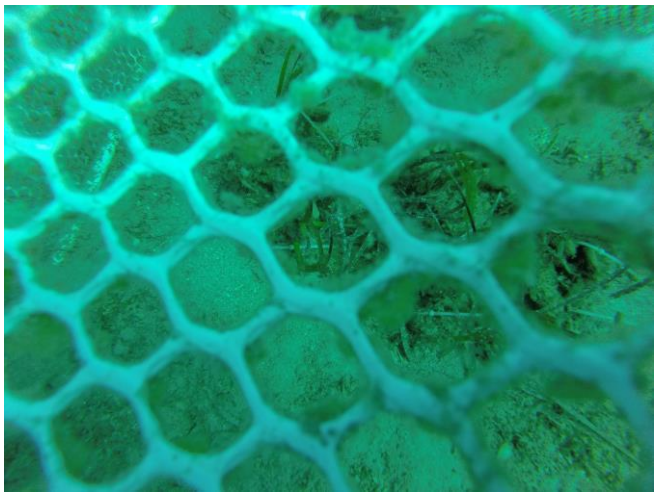


Fig. 2. Transplanted shoots under cage showing the zip ties used to station the shoots to the metal bar

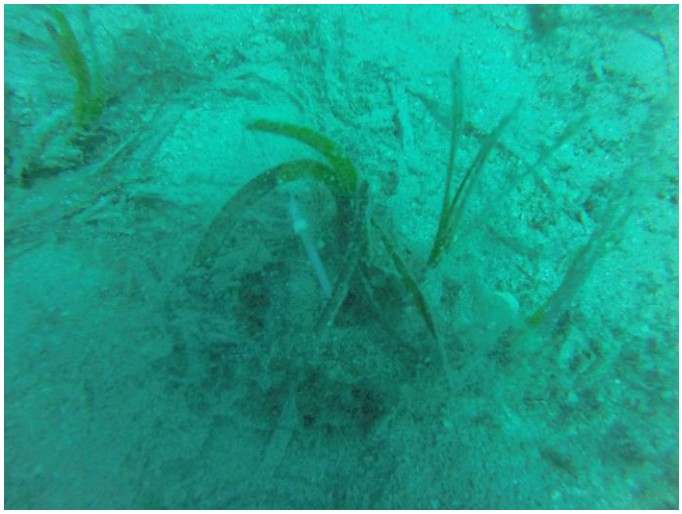


Fig. 3. Transplanted shoots without cage showing the zip ties used to station the shoots to the metal bar

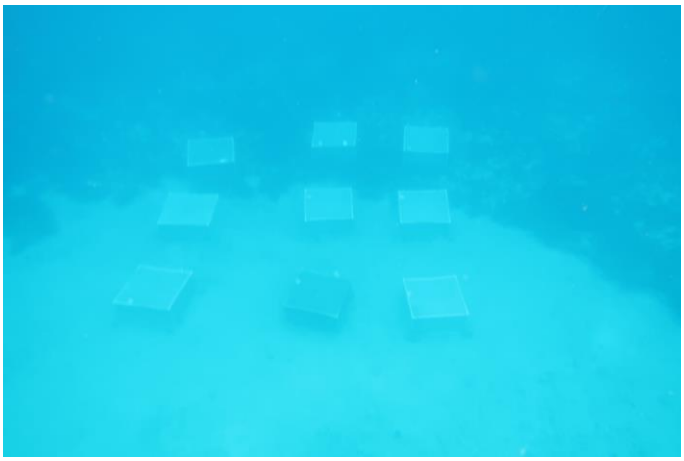


Fig. 4. 3 Replicate cages for each tested scenario

Fig. 5. Periodic cleaning of the cages against organism or sediment accumulation

6. Observations and Recommendations

- Shoot density in the natural *P. oceanica* meadows increased 45% and 11%, in plots with and without cages, respectively. Conversely, transplanted shoots decreased 29% in both cases. Additionally, cages protected transplants against anchoring damage.
- Cages can be an effective tool to protect the transplants against anchoring damage as well as protect the natural meadows against grazing.

7. Challenges and barriers

Though cages provided protection against grazing on natural meadows, an increased grazing on transplants was observed (Fig. 6). Cages need regular maintenance. In case of protecting transplants, it is not an effective method since it protects juvenile grazers from predation enabling juveniles to graze more efficiently. Additionally, presents and rapid growth and coverage of invasive *Halophila stipulacea* was observed around the edges and at bare patches of seagrass meadows indicating space competition between native and invasive species (Fig. 7).

Fig 6. Photo of transplants with grazing damage.

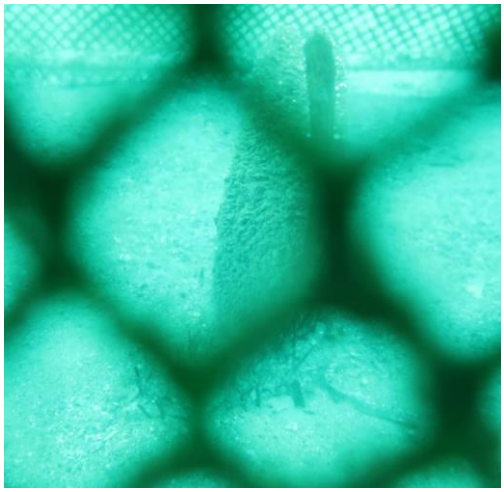


Fig 7. Photo of *Halophila stipulacea* growth under the cage

2.1.13 Protocol: Translocation of the noble pen shell *Pinna nobilis* as a species conservation action and a restorative measure for biogenic hard bottom habitat

1. Rationale

The strictly protected species *Pinna nobilis* (Linnaeus 1758) is an endemic, long-lived Mediterranean species and one of the largest bivalves in the world, reaching up to 120 cm in shell length (Zavodnik et al. 1991). As a suspension-feeding habitat-former it provides important biogeochemical functions of water clarification and biodeposition, and enhances local biodiversity (e.g. Addis et al. 2009; Trigos et al. 2014; Rabaoui et al. 2015). Anthropogenic and environmental threats such as habitat loss or degradation due to intense coastal development, anchoring, trawling, illegal extraction and most recently, a rapidly spreading disease (see review in Basso et al. 2015; Vázquez-Luis et al. 2017) have contributed to the decline of its populations across the Mediterranean. This bivalve is listed as endangered under the 1992 European Council Directive on the conservation of natural habitats and wild fauna and flora (92/43/EEC, Annex IV). It has been protected by the Protocol for Specially Protected Areas Biological Diversity in the Mediterranean (Barcelona Convention: UNEP) since 1996. It is also strictly protected by national laws. To avoid smothering of the noble pen shell population during construction of a new nautical centre in the Pula Harbour (North Adriatic Sea, Croatia), the environmental impact assessment prescribed translocation of this bivalve as a conservation measure. Since its shells provide substrate for diverse epibiontic community, at the same time such an action can be considered as a restorative measure for biogenic hard bottom habitat, that in addition enhance local diversity, i.e. ecosystem services of the host vegetated or unvegetated sediment bottoms.

2. Objectives

- To save population of an endangered and protected bivalve from smothering due to coastal construction;
- To test the translocation of *P. nobilis* as a previously suggested conservation action to protect the species and restore its shells as a biogenic hard bottom habitat.

3. Target species and habitats

Species: *Pinna nobilis*

Location: unvegetated sediment bottom at a donor site (Pula harbor) and seagrass (*Cymodocea nodosa*) meadow at the host site (Javorike and Pisak bays, Brijuni MPA, North Adriatic Sea, Croatia).

Criteria for site selection:

The nearby marine protected area (Brijuni MPA) was selected as a host site because it ensured protection of pen shells from adverse impacts of anchoring and illegal extraction and enabled monitoring of their survival. The selected bays within the MPA already harbor a sparse *P. nobilis* population within seagrass bed, and are located in the more sheltered part of MPA, not too exposed to hydrodynamism.

4. Materials

- Trowels
- Plastic boxes sub-divided by a rope
- Metal rods
- Tanks (preferably with constant supply of fresh seawater) or at least with air pumps
- Boat (preferably supplied with a water pump)/other mean of transportation, depending on a distance between donor and host location
- Diving equipment

5. Description of the protocol and activity

Step 1. Small trowels were used by SCUBA divers to carefully dig out pen shells from the sediment (Fig. 1a), in order to avoid (aggressive) pulling that could damage byssus gland responsible for the production of byssus threads used for pen shell attachment to the substrate.

Step 2. Pen shells were collected in sub-divided plastic boxes, used for their transportation by divers while in the sea (Fig. 1b).

Step 3. Until the last moment, pen shells were kept in the sea and were transferred to a larger boat at the time of departure (Fig. 1c). Pen shells can survive very short time periods out of the water, as sometimes happens in shallow areas during low tides. On the boat, pen shells were placed in large tanks with a constant supply of fresh seawater (Fig. 1d). We collected data on shell morphometry (total height and maximum shell width) for all rescued individuals.

Step 4. In the host habitat, we used a trowel or a metal rod to create holes in the sediment (Fig. 1e) where we translocated pen shells by inserting anterior part of the shell, covering

approximately 1/3 of the total shell height with sediment, as occurs naturally for this semi-infaunal bivalve (Fig. 1f).

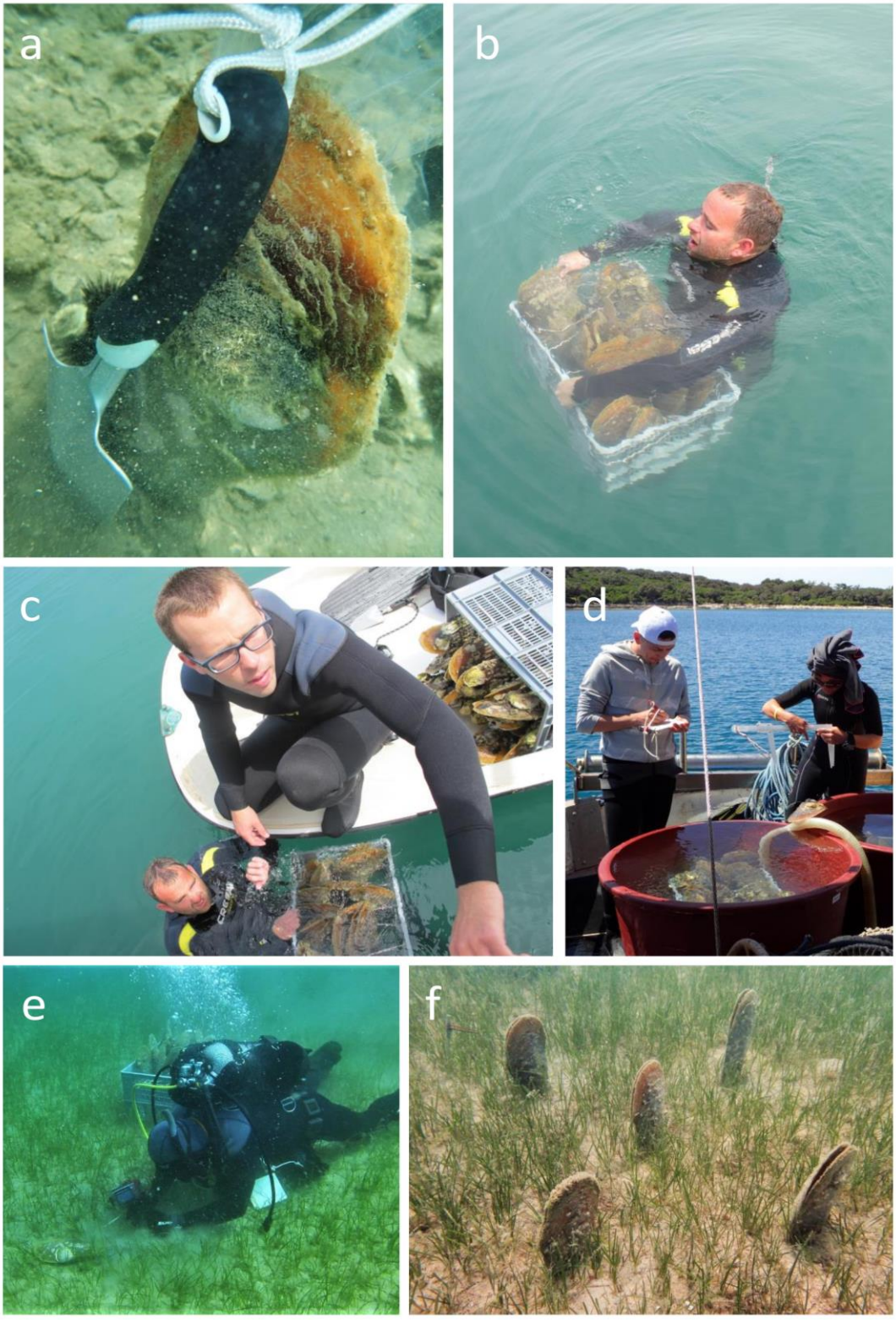


Fig. 1. Translocation of the noble pen shell *Pinna nobilis* in the Northern Adriatic (Croatian coast): a) digging out pen shells using trowels in Pula harbor; b) volunteer diver transporting pen shells; c) transferring pen shells on board a bigger vessel; d) transporting pen shells in tanks supplied by fresh seawater and measuring them on board; e) translocating pen shells in a host *Cymodocea nodosa* meadow in Javorike Bay (Brijuni MPA); f) translocated pen shells with their epibionts in a host location. (Photos credit a-c and e, S. Kipson; d and f, D. Petricioli).

6. Observations and Recommendations

Pen shell translocation was confirmed as an effective conservation method, resulting in high survival and increased function and services of the host habitat. The overall success of this action stems from cooperation and understanding of all involved parties (the investor, governmental bodies, national park authority, scientists and citizen scientists-volunteer divers). However, several major points need to be addressed before, during and after translocation in order to secure its effectiveness.

Prior to translocation:

- Need for securing the funds necessary for the action.
- Selection of appropriate donor population (especially important during ongoing disease alerts, see conclusions below), giving priority to larger, less vulnerable individuals (> 8 cm shell width, Katsanevakis 2016) and/or > 37 cm in total length, more likely to be already reproductive, Trigos et al. 2018).
- Selection of appropriate host location where pen shells' post-translocation survival would be enhanced. The best would be to select a location in which pen shells already live and preferably where there is a seagrass meadow e.g. *Cymodocea*; bare sand locations should be avoided as they could point to very strong hydrodynamism which could damage or dislodge translocated individuals. Additionally, selected sites should be devoid of adverse anthropogenic impacts such as anchoring, illegal harvesting, coastal construction.
- Selection of appropriate time of the year (sea and air temperatures should be similar during translocation process, and translocated individuals should have enough time prior to winter storms to re-grow byssus and firmly attach themselves to the bottom).
- Obtaining all the necessary permits, which can take considerable time.

During transplantation and transfer of organisms:

- Careful manipulation/digging out of pen shells not to damage byssus gland and to preserve byssus.
- Continuous water aeration during transport of pen shells to new location.
- Similar sea water and air temperature during transplantation process – not to additionally stress the organisms.
- Organised and well-trained diving team; this action offers a compelling case for the citizen-science and participation of volunteer divers is desired - however they need

to be well educated about the major points in the process, as this can influence the success of transplantation.

After the transplantation:

- Organisation of adequate monitoring (including securing necessary funds).

