BEST PRACTICES, MEASURES AND LESSONS LEARNT FOR BIODIVERSITY RESTORATION, ENSURING CARBON SINK OPTIMIZATION AND BUFFERING RESILIENCE TO CLIMATE EXTREMES

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For bibliographic purposes, this document may be cited as:
UNEP/MAP-SPA/RAC, 2024. *Best practices, measures and lessons learnt for biodiversity restoration, ensuring carbon sink optimization and buffering resilience to climate extremes.* By Monica Montefalcone. *Ed. SPA/RAC*, Tunis: 77 pp. ISBN 978-9938-79-373-4

This document was prepared with the collaboration of Chiara Robello, Alessia Marrocu, Ilaria Mancini, and Christine Pergent-Martini.

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1 Introduction

The Specially Protected Areas Regional Activity Centre (SPA/RAC) of the Mediterranean Action Plan (UNEP/MAP) is assisting the Contracting Parties to the Barcelona Convention in fulfilling their obligations under the SPA/BD Protocol, the Strategic Action Programme for the Conservation of Biological Diversity (SAP BIO) in the Mediterranean region and the regional Action plans for the conservation of threatened habitats and endangered species.

The Mediterranean Sea is a biodiversity hotspot, hosting a huge number of marine species, including several species that are unique (i.e., endemic) to the region. These species play important ecological roles, and their loss would represent a significant loss for the overall biodiversity of the basin. The Mediterranean Sea is also considered as a hotspot for human footprint, being one of the world's regions with the most heavily trafficked shipping routes, with a high level of industrial and commercial activity, and with the highest degrees of anthropization along its coastline. This has led to pollution, overfishing, and destruction of habitat, which have all had negative impacts on marine biodiversity.

Restoring the marine ecosystems would help to ensure that the Mediterranean Sea remains a healthy and productive environment for both marine life and human use. Restoring the marine ecosystems would help to preserve important species and their habitats. Further, the Mediterranean Sea provides numerous ecosystem services, such as carbon sequestration, nutrient cycling, and climate regulation. These services are essential for maintaining a healthy planet, and their loss could have far-reaching consequences.

The restoration of marine ecosystems in the Mediterranean Sea is an essential strategy for preserving its biodiversity, ensuring human well-being, and maintaining the planet's ecological balance. It is a complex and challenging task, but it is necessary for the long-term health of the Mediterranean Sea and the species that depend on it. Restoration is one of the Nature-Based Solutions essential to ensure more resilient ecosystems capable of providing ecosystem services and goods across the Mediterranean. Restoration of marine ecosystems is a complex and challenging process, but there are several lessons that have been learned in the Mediterranean Sea in recent years and best practices that have been established over the years through the implementation of various projects that focused on restoration of endemic Mediterranean species and habitats.

1.1 Ecological restoration

The Society for Ecological Restoration defined ecological restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Gann *et al.*, 2019). The term "recovery" refers to the objective of ecological restoration interventions. Their aim is to achieve conditions like those of the original state or like those of a reference ecosystem, in terms of the specific composition, structure, and functionality. Full recovery occurs when, after restoration, all essential elements of the ecosystem closely resemble those of the reference model. These elements comprise the absence of threats, species composition, community structure, physical conditions, ecosystem function, and external exchanges. Lower levels of recovery resulting from resource, technical, environmental, or social challenges are classified as partial recovery.

Restoration approaches include both active and passive methods, which are crucial components of a conservation strategy that seeks to optimise biodiversity conservation and ecosystem services provision. Passive restoration focuses mainly on habitat maintenance, management, and conservation, allowing natural processes to mitigate impacts with minimal to no human interference. It utilizes the ecosystem's natural resilience by eliminating stressors and disturbances while implementing safeguards, such as

Marine Protected Areas or Special Conservation Zones (SCZs). The main management action necessary to enable ecosystem recovery over time is the reduction of disturbance sources and mitigation of all human-induced factors that harm the ecosystem (Hawkins *et al.*, 1999). Due to the high intensity and frequency of human pressures on marine ecosystems, passive conservation is no longer enough, and severely degraded ecosystems might not be able to recover in a foreseeable time frame. Active restoration projects have thus been increasing all around the world, providing direct human intervention through transplantation and translocation that can be applied either *in-situ* or *ex-situ*. Active restoration includes interventions focused to replicate the original habitat, incorporating steps such as reducing social impacts, remediating, rehabilitating, and ecologically restoring. However, the idea of a total recovery that precisely restores the habitat to its original state (i.e., the historical reference condition, the baseline) is increasingly abandoned by experts and has been substituted by a most feasible target condition. Recovery actions should then be viewed as 'recovery accelerators', considering that the system might never fully recover to its pre-impact condition. In the Anthropocene epoch, significant changes in environmental conditions, known as "regime shifts", frequently indicate changes in the ecosystem state, which are commonly referred to as "phase shifts". When severe regime and phase shifts occur, ecosystems are unable to revert to their original state (Barnard and Midgley, 2009). Any effort to restore losses that are irreversible to their historical reference state is likely to be difficultly achieved or even impossible (Harris *et al.*, 2006; Montefalcone *et al*., 2007). Therefore, it is advisable to focus on the recovery of ecosystems that can tolerate future changes instead of attempting to replicate the historical environment (Choi, 2007). This is the context in which the target conditions should be identified. This notwithstanding, the importance of historical data and information documenting changes lies in their ability to aid in the comprehension of ongoing trends and in the prediction of future ecosystem configurations. This is crucial in establishing clear and feasible restoration goals. Without an exhaustive understanding of the reference conditions of species and habitats, as well as of the causes of their degradation, the identification of achievable restoration goals (i.e., the target conditions) and assessment of restoration operation success would be challenging (Fraschetti *et al.*, 2021).

To prevent further environmental degradation, a list of active restoration actions is possible, but the priority must always remain the protection (i.e., conservation of ecosystems in their good ecological status) and the non-degradation. When the ecosystem is already degraded, the priority is to eliminate the origin of the degradation before doing anything else, and active restoration should remain the last solution.

1.2 The UN Decade on Ecosystem Restoration 2021-2030

Growing awareness of the need for environmental restoration has led to an increase in global ecological restoration efforts (Gann *et al.*, 2019). To support this movement, on May 1, 2019, the United Nations launched the UN Decade on Ecosystem Restoration 2021-2030, a collaborative initiative between the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization of the United Nations (FAO) (Waltham *et al.*, 2020). It is a unified call for the protection and revival of worldwide ecosystems, benefiting both nature and humanity. The objective is to reverse the degradation of ecosystems and restore them to meet global conservation goals by 2030. Only robust ecosystems can enhance people's livelihoods, mitigate climate change, and avert biodiversity collapse.

The United Nations' Decade spans from 2021 until 2030, synchronizing with the time limit for achieving the Sustainable Development Goals. During this time, ten objectives must be achieved, which intend to halt and reverse the destruction and depletion of billions of hectares of ecosystems. Addressing this challenge requires empowering a global movement, financing on-the-ground restoration interventions, establishing appropriate incentives, celebrating leadership, changing behaviours, investing in research, building capacity, promoting a culture of restoration, shaping the future through education, and continuous listening and learning.

Following the UN Decade on Ecosystem Restoration and the EU Biodiversity Strategy for 2030 (EC, 2020), the European Union proposed the Nature Restoration Law (EC, 2022) that is the first continent-wide, comprehensive law that calls for binding targets to restore degraded ecosystems, in particular those with the most potential to capture and store carbon and to prevent and reduce the impact of natural disasters. The EU restoration strategy aims at achieving a continuous, long-term, and sustained recovery of biodiverse and resilient nature across the EU's land and sea areas by restoring ecosystems.

1.3 Purpose and aims

In the frame of the UN Decade on Ecosystem Restoration 2021-2030, with the aim to assist the Contracting Parties to the Barcelona Convention in fulfilling their obligations under the SPA/BD and SAP-BIO Protocols and under the regional Action plans for the conservation of threatened habitats and endangered species, SPA/RAC planned to elaborate and share the best practices, measures and lessons learnt from biodiversity restoration interventions carried out in the Mediterranean Sea, to provide a collective state of the art and recommend protocols and monitoring activities, for ensuring carbon sink optimization and buffering resilience to climate extremes.

The purpose of this guideline is to make an inventory of the most significant active restoration projects developed in the last decades in the Mediterranean Sea on marine habitats (i.e., seagrass meadows, coralligenous reefs, algal forests) and on threatened species (e.g., *Coralium rubrum*, *Pinna nobilis*), as well as to identify projects aimed to enhance biodiversity through indirect interventions (e.g., artificial reefs) or through the removal of marine debris and litter (e.g., ghost fishing gears removal).

The successful examples here presented will provide best practices, measures and lessons learnt to ensure carbon sink optimization and buffering resilience to climate extremes, also useful for scaling up restoration efforts at all levels, to guide public policies, Marine Protected Areas managers, decision makers, environmental protection associations and scientists. To this end, significant case studies have been selected showcasing good and best practices resulting from the implementation of restoration programs and projects to provide insights into the processes followed, lessons learned from successful and unsuccessful attempts, and conditions to make them transferable to other areas where restoration works might be foreseen. It does not mean, however, that these successful techniques enable compensation and/or large-scale transplantation projects.

Specifically considering restoration of the *Posidonia oceanica* seagrass meadows in the Mediterranean Sea, a comprehensive synthesis of the best practices and of the examples of long-term successful experiments has been recently prepared by the Mediterranean Posidonia Network (MPN) and can be found in Pergent-Marini *et al*. (2023). The guidelines here suggested for *P. oceanica* restoration are, thus, fully consistent with those underlined by the MPN.

2 Inventory of the restoration projects on species and habitats

2.1 *Patella ferruginea*

Patella ferruginea Gmelin, 1791 is a gastropod mollusc formerly widespread throughout the Mediterranean basin; its occurrence is currently limited to the western basin, as it appears to be the most endangered invertebrate species in the entire basin (Ramos, 1998), with a distribution restricted to specific populations in defined geographic regions, including the Maghreb coast, the Alboran Sea, the southern Spain, the northeastern Sardinia, the Tuscan islands, and Corsica (Figure 1). Due to the decline in its populations the species has been included in the Annex II of the SPAMI Protocol of the Barcelona Convention, in the Appendix 2 of the Bern Convention, and in the Annex IV of the Habitats Directive (92/43/EEC) of the European Union. Additionally, *Patella ferruginea* is a species targeted for the assessment of the good marine environmental status (GES) within the context of the European Marine Strategy Framework Directive (2008/56/EC).

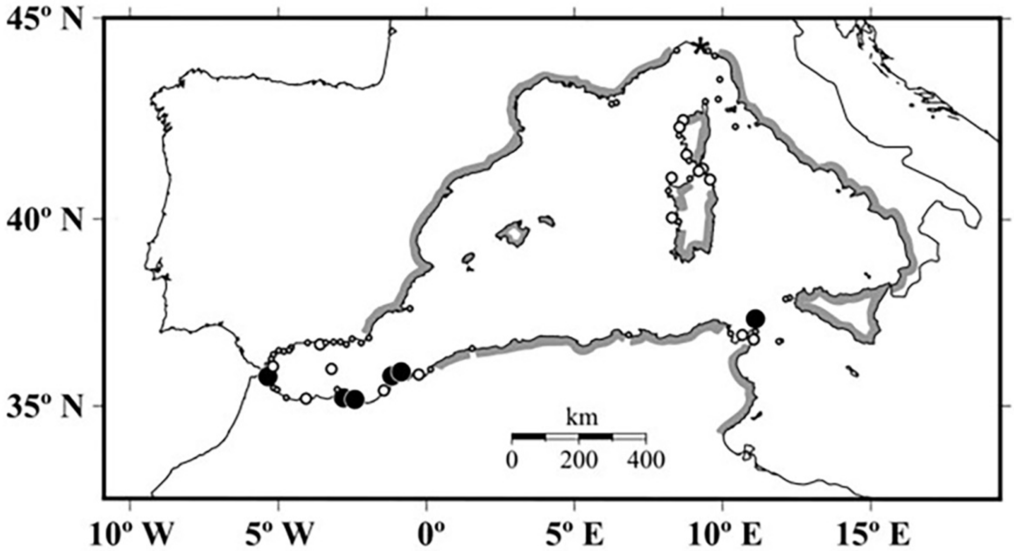

Figure 1. Synthesis of the distribution of *Patella ferruginea* in the Mediterranean Sea.
Grey bands: disappeared/not previously found. Small white dots: localities with some isolated specimens. Medium white dots: locations with populations numbering in the hundreds along kilometers of the coastline but with uncertain reproductive capacities. Black dots: hotspots of the species, locations with populations numbering in the thousands or ten thousands along kilometers of the coastline and with good reproductive capacity. Asterisk: location of the study area of the RE-LIFE project (map from Ferranti *et al.*, 2022).

In the last decade, this limpet species has attracted considerable interest and actions have been taken to repopulate endangered populations and re-establish populations in areas where the species is currently disappeared or locally extinct. *Patella ferruginea* is currently limited to a few populations in specific areas of the Mediterranean Sea (Espinosa *et al.*, 2009), likely due to its prevalence in easily accessible upper supralittoral and intertidal zones, where humans have been collecting *P. ferruginea* for food and as bait in recreational fishing for many years. Coastal infrastructure construction and water pollution, likely stemming from the presence of hydrocarbons, have significantly affected the current distribution of the species at the Mediterranean spatial scale. For a comprehensive overview on the state of *Patella ferruginea* in the Mediterranean, refer to the study by Espinosa *et al.* (2014) and Luque *et al.* (2018).

Most of the scientific contributions available in literature focus on either general or specific conservation concerns, on its geographical distribution, on population studies, but very few experiences have been carried out in the restoration of this species. In Spain, over the past 20 years, many technical and scientific projects were initiated to increase knowledge on *P. ferruginea* biology and to plan conservation strategies along the Spanish coast (MMAMRM, 2008; Luque *et al.*, 2018). In France, the RAMOGE agreement (http://www.ramoge.org) has been implemented to promote the collection of data on *P. ferruginea* along the coasts of the Ligurian Sea. In Italy, interest in this species has increased in recent years. In the context of the European Marine Strategy Framework Directive, the Regional Environmental Protection Agencies (ARPAs) have initiated surveys to collect data on the distribution of *P. ferruginea* in Italy, among other species of conservation interest.

One of the earliest recorded experiments on the reintroduction in nature of *P. ferruginea*, particularly for translocating adult specimens, was conducted by Laborel-Deguen and Laborel (1991). They translocated 188 adult individuals of *P. ferruginea* from Corsica to the marine protected area of Port Cros National Park (France); they experienced high mortality rates, resulting in very low survival rates for only 25% individuals after one year and 12% after two years. Since then, attempts to transfer *P. ferruginea* specimens from “donor sites”, where populations were still present and healthy, to “recipient sites” for reintroduction have continued, but the transplanted specimens have consistently experienced high mortality rates. The relocation of other limpet species, including *Patella vulgata*, has also been reported as a challenging process (Jenkins and Hartnoll, 2001).

Espinosa *et al.* (2008) conducted a study involving the transfer of 420 *P. ferruginea* specimens to six distinct recipient locations within Ceuta, located on the North African coastline in the Strait of Gibraltar, which has the highest densities of *P. ferruginea* in the Mediterranean area. Unfortunately, the transfer of specimens resulted in a high mortality in the following days, with a mortality of 50%. Zarrouk *et al.* (2018) similarly reported a high mortality rate few days after the translocation of 204 limpets to the Zembra Archipelago in Tunisia. On the contrary, when metal cages (Figure 2) were used to protect the translocated individuals, the mortality rate was only 18%, while without the cages the mortality rate was 35% after three days. These results suggested that the use of metal cages may have a significant protective effect, reducing mortality during the translocation process, especially in the first days after the relocation. However, both studies demonstrated that regardless of this, the translocation phase of the experiment was the most delicate.

The high mortality observed in the days immediately following the transfer of specimens in the receipt sites can be attributed to several factors. Primarily, desiccation stress can lead to the death of limpets. Particularly during the summer, due to high temperatures and intense solar irradiation, rocky substrates, especially horizontal ones, heat up, subjecting limpets to very high levels of stress (Williams and Morritt, 1995). Additionally, translocated limpets require several days to adapt to the new substrate and achieve secure attachment. During this period, wave action and tidal surges can also pose significant challenges. This explains the lower mortality of translocated limpets placed in cages, as observed by Zarrouk *et al.* (2018). Forty-eight days after translocation, the survival rate of limpets initially placed in cages was 71%, while the survival rate of those without cages dropped to 35%, as they were more exposed to waves and predation. Even over a more extended period (1-2 years), limpets translocated with cages exhibited higher survival than those translocated without cages, generally maintaining high survival rates. Survival rates of 58%, 25%, and 85% were observed for the cage-protected, cage-free, and control populations, respectively.

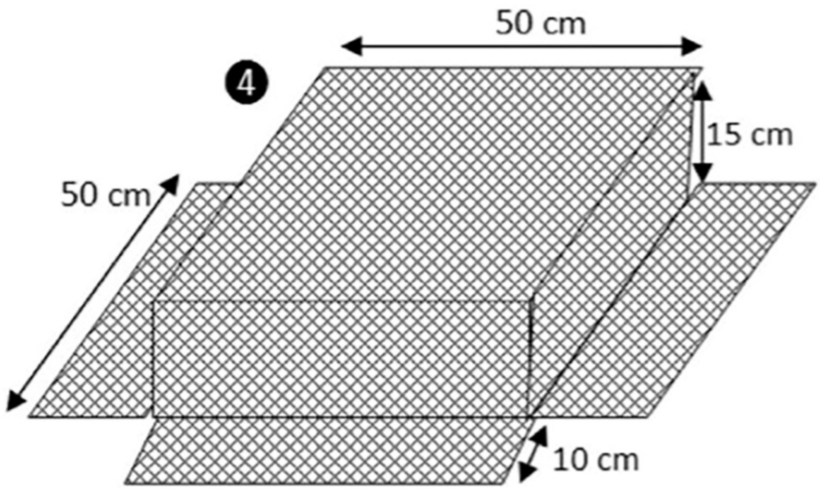

Figure 2. Protective metal cages employed in the experiment to shield the recently translocated *Patella ferruginea* specimens in the receiving sites (Zarrouk *et al*., 2018).

To overcome the limitation associated with the transfer of individual specimens, Fa *et al.* (2018) attempted to employ a method that did not involve detaching and subsequently relocating the individuals, a stressful process that, as mentioned above, resulted in high mortalities of individuals. The aim of their study was to relocate 97 *Patella ferruginea* specimens from a severely disturbed donor site to another receiving site with more favorable environmental conditions, but both located within a harbor. The selection of the receiving site was based on the presence of numerous individuals in the area, indicating the suitability of the site for the transfer of new individuals. The relocation was carried out by directly moving the large rocks to which the limpets were attached, rather than relocating any single individual alone. The authors believed that this method could offer various advantages, including transferring each limpet with its native "home" and with a portion of its feeding habitat, thus minimizing the stress typically associated with the detachment and the translocation of individual specimens to another substrate. Furthermore, this approach addressed the recent discovery of the species' "memory" concerning the surrounding topography, which develops during its foraging activities (Espinosa *et al.* 2008). Moving both the animal and the associated substrate would further reduce stress.

The results of the study by Fa *et al.* (2018) are highly encouraging. Ten months after the translocation, 84 out of the 97 limpets translocated to the receiving site were counted, resulting in a survival rate of 86.6%. This survival rate was consistent with the survival rate (79%) of the organisms already residing in the area at the time of the new specimens' transfer. The survival rate documented by Fa *et al.* (2018) exceeded all the rates recorded through the detachment of individuals from substrates, which resulted in a survival rate of approximately 60% after 10 months in Zembra (Zarrouk *et al.*, 2018), and 30-35% after one year and 18% after two years in the case of the Ceuta transfer (Espinosa *et al.*, 2008).

In the study by Espinosa *et al.* (2008), indeed, after an initial slowing of mortality, the number of transplanted individuals began to decrease over time. However, surviving specimens, mainly juveniles, showed a high growth rate immediately after translocation, and then the growth rate gradually stabilized over time. It was determined that growth rates of *P. ferruginea* were impacted by microalgal food availability, seasonality, and chlorophyll availability at the site. Smaller specimens displayed a higher growth rate, which was denoted by a clear ring on the shell edge, compared to larger specimens. This suggests that smaller specimens of *P. ferruginea* have a greater capacity to adapt to new environments after collection. On the other hand, in natural conditions, larger individuals have a greater chance of survival due to their enhanced resistance to predation.

Contrary to the findings by Espinosa *et al.* (2008), in the study by Zarrouk *et al.* (2018), the control group of limpets exhibited a higher growth rate compared to the translocated limpets, except for the 6-7 cm and the 8-9 cm size classes, which displayed similar growth rates. Another crucial factor to consider was the very high growth rates documented by Espinosa *et al.* (2008) in sites with a high chlorophyll concentration, indicating that the influence of this trophic resource, along with the other factors mentioned above, may represent a significant piece of the puzzle in supporting the growth of *P. ferruginea*.

Zarrouk *et al.* (2018) translocation experience underscored the importance of the cage protection and the initial size of limpets for their survival. The analysis of survival rates, categorized by size classes, revealed that the highest mortality rate was observed in the 4-5 cm size class after 380 days, whereas in Espinosa *et al.* (2008) study it has been hypothesized that relocating individuals of this size class would be preferable due to their greater adaptability to change.

The translocation method played a crucial role in the success of these experiments. Both the cage approach by Zarrouk *et al.* (2018) and the Fa *et al.* (2018) technique of relocating the entire rock on which the individuals resided have proven to be valid methods resulting in the highest long-term survival rates. Furthermore, given the success of *P. ferruginea* in establishing itself on man-made rocky coastal structures such as piers and breakwaters (Espinosa *et al.*, 2014), these findings also highlighted the potential to reintroduce adult *P. ferruginea* individuals from artificial coastal structures directly into natural habitats or in other port environments. This aligns with the suggestion by García-Gómez *et al.* (2011, 2015) that certain port structures could be used as “Artificial Marine Micro-Reserves” (AMMRs) for this species.

The translocation of individuals is thus a useful strategy for many species with limited geographical ranges that face local and regional threats (Hoegh-Guldberg *et al.*, 2008; Swan *et al.*, 2016). However, the use of this tool to facilitate species dispersion through assisted colonization is a subject of debate. As indicated by the studies by Espinosa *et al.* (2008), Fa *et al.* (2018), and Zarrouk *et al.* (2018), the expected mortality rates following translocation are quite high, even in the most successful experiments. Some authors also argued that, in the current context of climate change, translocations of narrowly distributed species should be planned considering their potential future habitats rather than solely relying on their historical locations, thus anticipating potential range shifts over time (Müller and Eriksson, 2013). Moreover, conservation efforts on *P. ferruginea* populations should primarily focus on safeguarding existing populations, particularly those in their natural habitats, as well as in artificial regions with abundant, well-maintained populations that can sustain the necessary genetic diversity.

For this reason, the strategy of the Spanish Administration for *P. ferruginea* advises against transferring specimens from natural populations and emphasizes the importance of doing it, only when necessary, and exclusively with specimens obtained through aquaculture techniques (MMAMRM, 2008). Currently, the recommended direction for maintaining and restoring the highly threatened species like *Patella ferruginea* is the controlled laboratory reproduction to minimize the impact on donor populations, which often are already under stress. However, a comprehensive understanding of all the fundamental biological aspects of the species is essential, and further research and conservation efforts are needed to enhance the success of similar translocation events.

Controlled reproduction in laboratory has been practiced for over two decades, although the outcomes have been only partially successful. At best, some juveniles have been obtained, which grew into adults, but this occurred not through the induction of egg deposition but rather through the dissection of female individuals, a lethal technique that sacrifices many specimens (Guallart *et al.*, 2020). This also resulted in low genetic diversity among the produced juveniles, whereas, conversely, a high genetic diversity is required for reintroduction purposes.