7. Challenges and barriers

In the light of recently reported pen shell mass mortalities due to a rapidly-spreading disease across the Mediterranean, every effort should be made to minimize more manageable impact *in situ* (e.g. of coastal construction, boat anchoring, trawling, illegal extraction) in order to support maintenance of its populations relying on survival of adults. However, given the current disease alert (September 2019), we strongly advise against any noble pen shell transplantations until one can exclude any doubt that targeted populations and individuals are affected. At the moment, greater knowledge on the factors involved in disease outbreaks is urgently needed in order to properly plan future conservation and restoration actions involving the noble pen shell.

2.2 Shallow hard bottom and mesophotic

2.2.1. Protocols for macroalgae and kelps

2.2.1.1 Protocol: Adults transplanting in the fringe: *Ericaria amentacea* (C.Agardh) Molinari & Guiry (previously known as *Cystoseira amentacea var. stricta* Montagne)

The transplant of adult specimens of *Cystoseira sensu latu* 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 multiple 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 disappeared. 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.

2.2.1.2 Protocol: *Ericaria amentacea* adult transplanting

2. Objectives

- to provide the rationale and synthetize the main techniques for the restoration of shallow hard bottoms according to the different species.
- to provide step by step indications to guide the application of proposed to techniques for restoration actions.

3 Target species and habitats

Species: *Cystoseira amentacea.*

Location: Salento coasts (Apulia, SE of Italy).

Criteria for site selection:

Historical presence of target species. Availability of data from scientific, 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 assemblages, bio-disturbance, presence of invasive species).

4 Material:

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

5 Description of the protocol and activity

Step 1. Prepare the necessary material:

a) build aluminum frames. These are structures made by a 30 × 30 cm aluminum frame with PVC strings, which will ensure and facilitate the attachment of *E. amentacea* thalli (Fig. 1).

Fig. 1. Aluminum frame and its installation on the field.

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

c) hammer and chisel, to remove *E. 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 (Fig. 2).

Fig. 2. Epoxy putty used to fix transplants.

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

f) build metal fences (Fig. 3). These are rectangular, parallelepiped structures (30 × 30 × 40-50 cm), made by metal mesh and plastic tighteners.

Fig. 3. Metal fence used to avoid grazing by mean of Salema fish (i.e. *Sarpa salpa*).

Step 2. In the donor sites, identify and mark with epoxy putty 30 × 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 × 30 cm plots at an appropriate depth. Plots should be cleaned to bare rock with hammer and chisel. Using an

underwater drill, aluminum 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 *E. amentacea* similar to that observed in healthy assemblages. Approximately 13 clumps of *E. amentacea*, during its maximum vegetative period, are sufficient to cover a 30 × 30 cm surface.
In the donor locations, clumps of *E. 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 *E. 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 (Fig. 4).

Fig. 4. Installation of *C. amentacea* clumps into the field.

Step 6. To separate the potential 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 *E. 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 aluminum frames in donor and recipient locations can be removed after the hardening of the epoxy putty used to fix transplanted thalli of *E. amentacea.*

6 Observations and recommendations

We recommend performing transplanting between April and June to avoid:

- the loss of *Cystoseira s.l.* fronds due to seasonality;
- months featured by a high frequency and intensity of hydrodynamic conditions (e.g. storms), which can substantially increase the loss of transplanted thalli;
- the proximity between donor and recipient sites may determine the feasibility of restoration intervention in order to reduce the stress due to transport and conservation of thalli.

7 Challenges and barriers

Adults transplants of *C. amentacea* was efficient at maintaining canopy cover comparable between donor and restoration sites. Yet, the study revealed spatially variable outcomes possibly due to dislodgement by intense hydrodynamism. However, it is widely recognized that the removal of adult individuals is hardly sustainable for existing *Cystoseira* beds and, due to the scarce resilience of compromised canopies, it would result in an irreversible disturbance of donor forests. This is why we prefer not to mention any relevant success rate for restoration efforts.

2.2.1.3. Protocol: Germling transplanting of *Ericaria amentacea*

1. Rationale

In the project MERCES, this technique has been used for *E. amentacea*. However, it can be adopted also for all species belonging to the genus of *Cystoseira s.l.*. *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; 2018; Sales et al. 2015; Verdura et al. 2018; Tamburello et al. 2019). 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 × 30 cm plots should be selected.

2. Objectives

- to provide the rationale and synthetize the main techniques for the restoration of shallow hard bottoms;
- to provide step by step indications to guide the application of proposed to techniques for restoration actions.

3. Target species and habitats: criteria for the selection of sites and list of species

Species: *Ericaria amentacea.*

Location: Salento coasts (Apulia, SE of Italy).

Criteria for site selection:

Historical presence of target species. Availability of data from scientific, 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, bio-disturbance, presence of invasive species).

4. Material

- *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,
- aluminum foil,
- seawater-wetted towels,
- fridges and ice blocks,
- clay dishes,
- Stosch's enriched seawater (VSE),
- sea-water filters,
- autoclave,
- air pumps,
- aquaria,
- brush.

5 Description of protocols and activity

Step 1. Several donor populations, characterized by dense *E. amentacea* cover, should be identified and monitored to identify the fertile period to get receptacles available (Fig. 1).

Fig. 1. Receptacle of *E. amentacea*.

Step 2. In the meantime, material and facilities at the recipient sites can be prepared.

Needed material:

a) build double-mesh metal cages (to be used in those areas where herbivory has been found a relevant driver, Fig. 2). These are 20 × 20 cm structures made with metal mesh

and wire, which will protect *E. 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 × 4 cm openings on each side, in order to allow the access of herbivores.

Fig. 2. Metal cages used to ensure the exclusion of grazers. On the right, an example of artifact control cage used in the experiment is also reported.

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 (Fig 3).

Fig. 3. Germling clay used for *E. amentacea* juveniles out planting.

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 (Fig. 4).

Fig. 4. Collection of mature receptacles in the field.

g) aluminum 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 × 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 *E. amentacea* can be transplanted in recipient plots from healthy populations. This require arranging anchoring facilities (aluminum frames with PVC) into metal cages.

Step 4. When *E. amentacea* fronds exhibite mature receptacles at donor sites, apexes need to be collected for fertilization and cultivation of germlings in the aquarium. Personal observations report 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 (Fig. 5). 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.

Fig. 5. Check and packaging of mature receptacles in the lab.

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 $m^{-2}s^{-1}$. 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 facilities, 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.

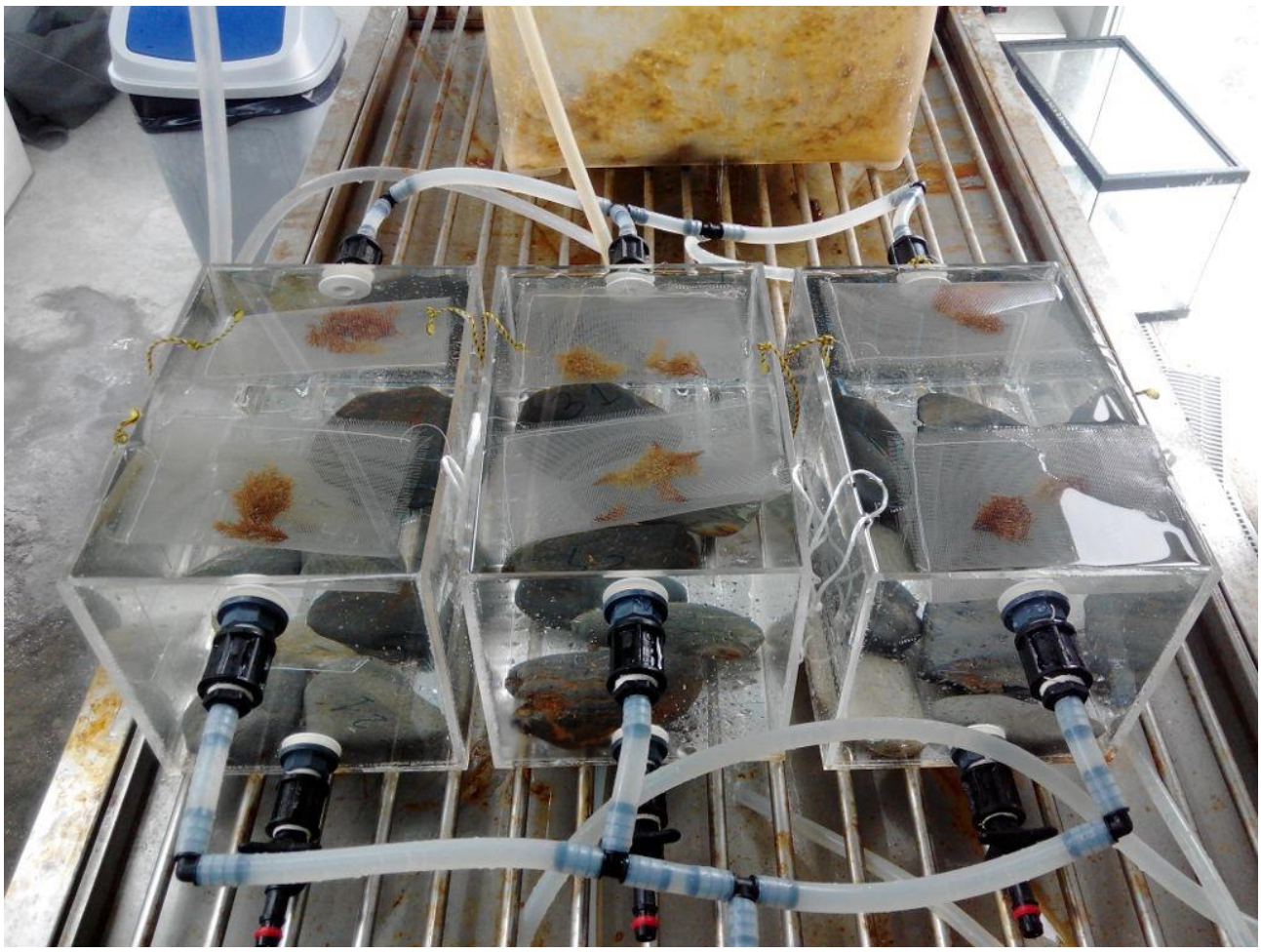


Figure 6. Fertile branches culturing to promote zygote release and recruit settlement.

Step 8. During germling culturing, adult thalli of *E. amentacea* can be transplanted at recipient sites, according to the "*E. amentacea* adult transplanting protocol".

Figure 7. Recruits cultured in aquaria facilities and transplant to the restoration site.

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 (Fig. 6).

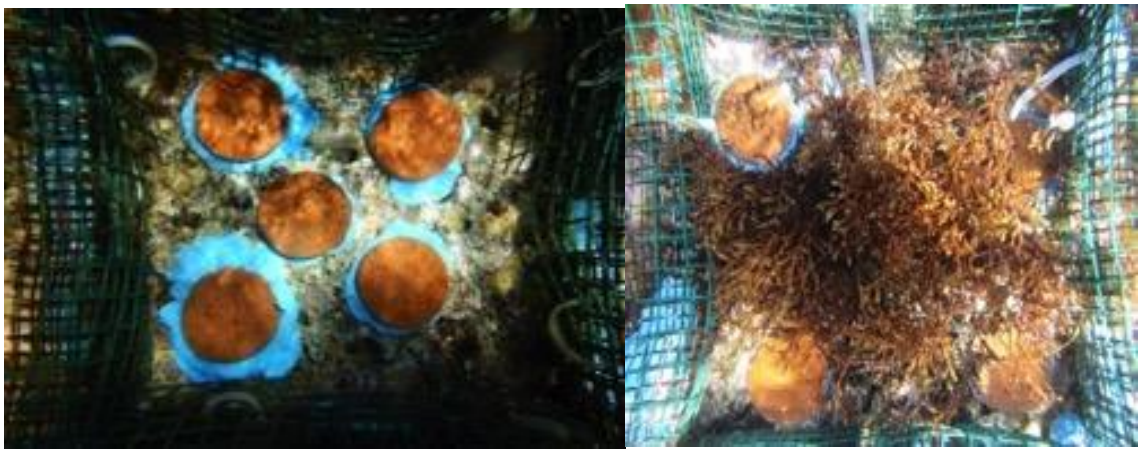


Fig. 6. Germling clays fixed inside the cages. Both conditions (i.e. with and without adults) are showed.

6 Observations and recommendations

- We recommend having prior knowledge of the phenology and recruitment periods of the species to be restored. Likewise, we recommend carrying out the restoration action coinciding with the peak of maximum fertility and recruitment of the species, in order to ensure a high contribution of zygotes and optimal environmental conditions for their settlement, survival and growth.
- For those species with long fertility and recruitment periods, we recommend carrying out the restoration action in spring (e.g. April - May), in order to reduce or avoid exposure to crowding and storms (e.g. summer and winter months) during the settlement and early stages of life.
- For the selection of the donor population, priority should be given to populations that present a good state of conservation (for example, dense *Cystoseira* s.l. coverage) and sufficient genetic variability to allow the provision of new recruits capable of adapting to environmental changes and avoiding inbreeding.
- *Monitoring the success of transplantation*
- We point out that the density (the total number of individuals per 0.04 m^2) and the structure of size classes (the length of the main axis) of the population, as well as the sexual maturity of the individuals should be established as the most appropriate indicators to evaluate the restoration success. During the first 6 months after the restoration action, the site should be visited monthly to evaluate the effectiveness of the technique, then the success monitoring should be done once or twice a year. These indicators of success should be compared with values from natural populations characterized by a good state of conservation that should be established as reference populations.

7 Challenges and barriers

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 (van 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 & Orth (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 intrinsic 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. 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.

2.2.1.4. Protocol: *In situ* seedling of *Cystoseira s.l.* species *(e.g. Gongolaria barbata* and *Ericaria crinita)*

1 Rationale

Recruitment enhancement by means of *in situ* seedling techniques, seems to be an appropriate and feasible option for threatened or endangered fucoid species. Using this technique, a large number of new recruits can be provided in the area to restore without impacting the natural donor populations. Under low hydrodynamic conditions, such as at depth below 10 m or inside lagoons, *in-situ* seedling has proved to be the most cost-effective technique (Verdura et al. 2018). In the MERCES project, this technique has been effectively used for *G. barbata* and *E. crinita*, although it can be appropriate for all fucoid species (Verdura et al. 2018; Medrano et al. 2020). 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 × 30 cm plots should be selected.

2. Objectives

- to provide the rationale and synthetize the main criteria for the elaboration of recruitment enhancement techniques for the restoration of fucoid populations (e.g. *Cystoseira s.l.*).
- To provide step by step indications to guide the application of proposed techniques for future restoration actions

3. Target species and habitats

Species: *G. barbata* and *E. crinite.*

Location: Menorca (Spain) and Catalan coast (Spain).

Criteria for site selection:

Current or historical presence of target species. Availability of data from scientific, grey literature.

Knowledge of stressors/causes of disappearance of target species and evaluation of actual mitigation/removal of the stressors.

Assessment of extant assemblage and identification of species, which could potentially influence the success of restoration (e.g. characterization of herbivore assemblage, bio-disturbance, presence of invasive species).

4. Material

- *Cystoseira s.l.* fertile thalli,
- hammer and chisel,
- mesh of 36% fiberglass and 64% PVC with a mesh size of 1.20 × 1.28 mm to build the dispersal bags (8 × 10 cm),
- pick,
- scissors,
- cooler,
- zip lock plastic bags.

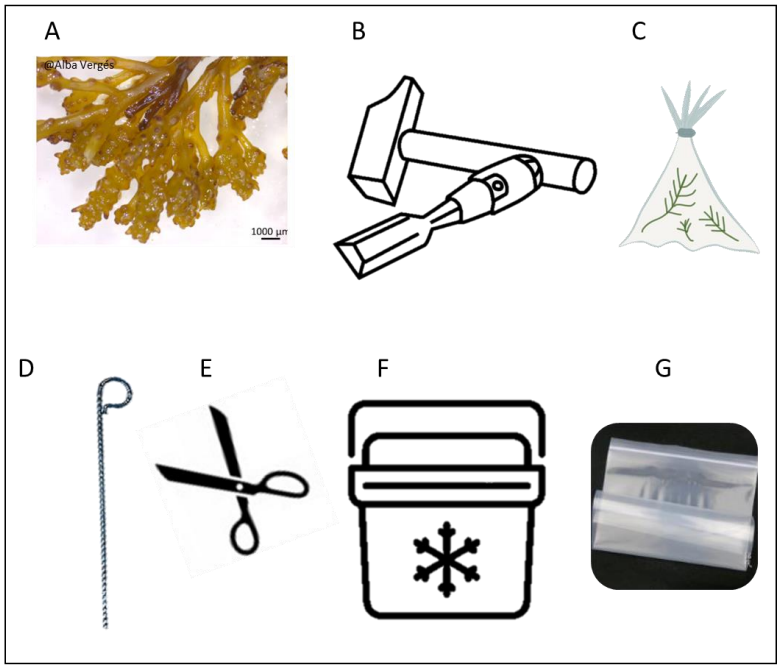


Fig. 1. List of needed material.

5. Description of protocols and activity

Step 1. During the fertile period of the target species collect fertile apical branches from the donor population. Around 100 fertile receptacles (mature apex) are required to restore an area of 25 m^2 with *E. crinita* or *G. barbata*. Cut 2-3 cm long fertile apical branches with a scissors (Fig. 2). In order to ensure a minimum degree of genetic variability and to avoid compromising the reproductive capability of exploited individuals, collect around 3 fertile apexes from each individual.

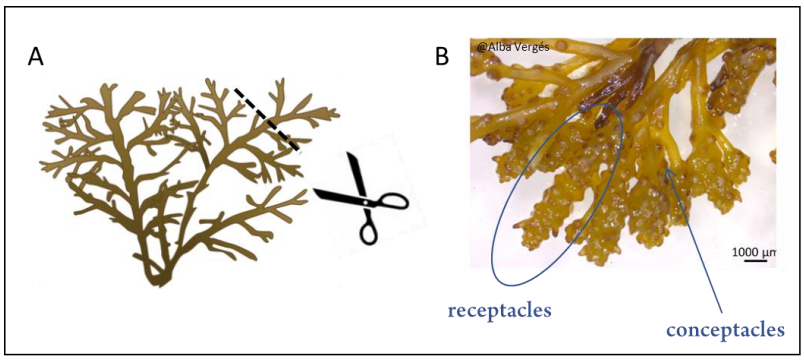


Fig. 2. A) Cut fertile apical branches of wild adult individuals (approx. 2-3 cm in length). B) Fertile apical branches with receptacles. Darker dots are conceptacles where the gametes are located.

Step 2. Transport the fertile branches to the restoration site. Transport should be done without water inside a plastic zip-lock bag and cold/fresh and dark conditions.

Step 3. Place the fertile branches in the dispersal bags, each bag should contain around twenty fertile receptacles. Dispersal bags (8 × 10 cm), made of 36% fiberglass and 64% PVC with a mesh size of 1.20 × 1.28 mm are recommended. Tie two dispersal bags to each pick (Fig. 3).

Fig. 3. Dispersal bags tied to a pick.

Step 4. The receiving area should be divided in several sites (from 25 - 30m^2) and 200m apart each other.

Step 5. For each receiving site (25m^2) eight dispersal bags containing fertile branches (two for each pick) should be placed interspaced and separated at distances of 2-3 m from each other. Fix each peak directly to the bottom with a hammer, ensuring that the dispersion bags remain floating at a vertical distance of 25 cm from the bottom (Fig. 4).

Fig. 4. Detail of the dispersal bags fixed *in situ* with the free substrate provided to promote *Cystoseira* recruitment.

Step 6. Provide free substrate close to the dispersal bags by placing flat stones deprived of any organisms, or by cleaning the rocky substrate with a metal brush to promote settlement of *Cystoseira s.l.* (Fig. 4). This is especially important if the area to restore is dominated by turf algae that can outcompete the new recruits.

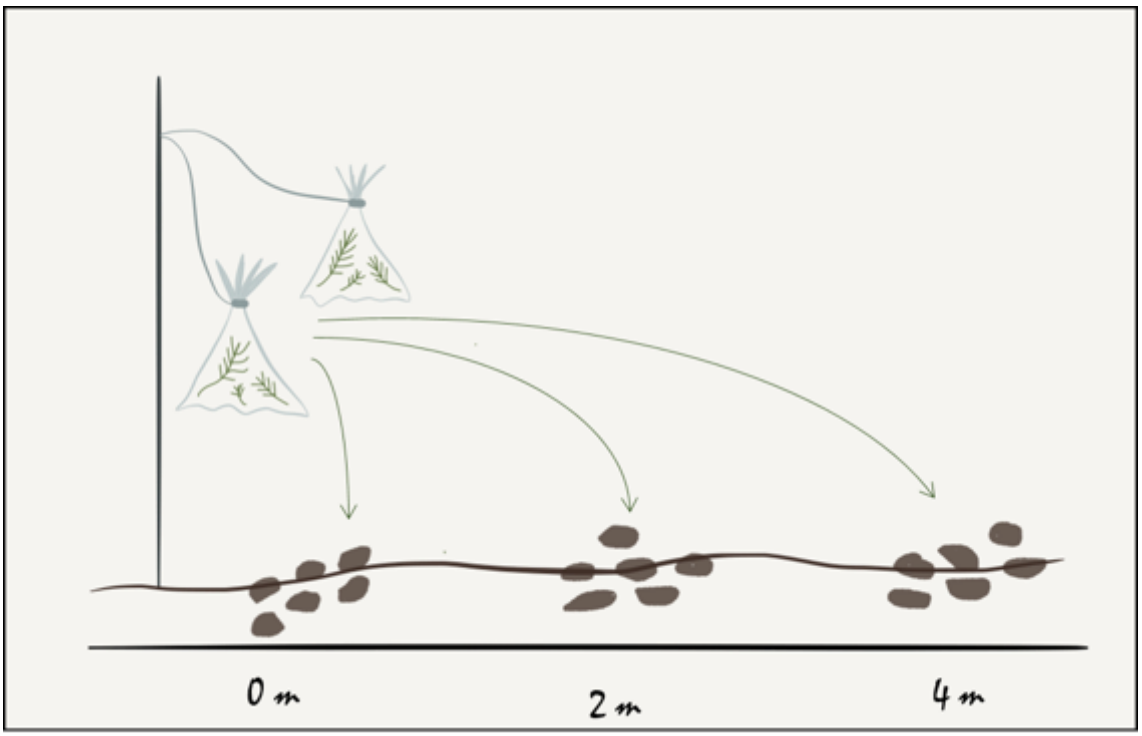


Fig. 5. Scheme of the *in situ* seedling technique, with the dispersal bags providing zygotes to the free substrate available.

Step 7. After 3 or 4 days of the establishment of the dispersal bags, they can be removed.

6. Observations and recommendations

- We recommend having prior knowledge of the phenology and recruitment periods of the species to be restored (Fig. 6). Likewise, we recommend carrying out the restoration action coinciding with the peak of maximum fertility and recruitment of the species, in order to ensure a high contribution of zygotes and optimal environmental conditions for their settlement, survival and growth.

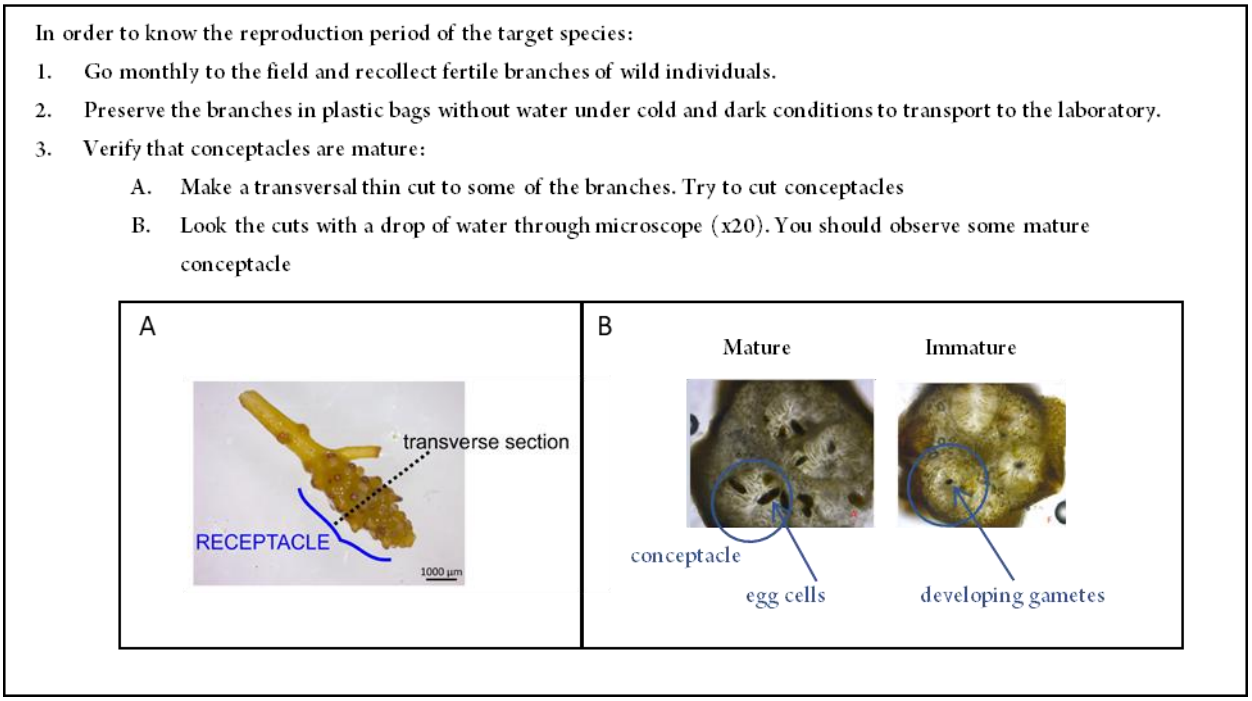


Fig. 6. Scheme of the procedure to determine the phenology of *Cystoseira* species

- For those species with long fertility and recruitment periods, we recommend carrying out the restoration action in spring (e.g. April, May), in order to reduce or avoid exposure to crowding and storms (e.g. summer and winter months) during the settlement and early stages of life.
- For the selection of the donor population, priority should be given to populations that present a good state of conservation (for example, dense *Cystoseira* coverage) and sufficient genetic variability to allow the provision of new recruits capable of adapting to environmental changes and avoiding inbreeding.
- The presence of herbivores (mainly sea urchins or Salema fish) is one of the main factors limiting the restoration success of macroalgal species. For these reasons, herbivory management, by means of herbivory exclusion (e.g. cages or fish deterrent devices) (Tamburello et al., 2019; Gianni et al. 2020) and/or sea urchins culling (Medrano et al. 2020) should be very appropriate as a complementary technique to the main restoration action.

Monitoring the success of transplantation

We point out that the density (the total number of individuals per 0.04 m^2) and the structure of size classes (the length of the main axis) of the population, as well as the sexual maturity of the individuals should be established as the most appropriate indicators to evaluate the restoration success. During the first 6 months after the restoration action, the site should be visited monthly to evaluate the effectiveness of the technique, then the

success monitoring should be done once or twice a year. These indicators of success should be compared with values from natural populations characterized by a good state of conservation that should be established as reference populations.

7. Challenges and barriers

In situ seedling technique is a cost-effective technique to restore *Cystoseira* species. One restoration action is enough to successfully restore a population of 25m^2. A high number of recruits can be provided with this technique, and while a large proportion of recruits usually die during the first year, similar densities between restored and reference sites can be achieved in the second year after the restoration action. However, a few more years (5 in our case) are needed to achieve comparable size-class structures to the reference populations. Since the reproductive capability of a species depends by several environmental conditions, zygotes/germlings availability could be extremely compromised and seed production in donor beds can vary dramatically from year to year. Therefore, it is crucial to operate as far as possible during the peak of reproductive season of the selected species. This fact, will allow us to collect an appropriate number of mature apexes, but harvesting a small proportion (< 5%) of reproductive fertile branches from wild individuals. Thus, the technique failure may not have severe ecological implications for the donor populations. However, fertile branches availability represents an intrinsic limit of the restoration technique, which cannot be repeated until the following reproductive period of the target species. A successful scaling up of macroalgal forest restoration is still impaired by the inherent uncertainty linked to the lack of large temporal and spatial scale restoration actions. As a result, indicators of success are always based on variables of the target species (e.g. recruits' survival or density) which is limiting the assessment of the recovery of ecosystem functioning. Investment on large-scale restoration projects and with long-term monitoring of success are needed to necessary in order to determine the real recovery of the functionality of the system. Furthermore, there is still a gap in knowledge about the current ecological status of macroalgal forests in some regions of the Mediterranean Sea, while often the main stressor driving population decline is not known. Filling these gaps is a big challenge that will represent a baseline of knowledge for scaling up macroalgal forests restoration. Viability of ecological restoration could be strongly compromised by accelerated environmental modifications associated with climate change. A promising, but as yet untapped, opportunity for enhancing the climate-resilience of restoration investments rests in the exploitation of natural genetic variability of key species (Prober et al. 2015). While the capacity of plants to adapt to environmental change through

plasticity, selection, or gene flow has been intensively explored (Prober et al. 2015), for marine habitats and species the available knowledge is still scarce. In addition, the impacts of climate change are highly variable geographically, and a place-based understanding of climate change threats to marine ecosystems is needed. Combined modeling approaches considering intrinsic adaptation of habitats and species, together with predictions of climate change trends and impacts, are essential to properly assess the fate that species, habitats and sites will follow when restored.

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

1. Rationale

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).

2. Target species and habitats

Species: *Laminaria hyperborea* and *Saccharina latissimi.*

Location: Norway.

Criteria for site selection:

Historical presence of target species. Availability of data from scientific, 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).

4. Material:

Collection procedure for kelp at the donor site

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

5. Description of the protocols and activity

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 have 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 (Fig. 1). 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; Peteiro et al. 2014).

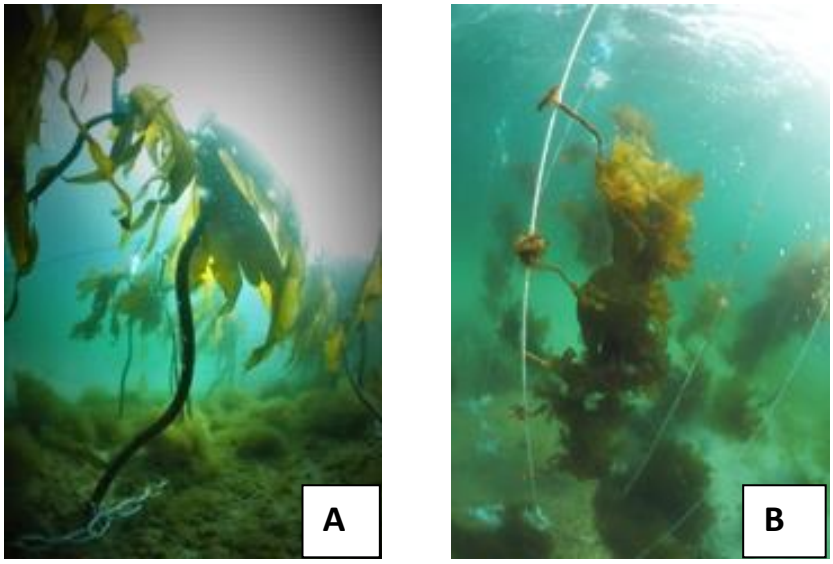


Fig. 1. Transplant set-up for A) *L. hyperborea* and B) *S. latissima* kelps.