The Re-Life project (Re-establishment of the Ribbed Limpet *Patella ferruginea* in Ligurian MPAs by Restocking and Controlled Reproduction, LIFE15 NAT/IT/000771) was a recently launched conservation strategy, funded by the EC program, which began in 2016 and ended in 2022, aimed to reintroduce the ribbed limpet *P. ferruginea* in the Ligurian Sea, an area of historical occurrence from where the species disappeared, also through the active introduction of young individuals obtained through controlled laboratory reproduction. In this context, Ferranti *et al.* (2022) provided evidence, for the first time, on the possibility to induce the reproduction of *P. ferruginea* in captivity and demonstrated the feasibility of obtaining young individuals under controlled conditions through low-invasive methods. The project also showed how the induction of reproduction and the subsequent release of eggs for fertilization had much more successful compared to the dissection technique. The fertilization rate reported by Ferranti *et al.* (2022) ranged between 97.4% and 98.9%. Thanks to the Re-Life project and to Ferranti *et al.* (2022) findings, they defined a protocol for the transfer of limpets from high- to low-density areas and they developed a preliminary protocol to induce the controlled reproduction of the species, thus paving the way for the possibility of repopulating and reintroducing *P. ferruginea* into its natural environment.

A portion of the juveniles obtained in the laboratory, big enough to be translocated, were released into the natural environment in October 2021. Before being translocated, the juveniles were transferred from the settling substrate to EARLs (Artificial Elements for Littoral Reintroduction). These substrates were polyethylene tiles measuring 12 × 8 × 0.5 cm that were used to transfer specimens from the laboratory to the natural environment. During transport, the EARLs were kept moist with a seawater-soaked cloth and cooled. Three different methods were used to implant the juveniles on the rock: the first method involved securing the EARL to the cliff with screws and wrapping it with a net; the second method involved placing the juveniles directly on the rock and protecting them with a 30 × 30 cm net, as was done with the adult specimens. The third method was a combination of the previous two (Figure 3). This third method proved to be the most effective as it allows juveniles to move out of the EARLs while still being protected by the net until they are large enough. Survival and growth rates of the relocated limpets in the natural environments are not available, thus preventing a definitive assessment of the effectiveness of this methodology for the moment.

Figure 3. Different methods used to implant the juveniles of *Patella ferruginea* on the rock in the Re-Life project: method 1 with EARL covered with a net (left panel), method 2 without EARL but only with 30 × 30 cm protective net (middle panel), and the combined method EARL + protective net 30 × 30 cm (right panel) (Ferranti *et al*., 2022).

Within the scope of the Re-Life project, it became evident that the compatibility between the donor and the recipient sites needs to be assessed to ensure the adaptation and well-being of individuals that will be translocated. This characterization particularly concerns all the biotic and abiotic characteristics that, as already highlighted, play a fundamental role in the success of individual translocation. The environmental status of the target sites in Re-Life project was assessed through the implementation of the CARLIT Index (Nikolić *et al.*, 2013), an ecological index developed in the frame of the Water Framework Directive (2000/60/EC) to evaluate the environmental quality of coastal waters through the adoption of macroalgae occurring in the infralittoral fringe (upper sub-littoral zone) as bioindicators.

2.2 *Pinna nobilis*

The fan mussel *Pinna nobilis* (Linnaeus, 1758) is the largest endemic mollusk bivalve of the Mediterranean Sea. *P. nobilis* occurs in soft-bottom habitats of transitional water ecosystems and in marine coastal zones at depths between 0.5 and 60 m, mostly in seagrass meadows of *Posidonia oceanica* and *Cymodocea nodosa* (Katsanevakis, 2006; Prado *et al.* 2014), but also in bare sandy bottoms and in algal forest beds (Katsanevakis and Thessalou-Legak, 2009). This is a long-lived species, with some individuals reaching over 20 years of age (Butler *et al.*, 1993) and an important benthic filter feeder contributing to water clarity. It is recognized as an iconic species playing the roles of flagship, key, and umbrella species. Due to its ecological relevance, *P. nobilis* has recently been suggested as being a reliable bioindicator for benthic coastal ecosystems according to the EU Marine Strategy Framework Directive (2008/56/EC). In addition, the fan mussel represents the host for two crustacean symbionts (i.e., *Pontonia pinnophylax* and *Nepinnotheres pinnotheres*) and it is also predated by other species, such as for instance *Octopus vulgaris*, playing a key role in the trophic web.

Pinna nobilis has undergone a significant decline in the last three decades due to multiple factors that nearly caused its extinction. Historically, *Pinna nobilis* has been exploited to produce high-quality fabrics derived from its byssus, although the impact on the species is not well understood. More recently, the most severe damages have been inflicted by recreational and commercial fishing for consumption and the collection of large shells for ornamental purposes (Addis *et al.*, 2009; Katsanevakis and Thessalou-Legaki, 2009). Like *Posidonia oceanica*, *Pinna nobilis* also faces a significant impact from trawling, dredging, and uncontrolled anchoring (Katsanevakis *et al.*, 2022). Consequently, *P. nobilis* is nowadays a protected species under the Annex IV of the EU Habitats Directive 92/43/EEC and the Annex II of the SPAMI Protocol of the Barcelona Convention, and by national laws in most of the Mediterranean countries. In few decades, this full regime protection led to a complete recovery of the species in the whole Mediterranean, as it was also evidenced by molecular analyses that examined mitochondrial DNA markers (Sanna *et al.*, 2013, 2014).

Since 2016, an additional severe negative impact has been added to its populations. A protozoan *Haplosporidium pinnae* (Catanese *et al.*, 2018) has caused a mass mortality event in the south-western Mediterranean (Vázquez-Luis *et al.*, 2017). Within a year from the arrival of these pathogens, 90% of the *P. nobilis* populations in Spain had disappeared, followed shortly by Italy, France, Turky, and Tunisia (Catanese *et al.*, 2018). This microorganism affects the digestive system of the mollusc progressively reducing the feeding of the animal and causing its death (Catanese *et al.*, 2018). *H. pinnae* appeared to exhibit high specificity for *P. nobilis*, as the mass mortality did not affect the other species *Pinna rudis*, despite belonging to the same genus (Vázquez-Luis *et al.*, 2017). Recently it has been discovered that this pathogen is not host specific of *P. nobilis*, as it was initially hypothesized by Catanese *et al.* (2018), and that it was already present in other bivalves of the Mediterranean basin since 2014 (Scarpa *et al.*, 2020). Several bacteria species have also been invoked as pathogens involved in the mass mortality of this species (Scarpa *et al.*, 2020), suggesting that the real causes of the mass mortality are not completely understood and that a multifactorial disease may be the most probable responsible factor.

Currently, surviving and resistant individuals are scarce and scattered throughout the Mediterranean (Figure 4). Consequently, *P. nobilis* is nowadays listed as “Critically Endangered” by the IUCN Red List of threatened species.

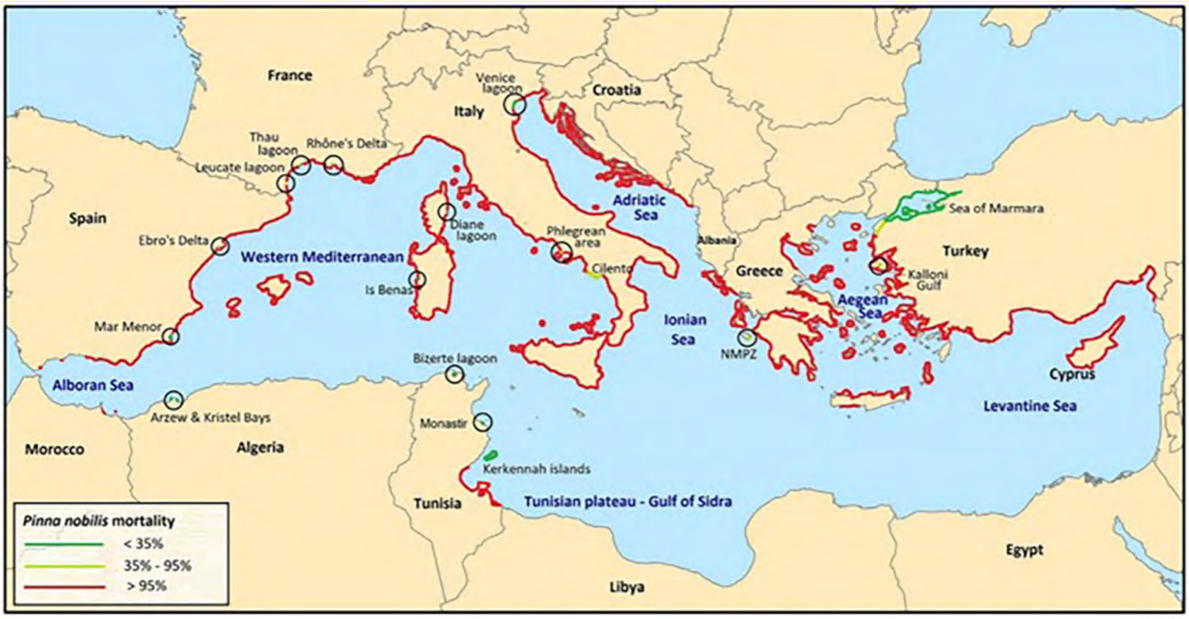

Figure 4. Status of *Pinna nobilis* populations in the Mediterranean Sea (November 2020) after the parasitic outbreak in 2016. In the sectors of the coastline with no color, the situation remains unknown. Even along the coastline indicated as areas of high mortality (red color), the possibility that yet unidentified healthy populations exist cannot be fully excluded (map from Katsanevakis *et al.*, 2022).

Survivors of *Pinna nobilis* are still present in some sheltered areas of the Mediterranean Sea. Survivors are likely characterized by a natural resistance to the pathogens responsible for the outbreak of the disease, and analyses of the level of pathogenic infection in the tissues of these individuals may be useful to identify the microorganisms that are involved in the disease. It would be also important to assess the level of contamination/infection depending on whether *P. nobilis* specimens die or survive. There are no records worldwide about a mass mortality event like the one that is currently leading *P. nobilis* populations on the brink of extinction. The loss of *P. nobilis* would not have the magnitude of a simple species extinction but it is a big issue to the whole Mediterranean biodiversity. In fact, for its ecological role, the loss of *P. nobilis* would represent one of the most dramatic events of our century in marine environments. Strong and decisive actions are thus needed to prevent this species loss.

The first recorded experiment of transplanting *Pinna nobilis* was carried out in 1955 with the objective of cultivating the species for food, byssus, and shell uses (Trigos and Vicente, 2016 and references therein). Subsequent experiments in the following years failed to produce positive results (Hignette, 1983; De Gaulejac and Vicente, 1990). In 2008, a few specimens were transplanted in Tavolara-Punta Coda Cavallo Marine Protected Area (Sardinia, Italy). In this study, some *P. nobilis* specimens uprooted from the seabed and lacking byssus were utilized but the transplantation was successful only for specimens with valve lengths ranging from 15 to 20 cm (Caronni *et al.*, 2008). Other studies demonstrated that young individuals have higher attachment capability and quicker regeneration of byssal filaments, attributable to their faster growth rate relative to older individuals (Katsanevakis, 2007). However, transplanting young and small individuals in some studies resulted in poor outcomes, as mortality rates were significantly higher for young individuals (10-30%) than for adult and small individuals (0-13%) (Basso *et al.*, 2015; Katsanevakis, 2016; Bottari *et al.*, 2017). To conclude, for ensuring the highest success of transplantation, small adult specimens (5.5-10 cm shell length, equivalent to 6-18 months of age) are preferred due to the reduced stress and predation pressure compared to younger and older individuals (Bottari *et al.*, 2017).

Another technique proposed for enhancing transplantation success involves the collection of *Pinna nobilis* larvae, raising them in suspended systems (Katsnevakis, 2016; Acarli, 2021). Young fan mussels are placed into containers, such as boxes, baskets, or lantern nets, which are suspended from a floating system. These containers can either be filled with sand with the young mussels planted vertically (Wu and Shin, 1998), or cultivation can be conducted without sand in the containers (Acarli *et al.*, 2011) (Figure 5).

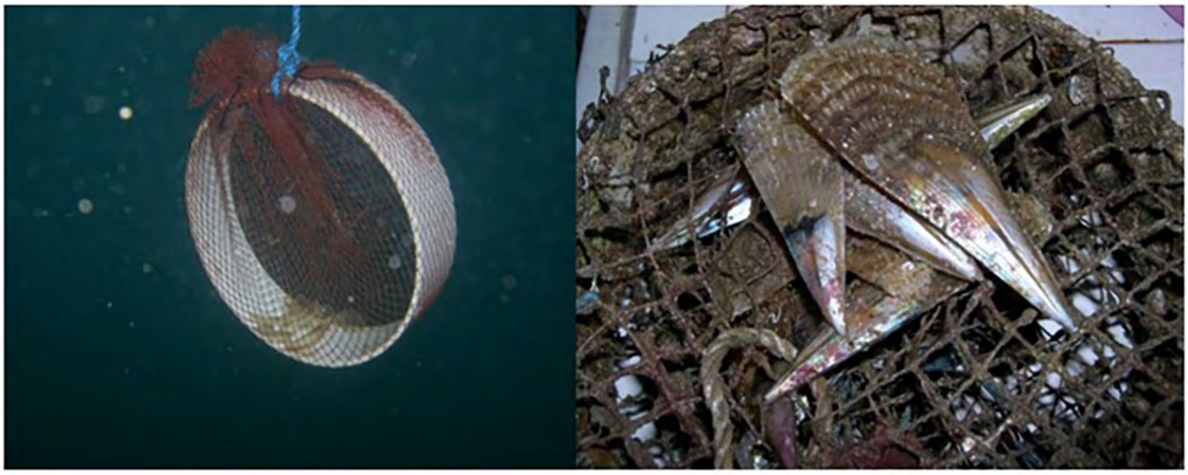

Figure 5. Culture trials of *Pinna nobilis* with suspended systems (Acarli *et al.*, 2011).

Once the juveniles have reached a sufficient size to better withstand external pressures, they can be transplanted to a suitable area (Katsanevakis, 2016). The suspended culture system showed a positive contribution to *Pinna nobilis* survival, with recorded survival rates of 78% (Acarli, 2021). The suspended system provided an opportunity for young specimens to grow at a distance from the seafloor, decreasing predation pressure and increasing survival rates (Wu and Shin, 1998). Several studies indicated that bivalves in bottom culture experience lower growth rates, with a significant number of deaths attributed to predation (Acarli, 2021 and references therein). Also, when individuals are mature, predation-related deaths become less frequent (Velasco and Borrero, 2004). However, a recent study suggested reducing predator pressure by selecting transplantation methods that offer protection once the organism is in the substrate. Newly transplanted individuals must be covered with appropriately sized nets in sheltered systems, effectively preventing predator access (Figure 6) (Acarli, 2021).

Figure 6. Individuals of *Pinna nobilis* planted into bottom after rearing trial in the Karantina Island (Izmir Bay, Aegean Sea) in December 2006 (Acarli, 2021).

The consulted literature is unanimous suggesting the vertical orientation of the shell as the optimal transplantation position for *Pinna nobilis* into the substrate. More specifically, the shell should be inserted into the substrate at least halfway (Trigos and Vicente, 2016), or while considering traces of mud or sand present on the shell (Acarli, 2021).

Positive results have been obtained from the limited experiments conducted so far on *Pinna nobilis* transplantation. Survival rates ranged from 100% to 66%, with an average of approximately 82%. The low number of individuals transplanted in some studies should also be considered, as it ranged from 6 to 53 individuals (Caronni *et al.*, 2007, 2008; Katsanevakis, 2016; Trigos and Vicente, 2016; Bottari *et al.*, 2017; Acarli, 2021). Another crucial aspect is the genetics: it is essential breeding and transplanting individuals that have developed resistance to diseases across generations, to ensure survival following transplantation into the natural environment (Acarli, 2021).

Resistance to diseases is one of the critical components of the Life Pinna project funded by EC, launched in October 2021 and still ongoing, which aims to explore the most effective techniques for monitoring and safeguarding existing *Pinna nobilis* populations. The project also aims to maintain and reproduce *P. nobilis* specimens in captivity to repopulate some selected areas where populations disappeared. The genetics of individual specimens are considered, focusing on reproducing only the strongest and the most resilient individuals to ensure higher survival rates for juvenile individuals after transplanting. The selection and characterization of both donor and receiving sites for implantation is also another crucial phase.

Donor sites where resistant individuals (i.e., survivors) of the fan mussel *P. nobilis* are still present have been selected according to the occurrence of a minimum number (about 30) of adult resistant individuals (e.g., in the Adriatic Sea), based on the available information collected during previous monitoring activities. Eligibility as a donor site required that, since the last monitoring, at least 10% of living specimens had survived. The environmental characterization and the mapping of the sites identified as donors has been carried out, based both on the literature review and on the results of the most recent monitoring activities.

Similarly, an environmental and ecological assessment of the pilot sites for restocking has been carried out based on the analysis of historical data reporting the presence of fun mussels in these sites and on the new environmental data collected through ex-ante monitoring actions. Pilot receiving sites should have characteristics that correspond to the natural requirements of *P. nobilis*, that are: i) physical and chemical (e.g., water temperature, salinity, nutrients); ii) geomorphological and geographic (best depth range, similar latitude, similar climatic and seasonal variations); iii) ecological (sites should show the best habitats for survival of *P. nobilis* populations, which might be different in the Mediterranean regions. In the western Mediterranean regions, the endemic *Posidonia oceanica* is considered as the main coastal habitat where fun mussels thrive, while in the northern Adriatic regions *P. nobilis* is also found on coarse sandy bottoms and in *Cymodocea nodosa* and *Zostera* spp. meadows).

At each donor and recipient site, the following activities must be performed:

1. Definition of the bathymetric range of seagrass development. Because of the well-described relationship between meadows lower limit depth and water clarity, lower limit depth is one of the best-known indicators of water quality in seagrass monitoring plans. Due to the availability of a standardized environmental quality classification in the literature (UNEP/MAP-RAC/SPA, 2011), the depth and type of the lower limit of *Posidonia oceanica* must be compared to reference values providing a measure of the ecological status of seagrass.
2. Cover, expressed as the percentage of the bottom covered by *Posidonia oceanica* relative to the area not covered by plants, will be visually estimated by divers in at least three replicates located approximately 10 m apart.
3. For *P. oceanica*, shoot density, expressed as the number of shoots per area (conventionally equal to 1 m^2), must be measured in nine replicates (three replicates of measurements in each of three areas located approximately 10 m apart). Shoot density is measured by counting the number of

shoots within a standardized area (20 cm × 20 cm square). Due to the availability of a standardized environmental quality classification in the literature (UNEP/MAP-RAC/SPA, 2011), *P. oceanica* shoot density must be compared to reference values to define the ecological status of meadows.

Additionally, prior to the transplantation of *Pinna nobilis* in receiving sites, sentinel species must be collected in the selected receiving locations to verify their appropriateness. Preparatory actions require the use of sentinel mussels as bioindicator (filter feeders that can be hosts for the same pathogens as *P. nobilis*). This preparatory phase will be useful for defining the exact localization of the pilot areas, as well as for the ongoing monitoring plan settled to monitor the spreading of pathogens over time, to ensure that the receiving sites will be always pathogen-free areas. Sentinels are mussels belonging to *Mytilus galloprovincialis* species or other bivalves, which provide information about the etiological agents present in coastal environments. Occurrence of pathogens in sentinel bivalves must be evaluated both in the sites where the fan mussel survivors occur (i.e., donor sites) and in the putative pilot sites of restocking (i.e., receiving sites). Constant molecular analysis of sentinel species organisms devoted to monitoring the infection level of pathogens responsible for *P. nobilis* mass mortality must be carried out for the entire duration of the whole project. This step allows obtaining a constant and updated picture of the pathogens in the areas, to quickly evidence anomalous values potentially dangerous for the survival of restocked *P. nobilis* (Scarpa *et al.*, 2020).

At least 100 naturally occurring sentinel organisms of the species *Mytilus galloprovincialis* (or other similar bivalves) must be collected in the wild, to provide a cognitive framework about the presence of etiological agents that could invalidate the restocking initiatives of juveniles. Constant molecular analysis of sentinel organisms, devoted to monitoring the infection level of pathogens is needed - at regular intervals of time (e.g., every 3-4 months) - in every site in which specimens of *Pinna nobilis* will be relocated.

A molecular characterization of surviving specimens in donor sites must be performed to select the best adult candidates to be transferred in the laboratory for reproduction in captivity. The same molecular characterization must be also carried out before transplanting both adult and juvenile individuals in the wild. This phase requires genetic analysis and pathogen research on adult specimens, following the authorization for non-lethal sampling of *Pinna nobilis* tissues, using a standardized sampling method that has been developed in the frame of the Life Pinna project. A 20-50 mg sample of mantle tissue is collected while maintaining the valves open, then stored in a 1.5 ml tube and preserved in 75% ethanol. A cotton or a sampling brush swab is gently rubbed to collect mucus samples from the soft tissues of *Pinna nobilis*. After that, the soft head of the swab is cut off, preserved in proper tubes filled with 90% ethanol and then stored at -20° C (Casu *et al.*, 2019). This sampling method has shown to cause very low level of invasiveness for the specimen.

Thanks to the Life Pinna project, a standardised protocol for the transport of both adult and juvenile individuals of *P. nobilis* is going to be defined, useful for transferring the surviving specimens from the donor sites to both the laboratory for reproduction experiments (i.e., the adults) and the receiving sites for restocking activities (i.e., the juveniles). Similarly, the project will also allow for the definition of a standardised protocol for the maintenance, the spawning, and the reproduction of *Pinna nobilis* in captivity.

The Life Pinnarca project is another EC funded project similarly focused on reintroducing *Pinna nobilis* in receiving areas. This project has installed larvae collectors with the purpose of capturing them and then growing them in a controlled environment for future active transplantation. This project also aims at gathering all existing information on the remaining populations and resistant individuals to include it into a database integrated within the project webpage. This will provide a more informed background to other countries planning mitigation and recovery actions.

Two additional projects have been addressing the restoration of *Pinna nobilis*. The 'MERCES – Restoring European Seas' project focuses on restoring several degraded marine habitats with the aim of i) assessing the potential of different technologies and approaches, ii) quantifying the returns in terms of ecosystems services and their socio-economic impacts, iii) defining the legal-policy and governance frameworks needed to optimize the effectiveness of the different restoration approaches. The MERCES project attempted to relocate several *Pinna nobilis* specimens to prevent suffocation during the construction of a new nautical center in the port of Pula (northern Adriatic Sea, Croatia). The receiving site, Javorike Bay in Brijuni National Park, is located in a protected area with a minimal exposure to hydrodynamics and already had a limited occurrence of *P. nobilis* within the *Cymodocea nodosa* meadow. A total of 154 specimens were translocated to depths between 6 to 12 m. Two years after the translocation, their survival rate was very high (86.4%) (Pajusalu *et al.*, 2019), which is consistent with the estimated natural mortality rate of 7% per year (Katsanevakis, 2016). Among all the dead individuals, the half died soon after the translocation, likely due to associated stress and/or inappropriate handling (Pajusalu *et al.*, 2019).

The 'RESTORFAN' project was activated in 2019 with the support of the MedPAN-network and is coordinated by the Miramare Marine Protected Area located in the Gulf of Trieste, in the northern Adriatic Sea (Italy). This latter project aimed to improve knowledge on *P. nobilis* by developing protocols for handling, capturing, and restoring the species through monitoring and census of specimens already present in the area.

To date, developing a standardized and effective protocol for *Pinna nobilis* transplantation remains a daunting challenge. Continued research is crucial to determine optimal practices, especially regarding aquaculture activities. Transplantation alone proved inadequate without understanding recruitment rates and larval and juvenile survival (Bottari *et al.*, 2017). Other similar mussel species, including *Pinna bicolor*, *Pinna rugosa*, and *Atrina maura*, continue to be commercially traded and consumed for their meat, with ongoing studies aimed at advancing sustainable reproduction in some countries. Research on these related species may offer valuable insights for enhancing *Pinna nobilis* studies (Acarli, 2021).