6. Observations and recommendations

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.

2.2.2 Protocols for coralligenous

2.2.2.1. Protocol: Transplant of adult arborescent macroinvertebrates species

1. Rationale

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. Here, we provide two restoration protocols based on adult transplants and one on recruitment enhancement.

2. Objectives

- Description of techniques used to transplant fragments of adult arborescent macroinvertebrates species dwelling in the coralligenous communities.

3. Target species and habitats

Species: *Corallium rubrum, Paramuricea clavata* and *Myriapora truncate.*

Location: Catalan coast (Spain), Gulf of Genoa (Italy) and Croatia.

Criteria for site selection:

Habitat suitability determined by the presence (current or determined from historical records) of target species and/or Coralligenous formations.

4. Materials

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

5. Description of the protocol and activity

Step 1. Prepare the material needed (Fig. 1):

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 annotate 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

Fig. 1. Summary of the material required for this protocol (see details in the text above for letters in the picture) (Photos credit: Silvija Kipson).

Step 2. Underwater, use scissors to collect 5-10 cm long apical fragments of mature, healthy donor specimen of selected species (Fig. 2a). 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 location identified for restoration (Fig. 2b). 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.

Fig. 2. Collection of colonies into the field from donor population, and maintenance of samples (Photo credits: MedRecover)

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 (Fig. 3). 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.

Fig. 3. Preparation of epoxy putty (Photo credits: MedRecover).

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 (Fig. 4). 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 spatially arrangement transplants use small PVC quadrats (e.g. 20 × 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).

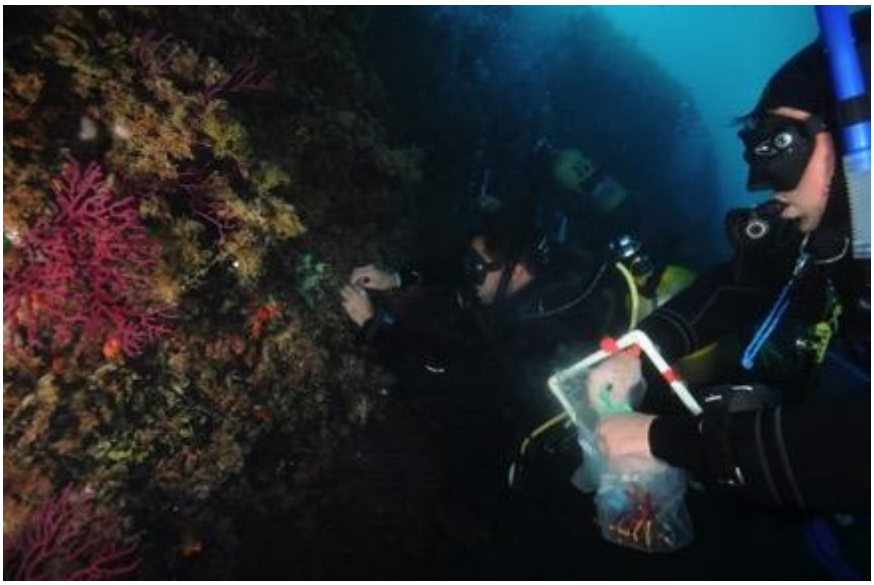


Fig. 4. Transplant of colonies into the field (Photo credits: MedRecover).

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 might seem 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 (Fig. 5).

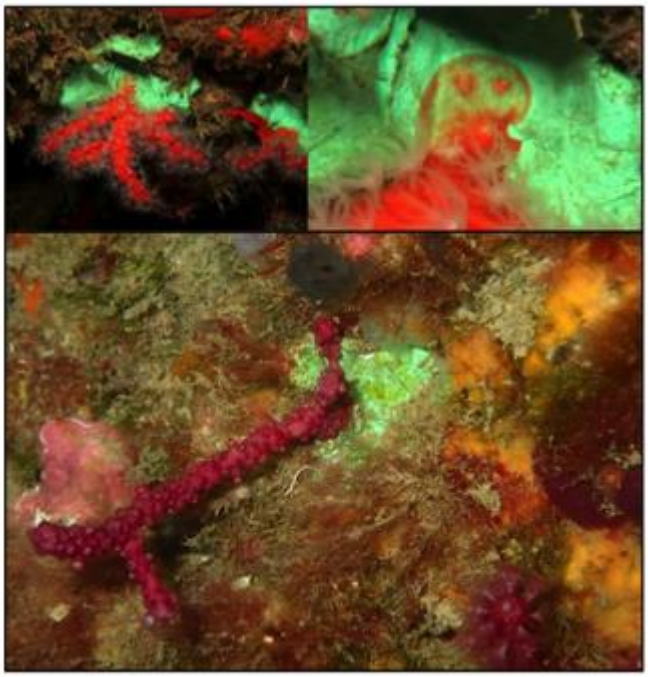


Fig. 5. Overgrowth of gorgonian specimen on epoxy putty (Photo credits: MedRecover).

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 (Fig. 6). 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.

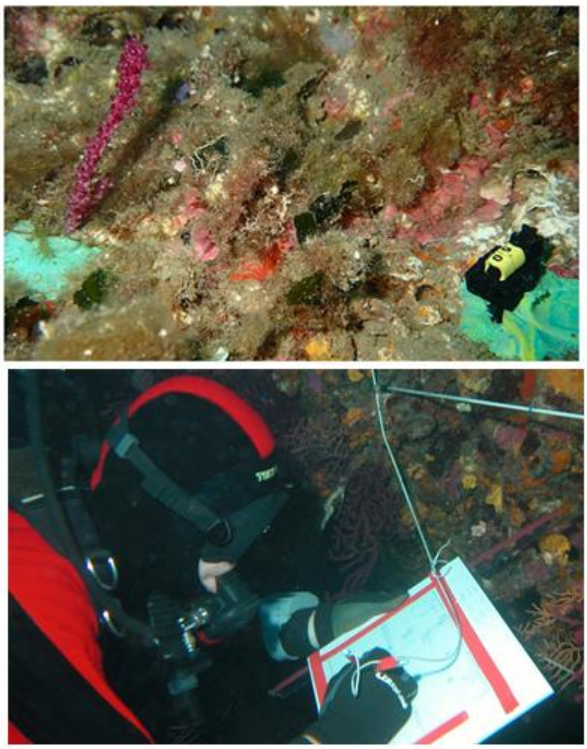


Fig. 6. Tagging and monitoring of transplanted colonies (Photo credits MedRecover).

To sum up:

Fig. 7**.** Summary of the different phases in gorgonian transplanting (Photo credits: 1 & 3 Silvija Kipson; 2,4,5 & 6: MedRecover).

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. Successful tests have been carried out for *Corallium rubrum* and *Paramuricea clavata* (Fig. 8).

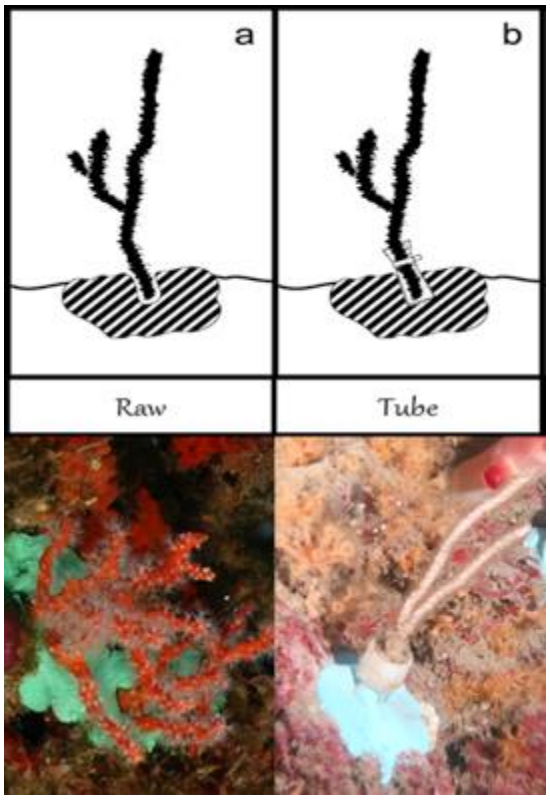


Fig. 8 Techniques adopted for the transplant of gorgonian colonies according to the skeleton features of the species involved.

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 (Fig. 9). In this way the basal branching immersed in the putty will securely anchor the transplant.

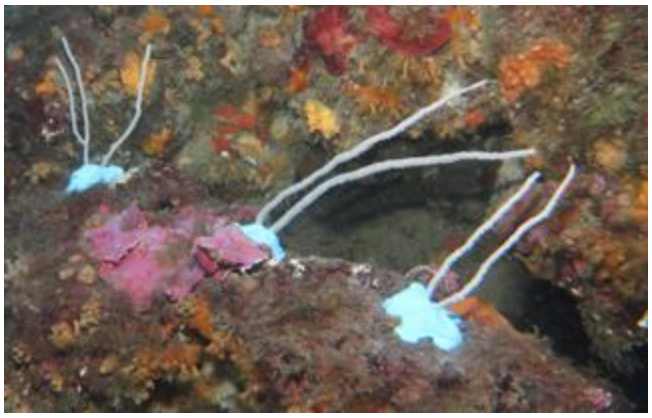


Fig. 9. V shaped gorgonian transplant.

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 (Fig. 10). In this case, the fragment should 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).

Fig. 10. Summary of the techniques adopted for the transplant of *M. truncata* (above) and *P. fascialis* (below).

6. Observations and recommendations

- We recommend performing the restoration action between April and September to avoid the winter months featured by a 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.
- 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 to 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.

  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.

7. Challenges and barriers

The adult of adult arborescent macroinvertebrates restoration protocol adapted to the different skeleton traits showed an excellent performance. The technique failure can cause 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 in the first month), the mortality due to attachment failure reached between 5 to 15% in the tested locations and species. However, in the second phase the survival of transplants is similar to that exhibited by natural colonies up to 90% of survival.

2.2.2.2. Protocol Adult sponge transplants

1. Rationale

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 (Fig. 1). 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.

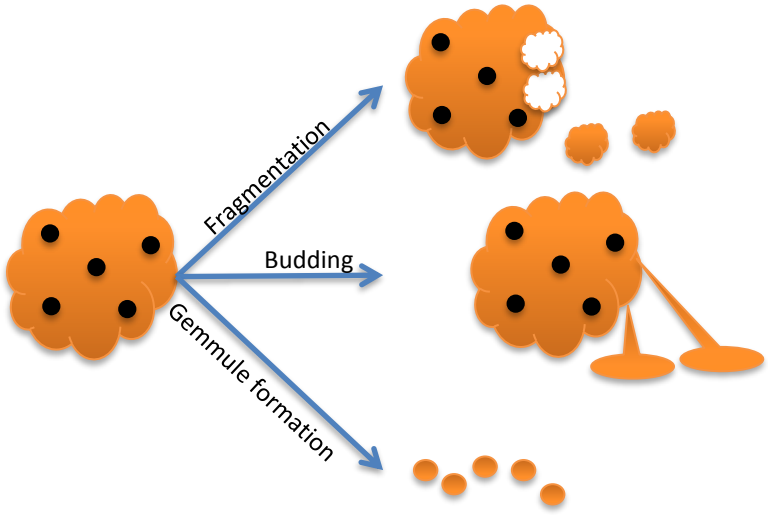


Fig. 1. Summary of the approaches adopted for sponge transplanting.

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.

2. Objectives

- Description of techniques used to transplant fragments of adult sponges dwelling in the coralligenous communities.

3. Target species and habitats

Species: *Petrosia ficiformis, Spongia* spp., *Sarcotragus* spp., *Ircinia* spp.

Location: Gulf of Genoa (Italy).

Criteria for site selection:

Habitat suitability determined by the presence (current or determined from historical records) of target species and/or Coralligenous formations.

4. 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).

5. Description of the protocol and activity

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* (Fig. 2).

Fig. 2. Transplant of sponges with hard skeleton (e.g. *P. ficiformis*) (Photo credit: Carlo Cerrano).

Step 1. Cutting of transplants should be done with a sharp blade, in order to minimize torsion and stretching of fragments during detachment.

Step 2. 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.

Step 3. 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.

Step 4. 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 (Fig. 3). 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 activities, the temperature at the time of sampling activities and so on.

Step 1. 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*.

Fig. 3. Transplant of sponges with soft skeleton (e.g. *Spongia*) (Photo credit Carlo Cerrano).

Step 2. Handling of transplants should always be 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 be always avoided. Abrupt changes in temperature, also over short periods 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, 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 Figure 4 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.

Fig. 4. Donor-specimen of *Spongia lamella* before manipulation, just after the cutting of the portion to be transplanted (Nov. 16) and one year later (Nov. 17). (Photo credit: Carlo Cerrano)

Furthermore, an example of the evolution of a transplant of the hard skeleton sponge *Petrosia ficiformis* is shown in Fig. 5.

Fig. 5. Example of the evolution of a transplant of the hard skeleton sponge *P. ficiformis* (Photo credit: Carlo Cerrano).

Lastly, the transplant of the soft skeleton sponge *Spongia lamella* is shown in different phases, using the technique 2: cicatrisation 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) (Fig. 6).

Fig.6. Example of the evolution of a transplant of the soft skeleton sponge *S. lamella* (Photo credit: Carlo Cerrano).

6. Observations and recommendations

- Early-winter months should be generally avoided as period to implement the restoration protocol. In fact, these months are characterized by high frequency of storms. This can substantially increase the loss of transplanted specimens. Months from March to June occurs just before the main reproductive period of many western Mediterranean sponges. These months could therefore be considered 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.
- For the monitoring, 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.

7. Challenges and barriers

The restoration technique of adult sponge transplants 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 up to 90%.

2.2.2.3. Protocol: Macroinvertebrate recruitment enhancement techniques

1. Rationale

As commented for the 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 (Fig. 1).

Fig. 1. Settling colony of *P. fascialis* on the skeleton of a gorgonian species (Photo credit: MedRecover).

2. Objectives

- Test techniques to enhance for macroinvertebrate recruitment dwelling in the coralligenous communities.

3. Target species and habitats

Species: *Pentapora fascialis*

Location: Catalan coast (Spain)

Criteria for site selection:

Habitat suitability determined by the presence (current or determined from historical records) of target species and/or Coralligenous formations.

4. Materials

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

5. Description of protocol and activity

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, thus ensuring 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 (Fig. 2).