2.3 Coralligenous habitat

The calcareous formations of biogenic origin in the Mediterranean Sea are represented by coralligenous reefs, vermetid reefs, cold water corals, *Lithophyllum byssoides* concretions/trottoirs, banks formed by the corals *Cladocora caespitosa* or *Astroides calycularis*, sabellariid and serpulid worm reefs, and rhodoliths seabeds. Among all, coralligenous reefs are the most important and widespread bioconstructions in the Mediterranean Sea that develop in the circalittoral zone, built-up by coralline algal frameworks that grow in dim light conditions (UNEP/MAP-RAC/SPA, 2008). Coralligenous is an endemic and characteristic habitat considered as the climax biocenosis of the circalittoral zone (Pérès and Picard, 1964). Coralligenous is characterised by high species richness, biomass, and carbonate deposition values comparable to tropical coral reefs (Bianchi, 2001), and economic values higher than seagrass meadows (Cánovas Molina *et al.*, 2014). Construction of coralligenous reefs started during the post-Würm transgression, about 15000 years ago, and develops on rocky and biodetritic bottoms in relatively constant conditions of temperature, currents, and salinity.

Coralligenous structure results from the dynamic equilibrium between bioconstruction, mainly made by encrusting calcified Rhodophyta belonging to Corallinales and Peyssonneliales (such as the genera *Lithophyllum*, *Lithothamnion*, *Mesophyllum*, *Neogoniolithon*, and *Peyssonnelia*), with an accessory contribution by serpulid polychaetes, bryozoans and scleractinian corals), and destruction processes (by borers and physical abrasion), which create a morphologically complex habitat where highly diverse benthic assemblages develop (Ballesteros, 2006). Although light represents the main factor limiting bioconstruction, coralligenous reefs can develop in dim light conditions (<3% of the surface irradiance), from about 20 m down to 120 m depth. Also, the upper mesophotic zone (where the light is still present, from 40 m to 120 m depth), embracing the continental shelf, is shaped by extremely rich and diverse coralligenous assemblages dominated by animal forests that grow over biogenic rocky reefs.

Coralligenous reefs provide different ecosystem services to humans (Paoli *et al.*, 2016, but are fragile ecosystem vulnerable to both global and local pressures (Montefalcone *et al.*, 2017). Coralligenous is threatened by direct human activities, such as recreational boat anchoring and bycatch resulting from the use of trammel nets or bottom trawl nets. Nets entangle in gorgonian branches and other erect calcareous organisms, breaking or entirely eradicating them. Harvesting and illegal exploitation of targeted species with high commercial value, such as the red coral *Corallium rubrum*, the date mussel (*Lithophaga lithophaga*), and some sponges, can cause huge damages to their populations. Also, poorly regulated pleasure diving activities can provoke significant physical damages to coralligenous because of its aesthetic touristic value.

Coralligenous is also vulnerable to the indirect effects of climate change (e.g., positive thermal anomalies and water warming), which are causing mass mortality events of stenothermic organisms and are enhancing the spread of highly harmful pathogens (Cerrano *et al.*, 2000; Garrabou *et al.*, 2001, 2009; Bramanti *et al.*, 2013). The excessive input of carbon dioxide from the atmosphere is causing ocean acidification, interfering with the production of calcium carbonate by bioconstructors and resulting in severe damages. Some invasive algal species (e.g., *Womersleyella setacea*, *Acrothamnion preissii*, *Caulerpa cylindracea*) can also pose a severe threat to these communities, either by forming dense carpets or by increasing sedimentation rate. Other significant pressures to coralligenous include sedimentation, which reduces water clarity and limits light penetration, and eutrophication, which not only further reduces water clarity but also reduces oxygen levels, harming photosynthetic processes.

Despite its high vulnerability to human disturbances and the occurrence of many species with high ecological value in its communities (some of which are also legally protected, e.g. *Savalia savaglia*, *Spongia officinalis*), coralligenous habitat is not listed among the priority habitats defined by the EU Habitat Directive (92/43/ EEC), even if it can be generally included under the habitat “1170 Reefs” of the Directive and it appear also in the Bern Convention. This implies that the most important Mediterranean bioconstruction remains without a formal protection, as it is not included within the list of Sites of Community Interest (SCIs). Few years after the adoption of the Habitat Directive, coralligenous reefs were listed among the “special habitat types” needing rigorous protection by the Protocol for Special Protected Areas (SPA/BIO) of the Barcelona Convention for the conservation of Mediterranean biodiversity (1995). Only recently, in the frame of the ‘‘Action Plan for the conservation of coralligenous and the other Mediterranean bioconstructions’’ (UNEP/MAP-RAC/SPA, 2008) adopted by Contracting Parties to Barcelona Convention Barcelona in 2008, encouraged the legal conservation of coralligenous assemblages by the establishment of marine protected areas and emphasized the need for standardised programs for its monitoring. Coralligenous has also been included in the European Red List of marine habitats, where it is classified as “data deficient” (Gubbay *et al.*, 2016), thus demonstrating the urgent need for thorough investigations and accurate monitoring plans. In the same year, the Marine Strategy Framework Directive (2008/56/EC) included “seafloor integrity” as one of the descriptors to be evaluated for assessing the good environmental state (GES) of the marine environment. Biogenic structures, such as the coralligenous reefs, have thus been recognized as important biological indicators of environmental quality.

Notwithstanding its high ecological importance and the many threats affecting its overall conservation status in the Mediterranean Sea, no attempts have been made at present to actively restore the biodiversity and the community structure of an impacted coralligenous habitat. To date, only experiments aimed at the reintroduction and the transplantation of individual target species in limited areas have been carried out. In particular, the targets of the few restoration interventions have been the red coral, a protected species with high conservation interest, the gorgonians, which are highly vulnerable structuring species currently suffering from global warming and mass mortalities, and the sponges, given their high commercial interest, both as simple “bath sponges” and for the numerous secondary metabolites they produce, which are very important in the medical and the pharmaceutical fields.

The only example found in literature of a restoration intervention made on coralligenous habitat comes from a private construction project that affected coralligenous outcrops in deep coastal environments, where the relocation of these bioconstructed habitats has been undertaken as a compensation measure to reduce the environmental impact of the project itself and to reduce the habitat loss (Casoli *et al.*, 2022a). The project included the planning, intervention, and monitoring phases following the Trans Adriatic Pipeline (TAP) laying along the Apulian coast (Adriatic Sea, Italy). Preliminary field activities encompassed morpho-bathymetric data (using high-resolution multibeam sonar imagery and side scan sonar), SCUBA and ROV observations to accurately map and characterize the coralligenous mesophotic reefs found in the area interested by the project. The portion of the seabed where the TAP pipeline route interfered with the outcrops was evaluated. They considered as interfered those outcrops located within the pipeline designed route, therefore, which would have been physically impacted by the TAP laying operations. The pipeline route interfered with 30 outcrops occurring between 50 and 80 m depth. A functional and conservative approach was adopted to recognize the taxa/morphological groups on which to focus the removal and the following relocation activities based on their abundance, conservation status, and functional traits defined from the ROV video footages. According to the existing bibliography, the expertise of the team members, and by consulting other experts a relocation score was assigned to each taxon/morphological group that allowed the identification of the taxa for which the relocation activities were of primary concern, mainly according to their frequency of occurrence, conservation status, and role in the growth and functioning of the reef.

The targets of the relocation interventions were bioconstruction portions (hereafter referred to as nuclei) mostly colonized by taxa/morphological groups of primary ecological/conservation concern defined through the application of the above-mentioned relocation score. A team of saturation divers, ROV pilots and technicians, and marine ecologists were boarded on a research vessel during the following operational phases, and all collaborated to minimize the physical impact and the loss of organisms due to the pipeline installation. A total of 899 living portions (nuclei) were manually removed from the 30 interfered coralligenous outcrops by using hammer and chisel to achieve a high degree of accuracy during the removal and reduce as much as possible damages to the benthic organisms (Figure 7). All the activities were followed remotely by onboard researchers that chose the nuclei to be removed by watching real-time diver and ROV cameras, leading saturation divers’ activities. Then the nuclei were temporarily placed on underwater iron grid tables, positioned at the same depths as the outcrops from which the nuclei were removed, to allow water flows to ensure filter-feeding, and waiting for pipeline laying and the following relocation (Figure 7).

After the pipeline laying the nuclei were relocated on the top of the pipeline using epoxy resin Milliput Standard Yellow-Grey type, which has been recently reported as effective and biocompatible epoxy putty to attach benthic colonial organisms (Casoli *et al.*, 2022b). The nuclei were relocated over 17 pipeline segments measuring 10 m in length close to the interfered bioconstruction from which they were removed. The following monitoring activities, carried out after fourteen months since the intervention, revealed a high mean survival rate (88.1%) and slight variations in the structure of the nuclei assemblages (Figure 7). This study represents a paradigmatic case of involvement and support of the private oil and gas sector to mitigate habitat loss in the Mediterranean Sea and stresses the need for integrated management involving different stakeholders to mitigate the effects of the exploitation of marine resources through *ante-operam* assessment and active restoration actions.

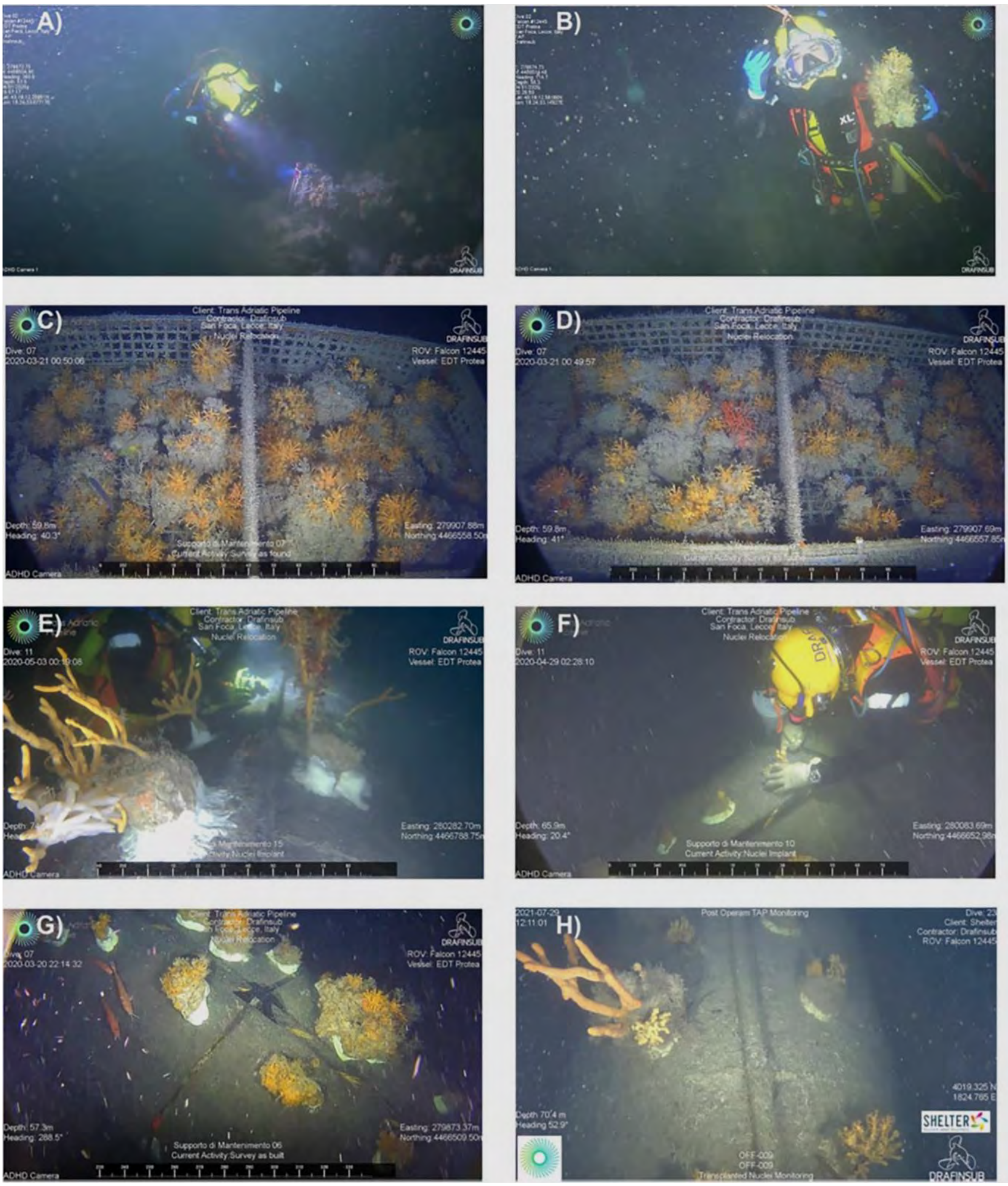

Figure 7. Photo-table showing the three different phases of the removal and relocation intervention, and the following monitoring: (A, B) saturation divers removed nuclei from the interfered outcrops; (C, D) the nuclei were temporarily placed on the iron grid tables; (E, F) saturation divers relocated the nuclei on the top of the pipeline by using epoxy resin on concrete coatings; (G, H) images acquired during monitoring activities (Casoli *et al.*, 2022a).

2.3.1 *Corallium rubrum*

The red coral *Corallium rubrum* (Linnaeus 1758) is considered an ecosystem engineer as it contributes to biodiversity through the creation of three-dimensional habitats. It is a long-lived species exhibiting an arborescent growth form, which can reach 50 cm in height (weight >2 kg). It can form true facies in semi-dark caves, with dense monospecific stands especially on the cave roof. It is also commonly found in enclave in the coralligenous habitat, where it colonizes overhangs and crevices throughout the Mediterranean Sea, and especially in the western basin; in the eastern basin it is rarer and occurs deeper.

For centuries the red coral has been overexploited due to its high desirability in the jewellery industry to produce ornaments. Over the years, fishing techniques have become increasingly efficient, and today almost all known red coral populations are declining or are even disappeared (Tsounis *et al.*, 2007, 2009). The huge fishing pressure, the very slow growth rate, the high vulnerability, and the remaining of only isolated populations, made *Corallium rubrum* a protected species listed in the Annex III of the SPAMI Protocol of the Barcelona Convention, in the Appendix 2 of the Bern Convention, and in the Annex V of the Habitats Directive (92/43/EEC) of the European Union. It is also considered an endangered species in the Red List of threatened species by the International Union for Conservation of Nature (IUCN), although it is not listed in the Appendix II of the Convention on International Trade in Endangered Species (CITES). Today, harvesting is allowed only by scuba divers and regulated by specific laws. Prohibition of coral harvesting in overexploited areas has been adopted by several countries. Colonies taller than 20 cm and thicker than 2 cm in basal diameter have become very rare because of the intensive harvesting. Minimum harvestable size (7 mm basal diameter) is reached in 30-40 years.

Because of the widespread decline in the red coral populations, active restoration actions became necessary to repopulate populations affected by intense fishing and to maintain the ecosystem services provided by this species. In recent years, numerous studies have been conducted to investigate the best restoration techniques for damaged red coral colonies. Most of the experiments involved colony fragments attached to the substrate using various techniques and the use of PVC tiles and other materials that allow larval settlement and growth.

The first transplantation experiment was conducted in shallow waters (10 m depth) and used red coral fragments inserted into rocks and secured with screws. No fragments survived after the transplantation (Weinberg, 1979). Numerous other experiments followed, employing various techniques and substrates, but none yielded significant results in terms of survival and success. In a recent study Villechanoux *et al.* (2022) compared the most common transplantation techniques used for red coral restoration in the western Ligurian Sea. They used six different techniques at the Gallinara Island (Savona, Italy), where the red coral is no longer present, and in the Portofino Marine Protected Area (Genoa, Italy), where the red coral still occurs. Two transplants secured the colonies in an upright position, as done in the most previously published studies. In one case, only epoxy resin was used as a support, while in another PVC grids were added. Additionally, two techniques involved transplanting colonies upside down within small crevices, using epoxy resin and polystyrene sheets in one case and PVC grids in the other. Two other techniques transplanted colonies collected from shallow waters to deep waters to observe their response to changes in the bathymetrical range; PVC tiles were used for the attachment of colonies and for the larval settlement on artificial substrates (Figure 8).

According to the experience reached to date, the most effective technique for red coral transplantation using colony fragments appears to be the use of epoxy resin to fix the colonies in an upright position (Cerrano *et al.*, 1997, 2000; Montero-Serra *et al.*, 2018; Villechanoux *et al.*, 2022). This technique was used for the first time in 1992 (Pais *et al.*, 1992) and then it has consistently produced the best outcomes. The estimated annual average survival rate is around 60.8% and, after 4 years of monitoring, a survival rate of about 99.1% was recorded (Montero-Serra *et al.*, 2018).

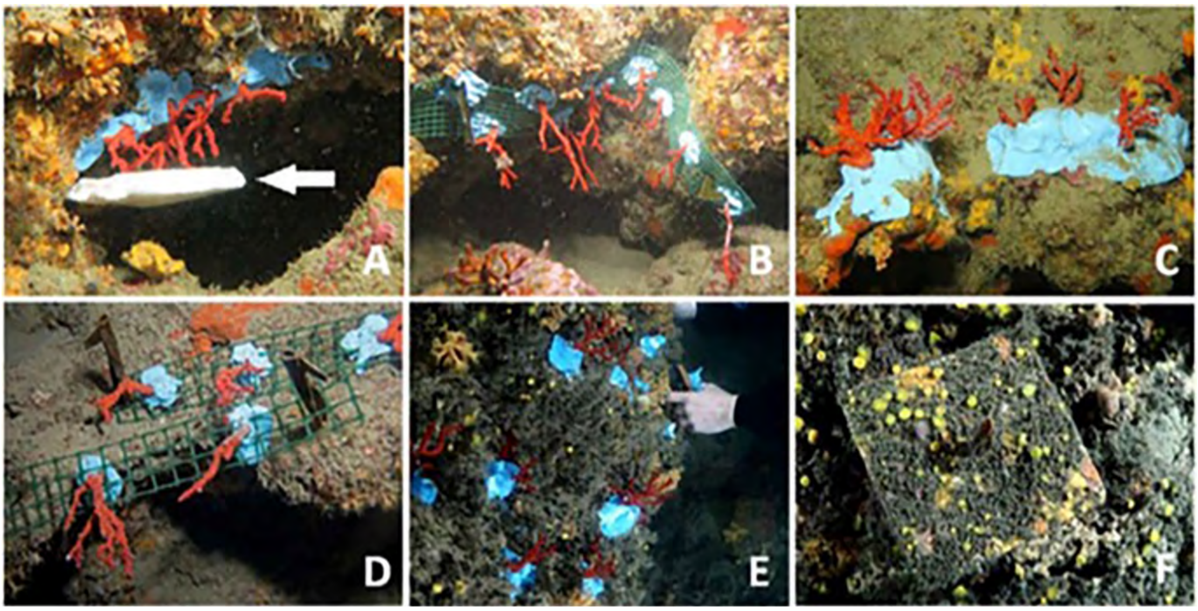

Figure 8. The six different transplantation techniques used in the experiment made by Villechanoux *et al*. (2022): (A) Polystyrene sheet; (B) Grid under cervices; (C) Epoxy putty on rocks; (D) Grid on rocks; (E) Shallow colonies transplanted to deep waters; (F) Larval enhancement experiment on PVC tiles.

Techniques employing PVC grids for support have also been widely used, but they have often experienced grid detachment, resulting in up to a quarter of the total transplants being lost (Villechanoux *et al.*, 2022). The technique of transplanting corals collected from the surface to deeper waters was also found to be effective (82% of survival; Villechanoux *et al.*, 2022). This result could be crucial for repopulating deeper areas where red coral has completely disappeared due to human harvesting, using colony fragments from shallower waters that are still alive.

The use of tiles (Figure 9) is a widely accepted technique, which has exhibited promising outcomes in terms of rates of larval settlement and recruitment density (Cerrano *et al.*, 2000; Garrabou and Harmelin, 2002; Bramanti *et al.*, 2005, 2007).

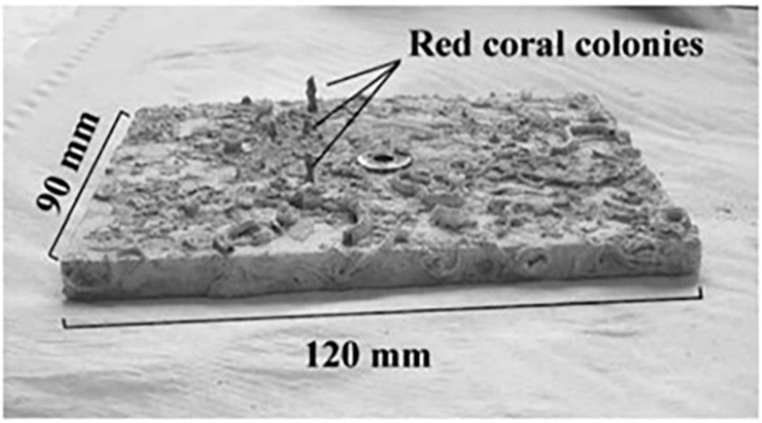

Figure 9. Marble tile collected 4 years after the placement (June 1998 - September 2002) on which four successive red coral cohorts settled (Bramanti *et al.*, 2005).

In a pioneer study conducted in Marseille (France) with the use of limestone tiles, the settlement of the red coral has been monitored for 21 years, reporting high mortality and low numbers of larval settlements

(Garrabou and Harmelin, 2002). However, in other studies conducted in Calafuria (Livorno, Italy) using marble tiles, the results were more successful: 3 to 19 settling larvae per square decimetre were recorded, although the monitoring activities lasted for no more than four years (Bramanti *et al.*, 2005, 2007; Benedetti *et al.*, 2011). According to literature, the Calafuria results were the most successful regarding colonization rates, and the high reproductive rate of the red coral population in this region can be considered as the main reason (Bramanti *et al.*, 2007). The high settlement density and the low mortality rates observed on many tiles planted in Calafuria indicated that these tiles could be a valuable tool for promoting repopulation in areas where red coral populations have significantly declined (Oren and Benayahu, 1997). Indeed, the dense population in Calafuria, characterized by a high reproductive rate, might demonstrate exceptional resilience in the years after an atypical mortality event or to a period of negative net recruitment lasting over a year, as suggested by Bramanti *et al.* (2005).

In the context of red coral transplantation, a comprehensive characterization of the study site, genetics, spatial and interpopulation differences, as well as sexual characteristics of the red coral populations under investigation is deemed indispensable. These variables could account for the diverse settlement rates and success percentages among different populations.

2.3.2 Gorgonians

In the Mediterranean Sea, gorgonians are the most typical facies in the upper layer of coralligenous habitats. They are arborescent and long-lived alcyonacean species, such as *Eunicella cavolini*, *E. singularis*, *E. verrucosa*, *Leptogorgia* spp., *Paramuricea clavata*, and *P. macrospina*. This facies creates the habitat usually known as animal forests. They usually develop in the circalittoral zone with dim light conditions and at depths between 25 m to about 130 m. Gorgonians usually grow on vertical slopes or on overhangs, but they can also develop on sub-vertical slopes or horizontal substrates being able to withstand a slight sedimentary deposit. In the circalittoral zone they can also be found on coralligenous outcrops and on coralligenous platforms. Some gorgonians can also be found at shallower depths in the infralittoral zone on rock. Through their role in supporting high biodiversity, gorgonians offer many ecosystem services to humans mainly due to the creation of a three-dimensional structure that amplifies the space available for other marine organisms. They also have a great aesthetic value for underwater tourism being mostly appreciated by divers and photographers.

Erect gorgonians are long-lived, slow growing and slow recruiting species and display a low resilience to human pressures. They are particularly damaged by fishing gears, bottom trawling, anchoring, and by diving activities (Mytilineou *et al.*, 2014). They are sensitive to entanglement by mucilage filaments and suffer for thermal anomalies. Severe diseases are triggered by a complex combination of pathogenic microbial and abnormally high seawater temperatures (Bo *et al.*, 2014a, b), and several mass mortality events have been recorded in the Mediterranean in coincidence with summer heat waves and the ongoing seawater warming trend (Cerrano *et al.*, 2000; Garrabou *et al.*, 2022). Gorgonians are often popularly collected for use in aquarium and as souvenirs. Filter feeders also suffer for the increase in the concentration of fine sediment and organic matter.

For these reasons, it became evident and essential to begin interventions aimed at restoring the most damaged gorgonian populations to mitigate the environmental damages caused by human pressures. Over the years, numerous studies have addressed gorgonian transplantation in the Mediterranean Sea. The most transplanted gorgonian species include *Eunicella singularis* (Esper, 1791), *Eunicella cavolini* (Koch, 1887), and *Paramuricea clavata* (Risso, 1827) (Weinberg, 1979; Linares *et al.*, 2008; Fava *et al.*, 2010; Monseny *et al.*, 2019, 2020; Casoli *et al.*, 2022b).

One of the earliest experiments on gorgonian transplantation was performed at Banyuls-sur-Mer (France). This experiment involved transplanting fragments from four different gorgonian species to shallow waters of the intertidal zone using two PVC support techniques to anchor the fragments to the substrate (Weinberg, 1979). The fragments were directly collected from living colonies residing within densely populated coralligenous habitats. The study reported a total loss of the transplanted fragments, which was ascribed to the unsuitable habitat of the intertidal zone for the survival of these organisms, and to the type of support used for anchoring to the substrate. Attaching fragments to the rock remains one of the critical aspects of gorgonian transplantation. Steel or PVC structures have been tested as supports for fragments (Fava *et al.*, 2010; Montseny *et al.*, 2019). However, previous studies unanimously agreed that the most effective method requires two-part adhesive to securely attach the fragments to the substrate. In a study performed at the Medes Islands, a Marine Protected Area located off the coast of Catalonia (Spain), three techniques were compared using two-part adhesive to fix the colonies (Linares *et al.*, 2008) (Figure 10). Some transplanted colonies were directly fixed on the rock by means of epoxy adhesive (the "raw" technique). Other colonies were placed inside a plastic tube to serve as a barrier preventing direct contact between living tissue and resin. The rest of the colonies were attached to the rock using a PVC support stick. The latter technique produced the best results, with a survival rate of 70% after one year, while the survival rates for the plastic tube method and the colonies directly attached to substrates were only 50% and 30%, respectively.

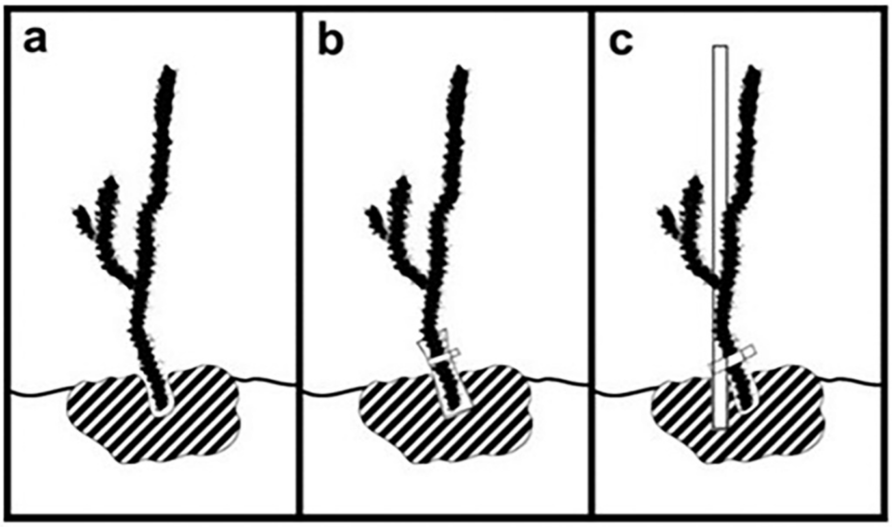

Figure 10. The three different techniques for gorgonian transplantation used by Linares *et al*. (2008). (a) the "raw" technique, where fragments are fixed directly to the substrate with epoxy; (b) fragments are transplanted using a plastic tube around the base of the colony to avoid direct contact with the epoxy; (c) fragments are transplanted using a PVC stick to hold up the colony in contact directly with the epoxy.

A study conducted between 2018 and 2019 in the Costa Concordia wreck area (Tuscany, Italy) retested the "raw technique" (Linares *et al.*, 2008) using colonies of *Eunicella cavolini*, *E. singularis*, and *Paramuricea clavata* (Casoli *et al.*, 2022b). All colonies used for transplantation were either collected from fishing net bycatch or found detached on the seafloor by divers. The transplantation survival rate after 2.5 years was 82.1%. Despite the successful outcome of the experiment, the detachment of resin from the substrate was found as the main cause of the colony loss (85%).

Another technique that involved the use of two-component resin has been called the "badminton method" (Monseny *et al.*, 2020). This experiment aimed at restoring a continental shelf habitat by launching from a boat *Eunicella cavolini* fragments attached to pebbles of varying sizes with resin (Figure 11). Gorgonians obtained from fishing bycatch were employed in this study.

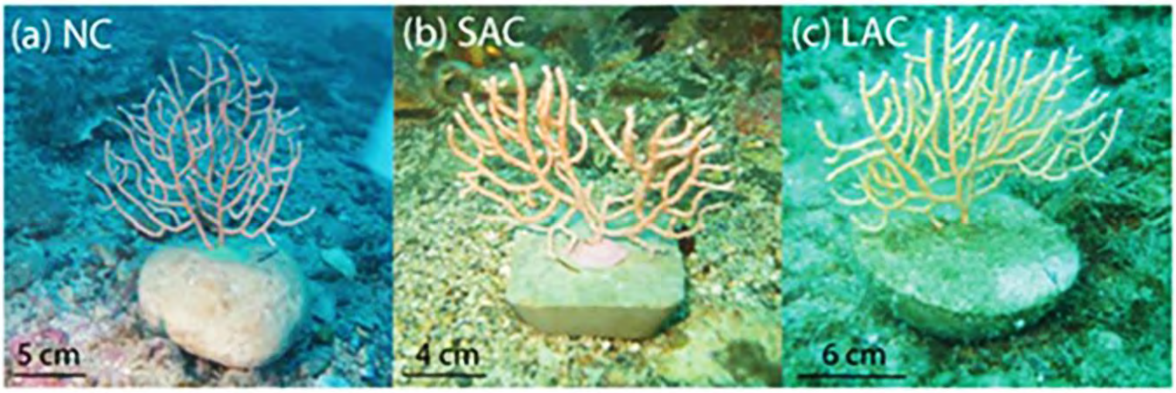

Figure 11. Gorgonian transplants with different types of cobbles. (a) NC = natural cobbles; (b) SAC = small artificial cobbles; and (c) LAC = large artificial cobbles (Monseny *et al*., 2020).

The overall efficiency of the "badminton method" appeared to be consistent with the results of the above experiments. This method may be economically advantageous for large-scale restoration of deep-sea habitats. In addition, the use of predominantly natural pebbles means that no artificial material is introduced into the environment. This last statement is consistent with the recommendation to use a natural substrate for any transplantation intervention, avoiding the introduction of artificial devices, which have been shown to be sometimes ineffective for this purpose (Weinberg, 1979).

Other important aspects to consider are the size of the fragments to be transplanted and the use of gorgonians from bycatch for transplantation. Some authors agree that smaller fragments are preferable to the whole colony or to large fragments, due to the greater resistance to water flow of the latter, which facilitates detachment from the substrate (Linares *et al.*, 2008). Furthermore, regarding the use of gorgonians from bycatch for habitat restoration, the use of colonies that have already been captured or detached from their support provides a practical resource and does not impose additional impacts on healthy donor colonies (Monseny *et al.*, 2019, 2020; Casoli *et al.*, 2022b).

In conclusion, a critical aspect to emphasize in future studies on gorgonian transplantation is the improvement of the implantation technique, especially the attachment of the transplants to the substrate (Linares *et al.*, 2008; Fa *et al.*, 2010). It has also been found that the survival rate of transplants is highly dependent on the time of the year when the transplantation is carried out, with the period from October to November being the most favorable for gorgonians' survival (Fava *et al.*, 2010).

2.3.3 Sponges

Sponges are a crucial component of marine benthic communities from shallow waters to bathyal depths, exhibiting remarkable diversity with an estimated 15,000 species worldwide (Hooper and Van Soest, 2002). Their species diversity, high abundance, biomass, and symbiotic relationships with other organisms contribute significantly to primary production and nitrification in the marine environment, making them essential elements of marine ecosystems. Additionally, these organisms affect the water column and related chemical processes through their capacity of water filtration and production of secondary metabolites (Diaz and Ruetzler, 2001).

Unfortunately, unregulated human harvesting and severe epidemic diseases have caused significant decreases in sponge populations over the course of the 20th century. These factors resulted in regional extinctions of some sponge species in certain areas (Gaino *et al.*, 1992; Webster, 2007; Bierwirth *et al.*, 2022). Due to their significant commercial interest as "bath sponges" and for the extraction of valuable secondary metabolites used in various fields from cosmetics to medicine, researchers are working to develop potential cultivation techniques to mitigate the impact of unregulated harvesting on natural habitats. Also, the techniques employed in sponge cultivation vary in terms of materials and methodologies. Numerous studies in the literature cultivated various sponge species on different substrates. These substrates ranged from horizontal to vertical ropes used as rearing structures (Pronzato, 1999; Corriero *et al.*, 2004; Osinga *et al.*, 2010) (Figure 12), to net panels (Van Treeck *et al.*, 2003), and to steel cages (Osinaga *et al.*, 2010). Additionally, natural supports like coralligenous rock and infralittoral rock, or artificial support like cement blocks, have been used.

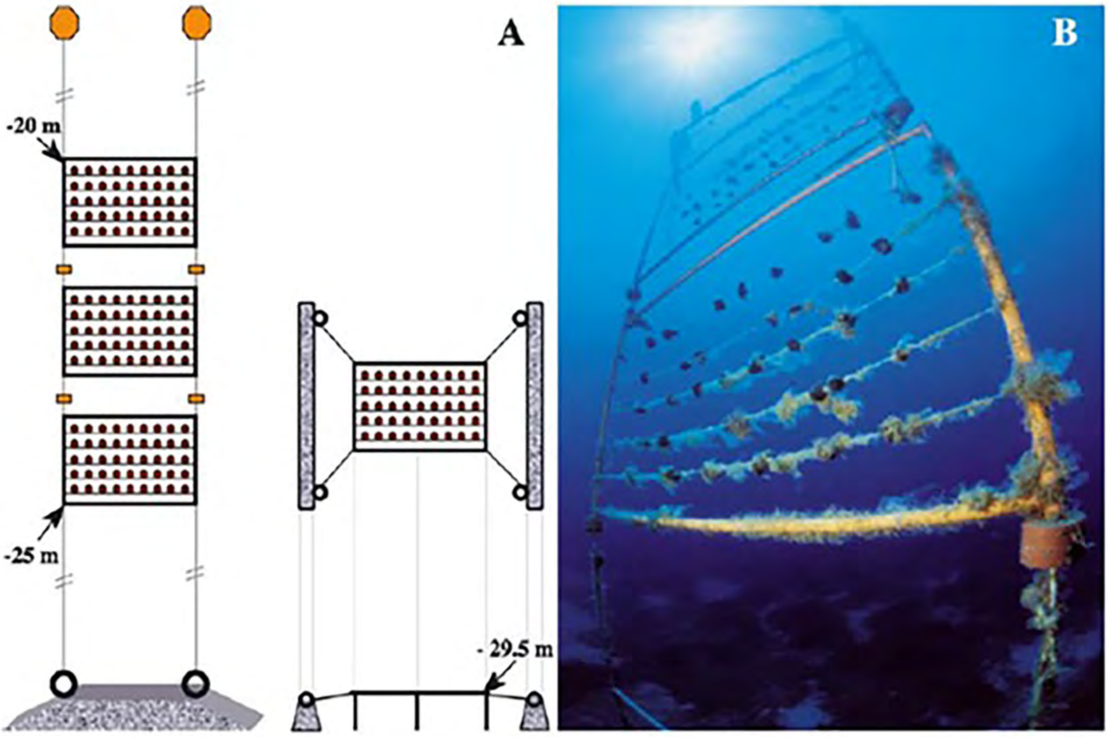

Figure 12. (A) Scheme of the vertical (left) and horizontal (right) rearing structures. (B)Underwater image of the vertical structure extended along the water-column (Corriero *et al.*, 2004).

Despite the numerous studies on this topic, there are still relatively few works focusing on the transplantation and growth of sponges to restore a damaged coralligenous habitat (Biggs, 2013), and no experiments have been conducted in the Mediterranean Sea. In a recent review on sponge cultivation, only 3 out of 40 studies analysed addressed the restoration of marine benthic communities, while all the others focused mainly on the production of bioactive metabolites and methods to produce "bath sponges" (Bierwirth *et al.*, 2022).

Recommendations for best practices in sponge transplantation are not yet available (Duckworth, 2009; Schippers *et al.*, 2012; Bierwirth *et al.*, 2022). This is likely due to the exceptionally high species and physiological diversity within the systematic group (e.g., skeletal consistency and composition). Looking at the results of various transplantation experiments, the most successful technique seems to be the use of artificial substrates, with an average survival rate of 77%. The mesh and rope systems also showed relatively high average survival rates, 72% and 69% respectively (Bierwirth *et al.*, 2022). This study also reported growth rates categorized by organism type and skeletal material, with the net panels appearing to be the best technique for reproducing sponges with siliceous skeletons. The study did not evaluate the success of transplantation on natural substrates due to the limited data available. However, the authors suggested that this substrate should be considered when transplanting for habitat restoration, as it may reduce uncertainties associated with artificial substrates.

The most used fixing technique generally involved fragments of sponges placed on a chosen substrate. This is also one of the most critical stages in the sponge transplantation. On some occasions, the survival rate recorded was low during the first year of transplantation. This can be attributed to two factors: one is the stress caused to the sponge during the manipulation in the initial phase (harvesting, cutting, and applying to the support), and the other depends on the fragility of the tissue during the healing process around the chosen supports (Corriero *et al.*, 2004). Furthermore, another reason for the low survival of the transplanted sponges may be the competition among encrusting organisms that colonized the cut area of the sponges and grow on the supports assigned to them (Corriero *et al.*, 2004).

In conclusion, significant progress has been made towards the restoration of target species of the coralligenous habitat. However, there is still a considerable amount of work that needs to be done. The current phase is experimental, focusing on identifying successful methods and techniques, while there are currently no established protocols for successful coralligenous habitat restoration. Therefore, future experimentation will necessarily refine the understanding of the best practices for restoring this habitat.

2.4 Algal forests habitat

Along the entire coastline of the Mediterranean Sea, rocky reefs in shallow waters are dominated by macroalgal forests mainly created by brown algae belonging to the Fucales order. These underwater forests are found from the midlittoral zone to the lower infralittoral zone, but some sciaphilic species may also reach the greatest depths in the upper circalittoral zone. The genus *Cystoseira sensu latu* (s.l.) includes algae belonging to the genera *Cystoseira* C. Agardh, 1820, *Ericaria* Stackhouse, 1809, and *Gongolaria* Boehmer, 1760. With about 42 species, some of which are also found in the Atlantic Ocean (Draisma *et al.*, 2010; Gianni *et al.*, 2013), they form diverse habitats and communities from the infralittoral fringe to the upper circalittoral zone (Ballesteros *et al.*, 1998, 2009; Hereu *et al.*, 2008). These macroalgae play a crucial role in forming habitats along the Mediterranean coasts (Feldmann, 1937; Giaccone and Bruni, 1973). Algal forests serve as a prominent hub of biodiversity by furnishing food and shelter to numerous fish and invertebrates. Moreover, they establish the underlying structure of benthic community (Mineur *et al.*, 2015), and are acknowledged as a CO_2 sink that amplifies coastal primary productivity (Ballesteros *et al.*, 2009; Sales *et al.*, 2012).

The decline of macroalgal forests on a global scale, especially in the Mediterranean region, is a cause of worry due to the potential negative impacts on biodiversity and ecosystem functions (Verdura *et al.*, 2023 in press). Their decline is attributed to multiple anthropogenic pressures including coastal urbanization, pollution and eutrophication, climate change, and overfishing. As a result of the latter pressure, herbivores are increasing due to a decrease in predators (Verlaque, 1984; Thibaut *et al.*, 2005, 2015; Blanfuné *et al.*, 2016). *Cystoseira* s.l. species are presently categorized as “species of community interest” in accordance with the Habitat Directive (92/43/EEC) and are used as biological indicators to assess the environmental quality of Mediterranean coastal waters, according to the Water Framework Directive (2000/60/EC). This attention reflects their ecological importance. Several species within the *Cystoseira* s.l. genus are safeguarded under the Bern Convention, prioritized by the Barcelona Convention, and have been designated as vulnerable by numerous international organizations, including IUCN, SPA/RAC, and MedPan.

Despite considerable conservation efforts, most of the degraded habitats still show no signs of recovery, underscoring the urgent need for active restoration interventions in endangered algal forest habitats (Marzinelli *et al.*, 2014). In addition, the genus *Cystoseira* is constrained in its growth and spread by its limited dispersal capacity due to rapid fertilization of eggs and rapid sinking of zygotes (Clayton, 1990; Johnson and Brawley, 1998; Gaylord *et al.*, 2002), which limits natural recovery in areas without existing adult specimens. For this reason, much attention has been paid in recent years to the active restoration of this habitat, especially in areas where it was historically present and where the main disturbance factors have been removed.

The first restoration experiments on *Cystoseira* s.l. in the Mediterranean Sea started in the early 2000s, and the most used methodology was the transplantation of adult thalli fixed with polyurethane foam or epoxy glue (Falace *et al.*, 2006; Susini *et al.*, 2007; Robvieux *et al.*, 2013) or the transfer of pebbles and rocks already colonized by juveniles (Perkol-Finkel and Airoldi, 2010; Perkol-Finkel *et al.*, 2012). However, the latter technique in both studies showed a high instability of the substrate, even when the rocks were fixed with resin, thus resulting in highly variable survival rates (from 0% to 100%; Perkol-Finkel *et al.*, 2012). The issue of attaching thalli to selected substrates, whether artificial or natural, has also been observed in other transplantation studies. Resins, epoxy adhesives, and polyurethane foams have been used to fix fronds in holes drilled in rocks, and although the technique appeared effective and inexpensive with good survival rates (57-87% with *Cystoseira barbata* in Falace *et al.*, 2006; 75% with *C. compressa* and *C. amentacea* in Susini *et al.*, 2007; 70% in low polluted sites in Sales *et al.*, 2011), it is not universally applicable for all the species within the genus *Cystoseira* s.l. For example, for *C. compressa* this technique is less effective because the thalli have a sympodial development with a densely ramified cauloid, which complicates the insertion into resinated holes (Falace *et al.*, 2006). This technique also showed low survival rates in some cases due to storm surges or strong waves occurred during and after the transplantation (Susini *et al.*, 2007). In addition, given the conservation status of *Cystoseira* s.l., any technique involving the removal of entire individuals for transplantation should be avoided, as it would cause further harm to donor populations and could be an additional impact factor on already severely degraded and endangered species (Cebrian *et al.*, 2021).

Experiments were conducted to test techniques that were less invasive for donor sites and did not involve thallus harvesting. Some experiments in 2010 investigated the ability of *C. barbata* to proliferate and recruit, as the success of transplantation depends on the reproductive capacity of the transplanted individuals (Perkol-Finkel *et al.*, 2010, 2012). Plates made by different materials (limestone, concrete, and clay) and with different levels of complexity (smooth surface or with deeper or shallower cracks) were used during these experiments. The density of young individuals displayed remarkable heterogeneity among the plates, ranging from 6 to 64 individuals. Considerable variability was observed among the plates: the density was lower in concrete than in limestone and clay plates. However, in each case, the composition of the substrate did not have a significant effect on the establishment of *C. barbata* (Perkol-Finkel *et al.*, 2012).

Other authors experimented out planting techniques producing juvenile individuals in the laboratory for transplantation at low cost and with low effort. The receptacles, which are the fertile parts located on the apical branches of *Cystoseira*, are harvested from healthy donor populations without causing any harm to each single individual. While this approach is advantageous for existing populations, it also poses a limitation for designing large-scale restoration actions (Falace *et al.*, 2018). In the frame of the ROC-POP Life project, which aimed to restore the 'Habitat 1170' (i.e., rocky reefs according to EC Habitats Directive) in two Italian Natura 2000 sites located in the Ligurian Sea and in the Northern Adriatic Sea, an *ex-situ* protocol to restore *Cystoseira amentacea* var. *stricta* Montagne, 1846 was developed for the first time. The protocol maximizes zygote attachment, minimizes embryo development time, and generates dense shoot production for subsequent implantation in selected natural sites (Falace *et al.*, 2018). The project involved the *ex-situ* cultivation of *Cystoseira* embryos for subsequent *in-situ* implantation, which was advantageous in terms of ecological impact on the donor sites, time, and costs. During this phase, it is crucial to test how the environmental variables, including light, temperature, and substrate, affect the attachment and growth of embryos for successful restoration. Any environmental fluctuation can significantly impact the mortality and the growth rates of seedlings (Falace *et al.*, 2018). Transporting samples in dark and cold conditions was advised to facilitate the immediate release of gametes, which prevented excessive thermal shock to the receptacles in the laboratory (Falace *et al.* 2018; Cebrian *et al.* 2021). It was also suggested that transportation be accomplished within 48 hours of collection to avoid considerable damage to the samples (Cebrian *et al.*, 2021).

When conducting experiments in the laboratory, it was crucial to ensure that the photoperiod replicates the seasonal conditions at the donor site and that the unique requirements of the species are considered (Verdura *et al.*, 2015; Liu *et al.*, 2017; Cebrian *et al.*, 2021; Rindi *et al.*, 2021). At the laboratory stage, it was crucial to enrich the growth substrate due to its effect on the photosynthetic capacity (Pérez-Lloréns *et al.*, 1996), the protein content (Vergara *et al.*, 1995), the photoprotection mechanisms (Huovinen *et al.*, 2006), and the relative growth rate of embryos (Chapman *et al.*, 1978; Falace *et al.*, 2018). Nutrient limitation can exacerbate these factors, making enrichment essential. Enriching the growth substrate can lead to a better growth of seedlings. The selected substrate must also ensure appropriate adhesion and promote the development of gametes and zygotes.

Various materials have been investigated for gamete adhesion, including *in situ* experiments (Perkol-Finkel *et al.*, 2012). In the laboratory, rougher tiles showed greater colonization compared to smoother pebbles (Falace *et al.*, 2018). According to Tamburello *et al.* (2019), stones should be preferred for optimal outcomes, with clay tiles being a backup option if stones are unavailable. However, the substrate did not have any significant effect on seedling growth or survival rate in any of the analyzed scenarios (Falace *et al.*, 2018).

For the subsequent laboratory maintenance, following the *ex-situ* cultivation protocol proposed by Falace *et al.* (2018), recommended values for maintaining irradiance and temperature are 125 µmol photons m^{-2} s^{-1} and 20°C, respectively. These conditions enhanced embryonic development and ensured robust and healthy growth of embryos.

After the laboratory growth period, the tiles housing the young organisms were moved to the field (Figure 13). Allowing the juveniles to develop before their transfer to the field prevented adverse effects on the samples during transportation, because juvenile individuals can tolerate transport better (Falace *et al.*, 2022). Then, the tiles are fastened securely to the rock with screws to withstand hydrodynamic forces. As a part of the ROC-POP Life project, a transitional phase was experimented between laboratory-based testing and tile anchorage. During this phase, tiles were situated inside suspended structures, left to float (see Figure 13), and then planted on the rock. The objective was to improve the survival rate of the tiles during the initial stages on the field, providing protection from storm surges and other elements. This method showed benefits by decreasing the duration spent in the nursery, which could lead to long-term financial expenses (Falace *et al.*, 2022).

Besides the *ex-situ* method, which includes the emission and growth of gametes in laboratory, there is also an *in-situ* approach for gamete release and growth (Verdura *et al.*, 2018; Medrano *et al.*, 2020). After obtaining mature receptacles, they are deposited in dispersal bags consisting of fiberglass and PVC with a mesh size of 1.20 mm × 1.28 mm. Then, they are stored in the dark and at low temperatures (Verdura *et al.*, 2018). Upon arrival at the receiving site, the bags are attached to the substrate (rocks or dedicated stakes) using epoxy glue. The distance between dispersal bags should be around 2-3 cm (Verdura *et al.*, 2018). Preliminary cleaning of the chosen receiving site from encrusting organisms or filamentous algae is crucial to enable zygotes to adhere better to the surrounding substrate and to face less competition.

Figure 13. Attaching the clay tiles on the rocky seafloor (a, b, and c) and on the floating structures (d) (Falace *et al*., 2022).