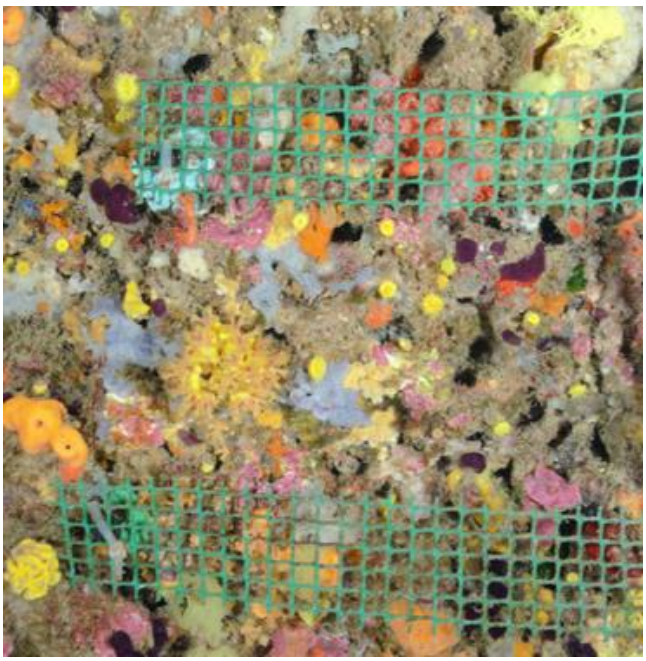


Fig. 2. Installation of artificial substrate (i.e., plastic mesh) to facilitate the recruitment of *P. fascialis* (Photo credit: MedRecover).

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 (Fig. 3).

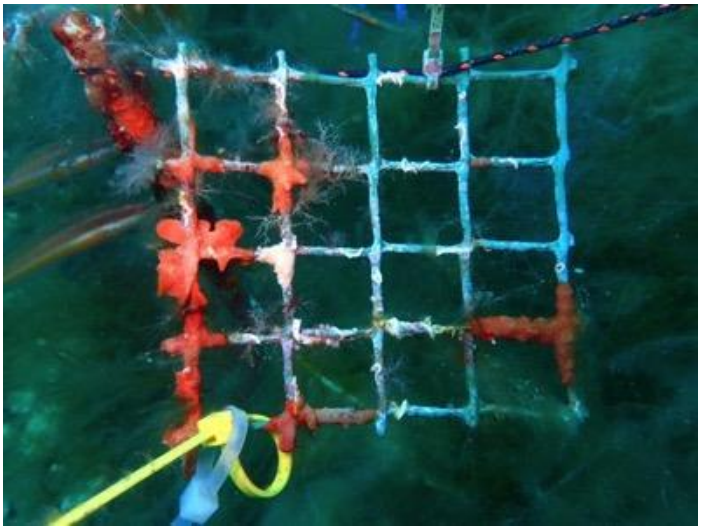


Fig. 3. *P. fascialis* recruitment on the mesh (Photo credit: MedRecover).

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 (Fig. 4).

Fig. 4. Suitable colonies of *P. fascialis* that can be transferred in a new site (Photo credit: MedRecover).

6. Observations and recommendations

- 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.
- To monitor restoration success, the 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.

7. Challenges and barriers

The recruitment enhancement tested for bryozoan in the Catalan coast showed good results given the high recruitment up to 6 recruits/10 cm^2. Considering that is a non-invasive methodology that represents no impact for natural populations. Besides since, the recruits are growing over the plastic meshes they can easily translocated to different locations in view to accelerate the restoration of selected sites. Thus, this technique is very promising to “culture” donor colonies to be used in restoration actions in the Coralligenous.

2.3 Deep-sea habitats

2.3.1 Active restoration of gorgonian populations on the Mediterranean continental shelf

1. Rationale

Coral gardens are among the most complex three-dimensional communities on the Mediterranean continental shelf, generating spatial heterogeneity and providing suitable habitat for hundreds of associated species (Bo et al. 2012; Gori et al. 2017; Grinyó et al. 2016 and references therein). Besides their bioengineering role, coral gardens provide important provisioning services such as fisheries resources and pharmaceutical compounds, regulation services such as carbon storage and nutrient remineralization, and cultural services for aesthetical, educational and scientific purposes (Thurber et al. 2014). However, they are highly exposed to impacts caused by bottom trawling as well as trammel net and longline fishing. Since gorgonians are long-lived and slow-growing species, impacts derived from this fishing activity can have far-reaching and long-lasting effects, jeopardizing their long-term viability. In addition, they are considered as sensitive communities and vulnerable marine ecosystems (VME). Thus, ecological restoration initiatives focusing on gorgonian populations on the continental shelf may be necessary to enhance and speed up their natural recovery. A first pilot action was performed to test the feasibility of recover bycatch colonies of *E. cavolini,* one of the most abundant species in the continental shelf of Cap de Creus (Western Mediterranean Sea), and return them back to their natural habitat onto artificial structures. Subsequently, a potentially large-scale and cost-effective method consisting in gorgonian transplant onto natural cobbles was also tested.

2. Objectives

To evaluate active restoration methods on gorgonian populations on the Mediterranean continental shelf (80-110 m depth).

- Evaluate the feasibility of recovering and returning to their natural environment bycatch gorgonians, by means of transplants onto artificial structures deployed on the continental shelf;
- Evaluate the feasibility of a potentially large-scale and cost-effective method for actively restoring gorgonian populations on the continental shelf by means of transplants onto natural cobbles.

3. Target species and habitats

Species: *Eunicella cavolini* (Koch, 1887) one of the most common Mediterranean gorgonian species (Carpine and Grasshoff 1975; Weinberg 1976) showing a wide bathymetric distribution (<10–220 m depth) (Russo 1985; Carpine 1963; Sini et al. 2015). Laboratory incubations showed *E. cavolini* colonies displaying a slow growth, a few mm per year (linear extension of apical branches) (Dominguez-Carrió et al. 2017).

Location: Cold-water coral gardens on offshore rocky bottoms at 80-110 m depth. Populations of the temperate gorgonian *Eunicella cavolinii*, our target species and one of the most abundant species in the continental shelf of Cap de Creus, are closely associated to high diversity of associated fauna (e.g. sponges *Suberites syringella*, *Stelligera stuposa*, *Raspailia viminalis*, *Haliclona elegans* and *Dysidea avara*, soft corals *Parazoanthus axinellae* and *Paralcyonium spinulosum*, and an extent list of bryozoans, hydrozoans and polychaetes) (Dominguez-Carrió 2014).

Criteria for the selection site:

Well-developed gorgonian populations of *Eunicella cavolini*: patches dominated by medium to large sized colonies and reaching densities of the order of 20 colonies m^{-2} (Dominguez-Carrió 2014).

Artisanal fishing pressure: Gorgonian colonies are among the most frequent bycatch species of longlines and trammel nets, which are largely employed in the Cap de Creus area.

4. Material

4.1. Artificial structures method

- Bycatch *E. cavolini* colonies recovered from artisanal fishing
- Plastic containers
- Aquarium facilities (tanks, filters, chillers, pumps…)
- Stainless steel structures:
    - Concrete plates
    - Stainless steel bars
    - Acoustic reflector
    - Stainless steel grid
        - Conical supports
        - Polyester fibreglass resin
        - Drill
    - Epoxy putty (Corafix SuperFast, GROTECH®)

- Diving material
- Buoys, ropes and a boat (for the deployment manoeuvre)
- Girona 500 autonomous underwater vehicle working as a hybrid ROV

4.2. Transplants onto natural cobbles method

- Bycatch *E. cavolini* colonies recovered from artisanal fishing
- Plastic containers
- Aquarium facilities (tanks, filters, chillers, pumps…)
- Natural rocky cobbles
- Driller
- Epoxy putty (Corafix SuperFast, GROTECH®)
- Boat
- Girona 500 autonomous underwater vehicle working as a hybrid HROV

5. Description of the protocol and activity

Step 1. For both methods, *Eunicella cavolini* colonies are recovered from artisanal fishermen's bycatch from Cap de Creus from a depth range of 70 to 100 m. Fishermen pick up gorgonians entangled in trammel nets and keep them in containers filled with surface sea water (~20–23°C).

Step 2. Once back on land (1-2 hr after collection), gorgonians colonies are transported and held on experimental aquarium facilities at the temperature of water on the continental shelf of Cap de Creus (14 ± 1.0°C) (Fig. 1). While being maintained in aquaria, gorgonian colonies recover from partial breakage and tissue abrasion that initially several had suffered.

Fig. 1: Aquarium facilities where gorgonians, recovered from fishers, remain until they are returned to the continental shelf (Photo credit: ICM - CSIC).

4.1 Artificial structures deployment method

Artificial structures are round stainless-steel structures, with an outer diameter of 2 m and an inner diameter of 1.5 m. They are composed of a base grid 10 (~10 cm^2) surrounded by four concrete plates and a central 1 m vertical axis holding an acoustic reflector (30 cm in diameter) supported by four stainless steel bars (12 mm in diameter) (Fig. 2). The grid has forty conical supports for the gorgonians (80 mm high, 20 mm diameter). The inside of the supports is filled by polyester fiberglass resin and, once dry, 8 mm boreholes are made in order to attach the gorgonians colonies with epoxy putty (Corafix SuperFast, GROTECH®). Each structure weigh 137 kg in the air.

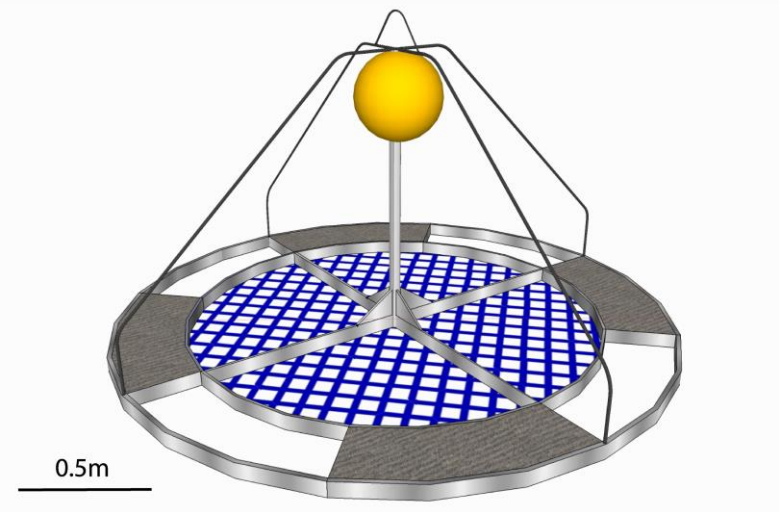


Fig. 2: Schematic figure of the stainless-steel structures used in the artificial structure deployment method (Montseny et al. 2019).

Step 1. Initially, 40 gorgonian colonies for each structure are transplanted to the supports by scuba divers in shallow waters (Fig. 3a and b).

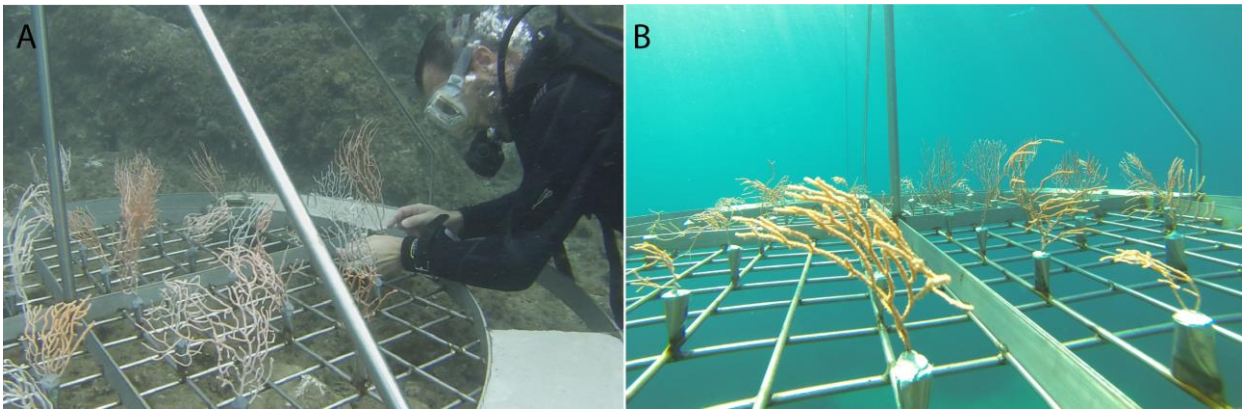


Fig. 3 (A) Diver attaching gorgonian colonies to the artificial structure at 6 m depth and (B) Detail of transplanted gorgonians attached to the artificial structures (Photo credit: ICM - CSIC).

Step 2. Then, structures are raised up to below the water surface by means of a buoy and transported by boat at a slow and constant speed (~0.5 kn) towards the continental shelf, where they are deployed (Fig. 4).

Fig. 4. Artificial structure with transplanted colonies deployed on the continental shelf (85 m depth) (Adapted from Montseny et al. 2019).

Step 3. Once on the continental shelf, structures have to be monitored in order to assess the success of the transplantation action. This monitoring is performed by consecutive surveys using the Girona 500 Autonomous Underwater Vehicle, working as a hybrid ROV (Carreras et al. 2016). The HROV use a sonar to locate the acoustic reflector and approach each structure. Images, with high resolution, are collected by encircling each of the structures, while maintaining the gorgonians in the center of the view. The robot maintains an approximately constant distance of 2 m between the camera and the center of the structure, enabling observations of the gorgonians from various directions with sufficient image quality to allow successful assessment of their survivorship. Besides, in order to obtain more information, three-dimensional (3D) reconstructions of the three structures with transplanted gorgonians can be made using an optical 3D reconstruction procedure, as described in Hernández et al. (2016).

4.2 Gorgonian transplants onto natural cobbles method

Step 1. Natural cobbles (about 9-10 cm width: 9-10cm, mean length: 12-13 cm length, 3 cm height and 400-500 g weight) are collected from the coastal areas and are drilled in order to allow colony attachment (Fig. 5a).

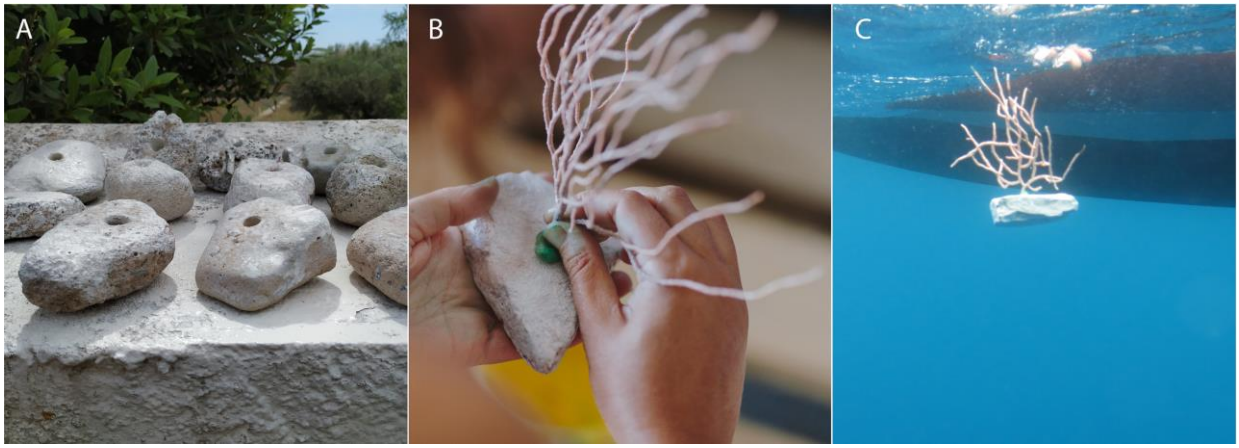


Fig. 5. (A) Drilled natural cobbles used in the gorgonian transplant onto natural cobbles method (Photo credit: ICM - CSIC). (B) One gorgonian colony being attached to a natural cobble using epoxy putty (Photo credit: L. Sabaté). (C) Gorgonian transplant gently thrown from a boat (Photo credit: N. Viladrich).