The *in-situ* technique offers cost advantages over preserving and growing zygotes within a laboratory. Both techniques, however, demonstrated high efficiency rates. Cebrian *et al.* (2021) synthesized methods and techniques for brown algae transplantation (Figure 14) and concluded that *in-situ* transplantation is best suited for species with high gamete dispersal capacity and in calm hydrodynamic conditions. Conversely, *ex-situ* transplantation is preferred for species with lower dispersal capacity and in significant hydrodynamic conditions.

In any case, the authors agreed on the importance of comprehending the phenology of donor populations and the seasonal timing of reproductive cycles in the various species. Additionally, it is critical to evaluate how environmental conditions affect species reproduction and to determine the most effective periods for restoration efforts (Cebrian *et al.*, 2021; Rindi *et al.*, 2023; Smith *et al.*, 2023). All these details can optimize brown algae reforestation efforts (Gianni *et al.*, 2013).

Genetic studies applied to transplantation interventions on macroalgae, while still relatively understudied, offered potential benefits. Natural genetic variability can be advantageous when countering the effects of climate change, for example (Prober *et al.*, 2015). The success rates of restoration intervention can be improved by utilizing resilient populations.

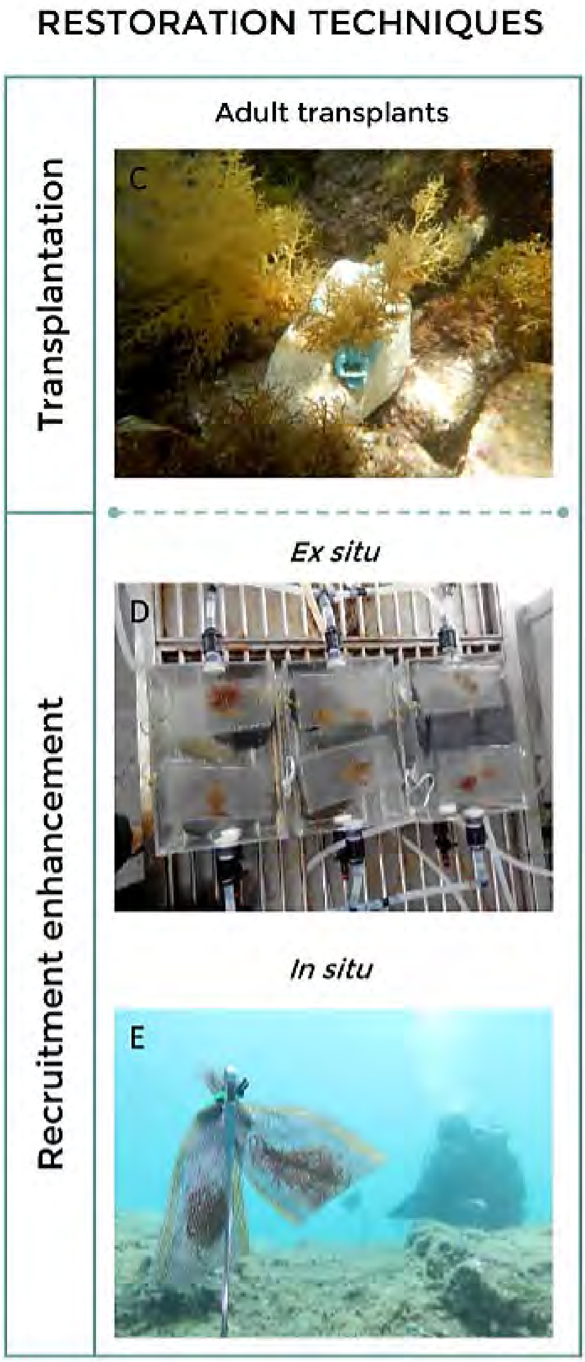

Figure 14. Different restoration techniques for *Cystoseira* spp. (Cebrian *et al.*, 2021).

In conclusion, identifying the most effective techniques for any algal species proved to be a highly intricate task due to the high variety of techniques and the morphological and physiological diversity among species (Rindi *et al.*, 2023). Certainly, the combination of active restoration strategies, including the removal of herbivorous predators like sea urchins and the promotion of algal settlement, along with passive conservation strategies like the establishment of Marine Protected Areas, will promote biodiversity conservation and has the potential to positively impact the restoration of ecological functions (Gianni *et al.*, 2013).

Is therefore crucial to maintain research efforts regarding the subject. Although protocols for restoration of algal forests have been disseminated in recent years, much remains to be accomplished (Falace *et* *al.*,2018; Cebrian *et al.*, 2021; Rindi *et al.*, 2023). To address this issue, a new European funded project, the REEForest Life, has recently been launched with the aim of improving the techniques currently used in the restoration of *Cystoseira* s.l. species. The project aims to reverse the degradation of the endangered *Cystoseira* Habitat 1170 by implementing active restoration and setting up monitoring plans in four Marine Protected Areas in Italy where the causes of degradation have been addressed. Capitalizing the outcomes of the ROC-POP Life, the REEForest Life project will restore the ecological status in the target MPAs through the implementation of specific conservation measures and cost-effective and sustainable reforestation activities, including *ex-situ* and *in-situ* recruitment enhancement. In addition, REEForest will provide concrete and robust methods that will be used to replicate and scale up restoration activities in other areas and with other species and will propose guidelines and best practices for Mediterranean marine forest restoration that are relevant to EU policies.

2.5 Seagrass habitat

Seagrass meadows are widely recognized as key habitats in tropical and temperate shallow coastal waters of the world (UNEP-MAP-Blue Plan, 2009). They form some of the most productive ecosystems on earth (McRoy and McMillan, 1977), shaping coastal seascapes and providing essential ecological and economic services (Green and Short, 2003; Vassallo *et al.*, 2013). They support high biodiverse associated communities, have high primary production, and contribute to nutrient cycling, sediment stabilization and protection of the littoral and, globally, to a significant sequestration of carbon (Waycott *et al.*, 2009 and references therein). A major economic value of over 17,000 $ per ha and per annum has been quantified for seagrass meadows worldwide (Costanza *et al.*, 1997).

Seagrass, like all Magnoliophytes, are marine flowering plants of terrestrial origin which returned to the marine environment approx. 120 to 100 million of years. The global species diversity of seagrass is low when compared to any other marine Phylum or Division, with less than sixty species throughout the world. However, they form extensive meadows that extend for thousands of kilometres of coastline between the surfaces to about 50 m depth in very clear marine waters or transitional waters (e.g., estuaries and lagoons). In the Mediterranean region six seagrass species occur: *Posidonia oceanica* (L.) Delile, 1813, *Cymodocea nodosa* (U.) Ascherson, 1870, *Halophila decipiens* Ostenfeld, 1902, *Halophila stipulacea* (F.) Ascherson, 1867, *Zostera noltei*, Hornemann, 1832, *Zostera marina*, Linnaeus, 1753. The endemic *Posidonia oceanica* is doubtless the dominant and the most import seagrass species (Green and Short, 2003), and the only one able to build a ‘‘matte’’, a monumental construction resulting from horizontal and vertical growth of rhizomes with entangled roots and entrapped sediment (Boudouresque *et al.*, 2006).

Physical damages resulting from intense human pressures, environmental alterations, global warming, reduction of water and sediment quality, disease, storms, anchoring, trawling, aquaculture, underwater cables and pipes, explosive, and non-indigenous species are causing structural degradation of seagrass meadows worldwide (Orth *et al.*, 2006). An alarming and accelerating decline of seagrass meadows has been reported in the Mediterranean Sea and mainly in the north-western side of the basin, where many meadows have already been lost during last decades (Boudouresque *et al.*, 2009; Pergent *et al.*, 2012; Marbà *et al.*, 2014; Burgos *et al.*, 2017; Boudouresque *et al.*, 2021).
Concerns about these declines have prompted efforts to protect legally these vegetated habitats in several countries. Control and reduction of the full suite of anthropogenic pressures via legislation and enforcement at both local and regional scales have been carried out in many countries (UNEP/MAP-RAC/SPA, 2019). Also, the establishment of marine protected areas (MPAs) locally enforces the level of protection on these priority habitats.

Enhanced conservation policies in recent decades have favoured the natural recovery in some meadows of the Mediterranean Sea, thus interrupting and sometimes reversing the general trend of decline observed in the last century (De Los Santos *et al.*, 2020). Occurrence of signs of natural recovery in degraded meadows should be the first requisite for planning successful restoration interventions in seagrass meadows.

2.5.1 *Posidonia oceanica*

Posidonia oceanica is an endemic seagrass of the Mediterranean Sea where it creates vast meadows on soft and hard substrates, ranging from the surface down to 40-45 m depth, depending on water clarity (Boudouresque *et al.*, 2009). It is an engineer and keystone species that provides several ecosystem services because of its high primary production, its biodiversity, and its ability to store and to sequester carbon for millennia (Monnier *et al.*, 2022). *P. oceanica* meadows cover about 1.5% of the total surface of the Mediterranean Sea (Pasqualini *et al.*, 1998). Meadows are mainly located in the western Mediterranean but are absent or rare in the northern Adriatic and in the southern coast of France, due to unsuitable salinity levels and/or adverse weather conditions (Figure 15). *P. oceanica* meadows are less abundant in the Levantine Sea and are scarce or absent in the Sea of Marmara and in the Black Sea (Boudouresque *et al.*, 2006).

Figure 15. Current distribution of *Posidonia oceanica* meadows in the Mediterranean Sea (from IUCN website).

Posidonia oceanica meadows are very sensitive to multiple pressures, both local and global. A widespread regression has been reported for *P. oceanica* meadows in the Mediterranean, because of water pollution, water turbidity, construction of coastal infrastructures, anchoring, trawling, and aquaculture facilities. Approximately 34% of its meadows have undergone a decrease in extent in the last 50 years, and even a higher value of 56% of decline was reported when considering the north-western sector of the Mediterranean (Telesca *et al.*, 2015). This species is listed as "Least Concern" by IUCN.

Posidonia oceanica displays a very slow-growth rate (averagely between 10 and 100 cm by century), recolonization through natural processes would require a long time in the absence of human activities (Marbà and Duarte, 1998). These considerations have led to the necessity of safeguarding *P. oceanica* meadows through conservation interventions.

Posidonia oceanica meadows are defined as priority natural habitats (Habitat 1120*) on Annex I of the EC Habitats Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora (EEC, 1992), which lists those natural habitat types whose conservation requires the designation of special areas of conservation, identified as Sites of Community Interest (SCIs). The species is protected by the Bern Convention, which classify *P. oceanica* as a strictly protected species in the Annex I. *P. oceanica* is also listed as a threatened species in the Annex II of the Barcelona Convention and is safeguarded by national laws in different countries (Pergent-Martini *et al.*, 2022). France provided legal protection for *Posidonia oceanica* through the Nature Conservation Act (July 10, 1976) and the implementing decree (November 25, 1977), which safeguards natural flora and fauna (Boudouresque *et al.*, 2006). Similarly, Law No. 175 (May 27, 1999) provided national protection of this species also in Italy. The species is included in the wildlife species legislation (R.D.139/2011 No. 46) in Spain. Certain autonomous regions, like Catalonia, Valencia, and the Balearic Islands, have implemented measures to safeguard *P. oceanica* and the other seagrass. *Posidonia oceanica* is safeguarded by the Catalonia ordinance DOGC No. 1479 12/08/91 (July 31, 1991), which specifically bans any activities that would lead to its regression, sale, purchase, or utilization. Impacts on seagrass meadows in the Valencia Region are also prohibited by DOGV No. 1724 (February 14, 1992). Additionally, fishing, shellfish, and aquaculture activities carried out on seagrass meadows within the Balearic Islands are regulated by a government ordinance (September 21, 2001). In Albania seagrass meadows are protected by a Council of Ministers decision on protected species (UNEP-MAP-RAC/SPA, 2007), in accordance with the country's legal framework. In Croatia, *Posidonia oceanica* is protected at the national level through the ordinance "Proclamation of wild taxa as protected or strictly protected" (Official Gazette No. 7/2006) (UNEP/MAP-RAC/SPA, 2007). In Montenegro, *P. oceanica* is protected by the national legislation as a rare or endangered species. Meadows are also protected from trawling by national fisheries ban (trawling is forbidden above 50 m depth and within two nautical miles from the coast) (National Gazette 55/03). *Posidonia oceanica* has been listed as a rare and endangered species in Slovenia since 2002 according to a government decree. In Turkey, *P. oceanica* meadows are also protected by the "Circular on sea and inland waters n°37/1" law (UNEP/MAP-RAC/SPA, 2007).

Characterization of receiving and donor sites

Along with the above-mentioned passive conservation efforts undertaken by the Mediterranean countries to protect *P. oceanica* meadows, active restoration interventions have increased in the last decades to support and even accelerate the natural recovery. The literature agrees on the necessity of performing a comprehensive characterization of both the receiving and the donor sites before planning any ecological restoration project. This crucial step should not be overlooked as it lays the foundation for success. Objective evaluations are pivotal and guarantee that results are consistent and will support the future restoration efforts.

In the selection of the suitable receiving restoration site, it is mandatory to evaluate where the species historically existed. Relocating a seagrass to a habitat that was not naturally occupied is likely to be a failure (Boudouresque *et al.*, 2021). The selected site must also provide evidence of natural recolonization. In the receiving site, any human-induced pressures that could be responsible of the regression of the meadow in the past should be eliminated, or at least significantly reduced and managed, as successful restoration interventions can be achieved only where sustainable environmental regimes occur.

The selection of the receiving sites within Marine Protected Areas or in areas subjected to any kind of legal protection (e.g., within the Special Zones of Conservation, in Natura 2000 sites, or in areas where the anchoring is banned) would guarantee the adequate level of protection that is particularly desirable in the first months after the restoration when the transplanted shoots appear more vulnerable to physical damages. Alternatively, the protection of the restored site must be monitored and could be regulated through buoy fields on the surface. Transplanting must always be integrated within an overall meadow management strategy at large spatial scale (Boudouresque *et al.*, 2021).

Posidonia oceanica is naturally affected by environmental factors, including light, salinity, and temperature, as well as by overgrazing and competition with other vegetated species. It is therefore essential to carefully evaluate the environmental setting of the receiving site to be sure that all the natural pressures that could undermine the survival of transplanted shoots range within the natural level of variability. Hydrodynamics is another important factor to consider, particularly during the early stages of transplantation. Restoration interventions made in areas subjected to strong hydrodynamics (even seasonal) could result in comparatively lower survival rates. This is why most of the reported examples of restoration on *P. oceanica* have been carried out at depths between 10 m and 18 m depth, which is considered the best depth range where a meadow can thrive ensuring a good compromise between enough light intensity and low hydrodynamics. The context (i.e., degree of human pressures, environmental features, and habitat type) where the restoration activity is undertaken has been shown to be much more relevant for a successful outcome than the methodology adopted (Fraschetti *et al.*, 2021).

On the contrary, the aforementioned factors are not relevant for the donor sites, as restoration success does not seem to be affected by the stressors acting on the donor meadows. Typically, the restoration successes increase when the proximity to the donor site diminishes, likely due to genetic diversity (Reynolds *et al.*, 2013; Tan *et al.*, 2020). Furthermore, there is amplified success when the receiving and the donor sites had the same depths (Dattolo *et al.*, 2013), or with a slight decrease between the donor and the receiving site.

Plant material and substrate selection

Posidonia oceanica can reproduce either vegetatively or sexually. Through vegetative reproduction the plant propagates via rhizome fragments dispersion or by lateral branch formation, thus contributing to the meadow enlargement. With the sexual reproduction through flowers, fruits, and seeds the genetic diversity is ensured. However, this phenomenon is rare and sporadic and depends on the direction of currents and on the appropriate substrate for seed germination.

For *P. oceanica* active restoration, both fragments of rhizomes (i.e., cuttings) and seedlings can be used. Fragments collected (i.e., cut) from donor meadows are frequently used in most of the *P. oceanica* restoration attempts since they are easily accessible (e.g., Molenaar *et al.*, 1993, Piazzi *et al.*, 1998, Pirrota *et al.*, 2015). Fragments can be orthotropic (i.e., with a vertical growth) or plagiotropic (i.e., with a horizontal growth). Nonetheless, this harvesting method may affect the donor meadows and the current suggestion is to obtain less than 2 cuttings per m^2 to preserve existing meadows (Boudouresque *et al.*, 2021). A less harmful and preferred option is to collect drifting cuttings that have already been uprooted from meadows, are floating near the seafloor, or are stagnant in sedimentation areas (e.g., Castejón-Silvo and Terrados, 2021; Mancini *et al.*, 2021; Piazzi *et al.*, 2021) (Figure 16), or can be found in beach cast after storms (Balestri *et al.*, 2011). Experimental studies to compare the success of transplants utilizing drifting cuttings or those obtained from direct harvesting of the donor meadows are on-going. The preliminary results showed a slight decrease in term of survival rate after one year in the drifting cuttings compared to the harvesting ones. Difference in carbohydrates contents could be at the origin of these “less” successful results (Pergent G., *Pers. obs*.).

Once rhizome fragments are cut from a donor meadow or collected from drifting material, they are subsequently prepared, cleaned, and cut to a predefined size; resulting cuttings may include one, two, three or more “foliar shoots” (named also foliar bundles or leaf bundles) (Figure 17). Some researchers suggested to plant cuttings immediately after collection, but the preparation of cuttings before transplantation is likely to stimulate the roots germination and growth once the cuttings are transplanted and anchored in the substrate of the receiving site (Piazzi *et al.*, 2021). Using plagiotropic cuttings with a minimum of three shoots per cutting (1 plagiotropic ad 2 orthotropic) is recommended to ensure a higher survival rate. For *P. oceanica* the best option is transplanting cuttings on dead matte substrate (i.e., the remains of interlaced rhizomes and roots within the sediment).

Figure 16. Scientific diver collecting drifting cuttings that are found floating near the seafloor (Photo: Fabio Benelli).

Restoring *Posidonia oceanica* through laboratory cultivated seeds is undoubtedly a methodology that avoids any disturbance at the donor site and takes advantage of the plant's ability to perform sexual reproduction. However, availability of seedlings for transplantation is a significant issue in seagrass restoration, particularly for large-scale interventions. An increasing number of experts are recognizing the efficacy of using seeds to ensure a high quantity of plants to be transplanted (Terrados *et al.*, 2013). The use of seeds may also enhance genetic diversity, resulting in a greater success rate for the restored seagrass meadow (Procaccini and Piazzi, 2001). However, it is challenging to forecast *Posidonia oceanica* flowering events. The continuous rise in temperatures due to the predicted scenario of global warming may induce sexual reproduction, resulting in high seed availability in the coming years (Marín-Guirao *et al.*, 2019).

During sporadic mass flowering events, large quantities of fruits can be found in beach cast on the shores. After collection, seeds can either germinate in the laboratory before transplantation (Terrados *et al.*, 2013) or directly transplanted into the substrate and left to grow undisturbed. Metal nets can be used to prevent grazers. Tested experiences of seeds directly planted *in-situ* are not available. This approach requires a considerable effort to regulate several parameters during the germination and growth phases of the seedlings. It involves additional critical phases, such as the transportation and planting of the seedlings in a receiving site.

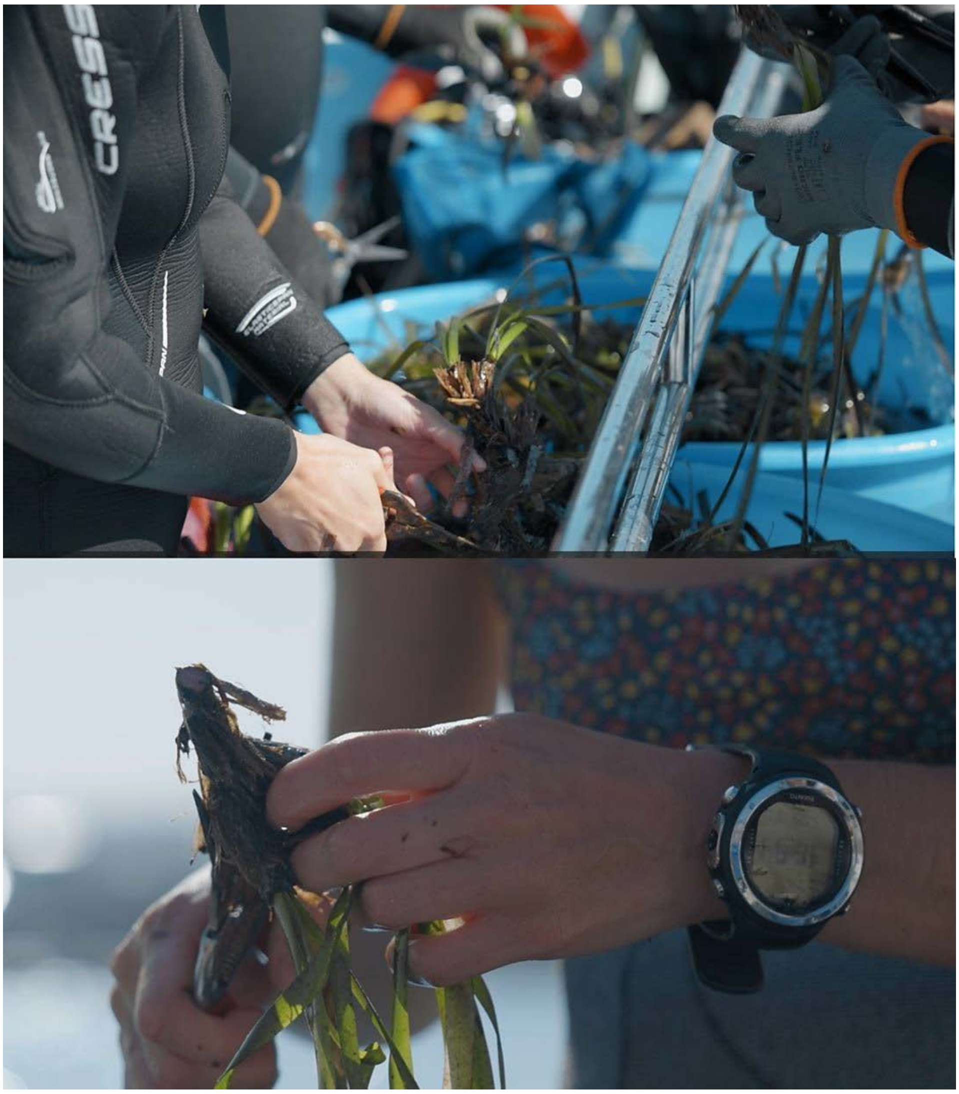

Figure 17. Rhizome fragments prepared and cut to a predefined size before the transplantation (® Fabio Benelli).

Replicate the same natural environmental conditions when seeds are cultivated in the laboratory is often difficult. The substrate selection also plays an important role in seedling growth and sustainability. Multiple *ex-situ* experiments were carried out using seeds of *Posidonia oceanica*, revealing that the substrates composed by dead matte and rock had the highest survival and anchoring rates. These two substrates were also better than the natural substrate made by living *P. oceanica* and by sand. Also the *in-situ* experiments confirmed that the dead matte and the rock are the optimal options for anchoring the seedlings. The survival rates of seedlings ranged from 92% to 67% on dead matte. The survival rates resulted 50% for bare rock, 2% for coarse gravel covered with macroalgae, and even lower values for sand and bare coarse gravel (Pereda-Briones *et al.*, 2020).

Alagna *et al.* (2020) analysed the anchoring rates of seedlings to rocky substrates with varying complexity. The experiment revealed that substrates with larger cracks resulted in 100% anchoring after six weeks, while completely smooth substrates displayed 0% anchoring.

Another experiment incorporated different diameters of coarse sand and rocks to create substrates with low, medium, and high complexity (Alagna *et al.*, 2015). The sand substrate did not show any anchorage of seedlings, while the other two substrate typologies had an anchorage that ranged between 84±5% for the least complex to 89±4% for the most complex. This difference can be explained by the greater substrate complexity that enhances retention of seeds and inhibits hydrodynamics impact, while not affecting formation of roots and root hairs.

Other studies found that the dead matte substrate (with survival rates of 69.6% at 10 m and 40.5% at 2 m depth) and the rock substrate (46.4% at 10 m depth) are optimal for seedling survival (Piazzi *et al.*, 1998). Additionally, substrate was identified as the most critical factor for seedling survival, whilst depth was found to be critical for growth rather than for initial survival.

Terrados *et al.* (2013) conducted two experiments. In the first they analysed the effect of the substrate (dead matte or living meadow) and the planting level (above or below the substrate), while in the second they explored the type of anchoring for the seedlings. Their findings indicated that seedling survival rates were notably higher (75±5%) in the dead matte substrate, although the survival rate declined to 44±6% after two years from the transplantation. The type of anchoring and the planting level did not affect survival rates.

According to a study by Infantes *et al.* (2011), *Posidonia oceanica* seedlings require 0.35 times the square root of their total leaf area to remain anchored to the substrate in case of strong hydrodynamics. On the contrary, Zenone *et al.* (2022) suggested that the adhesion strength depends on the number of roots rather than their average adhesion length.

Transplantation techniques

One of the earliest experiments in transplanting *Posidonia oceanica* was conducted in 1971, where six perforated concrete slabs were positioned on a sand area in the Gulf of Giens (France) at 8 m depth (Maggi, 1973). These slabs, measuring 100 × 100 × 10 cm, contained 36 cylindrical holes, each with a 10 cm diameter and with low organic content (Figure 18). These concrete slabs were designed to function as a safeguard against currents dislodging the cuttings before they were fixed in place. After transplanting, the cuttings reached 40-60% of coverage after 12-17 months, with the cuttings planted during spring reaching the highest values due to their faster growth rate in this season. Transplanting *P. oceanica* on a sandy substrate showed feasible according to this experiment, but only because the concrete slabs reduced hydrodynamics. However, implementing this approach on a large scale is considered costly and inflexible.

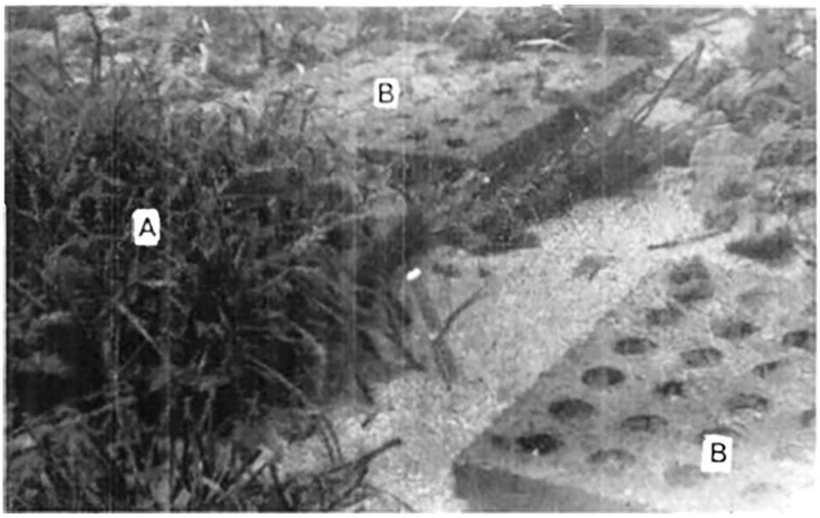

Figure 18. Perforated concrete slabs used to transplant *Posidonia oceanica* in the Gulf of Giens in 1971 (Maggi, 1973).

Carannante (2011) conducted an experiment on the persistence of non-biodegradable materials in the sea at two locations in Italy. The study employed multiple techniques including: 1) metal pegs in sand and dead matte; 2) metal mesh fixed to the sandy bottom with aluminium pegs; and 3) concrete and wooden crosses (Figure 19). The growth of transplants was then observed over a period of five years. An average survival rate of 91% for the implanted cuttings was recorded, with the lowest values reached on the receiving site where the environmental conditions were more disturbed; in particular, the high turbidity of the water column negatively affected growth and spread of the transplanted bundles. Additionally, the unstable sediments and the strong waves near the bottom resulted in damage to both the transplanted plant and the natural meadow. The use of non-biodegradable materials raises concerns regarding the persistence of such materials in the environmental. These methods are thus considered not environmental-friendly.

Figure 19. Transplanting experiment conducted with non-biodegradable materials in Italy (Carannante, 2011).

An alternative approach is the use of grids, the design of which vary depending on the material chosen. Metallic grids have been utilized in numerous experiences (Piazzi *et al.*, 1998; Gobert *et al.*, 2005; Pirrotta *et al.*, 2015). Survival rates ranged from 65% to 84%, with the highest values reached when plagiotropic rhizomes have been used. Most transplanted shoots displayed outstanding vegetative resilience by producing new rhizomes, leaves, and roots within several months from the transplantation, demonstrating high adaptability of cuttings to different environmental conditions at the receiving location. However, the high variability observed in the different sites highlighted the importance of a careful characterization of the recipient site (Acunto *et al.*, 2015). The combination of metallic grids and sand-filled mattresses has been shown to be an effective method for medium-scale projects, as demonstrated by experiments carried out on a 0.1 km^2 scale.

Plastic has been employed as an alternative material for the grids (Molenaar, 1992). A survival rate between 93-100% was recorded between 3-20 m depth, while the survival rate dropped to 72% at 36 m after 11 months. Similarly, bamboo has been used (Gobert *et al.*, 2005), with an initial density of 100 cuttings/m^2. Over 27 months, the number of leaf bundles increased by 1.4, despite a 20% loss of transplant during this period. The initial condition of the cuttings, the decay of links connecting the shoots within the grids after one year, and the insufficient root development (causing an inadequate nutrition for the sprouts) have been considered the main causes for growth reduction of the cuttings.

A widely employed method for transplanting *P. oceanica* cuttings utilizes the natural coconut fiber nets "R.E.C.S.® - Cocco" (Reinforced Erosion Control System) attached to a metal reinforcing element consisting of a double twisted hexagonal net (8 × 10 cm with 2.70 mm diameter wire) (Figure 20).

Figure 20. Biomats made by a natural coconut fiber net (® Monica Montefalcone).

The biodegradable mats, which range from 5 m to 12.5 m in length and 2 m in width, provided excellent stability and tightness. The biomats are laid on the seafloor (possibly adjacent to an existing meadow to ensure the habitat continuity) and secured to the substrate by metal stakes with a minimum length of 120 cm and a diameter of 1.4 cm. The many interventions carried out up to date in different areas of Italy and France involved areas of about 100-400 m^2 at about 15 m depth (Acunto *et al.*, 2021; Maltese *et al.*, 2021; Piazzi *et al.*, 2021). The minimum plot of intervention had a total surface area of 100 m^2, where ten biodegradable mats have been used (each 5 m in length and 2 m in width), each containing about 200 cuttings for a total of 2,000 cuttings (Figure 21). Cuttings mainly consisted of plagiotropic rhizomes, arranged in cores of at least 20 cuttings $\cdot$ m^{-2}. Cuttings are inserted by hand in the coconut net by the scientific diver (Figure 22). Six months after transplantation, the survival rate was 57.6%, with a significant increase after two years (87.6%) (Acunto *et al.*, 2021; Piazzi *et al.*, 2021).

Figure 21. Restoration intervention made with the biomats technique (® Fabio Benelli).

Figure 22. Cuttings of *Posidonia oceanica* inserted by hand in the coconut net by the scientific diver (® Fabio Benelli).

This technique can be applied to eroding seafloors with the presence of *Posidonia* meadows. However, it must be ensured that there is no high hydrodynamics and that it is not applied on very irregular or rocky seafloors. The best results were obtained on dead mats substrates. Care must be taken to ensure that the biomat structure is placed close to the bottom as the presence of wire mesh can cause problems for wildlife. The benefits of using biomats are numerous: they are highly resistant to erosion, have high permeability that prevents the formation of negative pressure, and are rapidly colonized by structured algae (Bacci *et al.*, 2014).

Other experiments employed steel supports to fix cuttings directly to the substrate, with a survival rate exceeded 75% (Molenaar and Pey, 1992), and biodegradable materials, specifically coconut fiber plant pots and bamboo shoots (Ward *et al.*, 2020) (Figure 23). Plagiotropic fragments planted using bamboo displayed a greater settlement rate (89±0.1%) compared to orthotropic fragments with the same properties (66.5±6.5%). On the other hand, plagiotropic fragments planted underneath coconut fibers demonstrated a reduced settlement rate (51±11%) compared to orthotropic fragments (79±7%). Given the recent progress made in generating plagiotropic fragments utilizing the bamboo method, it is advisable to adopt a mixed replanting approach that involves different types of fragment growth. Such an approach is essential to promote the horizontal growth of the specimens, which is crucial in colonizing the bare substrate surrounding replanted regions (Ward *et al.*, 2020).

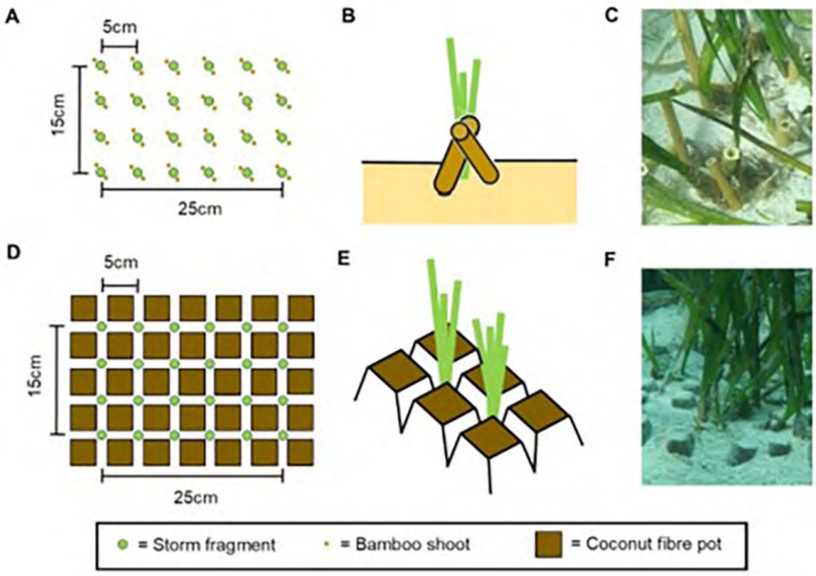

Figure 23. Transplantation experiments of *Posidonia oceanica* made by using biodegradable materials such as coconut fiber plant pots and bamboo shoots (Ward *et al*., 2020).

An alternative technique used a biobased plastic radial structure made with starch, with five arms to accommodate cuttings, which can be fixed to the seafloor with a quick-fix stake (Figure 24).

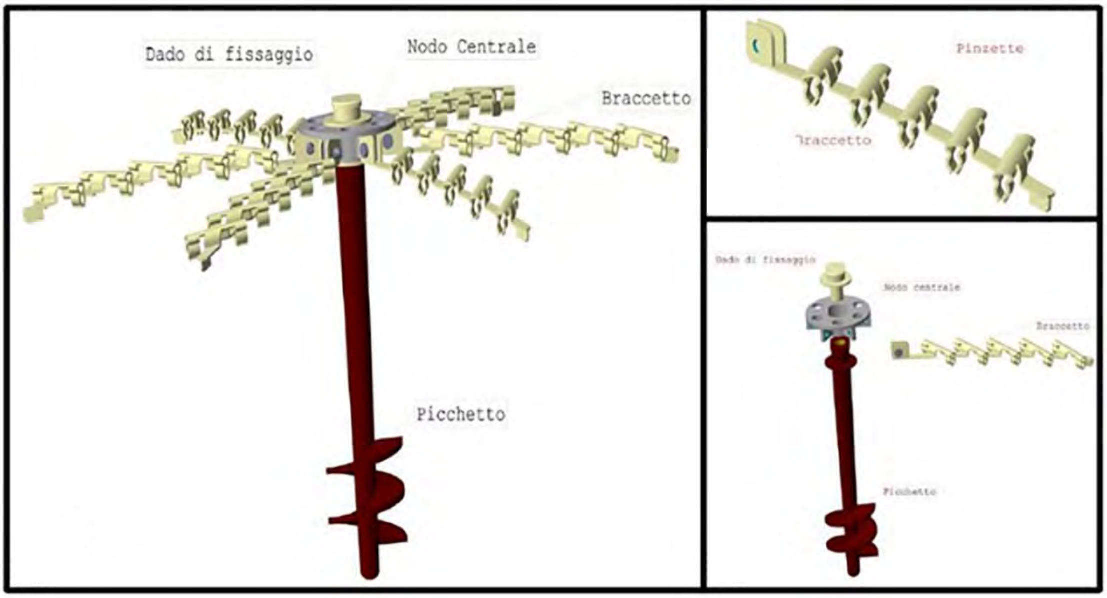

Figure 24. Biobased plastic radial structure (Bacci *et al*., 2014).

This approach has been applied in three sites in Italy (Bacci *et al.*, 2014, 2019; Scannavino *et al.*, 2014). The transplantations had survival rates ranging from 80% to 94% after 20 months. In some transplanted areas, however, about one third of the stands were lost due to recreational and commercial fishing activities, as well as to anchoring.

Some experiments transplanted *Posidonia oceanica* by extracting clods from meadows at the donor site and transporting them underwater with floating balloons (Sánchez-Lizaso *et al.*, 2009), containers (Descamp *et al.*, 2017), or placing clods in an overturned barge that gradually fills with water when opened (Bedini *et al.*, 2020) (Figure 25). Plants transplanted in the first seven months had a mortality rate of 85%, attributed to infections or losses resulting from poor anchoring or stress experienced during transportation, including sediment washing (Sánchez-Lizaso *et al.*, 2009). Transporting some sediment from the site with *P. oceanica* clods resulted in high survival rate (92%) (Bedini *et al.*, 2020). Most of these studies are too recent to provide inferences about the effectiveness of this transplanting methodology. It is, however, important to note that this method is destructive and causes significant damage to the donor site. Nevertheless, it may prove to be a useful solution in situations where the donor site will be disturbed or destroyed due to infrastructure constructions (Bedini *et al.*, 2020).

Figure 25. Experiments transplanted *Posidonia oceanica* by extracting clods from meadows at the donor site and transporting them underwater with containers (Descamp *et al.*, 2017).

Several *in-situ* experiments were conducted by transplanting seedlings. Bedini *et al.* (2013) attempted to increase the surface area of *Posidonia oceanica* meadows by directly planting seeds with nylon meshes with 1 ×1 cm openings, attached to a 1×1 m iron frame to ensure that the seeds remained in contact with the seabed. The meshes were divided into sections and anchored to the seabed with steel stakes. Four years later, the patches of *Posidonia oceanica*, freed from their plastic nets, were fully acclimated and were integrating with their neighboring meadows. This approach seems effective in areas where meadows are thriving, and the optimal environmental conditions are conducive to seed development.

Balestri *et al.* (1998) used grids with five evenly spaced seedlings, secured at the corners with 20 cm steel bars. After three years, 70% of the initially transplanted seedlings survived on the dead matte.

In the experiment by Terrados *et al.* (2013), after growing seeds in the laboratory, they planted the seedlings on two different substrates. Dead matte was the best substrate (44% survival after 3 years), while in the living meadow the results were significantly lower (22% survival after 1 year and nearly 0% of survival after 2 years). The type of anchorage, on the other hand, did not affect the survival of seedlings.

Although the very positive results reached with seedlings transplantation, the use of seeds in large quantities to regenerate large areas of meadow is not always feasible due to the limited availability of sexual reproduction in *P. oceanica*.

International projects on Posidonia oceanica restoration

Seposso LIFE

The Seposso LIFE project “Supporting environmental governance for *Posidonia oceanica* sustainable transplanting operations” (LIFE16 GIE/IT/000761) was funded by the EC in 2016 and aimed at analysing and sharing technical and scientific information, including performance data, related to the *P. oceanica* transplanting sites studied within the project (La Porta and Bacci, 2022).

Three Italian sites were monitored in the frame of the project. One site was in Mondello (Sicily, Italy), where biobased plastic structure and concrete supports were used. Monitoring four years after the transplantation showed a decrease in the survival rate to 35.9% in the case of biodegradable plastic structures, highlighting that many modules had been destroyed. When concrete supports were used, a survival rate of 90% was reached. Concrete supports would be preferable to biobased plastic supports, even though they are undoubtedly made by a more resistant material, are heavier and therefore more stable on the substrate.

Biobased plastic stakes have been tested in Syracuse (Sicily, Italy) in the second site monitored. In 2016, a total of 2,520 cuttings were planted in 252 anchor modules. Three years later, the survival rate of the cuttings was monitored in 18 modules in three different areas. Survival rates varied from 0%, possibly due to planting stress and anchoring problems, to approximately 60%, with an average survival rate of 46%. Subsequent monitoring was conducted on 28 modules evaluated in three different areas. The average survival rate was 52.8%, with values ranging from 37.3% to 64%.

The third monitoring site at Elba Island (Tuscany, Italy) involved the use of biodegradable coconut biomats fixed on a dead matte substrate in two separate areas. A total of 30,000 cuttings were transplanted. The initial monitoring showed an average survival rate of 63% in both areas. Subsequent monitoring at 12, 20, and 34 months showed survival rates of 85%, 63%, and 51%, respectively. This latter technique using coconut fiber biomats had proven to be an excellent transplantation method with consistently high survival rates, as also confirmed by the numerous transplant studies performed with this technique (Piazzi *et al.*, 2021).

SeaForest LIFE

The SeaForest LIFE project “Posidonia seagrass meadows as carbon sinks in the Mediterranean” (LIFE17 CCM/IT/000121) was recently funded by the EC in 2021 under the Climate Change priority (Climate Actions 2017 sub-program). Its main objective is to increase the carbon sequestration capacity of *Posidonia oceanica* seagrass meadows by implementing measures to limit degradation and facilitate the conservation of *P oceanica* meadows. Some of the most effective techniques for transplanting *Posidonia oceanica* are employed as outlined in the Action C.5.1 of the SeaForest LIFE manual (Maltese *et al.*, 2021), which include the use of coconut fiber biomats and durable, biodegradable supports to secure anchoring of cuttings.

Long-term experiments

Historical series of monitoring activities to adequately evaluate the fate of a restoration initiative is mandatory to understand the forcing factors that influenced its evolution and to verify the trajectories taken by the system through time. In the literature, there are only a few examples available on transplants that have been monitored over a long-term period. Six case studies can be considered as successful, and they encompass a monitoring period between 4 years and 35 years.

1) From 1988 to 1995, 301 plagiotropic and orthotropic cuttings and some seedlings of *Posidonia oceanica*, taken from various sites in the Mediterranean basin, were transplanted on dead matte between 13 and 15 m depth within the Port-Cros National Park (France), covering a total surface area of 625 m² (details can be found in Pergent-Martini *et al.*, 2023). The fixing method included mesh, plastic-coated vertical stake, or galvanized steel stake, curved at one end to insert the rhizome in a horizontal position with the stem of the stake pressed into the substrate). Plagiotropic cuttings with 3 to 6 leaf bundles showed the best survival results, with the number of leaf bundles that increased approximately 87 times more in 2023, 35 years after their transplantation. This is a unique experience due to the very long-term monitoring of cuttings and seedlings.

2) An experimental transplant was conducted in a heavily anthropized area in Rapallo (Liguria, Italy) in 1996 (Bavestrello and Cattaneo-Vietti, 1997). Two techniques were utilized to fix the 500 cuttings,

collected from a nearby donor meadow, to the substrate: metal stakes and metal grids. The metal stakes were widely utilized during the late 1990s and was anticipated by many long-term studies. After one year from the transplantation, both techniques showed positive results in terms of shoot survival and rhizome length. Cuttings over the grids recorded a loss of about 15%, while those fixed by stakes had a loss of about 50%. Monitoring activities carried out in 2019, 23 years later (Robello *et al.*, 2023), showed an enlargement of the area covered by the patch of *P. oceanica* transplanted, from 20 m^2 to 24 m^2, and the density of the leaf bundles was about three times higher than at the time of transplanting (from 61 shoots · m^{-2} to 195 shoots · m^{-2}). The metallic grids used in the transplanting were still visible on the bottom (Figure 26), whilst the stakes were not found. The location of this transplanted meadow within a touristic marina must be considered when discussing the success of this pioneering intervention. Since it is in a heavily anthropized area that is often exposed to high water turbidity and intense hydrodynamics, the success of this transplanting is even more remarkable.

Figure 26. Metal grids used during the transplanting intervention in Rapallo (Italy) 23 years ago and still visible on the bottom (® Federico Betti).

3) A third long-term example reports on the restoration of degraded *Posidonia oceanica* meadows in the Gulf of Palermo (Sicily, Italy). For the site selection a model for identification of suitable areas to be prioritized for restoration has been developed. The model included the integration of the Preliminary Transplant Suitability Index (PTSI), the Transplant Suitability Index (TSI), and multiple transplant pilot sites at approximately 13 m depth (covering a total area of 15 m^2) (Pirrotta *et al.*, 2015). Both indices are based on the calculation of multiple parameters and relative assessment in a GIS environment. Recently, the PTSI has been further implemented with the introduction of parameters obtained from satellite data and the development of a freely downloadable tool (for details see Calvo *et al.*, 2021, 2022). A total of 66 shoots · m^{-2} were fixed on dead matte by metal grids. After twelve years from the transplantation, the density of the meadow was 331.6±17.7 shoots · m^{-2} (five times higher than the initial value), which was about the same density as the nearest natural meadow. In the same area, 22,000 *Posidonia oceanica* shoots from a donor meadow were transplanted in 2021 on a dead matte substrate, using the biobased anchoring modular system (Mater-Bi, European Design No. 003000686-0001/2016 and Italian Patent No. 10201500008182/2018) (Figure 27), covering a total area of 1,200 m^2 (Calvo *et al.*, 2022). During the first year after transplantation, the plant performance, in terms of cuttings detachment and survival, was better than the previous intervention carried out in the same area with traditional anchoring supports (metal grids), suggesting an improvement due to the new technology employed.

Figure 27. Modular biobased (Mater-Bi) anchoring system for *Posidonia oceanica* cuttings (Calvo *et al.*, 2022).

4) Transplant interventions were carried out at the Giglio Island (Tuscany, Italy) where the Concordia shipwreck occurred in 2012 (Mancini *et al.*, 2021; Ardizzone *et al.*, 2023). Based on preliminary high resolution cartographic analysis, three areas of intervention for the transplantation of *P. oceanica* were identified on approximately 2000 m^2 of dead matte substrate. Although the impact on the meadow affected the seabed up to 30 m depth, restoration was carried out only between 10 m and 23 m depth, excluding both the shallow (due to the high hydrodynamism) and the deep waters (due to the low intensity of the light that reaches the seabed, and which could have created problems to the growth of the transplanted plants). Transplanted cuttings were fixed to the seafloor with iron stakes capable of degrading in a few years (within 8-24 years) once the complete rooting of the plant has been reached. The stakes were specially designed and built to be easily inserted and hold the rhizomes in the dead matte (Figure 28). Once planted in the substrate, the stakes are almost invisible.

The plant material comprised both orthotropic and plagiotropic shoots, mostly derived from clods naturally detached due to storms and erosional events along the lower limits of the meadows, as well as from detached clods from boat anchoring. Before transplantation, the clods were cleaned and dead or damaged parts were removed. The larger cuttings were divided into smaller pieces with several foliar shoots and roots. The optimal material preferably comprises 10-30 cm long plagiotropic rhizomes, each with 2-4 foliar shoots and roots in good condition. Underwater operators implanted each cutting manually, fixing them using one or two stakes, depending on their length. The density of the cuttings and shoots in the area transplanted on Giglio was 5-9 cuttings · m^2, corresponding to 26-33 shoots · m^2. Monitoring activities included both shoot density counts and underwater photogrammetry to reconstruct accurate photomosaic and 3D models of the areas (Ventura *et al.*, 2021).

An initial loss of shoots during the first year was observed, followed by increased densities after four years. The experimental area made in 2017 showed an increase of 250% of the shoot density in 90 months, indicating the good success of the transplant.

Figure 28. Stake of 0.6 cm diameter iron rods welded together in one or more points, each curved at one end to form two curved arms (crescents) holding the *P. oceanica* rhizomes (Ardizzone *et al*., 2023).