Step 2. Then, gorgonian colonies held in aquaria facilities (Fig. 1) are attached to supporting drilled cobbles, using epoxy putty (Corafix SuperFast, GROTECH®) (Fig. 5b).

Step 3. Subsequently, the transplants are transported in portable plastic fridges filled with seawater (ca14 ºC) and gently thrown to the continental shelf from a boat (Fig. 5c).

Step 4. The fan-shaped morphology of the gorgonian colonies attached to the cobbles makes them act as a badminton shuttlecock, slowing down the fall and facilitating an upright landing.

Step 5. Once on the bottom, transplants have to be monitored in order to assess the success of the transplantation action. The same HROV is used to acquire videos and photo-mosaics of the gorgonians in the restored area. Images are posteriorly analyzed to assess the survival of transplants and the area extension they have achieved.

6. Observations and recommendations

- Both protocols allow to successfully return bycatch cold-water gorgonians recovered from artisanal fishery to their natural environment on the Mediterranean continental shelf, using transplantation techniques. Thus, highlighting the feasibility of restoring cold-water coral gardens.
- By using bycatch colonies no additional impact to healthy donor coral gardens will be generated, while a viable output for those gorgonians already fished by artisanal

fishers is provided. Moreover, directly involving professional fishers in restoration actions also increase the awareness of local society about the need for the protection of cold-water coral gardens and facilitate the application of this methodology in an extensive manner, which is crucial for the restoration success (Gobster & Hull 2000; Yap 2000).

7. Challenges and barriers

The deployment of artificial structures protocol entails high economic cost, mainly related to manufacturing and deployment of artificial structures and the underwater technology needed for the monitoring of transplants. Moreover, the spatial scale of application is very restricted, while the main stressors affecting the continental shelf and deep waters are widespread (Halpern et al. 2008). Regarding this, the second protocol using natural cobbles as support for gorgonians transplants allows for restoration of high number of gorgonians colonies over extended area, avoiding high investment in build artificial structures. Moreover, by using natural substrates, no additional artificial material is introduced to the environment. The large number of suspended particles present in the study habitat make difficult to acquire high resolution images, making difficult to analyze the potential growth or recruitment of the transplanted gorgonians. Bearing in mind this, it is crucial to continue improving in the development of supporting underwater technology. Finally, to achieve successful restoration outcomes, it is important to highlight that restoration actions should be supported by proper long-term monitoring together with a high enforcement of the protection of the restored area, in order to minimize its drivers of degradation and ensure its development.

2.3.2 Coral transplantation techniques and deployment of artificial substrates for the active restoration of deep-sea cold-water coral gardens on seamounts

1. Rationale

Among the most important deep sea habitats in Azores are cold-water coral gardens formed mainly by octocorals, occurring predominantly between 200 and 1000m depth (Braga-Henriques et al. 2013). The structural complexity of coral gardens provides essential habitat for many different organisms, including invertebrates and commercially important fish species (Pham et al. 2015; Gomes-Pereira et al. 2017). Thus, coral gardens are often found in traditional fishing grounds and coral colonies are accidentally caught as bycatch during fisheries operations (Sampaio et al. 2012; Pham et al. 2014). Longline fishing impacts mostly organisms with complex morphology, which may eventually threaten their population health since growth and recruitment may be outbalanced by the amount removed and population recovery is highly unlikely. This is turn will reduce the habitat for associated species, resulting in overall loss of biodiversity and the ecosystem services they provide. Recognizing their structural complexity, functional significance, fragility and low recovery potential from fishing impacts, coral gardens are classified as Vulnerable Marine Ecosystems (FAO 2009; 2016) and as priority habitats in need of protection (OSPAR 2010). Indeed, because corals are long-lived and slow-growing species, impacts derived from this fishing activity can have far-reaching and long-lasting effects. Therefore, restoration initiatives may be necessary to enhance and speed up their natural recovery. The pilot restoration action in the Azores consisted in testing the use of coral transplantation techniques, using corals recovered form fisheries bycatch, and the deployment of artificial substrates as assisted regeneration tools to aid the recovery of degraded coral gardens.

2. Objectives

- Main objective: Feasibility of coral transplantation techniques and the deployment of artificial substrates for the active restoration of coral gardens on seamounts.
- Evaluate the feasibility of recovering and returning to their natural environment cold-water gorgonians accidentally caught during hook-and-line fisheries operations, by means of transplants onto artificial structures deployed on a seamount;
- Evaluate the restoration potential of different coral species and different coral conditions (intact vs. injured or damaged colonies);

- Evaluate the use of artificial substrates as a method to enhance coral recruitment in restoration sites

3. Target species and habitats:

Target habitat: Coral gardens on rocky bottoms at the summit of the Condor Seamount at 185-210 m depth. The Condor seamount is an elongated volcanic ridge, rising from 1700 m to a flat summit at ca. 200 m depth located 17 km southwest of Faial Island. A high number of associated sessile (e.g. zoantharians, anemones, hydroids) and vagile (e.g. polychaetes, echinoderms, crustaceans, fish) species use coral gardens as refuge, source of food, spawning and nursery areas (Braga-Henriques et al. 2015; Pham et al. 2015). Several commercial fish species inhabit the seamount, including the species *Helicolenus dactylopterus, Polyprion americanus, Pagellus bogaraveo*.

Target species: The corals used in the transplantation pilot action were chosen based upon the native coral species that can be found on Condor seamount, susceptibility to fishing activities, and survivability of corals in aquaria. Five gorgonian species were selected - *Acanthogorgia armata, Callogorgia verticillata, Dentomuricea* aff. *meteor, Paracalyptrophora josephinae and Viminella flagellum* (Fig. 1).

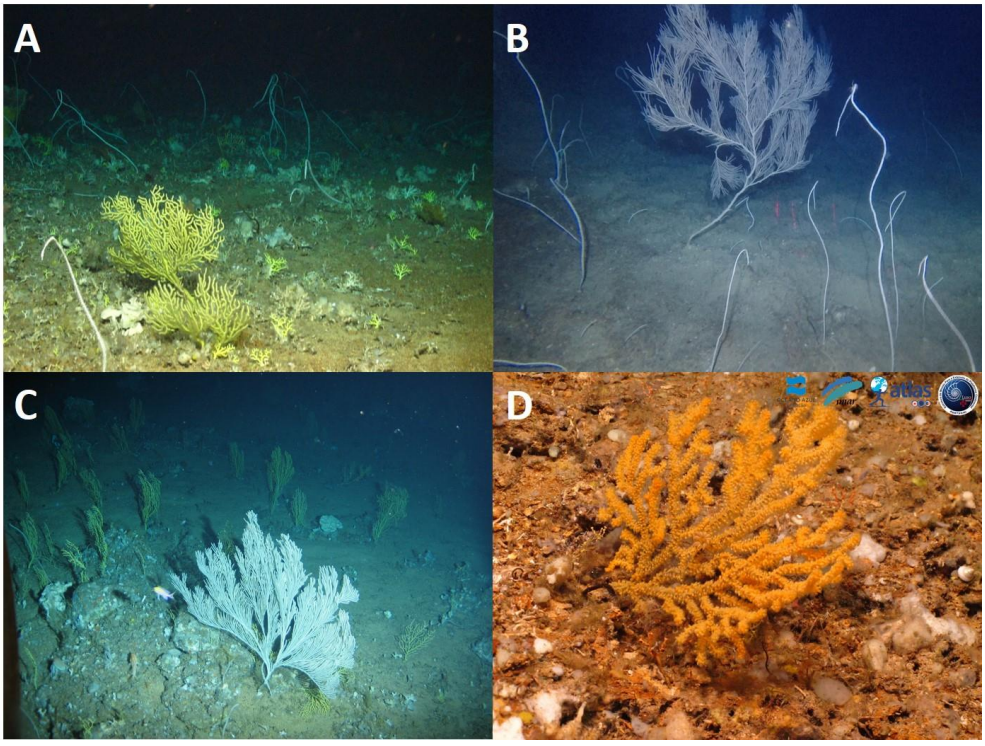


Fig. 1. Coral gardens in the Condor seamount. (A) Coral garden formed by the octocorals *Viminella flagellum* and *Dentomuricea aff. meteor*; (B) large colonies of the octocoral *Callogorgia verticillata* and (c) *Paracalypthrophora josephinae*; (D) small *Acanthogorgia sp.*

Criteria for the selection site

Coral transplantation studies were conducted in the Condor seamount because it has been close to fishing since 2010 (Morato et al. 2010) and thus coral landers were not at risk of being accidental removed by fishing or other activities.

Condor seamount has dense coral gardens in its summit related to the availability of hard substrates and suitable oceanographic conditions for corals, e.g. accelerated current flow and potentially high food input.

4. Material

4.1. Landers for coral transplants deployment

- Bycatch gorgonian colonies recovered from longline fishing
- Coolers to transport bycaught corals from fishing vessels to aquaria
- Aquarium facilities (tanks, filters, chillers, pumps…)
- Lander structures:
    - Grid approximately 58 × 58 cm wide and 3 cm height, made of Glassfiber Reinforced Plastic (GRP) grating ISO 30 with a polyester resin matrix and a glassfiber content of approximately 35%.
    - 12-16 mm Ø PVC rods
    - PVC threaded water pipping parts (4cm short threaded 16mm Ø pipe tips and T-shaped junctions) at each of the four tips to easily and quickly attach it on board to the GRP grating base
    - 20 pieces 10 × 3 cm of ceramic tiles around the edge of the grating base of the structure (5 units on each side) tied with a zip tie to add weight to the structure
- Epoxy putty (Milliput yellow standard)
- Cable ties
- Buoy (sonar reflector), ropes and a boat (for the deployment manoeuvre)
- Submersible or ROV for lander recovery

4.2. Lander for larvae settlement plates

- 10 mm thick and 60 × 52 cm PVC tray
- Four 20 cm long and 10 cm Ø pipes as legs at the corners
- Ø 10 mm rope
- Basalt tiles 10 × 10 cm and 1 cm thick stone (25 tiles per lander)
- 8M nylon screws and bolts to screw tiles to the PVC tray
- Plastic mesh

- Drill
- Cable ties
- Boat for deployment
- Buoys (sonar reflector), ropes and a boat (for the deployment manoeuvre)
- Submersible or ROV for lander recovery

5. Description of the protocol and activity

5.1 Landers for coral transplants deployment

Step 1. A first set of lander used for coral transplantation, were built with stainless steel but due to the high corrosion in Azorean seawater, were replaced with plastic landers in the second lander deployment occasion. Landers consisted of a squared base grid and a squared base pyramidal structure made out of two bended PVC rods and PVC threaded water pipping parts at each of the four edges to easily and quickly attach it on board to the grating base (Fig. 2a and b). Each lander weighed around 7 kg.

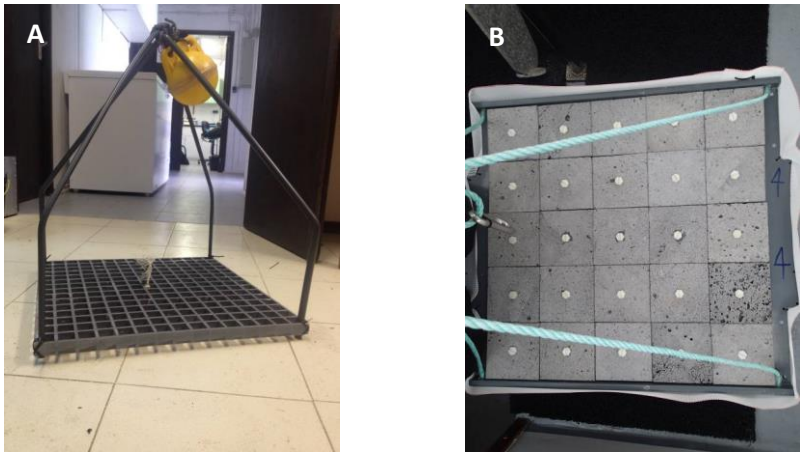


Fig 2. Example of the landers (A) and settlement plate structure (B) used in the coral restoration experiment at Condor Seamount, Azores.

Step 2. Coral colonies accidentally caught during longline and hand-line fisheries from a depth of 180 to 700 m depth, were recovered fisheries observers and kept on board in cooler boxes with chilled seawater (15-20° C). Upon arrival to shore, they were transferred to the Deep-SeaLab facilities where they were maintained in aquaria. Collected colonies were fragmented and maintained at the DeepSeaLab under natural temperature conditions (13 ± 1.0°C) until an enough number of fragments were obtained for re-deployment. Each coral fragment was attached to the lander structure using a zip tie that was embedded in the epoxy base. The landers with the coral fragments were kept in containers with chilled seawater onboard the vessel until deployment (Fig. 3a,b).

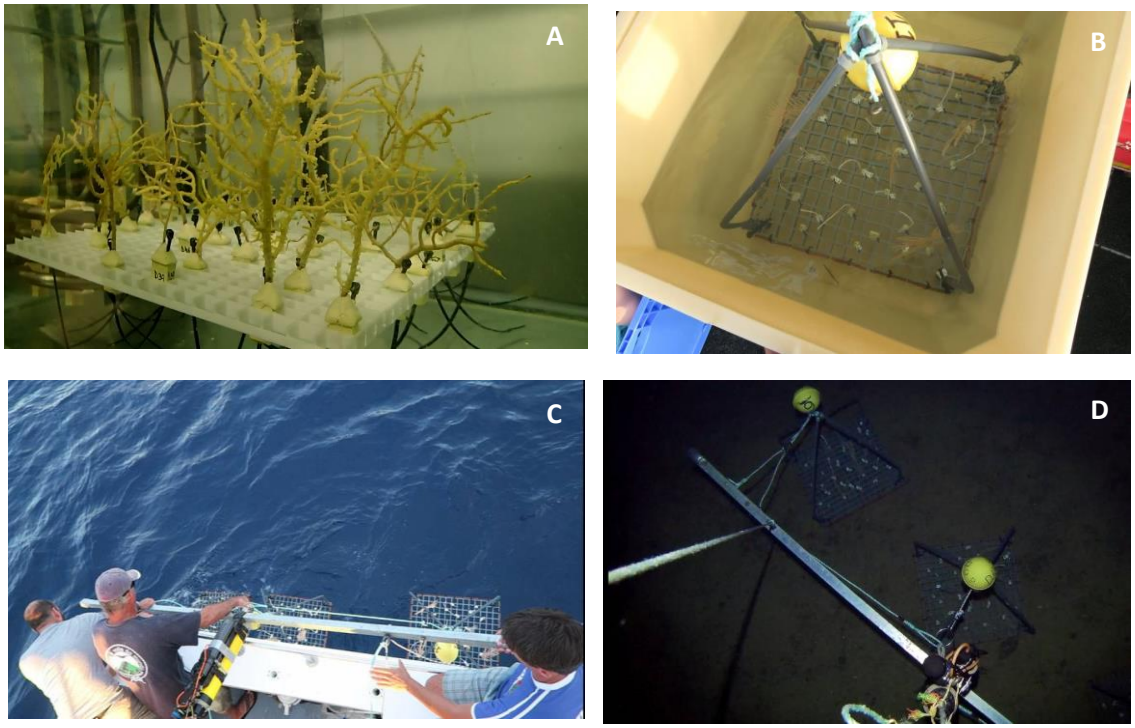


Fig 3. A) Gorgonian *Dentomuricea meteor* fragments after attaching to the epoxy base at the DeepSeaLab aquaria facilities; B) lander prepared for deployment; C) and D) Deployment structure including 3 coral landers, a metal bar and an acoustic releaser.