5) Transplant interventions were carried out at Balearic Islands (Spain) in a shallow (depth <5 m) area, sheltered from waves. Substratum was dead matte colonized by *Cymodocea nodosa*, *Caulerpa prolifera*, and other photophilic macroalgae (Terrados and Castejón-Silvo, personal communication). The presence of natural recruits (seedlings) in the transplanting area and the observation of active growth of rhizomes at the edge of the extant *P. oceanica* meadow indicated that natural recolonization was taking place. The plant material used for transplanting was plagiotropic rhizome fragments of *P. oceanica* produced by natural processes (storms), which were collected manually by divers from drifting material accumulated in meadow gaps. The fragments selected for transplanting had a minimum of one apical (plagiotropic, horizontal) and two vertical shoots. The fragments were anchored individually using a staple made of 6 mm in diameter corrugated iron bar with a length of 60 cm and bended in the shape of a "U". The fragment is tied to the staple with a piece of synthetic fiber cord and two cable ties. This system provided anchoring capacity to the rhizome fragment until it produces roots (Castejón-Silvo and Terrados, 2021).

From 2018 a total of 12,800 fragments were planted manually by divers in groups (patches) of 16 (four lines of four fragments) and the distance among fragments was 20 cm; 800 patches were established over an area of 2 ha. The transplanting area has been delimited with surface buoys to prevent disturbance by anchoring from recreational boats. Fragments survival during the first 3.5-5.5 years after transplanting was higher than 90%.

6) Transplanting interventions were conducted in Santa Marinella (Lazio, Italy) in 2004 and in Ischia (Campania, Italy) in 2009 as part of the Life SEPOSSO Project (Bacci *et al.*, 2019). In both locations, transplanting was implemented to compensate for damages caused by coastal works and/or infrastructures. The technique used was identical and involved concrete frames measuring 50 cm × 50 cm, with a thickness of 6-8 cm and with an internal lumen of 40 cm × 40 cm. The frames were reinforced with galvanized iron mesh, which is necessary to hold the cuttings.

In Santa Marinella, several clearings within the seagrass meadow were selected for transplanting, totaling a surface of 10000 m^2. About 40000 concrete modules were planted in these clearings. Subsequently, 800 modules were selected and distributed to 40 transplanting stations to periodically monitor the seagrass meadow and to assess the state of the transplanting over time. The monitoring was carried out for 13 years and showed an average survival rate of over 90%. Some damages have been observed, resulting in significant loss of replanted area, particularly in the shallower clearings, some of which were completely lost due to storm surges.

At the Ischia site, 6400 modules were allocated for replanting cuttings, covering an area of approximately 1600 m^2. After selecting 120 modules distributed over 6 stations for periodic monitoring, the transplant survival rate was found to be greater than 200%. One station, however, experienced complete loss only one year after the transplanting. The damage was mainly caused by storm surges, but anchorage damage and other issues due to defective wire mesh in the replanting modules have been also identified (Bacci *et al.*, 2019).

Significant progress has been made in the field of *Posidonia oceanica* restoration over the last fifty years. Long series of data are crucial in guiding future transplantation interventions. Despite the greater number of studies on this species compared to others, it is still not easy to clearly define the best or the worst transplantation techniques, mainly due to the limited availability of data in the literature, especially regarding long-term transplants. The chosen restoration technique must be closely linked to the specific characteristics of the restoration site, such as hydrodynamics and substrate type, which can be very different between studies.

A crucial aspect that will require prioritized attention in the coming years is the selection of materials for supporting cuttings. Currently, there is a trend towards using degradable supports. However, it is important to carefully assess the biocompatibility of these materials with the plant.

2.5.2 *Cymodocea nodosa*

Cymodocea nodosa (Ucria) Ascherson is another common seagrass distributed in the Mediterranean Sea and along the eastern Atlantic coasts, including the Macaronesian oceanic archipelagos of Madeira and the Canary Islands (Figure 29), where it plays the key role of habitat engineer. It is generally found along the eastern and the southern coasts of the islands, sheltered from the dominant ocean swells (Mascaró *et al.*, 2009). This seagrass forms extensive, but often fragmented, subtidal meadows on sandy and muddy bottoms, up to 40 m depth. A decreasing trend of *Cymodocea nodosa* meadows on the island of Gran Canaria has been reported (Tuya *et al.*, 2013). Similarly to *Posidonia oceanica*, also *Cymodocea nodosa* is affected by uncontrolled human activities along the coast and by the introduction of alien species, mainly *Caulerpa taxifolia* and *C. cylindracea*, which have led to the regression of the plant. This species is listed as "Least Concern" by IUCN. While *P. oceanica* was declining in many areas of the Mediterranean Sea, *C. nodosa* showed an increase over time in some highly urbanized areas of the Ligurian Sea (Burgos *et al.*, 2017).

Cymodocea nodosa is protected in different countries of the Mediterranean. In addition, the species is included in the Annex II of the SPA/BIO Protocol of the Barcelona Convention and in the Annex I of the Bern Convention.

As *C. nodosa* is favoured by sea water warming (Boudouresque *et al.*, 2021), its seeds germinate very quickly, and meadows grow fast (up to 70 mm/day), it is an ideal species for active restoration interventions. This notwithstanding, examples of transplants of this species are fewer in the Mediterranean compared to *Posidonia oceanica*.

Figure 29. Distribution of the seagrass *Cymodocea nodosa* (from IUCN web site).

Transplantation techniques

Cymodocea nodosa can propagate through the production of new shoots from the horizontal rhizome, while sexual reproduction may occur during spring. One of the first examples of transplantation used seeds of *Cymodocea nodosa* collected from 16 randomly selected areas near Livorno (Italy), and then cultivated at sea in a controlled environment (Balestri and Lardicci, 2012). Seeds were first allowed to grow to produce seedlings, which were then given time to asexually reproduce a larger number of plants (called as mother plants after 5 years) to be transplanted individually at a later stage (Figure 30). After their development, they were transplanted into the sea in a mixed substrate of sand and pebbles using metal pins that were removed immediately after anchoring. The experiment showed very high survival rates in the two selected recipient sites (100% and 75%). The advantages of this technique include genetic diversity and the fact that several plants were obtained from a single seed, since they were given time to grow and reproduce before transplanting, unlike the classical methods that involve the direct growth of plants from a single seed. On the other hand, it was a very expensive method in terms of equipment, personnel, and time.

Following studies showed the combination of techniques using materials such as nylon netting or stainless-steel rods, along with biodegradable components such as cornstarch bags incorporated into containers made of rice husks (Da Ros *et al.*, 2021) or trays made by compressing coconut fiber powder (Zarranz *et al.*, 2010). Nylon netting approaches resulted in the death of seeds adhering to the nylon nets, while the use of biodegradable trays resulted in a survival rate of approximately 40% when transplanted seeds were placed directly in the substrate.

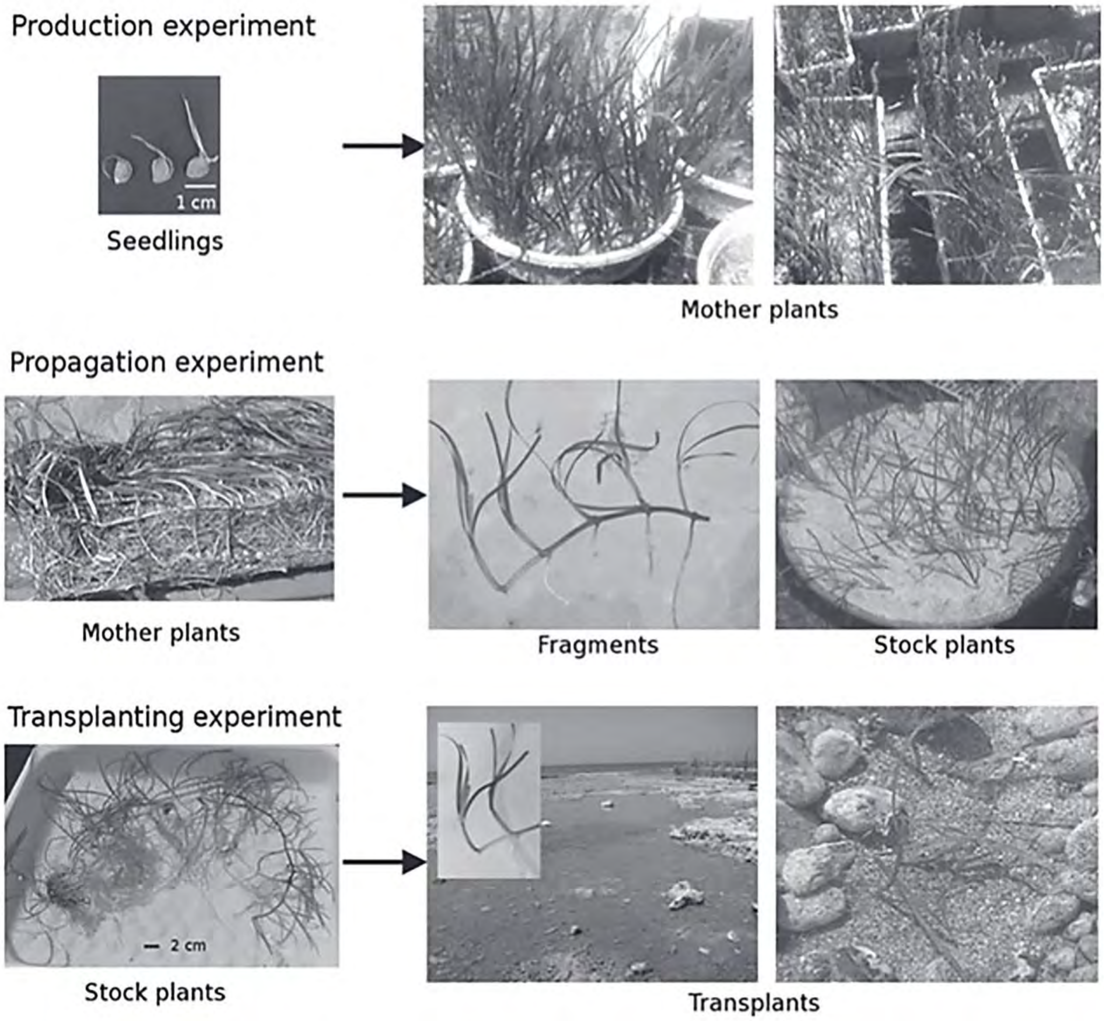

Figure 30. Transplantation interventions with seeds of *Cymodocea nodosa* at Livorno (Italy) (Balestri and Lardicci, 2012).

In a study conducted in 1994 two different techniques (non-anchoring method and anchoring method) were tested in five stations with *Cymodocea nodosa* in the lagoon of Venice (Italy) (Curiel, *et al.*, 1995, 2003). The non-anchoring method used clods, i.e. plants with substrate intact, which were harvested using a 30 cm high by 23 cm diameter metallic corer. The anchoring method used bundles of rhizomes with shoots held in the superficial sediments with plastic clips. Rhizomes with an average length of 30-50 cm were collected using a water jet to minimize damage to the plants. After two growing seasons, both transplanting methods showed good success. Most of the transplanted units still had seagrass and the coverage ranged from 76% to 86%. Compared to the initial densities the increase of the transplanting was 15.1 times greater for clod method and 42 times greater for rhizome method. In comparison with the control site, for both methods after 17 months the density of *C. nodosa* reached about 50% and biomass the 16-36% of the control site values.

In a recent experiment, a new technique of transplant using biodegradable bags and containers (made with rice husks, Figure 31) was tested in the Adriatic Sea in 2018 (Da Ros *et al.*, 2021).

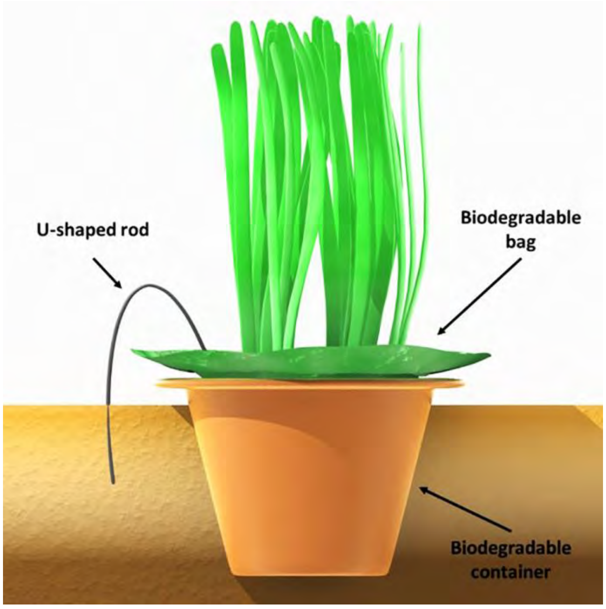

Figure 31. Biodegradable bag inserted into a biodegradable container anchored with U-shaped stainless-steel rods used for the *Cymodocea nodosa* transplantation in the Adriatic Sea (Da Ros *et al*., 2021).

These bags were located at similar depth (0.9-1.3 m) adjacent to breakwaters and were anchored with U-shaped stainless-steel rods. A manual stainless-steel corer was used to dig a clod from the donor seagrass meadow, avoiding any damage to the roots and leaves. This clod was immediately inserted in a biodegradable bag and the bag was inserted in the biodegradable container to maintain the consistency of the clod. The container was then inserted in the receiving sediments and anchored with a U-shaped stainless-steel rod. To limit the potential impact of shoots removal from the donor meadow, the final shoot density of the transplanted plots was 10% of the density assessed in the donor meadow. 13 containers, each containing 11-13 shoots of seagrass, were planted in each transplanted seagrass plots. This restoration technique was successful, enabling the seagrass survival (approximately 30%) even in high-energy conditions occurring in winter. Transplantation reached 70% of the density and 35% of the biomass with respect to the donor meadow. The sediments hosting the transplanted *C. nodosa* also showed an increase of trophic availability and of rates of organic matter cycling. The experiment was conducted in spring because this season has been reported as the most suitable period in temperate ecosystems to successfully conduct seagrass transplantation.

In Le Brusc Lagoon (France), cuttings of *C. nodosa* were fixed to the bottom by means of staples; the 1 m^2 transplant areas (80 m^2 in total) were covered by a low (20 cm in height) wire cage made of 5 cm mesh, to preserve the transplants from grazing by the fish *Sarpa salpa* (Figure 32) (Couvray *et al.*, 2020).

The LIFE-TRANSFER project, "Seagrass transplantation for transitional Ecosystem Recovery" (LIFE19 NAT/IT/000264), funded by the EC in 2019, aimed at improving the conservation status of the 'Coastal lagoon' habitat of the EU Habitats Directive (Habitat 1150*) in eight Natura 2000 sites, four in Italy, two in Greece and two in Spain. The project will favour the process of recolonisation of aquatic seagrass by transplanting small clods and individual rhizomes of species previously present in each area. In particular, it aimed at restoring meadows of *Zostera marina*, *Zostera noltei*, *Ruppia cirrhosa*, and *Cymodocea nodosa* to promote the natural propagation capacity of these plants through seed production and dispersion.

Figure 32. Transplantation of *Cymodocea nodosa* cuttings in Le Brusc Lagoon (eastern Provence). A wire cage preserved cuttings from *Sarpa salpa* grazing. For the purposes of photography and monitoring, the cage was raised vertically (Couvray *et al.*, 2020).

2.5.3 *Zostera noltei*

Zostera noltei is a small seagrass species found in intertidal and shallow subtidal waters (Hartog, 1970). The species is mainly found in areas with soft sediments in estuaries and coastal lagoons and along the coast and can only tolerate depths up to 10 m. It can also survive in permanent subtidal conditions in small brackish creeks and coastal lagoons with euryhaline characteristics, tolerating salinities up to 25-51 PSU (Green and Short, 2003). Its tolerance for burial and erosion is extremely low, ranging from 4 to 8 cm, due to its small size and lack of vertical rhizomes (Cabaço and Santos, 2007). *Zostera noltei* occurs in the eastern Atlantic, as well as in the Baltic, Mediterranean, Black, Caspian, and Aral Seas. *Zostera noltei* is also found in West Africa in Mauritania and in the Canary and Cape Verde Islands (Figure 33).

There are no significant threats to this seagrass. However, there have been localized declines in certain regions due to reduced water clarity from sedimentation, coastal development, and wasting disease. *Zostera noltei* appears to be sensitive to eutrophication (Short *et al.*, 1995). This species is listed as "Least Concern" by IUCN.

There are no specific conservation measures for *Zostera noltei*. The species is listed in the Rio Declaration as a diverse habitat in need of conservation and monitoring. It is found in two national nature reserves in the Caspian Sea and may be found in other marine protected areas (Green and Short, 2003).

Transplantation techniques

The study carried out by Valle *et al.* (2015) in two estuaries on the Basque coast (Spain) used the method of extracting seeding and clods having an area of 1026 cm^2, which consisted of roots, rhizomes, and shoots, together with associated sediments (10-15 cm width). Although sandy sediments appeared to be more conducive to root growth in the first few months, muddy sediments were more conducive to the establishment of transplanted protected unit in the long term. After 5.5 years, a single transplanted unit survived in the muddy site, representing 25% of the initial transplanted units. Its area increased 8-fold, representing a 200% increase in the transplanted area, demonstrating a positive growth trend.

A transplant of *Zostera noltei* occurred in the Venice Lagoon (Italy) in 2014 (Sfriso *et al.*, 2019) in the frame of the LIFE SeResto project (see below). Transplanting was carried out in stations of 100 m^2 (10 × 10 m) using clods that were approximately 20-30 cm in diameter, which were collected with a manual corer and arranged in groups of three for a total of nine clods per station. The cylindrical clods included plants, roots, and sediment. The depth of the intervention area was generally less than 1 m on the average tide. Hundreds of full-grown rhizomes were also transplanted individually at each station using pliers with a handle of approximately 1 m length. Plant rooting was successful where the waters were clear and the trophic status low. But, near the outflows of freshwater rich in nutrients and suspended particulate matter, the action failed. Similar experiments were conducted in France (Bernard *et al.*, 2013).

Figure 33. Distribution of *Zostera noltei* (from IUCN web site).

Zostera noltei was one the target species of the LIFE SeResto project “Habitat 1150* (Coastal lagoon) recovery by seagrass restoration. A new strategic approach to meet HD & WFD objectives” (LIFE12 NAT/IT/000331), which aimed to trigger a process of aquatic angiosperm recolonization in the Northern Lagoon of Venice (Italy), mainly through its transplantation in small sites distributed throughout the area. The proposed intervention technique involved transplanting a small number of plants, with advantages in terms of low costs and impact on the donor sites (Sfriso *et al.*, 2019). The technique was also suited to large-scale application. *Zostera noltei* was also one the target species of the recent LIFE-TRANSFER project “Seagrass transplantation for transitional Ecosystem Recovery” (LIFE19 NAT/IT/000264) (see above in *Cymodocea nodosa*).

2.5.4 *Zostera marina*

Zostera marina is a seagrass with dark green, long, narrow, ribbon shaped leaves 20-50 cm in length. Leaves and rhizomes contain air spaces that aid buoyancy. Numerous flowers occur on a reproductive shoot like those of terrestrial grasses. It forms dense meadows in the subtidal zone, on sand to fine gravel sediments, typically down to 4 m in sheltered waters such as shallow inlets, bays, estuaries, and saline lagoons. It supports a diverse fauna and flora and may act as a nursery for fish and shellfish. *Zostera marina* is distributed throughout the northern Atlantic and Pacific Oceans, as well as the Mediterranean and Black Seas. Its distribution extends from the Arctic region, including Alaska, Canada, Greenland, and northern Europe, to Baja California and Mexico (Figure 34).

Zostera marina is experiencing a severe decline in some regions due to wasting disease and pollution threats, especially in developed and populated regions of Europe and North America. There are regions where its populations are thriving, and in some areas *Zostera marina* has become completely extinct.

This species is listed as "Least Concern" by IUCN. There are currently no specific conservation measures for this species, but it is subject to general protection regulations for coastal habitats and protected areas.

Figure 34. Distribution of *Zostera marina* (from IUCN web site).

Transplantation techniques

In April 1994 two different techniques (non-anchoring method and anchoring method) were tested in five stations with *Zostera marina* in the lagoon of Venice (Italy) (Curiel, *et al.*, 1995, 2003). The non-anchoring method used clods, i.e. plants with substrate intact, which were harvested using a 30 cm high by 23 cm diameter metallic corer. The anchoring method used bundles of rhizomes with shoots held in the superficial sediments with plastic clips. Rhizomes with an average length of 30-50 cm were collected using a water jet to minimize damage to the plants. After two growing seasons, both transplanting methods showed good success. Most of the transplanted units still had seagrass and the coverage ranged from 70% to 74.4%. Compared to the initial densities the increase of the transplanting was 6.6 times greater for clod method and 16.7 times greater for rhizome method. In comparison with the control site, for both methods after 17 months the density of *Z. marina* reached about 50% and biomass the 34-54% of the control site values.

Park and Lee (2007) used three transplantation methods with one donor site and three recipient sites. In all cases, vegetative shoots were harvested by hand to minimize damage. The first method 1 used two shoots placed with the rhizomes aligned in parallel but opposite directions and secured with a bent wire staple, and then pressed horizontally into the sediment using 15 cm V-shaped wires (Davis and Short, 1997). Ten units with a total of 20 shoots were planted in each 50 × 50 cm plot to achieve 80 shoots per m^2, and four plots were planted at each site. The second method used 72 mature shoots on a 60 × 60 cm metal frame to achieve 200 shoots per m2. Seagrass rhizomes were attached to the frame with paper ties. Four frames were distributed at each site and then recovered by diving after an appropriate rooting period of 2 months. With the third method shoots were anchored to an oyster shell. 15 units with a total of 30 shoots were placed in each 50 × 50 cm plot to obtain 120 shoots per m^2, and 4 plots were planted at each site.

Most of the transplants planted during the summer using all three methods did not survive beyond three months. A decrease in shoot density of transplants was observed during the first four months after transplanting due to initial transplant shock. Typically, survival of transplants planted from fall to spring exceeded 60%. The first method had the highest survival rate (93.8% to 77.1% during winter); however, it was labour intensive and time consuming as it required the use of divers. The second method had a lower survival rate but was processed to minimize diver effort and was therefore more suitable for large-scale restoration. However, the frames need to be recovered after 1-2 months. The third method had a lower survival rate in sandy substrates, but was both cost effective and labour intensive, although it took the longest to establish. The shell method was highly sediment-dependent: it had a high survival rate in sites with clay and loamy sediments (75%), whereas only 5% of the transplants thrived in sandy sites, since eelgrass rhizomes planted by the shell method were not strongly pressed into the sediment. As a result, transplants can be moved by water currents, especially in sandy sediment sites. Because the shells used as anchors were harvested directly from the marine environment, they did not require retrieval and did not deposit hazardous materials in the planting areas. Ultimately, the authors agreed that the paper clip method was superior to both the metal frames and the shell methods when transplanting on sandy sediments due to its ability to maintain a firmer attachment. While both the metal frames and shell methods proved to be cost and labour efficient, they are better suited for large-scale restoration efforts. However, in cases where sediments are muddy, the use of the shell method should be limited. The optimal time for transplanting is after the peak of seasonal stress, as plants have a longer period to recover from damage before the next stress period. It is recommended that eelgrass is not transplanted during the summer, as transplants may be exposed to lethal high-water temperatures prior to establishment. In contrast, transplant survival was not significantly affected by low water temperatures.

A transplant of *Zostera marina* occurred in the Venice Lagoon (Italy) in 2014 (Sfriso *et al.*, 2019) in the frame of the LIFE SeResto project "Habitat 1150* (Coastal lagoon) recovery by seagrass restoration. A new strategic approach to meet HD & WFD objectives" (LIFE12 NAT/IT/000331) (see *Z. noltei* for details). *Z. marina* was also one of the target species of the LIFE-TRANSFER project "Seagrass transplantation for transitional Ecosystem Recovery" (LIFE19 NAT/IT/000264) (see above in *Zostera noltei*).

2.6 Artificial reefs

Artificial reefs are recognized as a valuable solution to boost marine biodiversity, prevent illegal trawling, bolster fish populations, increase marine productivity, enhance small-scale coastal fisheries, and promote recreational diving (Fabi *et al.*, 2011). Indirectly, artificial reefs may thus represent an intervention of active restoration as they help the recovery of biodiversity in marine ecosystems.