5.2 Landers for larvae settlement plates

Step 1. The landers consisted of a horizontal PVC tray with four legs built with PVC pipes (Fig. 2b). Twenty five basalt tiles were fixed to the PVC tray using nylon screws and bolts attached through the center of each tile. A plastic mesh 7 cm high was attached around the edge of the PVC tray to potentially contain benthic associate fauna present at the time of recovery of the structures (Fig. 2b). A pyramid shaped rope system fixed at the corners of the PVC tray was tied to a buoy for lander deployment and recovery. Each lander weighed around 20 kg.

Step 2. For the deployment of the coral landers and settlement plates, we used a 3 m long metal bar where we attached 3 structures at a time. The bar was connected to an acoustic releaser that would liberate the structures at the bottom (Fig. 3c,d), a camera system to document the deployment, and a pinger that was transmitting the position during the deployment. Landers were recovered using the Lula submersible (Rebikoff-Niggeler Foundation) after 21 months deployment.

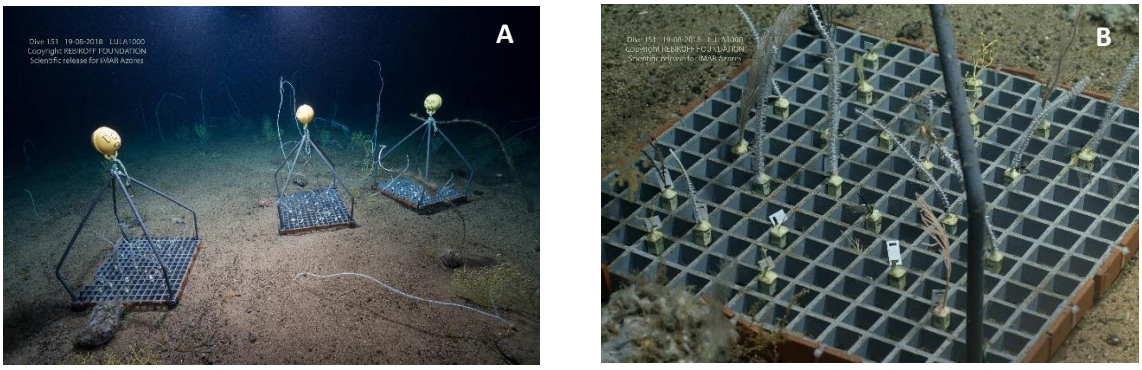


Fig. 4.·(A) Landers used in the gorgonian transplantation studies; (B) close-up of coral nubbins used in the landers.

Step 3. Coral survival was evaluated from the photographs *in situ* with the submersible as well as directly counted from the recovered landers. Immediately upon recovery, living coral fragments were moved to chilled aquaria and subsequently transferred to DeepSeaLab facilities to obtain 3D photographs of the fragments. Small fragments were stored in liquid nitrogen for enzymatic bioessays and in 10% buffered formalin for reproduction studies.

Upon recovery of the settlement structures, basalt tiles were removed and individually placed in containers with 30 % ethanol and later examined with a dissecting microscope for identification of settled organisms.

6. Observations and recommendations

- Tested coral transplantation protocols were successful in returning bycaught cold-water gorgonians recovered from bottom long-line fisheries to their natural environment. Thus, demonstrating that coral transplantation is a reliable restoration technique for cold-water coral gardens.
- The survival of transplanted corals depends on the octocoral species, the condition of coral colonies (intact vs. injured or damaged colonies) and the location of the restoration action. Therefore, care should be taken in selecting healthy undamaged coral fragments for transplantation and having prior knowledge of the oceanographic conditions and abundance of natural food on the site where the restoration action will take place.
- Using bycaught coral colonies minimizes the impact on natural potential donor coral populations, and overcomes the need for expensive technology for coral collection, reducing the overall cost of the restoration action. In addition, the use of fragments of adult bycaught coral colonies instead of rearing coral early life stages ensures immediate recovery of the three-dimensional structure, facilitating the recovery of

habitat-forming functions as structural habitat for associated species. Moreover, directly involving professional fishers in restoration actions increases awareness by the fishermen of the need to mitigate the impacts of their activities on coral gardens and preserve and restore these fragile ecosystems.

7. Challenges and barriers

Assisted regeneration (such as transplantation) may be used for some species, while natural regeneration (through fisheries closures, marine protected areas) at large scales may be needed to assist individual native species that cannot be transplanted and may take longer to recover. The lack of recruitment by coral larvae on artificial substrates after nearly 2 years deployment, points out to the long-time scales required for the restoration of impacted coral garden habitats targeting natural recruitment. The spatial scale of application of transplantation techniques for the recovery of coral gardens is very restricted further emphasizing the need for the combine assisted and natural spontaneous regeneration strategies. Finally, given the life history traits of corals, short-term monitoring (i.e. within the lifetime of the MERCES project) cannot be expected to reveal fully restored habitats. Therefore, management measures should be taken to ensure the long-term monitoring.

2.3.3 Artificial Structures for Deep-sea species recruitment and Ecosystem Restoration

1. Rationale

Deep-sea habitats can be subjected to a several anthropogenic impacts that can cause habitat loss and the reduction of their biodiversity. This is particularly evident in deep-sea areas subjected to trawling, oil and gas extraction, pipeline and cable disposal, sewage dumping. The Dohrn Canyon is ~12 nautical miles off the Naples metropolitan area. It is the main canyon crossing the Gulf of Naples, eroding the slope down to 1000 m-depth, and is articulated in two branches (eastern and western) merging in a NE-SW direction, bounded in the south by the Capri Basin. The Dohrn Canyon is considered a hotspot of deep-sea benthic biodiversity of sessile fauna at ca. 400 m depth. The hard bottoms are characterized by a high abundance of charismatic species, such as the habitat forming cold-water corals *Madrepora oculata, Lophelia pertusa (Desmophyllum pertusum), Desmophyllum dianthus* in association with the large size bivalves *Acesta excavata* and *Neopycnodonte zibrowii*. Over many decades the canyon has been subjected to high intensity human uses linked to coastal zone pressures such as illegal dumping and fishery malpractices, as well as trawling in shallower parts. This has resulted in environmental degradation and large amounts of litter including lost fishing gears and plastic waste along the canyon axis and walls (e.g. Taviani et al. 2019). The anthropogenic (human related) activities influence the biodiversity of benthic fauna associated to the canyon system with different impacts when different benthic groups are considered.

2. Objectives

- Test a new device to facilitate the restoration of deep-sea degraded habitats based on the use of artificial substrates.

3. Target species and habitats

Species: cold-water corals: *Madrepora oculata*, *Lophelia pertusa (Desmophyllum pertusum)*, and *Desmophyllum dianthus*, large size bivalves *Acesta excavata* and *Neopycnodonte zibrowii*.

Habitat: Dohrn Canyon

Criteria for the selection of sites

similar environmental conditions between the donor and receiving site;

avoid the presence of anthropogenic activities.

4. Material

- ASDER (Artificial Structures for Deep-sea species recruitment and Ecosystem Restoration)
- ARMS (Autonomous Reef Monitoring Structures)
- ROV (Remotely operated underwater vehicle)
- CTD (conductivity, temperature, and depth) probe
- Photo and video cameras
- hydrophone

5. Description of the protocol and activity

Step 1. Preparation of the artificial substrates. The Artificial Structures for Deep-sea species recruitment and Ecosystem Restoration (ASDER), is designed with a triangular-based structure (1m × 1m × 1m) to provide the support for anchoring 3/6 Autonomous Reef Monitoring Structures (ARMS). These latter are cubic, long-term collecting structures designed to mimic the structural complexity of a three-dimensional habitats and to attract colonizing invertebrates. The lander (ASDER + ARMS) can be assembled on the deck of the research vessel (Fig. 1a and b).

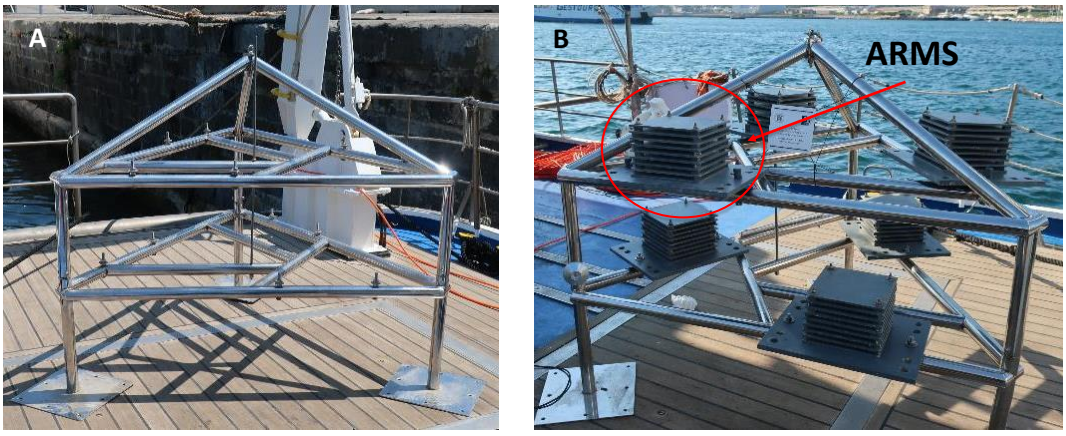


Fig. 1 Preparation of the artificial substrate: A) the triangular-based structure and B) ARMS anchored on the ASDER (Photo credit: Cristina Gambi)

Step 2. The ARMS can also host high resolution cameras to collect photos and short videos on the different stages of the fauna recruitment at scheduled temporal intervals; a sensor to monitor environmental conditions such as the temperature, conductivity, pressure and oxygen; a hydrophone to sample the sound seascape (Fig 2).

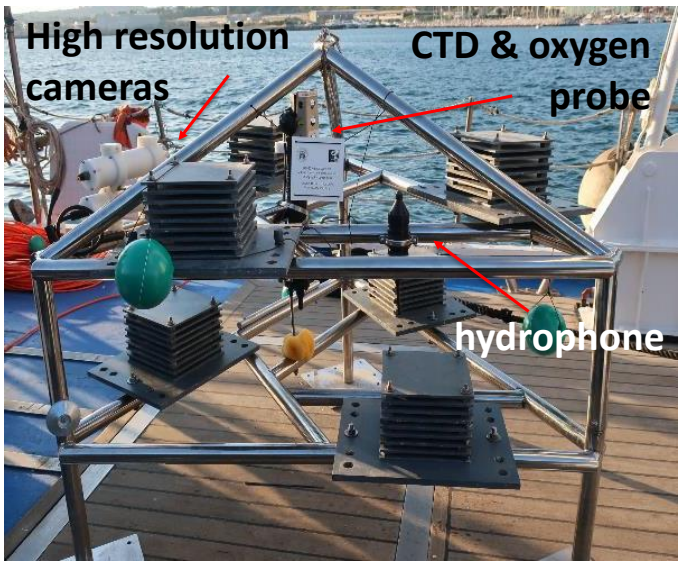


Fig. 2 Artificial substrate hosting ARMS, high-resolution cameras, CTD and oxygen probe (Photo credit: Cristina Gambi)

Step 3. The device can be easily deployed in different deep-sea habitats at different depths. The deployment of the ASDR can be made with the support of a ROV to verify the integrity of the structure during the transfer along the water column and the final step related to the position on the seafloor.

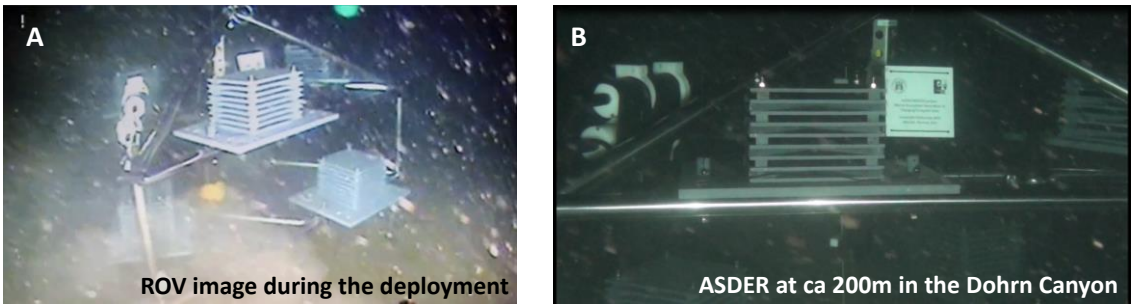


Fig. 3 A) ASDR deployment and B) ROV survey after the position on the seafloor (Proto credit: Cristina Gambi)

Step 4. The system is highly complex in terms of 3D structure, thus allowing the recruitment of benthic organisms with different habitat requirements. Once the ASDER is colonized by organisms, these video-monitored structures can be transferred to degraded areas in order to promote a faster recolonization of benthic organisms. All steps require the support of a ROV for video monitoring.

6. Observations and recommendations

- ASDERs are potentially effective and low-cost devices to support active restoration initiatives in deep-sea ecosystems, which traditionally have very high costs.
- The assemblage of the different components (ARMS, hydrophone and multiparametric probe) on the ASDER can be finalized on the deck of the research vessel.
- The structure can be easily assembled and di-assembled by the scientific personnel.
- Different devices (ARMS, hydrophone and multiparametric probe) can be hosted on the ASDER. The selection is mainly driven by the aims of the actions: restoration and/or long-term monitoring.

7. Challenges and barriers

The artificial structure for deep-sea species recruitment and ecosystem restoration can be removed or partially buried in the presence of trawling activities. The presence of anthropogenic activities can compromise the functionality of the device and contribute to the failure of the restoration action. A preliminary survey was recommended to identify the best site for deep-sea species recruitment and ecosystem recovery to avoid any activities that could alter the structure. The relatively simple structure of this artificial substrate can represent a good compromise on the efficiency of restoration and the scalability at larger spatial scale.

2.3.4 Transplantation of replacement organic substrates at the seafloor

1. Rationale

Since 1970, Norway has seen a dramatic reduction in the biomass of its kelp forests. It is estimated that 9500 km^2 has been lost during this period; much of this has been attributed to catastrophic urchin grazing events, eutrophication, as well as the multiple complex stressors associated with climate change. In addition, recent decades have brought a resurgence of interest in kelp harvesting in Norway, previously thought not to be economically viable due to habitat loss. Today, mechanised harvesting operations are removing 130,000–180,000 tonnes wet weight annually. The loss and removal of large volumes of kelp material from coastal systems is likely to greatly reduce the transport of this material to the deep-sea and its availability to deep-sea benthic consumers. At the same time, the size of terrestrial boreal forests in Norway is rising year after year, with several government sponsored programmes encouraging reforestation and banning land clearance. As such, the increase in forestation is likely to have led to an increase in the transport of wood and other organic forest detritus into deep fjords which are likely also being exposed to decreasing kelp biomass input. In MERCES WP4, we assessed whether the input of wood material as a response to increased land forestation could restore essential hard-substrate habitat and provide an alternative substrate to kelp, thereby reducing deep-sea benthic biodiversity loss in areas exposed to kelp forest reductions. Despite numerous studies investigating the fauna colonising kelp substrates in shallow environments, we know of no study that has ever investigated benthic biodiversity and ecosystem functioning on wood and kelp falls in Norwegian fjords. With an unprecedented loss of kelp from coastal systems and increased forest coverage along the Norwegian coastline, this work was of vital importance to assessing the future of these inadequately understood deep-sea ecosystems.

2. Objectives

To document the faunal taxa colonizing wood and kelp substrates in the deep Norwegian Sea to assess if the placement of wood material at the seafloor can act as a like-for-like replacement for kelp material and aid in recovery of deep-sea fjord systems subjected to significant kelp loss.

3. Target species and habitats

The target sites should be deep-sea habitats where significant kelp loss has occurred and where forestation activity along the coastline is significant. For our study, we selected the deep Osterfjord in Bergen (Fig. 1).

The target fauna are organic enrichment opportunists known to colonise kelp detritus such as:

- Dorvelleid, capitellid and spionid polychaetes
- Cumaceans

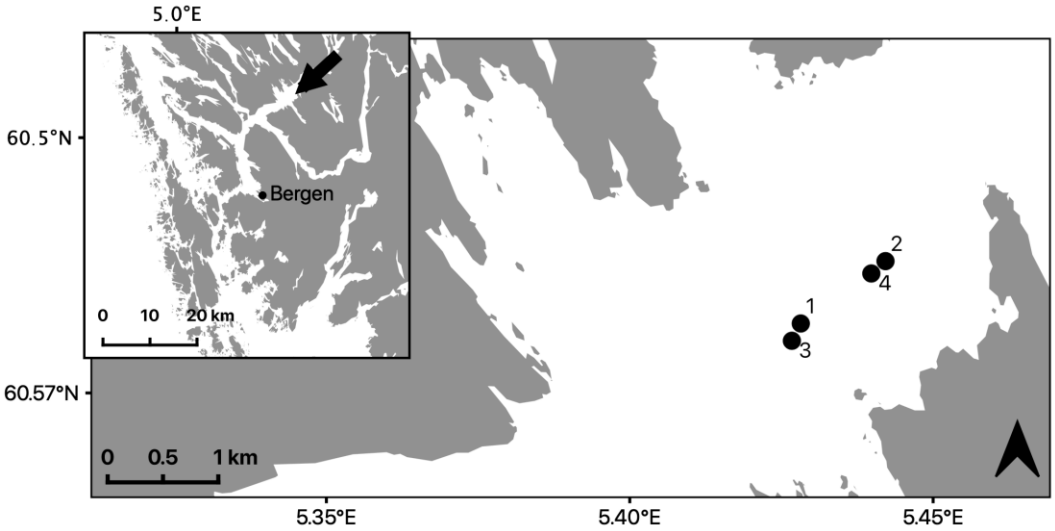


Fig 1 The positions of the four benthic landers deployed in Osterfjorden, Norway (from Harbour et al. in press).

4. Material

- Kelp parcels wrapped into a bundle
- Wood parcels
- ROV to place parcels at the seafloor

5. Description of the protocol and activity

We deployed four benthic landers (BOWLs – Bone and Wood Landers) for ten months between May 2017 and March 2018, in Osterfjorden, Norway. The experimental deployment sites were located at a depth of ~530 m where the temperature remains a relatively constant 8 ℃ year-round. The seafloor is bathed in well oxygenated (~135 µmol) seawater. The BOWLs landers were deployed at similar depths and placed in pairs. BOWLs 5 and 2 were placed closer to the fjord entrance, and BOWLs 6 and 3 were placed further from the entrance to the fjord in order to cover a range of potential flow conditions.

Step 1. Each triangular-shaped lander (Fig. 2) was constructed of aluminium and had nine 40 cm^3 fine-mesh bins (arranged in sections of three) that were used to attach substrates. At the centre of each lander was an acoustic release, connected to a steel weight. Attached by a rope and chain to the frame of each lander were 6 glass floatation spheres. Each set of three bins had a plastic lid which was held open by a releasing mechanism; when the lander recalled to the surface, the lid closed so that the contents of the bin were not lost during the ascent and subsequent recovery.

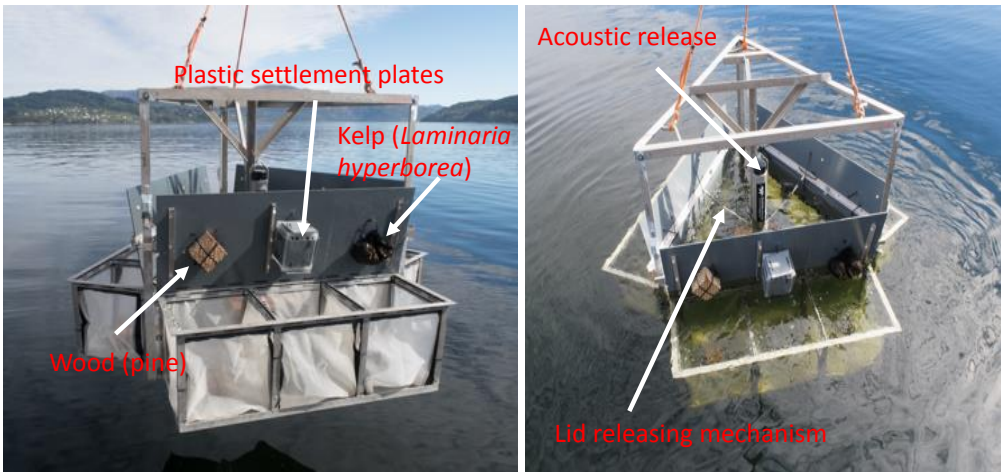


Fig 2. The triangular benthic landers showing the different substrates within each bin.

Substrates - Blocks of untreated wood measuring approximately 15 cm^3 were cut from a felled pine tree (*Pinus* spp.) found in an area close to the location of the lander deployments. Volume measurements were made from each block using water displacement. The blocks were wrapped in 2 cm gauge, nylon knotted netting and fastened with cable ties to both the underside of the lid and the bottom of each allocated bin. Kelp (*Laminaria hyperborea*) was collected by hand from a depth of approximately 2 m from a rocky outcrop in a fjord close to Bergen, Norway. The stipes were scraped of epiphytes using a pocketknife and cut into pieces measuring approximately 15 cm, and the kelp blades were separated. Bundles of kelp stipes and blades were wrapped in 2 cm gauge, nylon knotted netting and weighed while wet. The kelp bundles, weighing approximately 2 kg, were fastened to the underside of the lid and the bottom of each allocated bin. In addition, four tiles made from plastic and one of stone were attached to the outside of a plastic box and attached to the lander, so that when these boxes were attached to the underside of the lid and the bottom of each allocated bin, a complete range of settlement angles were covered. The tiles measured approximately 15 cm^2. The settlement tiles were placed in the central bins on each side of the lander (bins 2, 5 and 8)

and the wood and kelp were attached inside randomly allocated bins either side of them to maintain separation of organic substrates. Once each substrate was attached to the lander, a reference photograph was taken (Fig. 3).

Fig. 3. (Left to right) Inorganic settlement tiles, a kelp parcel and a wood block attached to the lids and bottoms of the lander bins before the lander was deployed (Harbour et al. in press).

Step 2. *Lander recovery and post-recovery sample processing* - The four landers were retrieved from Osterfjorden, Norway in the middle of March 2018. The landers were recalled to the surface by an acoustic signal. Once onboard, reference photographs were taken of each substrate before detachment from the lander. Following this, the substrates were removed and placed into buckets of filtered seawater for transport to Bergen Marine Station laboratory for processing. During the transit, each of the mesh bags was also washed with filtered seawater, its contents sieved onto a 300 µm sieve and stored in 500 ml HDPE jars. These samples were referred to as the 'bag wash'. Back in the Bergen Marine Station laboratory, a total of 10-20 individuals from each of the dominant taxa were picked out of the bag wash samples for stable isotope analysis, DNA barcoding and genetics. The stable isotope samples were frozen in cryotubes and the DNA samples were fixed in 95% ethanol - all samples were then stored at -80 °C.

The remaining fauna and detritus were put into HDPE jars and fixed with 4% buffered formalin. Each bucket used to store the substrate sample temporarily during transit was rinsed with filtered seawater over a 300 µm sieve; the contents of the sieve were then transferred into another container and fixed with 4% buffered formalin. Each wood block was laid in a tray and photographed from each side before being cut into one half and two quarters with a reciprocating saw. Xylophagid molluscs were removed from a number of wood blocks; half were frozen in cryotubes for stable isotope analysis and half were fixed in 95% ethanol for genetics and DNA barcoding. The two wood block quarters were frozen, and the remaining half was preserved in 4% buffered formalin. A single whole

wood block was also preserved in 95% ethanol. Wood fragments and xylophagid faeces were also sampled from the wood blocks and frozen separately for stable isotope analysis as these were probable food-sources for various taxa.

Kelp parcels were washed over a 300 µm sieve. The fauna were fixed in HDPE jars with 4% buffered formalin. The remaining kelp detritus was placed in 3L buckets and fixed with 4% buffered formalin. Blade, stipe and gel material from the centre of the kelp stipes were also sampled from the kelp bundles and frozen separately for stable isotope analysis as these were probable food-sources as well. In almost all cases, no visible macrofauna was present on the settlement tiles. Some tiles were rinsed and sieved over a 300 µm sieve and preserved in 4% formalin but the majority were carefully checked under a microscope before being discarded. Back in the laboratory, fauna were enumerated, identified and weighed for biomass measurements so secondary production estimates could be determined. Data were analysed by univariate and multivariate analyses to check for significant differences between substrates.

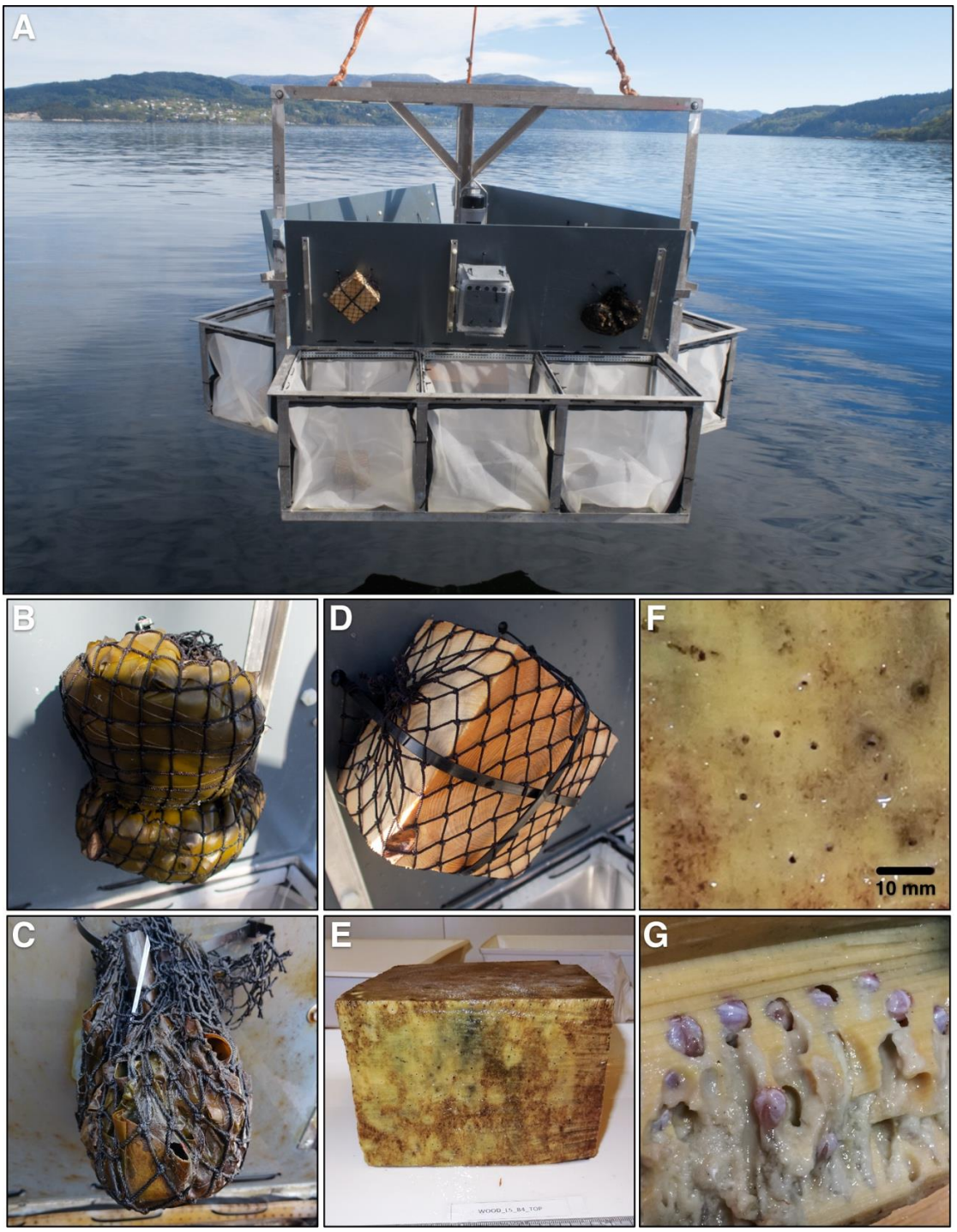
6. Observations and recommendations

- Our results revealed that while wood and kelp falls can support a similar number of species and abundance of fauna, they support significantly different faunal communities. Biomass and secondary production on both wood and kelp substrates was significantly greater than in the control samples.
- Secondary production estimates were similar or higher than those reported from soft-sediment ecosystems at shallower European marine sites. Biological trait analysis showed that macrofaunal assemblages were distinct between the kelp and wood, providing evidence for differences in ecosystem function between the substrates.
- This case study from a deep-sea fjord in Norway therefore provides clear evidence that while wood and kelp organic falls can support similar abundances of fauna, the associated benthic biodiversity, community structure and ecosystem functioning can be dramatically different between these substrates, and as such wood does not provide a like-for-like replacement substrate for kelp.
- It is possible that as wood gets more degraded the organic material attracts more fauna that show closer taxonomic affiliation (i.e., the community transitioned to a more polychaete-dominated community) with the communities that develop around kelp-falls, but secondary production is likely to still be much greater on the wood than on the kelp overall.

- We therefore suggest more studies are conducted to look at colonisation of both substrate types at the deep-sea floor over a longer time period (>10 months).

7. Challenges and barriers

Wood does not appear to act as a like-for-like substitute for kelp detritus, and if greater amounts of wood material are placed at the seafloor to replace kelp material it will do little to mitigate the effects of kelp loss in deep-sea communities. Nevertheless, wood falls appear to be an important habitat for a unique community of fauna, including some specialists that depend on them completely e.g., xylophagaid molluscs. Furthermore, communities living on wood samples exhibited secondary production estimates twice that of communities living on kelp falls, largely due to high abundances of xylophagaid molluscs. When normalized to 1 m^2, secondary production for both wood and kelp falls was found to be similar or higher than that of shallower, soft sediment sites in European seas, illustrating the importance of both these substrates in the transfer of energy from primary production in terrestrial and shallow-ocean ecosystems to higher trophic level consumers in deep-sea habitats.



(A) One of four identical benthic landers during deployment in May 2017; each lander consisted of nine 40 x 40 x 40 cm fine-mesh bins, each containing one of three types of experimental substrate (wood block, settlement tile control, kelp parcel). (B) Fresh kelp parcel, (C) Kelp parcel after deployment, (D) Fresh wood block, (E) Wood block after deployment, (F) *X. dorsalis* boreholes in the wood, (G) *X. dorsalis* molluscs and burrows inside the wood block. (Credit: **Rob P. Harbour**, Craig R. Smith, Cornelia Simon-Nutbrown, Marta Cecchetto, Emily Young, Caterina Coral, **Andrew K. Sweetman**, Biodiversity, community structure and ecosystem function on kelp and wood falls in the Norwegian deep sea. *Marine Ecology Progress Series*. In press)

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