Several researchers characterized artificial reefs as either man-made structures or natural objects strategically positioned underwater in specific areas with the aim of establishing marine habitats and enhancing heterogeneity (Buchanan, 1972; Bombace, 1989). The UNEP/MAP program "Integrated Coastal Area Management in Cyprus: Biodiversity Concerns" developed in 2007 defined artificial reefs as one of the most effective actions to stop illegal trawling. Artificial reefs deployment falls under some international regulations concerning the protection of the sea against pollution, like the Barcelona Convention (Convention for the Protection of the Mediterranean Sea against Pollution, 1977) and the London Convention (Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, 1972 and 1996). Specialized regional plans governing the implementation of artificial reefs in marine environments and guidelines for their construction have been formulated based on some key regulations. Comprehensive national and/or regional initiatives for deploying artificial reefs and integrating them into the management plans of coastal zones are becoming operational in many Mediterranean countries. In contrast, only a limited number of projects have been initiated in other European regions. Furthermore, there is a notable absence of post-deployment management plans for artificial reefs in many countries (Fabi *et al.*, 2011).

Before the installation of an artificial reef, it is important to formulate, and have approved, a comprehensive management plan outlining the strategies for controlling the reef throughout its designated operational lifespan. This management plan should provide in-depth details on the methodologies employed to evaluate the reef's effectiveness, the proposed measures for mitigating any adverse environmental impact, and the identification of the users or non-target stakeholders of the reef.

Another important aspect for managing and monitoring artificial reefs after installation includes the evaluation of the ecological conditions in areas where the artificial reefs are positioned during the post-operation phases of their life cycle. Most structures in this category lack provisions for maintenance and repair. Consequently, over time, the ecological and ameliorative benefits of artificial reefs frequently diminish (Suzdaleva and Beznosov, 2021).

According with Suzdaleva and Beznosov (2021), classifying artificial reefs allows for a unified approach to evaluating the environmental impact of these structures throughout their entire life cycle. They analysed different artificial structures. 1) Removable artificial reefs are deployed in the aquatic environment for a defined period for the farming of various aquaculture objects. 2) Self-destructive artificial reefs are designed to fulfil a specific task and their materials undergo dissolution and corrosion in seawater, leading to their eventual destruction. An example is the openwork structure made by 1.5-2.0 m long iron rods connected at the ends, utilized to improve the water quality in the coastal area of Anapa (Black Sea). Biodegradable ropes were added between the rods to increase the surface suitable for organism colonization. Molluscs and algae growing on these structures effectively removed a significant amount of contaminants accumulated during periods of excessive recreational activity. 3) Transforming artificial reefs are intended to evolve over time into substrates resembling natural soils and have been utilized in the engineering and environmental development of the Anapa resort area. In the mid-20th century, realization of artificial beaches contributed to the destruction of coastal aquatic vegetation, particularly of macrophytes. The disappearance of this natural biofilter resulted in a decline of the overall coastal water quality. To restore the algal belts, artificial reefs in the form of underwater rocks were deployed on the seafloor along the beach. Within 2-3 years, new algal belts formed in these areas. 4) Another type of artificial reefs is represented by dismountable artificial reefs, hydraulic structures created for extended service life, comprising elements that can be individually removed from the aquatic environment when needed. 5) Hard-to-dismantle artificial reefs are typically large reinforced concrete structures; usually their main purpose is coastal protection, but they can simultaneously act as substrates for organism development, aiding in the absorption of contaminants.

Since the 1970s, artificial reefs have been strategically placed along the Mediterranean coast to safeguard coastal habitats and support small-scale fisheries, particularly in conflict-prone areas affected by illegal trawling and hydraulic dredging. One of the first example of artificial reef in the Mediterranean Sea was conducted by Relini *et al.* (1994). They investigated the short-term (monthly) and the long-term (3-years) evolution of benthic communities settling on hard artificial substrates at different depths off Loano (Ligurian Sea, Italy). The artificial site was created for protecting the area from illegal trawling and for restocking fish populations. The artificial reef comprised large concrete blocks (2 × 2 × 2 m) arranged in pyramids and smaller concrete blocks (1.2 × 1.2 × 1.2 m). Short-term observations revealed seasonal changes in the settlement periods for exploitable resources, such as the oysters (*Ostrea edulis*). Long-term investigations illustrated the temporal pattern of benthic assemblages, the climax stages, and the interactions with fishes. Results indicated increases in biomass, cover, and number of sessile species over time. However, five years after the immersion of the concrete blocks, a climax stage had not yet been reached and the community continued to change, especially with an increasing abundance of macroalgae and sponges. The Loano artificial reef represented an active restoration intervention useful to diversify the environment, offering organisms the diverse habitats they required. This contribution sustained a rich biodiversity within confined spaces and facilitated the creation of new ecological niches and intricate food webs. Additionally, it is important to note that the communities thriving on artificial reefs can be also employed for transplants to degraded areas that require restoration.

In the Adriatic Sea, where clam fisheries operate with hydraulic dredges, conflicts arise due to resource competition and damage to gear caused by illegal trawling and hydraulic dredges. To address these issues, large-scale multipurpose artificial reefs, located approximately 5.5 km offshore, were constructed to allocate space and resources. The deployed modules had three typologies: 1) protection modules; 2) production modules; and 3) mixed modules (Fabi *et al.*, 2015). Anti-trawling structures, often combined with production units or mixed modules, were strategically positioned to deter trawlers from entering prohibited zones (Bombace *et al.*, 2000; Fabi *et al.*, 2006). The layout of rectangular zones, oriented horizontally with respect to the coast, was designed based on otter-board openings. These artificial reefs played a crucial role in mitigating conflicts among fishers by providing a suitable area for small-scale fisheries to conduct seasonal activities in harmony with the eco-ethology of different reef-inhabiting species. This cooperative management approach contributed to the sustainable utilization of reef areas and their resources.

In the past decade, numerous new projects took place off the coast of France, including the largest artificial reef project known as “récifs PRADO” carried out in the Bay of Marseille in 2007. The project involved the deployment of 27,300 m^3 of artificial reefs. The primary goal was to actively restore artisanal fishery in an area where *Posidonia oceanica* beds had been previously destroyed. The project focused on replicating productive natural benthic habitats to enhance artificial reef biological efficiency. Architectural complexity, module design, and urban layout were considered crucial for the artificial reef effectiveness, achieved by creating horizontal and vertical discontinuities using a variety of reef types, shapes, and arrangements. The project faced three years of planning, overcoming technical and administrative challenges. Six types of modules, diverse in shapes, sizes, volumes, and materials, were designed (Charbonnel *et al.*, 2011): 1) the “metal basket” is a substantial reef (187 m^3) made by a metal frame consisting of three baskets measuring 5 × 5 × 3 m, filled with various materials of different sizes (including 4 concrete cubes of 1.7 m^3, 10 concrete piles, and 200 breeze blocks per basket); 2) the “fakir basket” (82 m^3, 5 × 5 × 3 m) is constructed with 26 peripheral concrete piles, filled with materials of varying sizes (including 16 concrete cubes of 1.7 m^3 and 200 breeze blocks); 3) the “chicane” (19 m^3, 4 × 2.4 × 2 m) features a concrete frame with two floors of maze-like galleries connected by five holes, which is specifically designed for sea breams; 4) the “floating ropes” (6 × 6 × 7 m) consists of a concrete frame with two floors of interconnected floating ropes, designed to attract pelagic species, small planktivorous fishes, and their predators; 5) the “cube pile” (10 m^3, 2 m high) is an assembly of six concrete cubes of 1.7 m^3 with small void spaces between the cubes; 6) the “quarry rocks” (300 tons, 160 m^3, 20 × 4 × 2 m) is composed of piles of quarry blocks of three different sizes, and is particularly suitable for many target species such as groupers and sea breams.

To maximize habitat diversity, modules were made more complex with additional small filling materials and floating immersed ropes. The inclusion of quarried blocks reconstituted natural rocky boulders, ideal habitats for target species. An innovative aspect of this intervention was grouping modules into “hamlets” and “villages” to create biological corridors and stepping stones, considering the locations of natural habitats for rapid artificial reef colonization. The reef structures were deployed in two areas, one closed to all fishing areas and the other restricted to artisanal fishing areas, between October 2007 and July 2008. Despite the need for compromises due to legal, economic, environmental, and social constraints, the project showed effective in terms of collaboration among various stakeholders, including marine biologists, planners, fishermen, and authorities; it adapted the reef structures to the local context and achieved multiple objectives.

More recently bio-mimetic artificial reefs were deployed in Ajaccio Bay (Corsica, France) by the Corsica’s Environmental Office in 2017. These reefs utilized bio-mimetic technology to replicate the texture of a natural reef and employed a chemical reaction to consolidate natural sedimentary particles (Salaün *et al.*, 2020). Various materials, including concrete, metal, and broken shells were chosen for constructing the artificial reefs, each designed with specific fish targets. Another project involved artificial reefs as habitat restoration initiative in Cortiou’s Bay (Marseille, France) in 2018 (Salaün *et al.*, 2020). In the Marine Protected Area of the “Parc National des Calanques”, 36 artificial reefs were submersed at the termination point of an old sewerage pipe in 2013, through a call for sustainable ideas. The 36 artificial reefs, each featuring distinct designs, utilized bio-mimetic technology to replicate the natural habitat's shape. These artificial reefs were strategically deployed in four sites located at varying distances from the pipe's end. Ongoing monitoring was scheduled to assess the colonization efficiency of the artificial reefs and the impact of this new habitat on local biodiversity.

Artificial reefs are increasingly influential in shaping the condition of coastal waters. This underscores the need to establish guidelines for ensuring the environmental safety of these structures throughout their entire life cycle. Therefore, it should be a priority evaluating the environmental effects of artificial reefs, their potentiality to restore natural habitats and their capacity to function as ecological regulators able to establish a controllable natural-technical system in their designated locations (Suzdaleva and Beznosov, 2021). The significance of artificial reefs is expected to increase in the upcoming years, aligning with the heightened emphasis on integrating fisheries and environmental policies into fisheries management, research, and habitat restoration.

2.7 Marine litter

There have been numerous anthropogenic-driven changes to our planet in the last century, which supported the definition of the new geological epoch in which we are currently living that is the Anthropocene. One of the most evident pressures is the ubiquity and abundance of litter in the marine environment. The escalation of marine litter globally is recognized as a growing concern that poses a threat to marine biodiversity (Barnes *et al.*, 2009). According to the United Nations Environment Program (UNEP), marine litter is defined as "any persistent, manufactured, or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment". UNEP estimated that annually 6.4 million tonnes of litter enter the oceans, with the entire Mediterranean basin currently hosting 62 million macrolitter items afloat (Suaria and Aliani, 2014).

The Mediterranean Sea is one of the most polluted and threatened semi-enclosed seas worldwide (Costello *et al.*, 2010; Deudero and Alomar, 2015). In the Mediterranean Sea, plastics constitute the most widespread litter on deep seafloors (Pham *et al.*, 2014), and similarly plastic objects make up 82% of all man-made floating items (Suaria and Aliani, 2014). The consequences of this pervasive 'Plastic Era' are evident at various levels, and efforts to address these issues are still in the early stages of development. Additionally, diverse types of litter are accumulating in the marine environment, including glass, paper, cardboard, metal, cloth, rubber, fishing-related waste, munitions, wood, cigarette filters, sanitary and sewage-related litter, ropes, toys, and strapping bands (UNEP, 2011). Litter enters the marine environment, propagates, migrates, and accumulates in natural habitats globally, as well as within tissues of animals.

Globally, up to 80% of ocean debris originates from sources on land (land-based) (Sheavly, 2005; UNEP, 2005; McIlgorm *et al.*, 2011). This includes discharges from rivers and estuaries, stormwater runoff, industrial outfalls, landfill activities, and tourism. Debris from activities directly connected to the ocean (ocean-based), such as commercial fishing, shipping, oil-related operations, as well as recreational boating and military vessels, constitutes the remaining portion (Allsopp *et al.*, 2006). Marine debris has been identified at both regional and local levels within the Mediterranean Sea, manifesting along shorelines, drifting on the sea's surface, existing within the water column, and accumulating on the seafloor (UNEP, 2011; Fossi *et al.*, 2018).

As documented by various studies marine litter encompasses physical, chemical, and biological implications, as well as economical ones (McIlgorm *et al.*, 2011; Vlachogianni, 2017; Madricardo *et al.*, 2020). Reports on the impacts of marine litter in the Mediterranean Sea extend to 134 species that are threatened in different ways, ranging from the detrimental effects of entanglement, smothering, consumption, and pollution (Deudero and Alomar, 2015). Beyond the immediate threats to marine organisms, marine litter has also been proposed to facilitate the spread of non-indigenous species (Lewis *et al.*, 2005). While attention has primarily been focused on charismatic animals such as marine birds, turtles, and mammals, the effects on other animals like fish, invertebrates, and other habitat forming species are becoming more evident. Ingestion of floating waste and entanglement in discarded or lost fishing gear and ropes can impact the survival of many species, often leading to direct mortality (Kühn *et al.*, 2015).

To minimize its negative impacts, a plethora of instruments has been developed at international, regional, and national levels to prevent, reduce, and manage marine litter. They represent a wide range of international, regional, and national efforts devoted to combat marine litter. These mechanisms typically encompass regional agreements, regional or national initiatives, legislation, or specific programs addressing various facets of marine litter issues. Examples include the Barcelona Convention, the EU Marine Strategy Framework Directive (2008/56/EC) that establishes a framework within which EU Member States shall take action to achieve or maintain the good environmental status (which also includes the management of marine litter), numerous coastal cleanup campaigns, and various relevant national laws. The UNEP Regional Sea Programme and the Global Programme of Action (GPA22) launched a global initiative on marine litter in 2003 (Bergmann *et al.*, 2015). This initiative has effectively coordinated and executed regional initiatives on marine litter across the globe. Activities centred on the management of marine litter were organized through individual agreements in 12 Regional Seas, including the Mediterranean Sea.

Efforts aimed at minimizing marine litter are diverse and encompass a broad spectrum of initiatives. These initiatives include altering consumer behaviour, introducing innovative technologies and materials, executing and upholding plans, policies, and laws, reevaluating current production and consumption practices (such as transitioning to a circular economy), and improving waste management. The reduction of marine litter calls for the active participation of various entities and stakeholders, including consumers, producers, policymakers, managers, residents, tourists, industries, and the fishing sector (Ronchi *et al.*, 2019). Addressing this issue requires minimizing the creation of debris and preventing its entry into the sea. Strategies encompassed in this approach include source reduction, the reutilization and recycling of waste, converting waste into energy, implementing port reception facilities, gear marking, containing debris at points of entry into receiving waters, and implementing diverse waste management initiatives on land (Bergmann *et al.*, 2015).

Removal measures on litter, which represent active restoration interventions, should be designed to eliminate debris already existing in the marine environment. Beach cleanups are a frequently used method, but these active removal measures proved to be time-consuming and expensive (Newman *et al.*, 2015). Furthermore, such efforts only manage to capture a fraction of the total debris. As an illustration of the financial commitment involved, UK municipalities, for instance, expend around €18 million annually on beach litter removal (Bergmann *et al.*, 2015). The collaboration between The Society for the Protection of Nature in Israel and The Israeli Diving Federation resulted in the creation of the volunteer program "Sea Guard". This program actively contributes to marine conservation through citizen science. Divers enrolled in the program undergo training in marine ecology and survey techniques, empowering them to conduct autonomous surveys and lead underwater cleanup initiatives to remove the litter encountered during dives (Pasternak *et al.*, 2019). Another initiative that aims at litter removal is represents by "Fishing For Litter" (FFL), which involves the fishing industry, and is focused on cleaning up the seafloor by retrieving marine litter: fishing vessels gather the debris caught in their nets during fishing operations and properly dispose of it at the jetty. The Regional Plan for Marine Litter Management in the Mediterranean Sea (MLRP), ratified by the Mediterranean countries in 2013, recognized the FFL initiative as a crucial measure with the potential to significantly reduce marine litter volumes at sea. Between 2014 and 2016, 15 ports in the Adriatic-Ionian region, spanning Italy, Slovenia, Croatia, Montenegro, and Greece, initiated pilot FFL projects. A study conducted by Ronchi *et al.* (2019) investigated the strength, weaknesses, opportunities, and threats (through a SWOT analysis) of these pilot projects. Strengths observed in the Adriatic-Ionian region primarily revolved around collaboration and governance procedures. Positive engagements among coastal municipalities, Port Authorities, and relevant ministries (such as Environment and Fisheries) in addressing marine litter issues were established during these pilot projects.

Additionally, there was willingness among fishermen across all countries to collaborate with scientific institutions. The study highlighted anyway notable weaknesses associated with the legislative domain. These included the absence of a comprehensive policy or legal framework for managing marine litter and a convoluted bureaucracy with unclear divisions of responsibilities among authorities. A significant concern was the lack of specific national laws for quantifying marine litter. As a result, the legal status of marine litter remained undefined, categorizing it as "special waste" and implying the necessity for specialized management schemes, consequently suggesting fishermen bear responsibility for its production. The opportunities for implementing the FFL scheme in the Adriatic-Ionian region were primarily associated with the growing concern of EU Governments and Institutions regarding marine litter. This heightened interest has bolstered the current political momentum for decisive action. Additionally, intergovernmental organizations, such as UNEP, acknowledged the significance of the FFL scheme in mitigating marine litter. This SWOT analysis revealed various external factors that could jeopardize the success of marine litter removal projects. Global plastic production is on the rise, and without advancements in waste management, the volume of plastic waste entering the ocean is expected to surge significantly by 2025. This escalation may potentially diminish the effectiveness of the scheme. Another potential threat lies in the widespread perception that the fishing sector is the major contributor to marine litter, leading to public reluctance in supporting FFL projects with tax funding.

In the Mediterranean Sea, where plastic pollution is a predominant concern, the presence of lost or abandoned fishing gears (known as ghost nets) poses a significant threat to diverse habitats (Enrichetti *et al.*, 2021). One of the habitats mostly affected by fishing gears, such as trawling nets and longlines, is the coralligenous because of the physical damages that the nets can provoke both during fishing activities and even when they are lost and remain abandoned on the seafloor (Enrichetti *et al.*, 2021). Little is known about the recovery capacity of coralligenous in response to impact from fishing gears (Auster and Langton, 1999). However, the slow growth rates and the low resilience of the most abundant and structuring coralligenous species (e.g., gorgonians and bryozoans) suggest a reduced capacity of this habitat to recover after extensive mechanical damage (Piazzi *et al.*, 2012).

In this context, active restoration actions should be taken, including the removal of abandoned tools at the bottom. In many countries of the Mediterranean Sea, specific projects aimed at removing the abandoned fishing gears (AFG) on the bottom have recently been undertaken, with the economic support by either national institutions or private companies. This notwithstanding, a standardised and shared protocol with instructions on where to remove, how to remove, and how to monitor the effects of the removal of AFG is not yet available.

In the Capo Carbonara Marine Protected Area (south-east Sardinia, Italy), the Blue Foundation and the Capellino Foundation supported the activities planned for the project "Cleaning coralligenous reefs from abandoned fishing gears", with the aim of implementing the provisions of the Nature Directives (Habitats Directive 92/43/EEC, Birds Directive 2009/147/EC) for the EU habitats and species of special interest. In this project, the ecological status of the Capo Carbonara coralligenous assemblages affected by AFG was compared through time to nearby unaffected coralligenous assemblages. With this aim, the COARSE index (Coralligenous Assessment by Reef Scape Estimate; Gatti *et al.*, 2015) was applied to compare the ecological status of coralligenous before and after the AFG removal. The BACI (Before-After/Control-Impact; Underwood, 1992) sampling design was used to evaluate the resilience of coralligenous to this type of impact after the removal of AFG (Azzola and Montefalcone, 2023). The removal of abandoned fishing gears was successfully completed at the six sites selected within the MPA, where preliminary monitoring activities identified these coralligenous as adequate for the subsequent removal of the gears or the ropes. In these selected sites, the AFG were placed or were entangled on the assemblages, but visual surveys established that the ghost gears could be carefully removed without causing significant impacts on the entangled species. The removal was carried out by the Carabinieri Diving Unit and by some volunteer divers, always under the supervision of the staff of the MPA to ensure that no severe damage was done to the environment and to the entangled species. When a gorgonian species was found entangled in the net, it was carefully detached from the net and then replanted on the nearby coralligenous rock, using quick-setting glue.

The monitoring of the ecological status of coralligenous was firstly carried out during the preliminary monitoring activities (i.e., before), both on coralligenous assemblages affected by AFG (i.e., impact) and on the nearby unaffected coralligenous (i.e., control), to compare their status according to AFG presence. Similarly, monitoring activities were performed also immediately after the removal of the AFG (i.e., after, few days later) on both the control and the impact sites, to evaluate the effect of the removal on coralligenous assemblages. The last monitoring activities have been carried out one year after the AFG removal (i.e., after 1 year) to evaluate the capacity of coralligenous to recover from the impact of the ghost gears. To quantify the impact of AFG, the staff of the MPA also collected quali-quantitative information on species entangled in the gears by analysing the retrieved fishing gears after their removal. When possible, length and width of AFG have been measured, entangled/encrusted species have been recognized at the highest taxonomical resolution as possible, and their percentage cover on the total surface of the gears has been visually estimated. Application of the COARSE index revealed an overall good ecological status of the Capo Carbonara coralligenous assemblages in almost all the sites (both impact and control) investigated before the AFG removal. This preliminary result supported the choice of removing the gears from the impact sites, as they did not have significantly affected yet the status of reefs (or they had only a limited impact). A reduction in the ecological status of coralligenous immediately after the removal of the AFG was observed only in two impact sites, which shifted to a moderate ecological status. After the AFG removal, no significant changes in the coralligenous assemblages were observed, excluding a reduction in the number of some vulnerable species of the intermediate layer (such as calcified bryozoans and erect algae), which were grown directly on the AFG (Azzola and Montefalcone, 2023). The monitoring activities carried out one year after the AFG removal did not show a significant recovery of the ecological quality of coralligenous in the impact sites, thus supporting the hypothesis of a low resilience of coralligenous reefs. A longer time (i.e., tens of years) will be necessary for most of the structuring species (i.e., gorgonians) and for calcified organisms (i.e., bryozoans and scleractinians) to recover from the physical impact of gears.

The monitoring activities carried out at Capo Carbonara MPA represent an effective example on how the AFG removal is recommended when it is done by trained operators and in a way that does not create further damages to the sessile communities. In addition to its natural and ecological value, coralligenous habitat also has an aesthetic and economic value, as it represents an attractive for divers (Rodrigues *et al.*, 2016). The maintenance (or restoration) of the seascape integrity, as requested by the Marine Strategy Framework Directive (2008/56/EC), must always include the cleaning of AFG from the bottom. Similarly, the removal of marine litter (including AFG) must be considered in the conservation activities of any Marine Protected Area.

Before planning any activity for litter removal to restore the natural features of marine environment and to recover the seafloor integrity, a comprehensive characterization and recognition of all the anthropogenic items occurring into the sea would be mandatory. This requires underwater visual surveys to identify, firstly, their nature and abundance and, then, to precisely map their geographical location, all information needed to plan the following removal interventions. Again, no standardised protocols exist yet in the Mediterranean to lead these kinds of initiatives. A recent example of monitoring activities required in the frame of an Environmental Impact Assessment (EIA) procedure for an infrastructure construction at sea in the Ligurian Sea (Genoa, Italy), included the monitoring of all the descriptors suggested by the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC) to evaluate the good environmental status (Montefalcone and Mancini, 2023), including the management of marine litter (Descriptor 10). During the monitoring activities of the *ante-operam* phase, remote and georeferenced visual surveys through Remote Operated Vehicle (ROV) and sea truth activities conducted by scuba diving were carried out to define the presence of marine litter on the seafloor of the monitored area. These surveys allowed recording the presence and the abundance of objects abandoned on the bottom, easily visible and identifiable through visual surveys. The proposed protocol required a qualitative and quantitative visual census of the waste present along the video transects (underwater ROV video) conducted in a bathymetric range of approximately 0-60 m. Data collected directly underwater by divers (between 0 to 40 m depth) during sea truth activities and analysis of the ROV video footages made it possible to develop a new multimetric ecological index to evaluate the impact of the litter on benthic habitats. The index considered three different indicators: 1) type of waste; 2) abundance of waste; and 3) size of the waste. The index provided a synthetic evaluation and measure of the pressure of marine litter on the bottom (Montefalcone and Mancini, 2023).

Comprehensive ongoing monitoring programs allow us to evaluate the efficacy of legislation and coastal management policies. These programs have the potential to support site-specific management and produce extensive pollution maps on a large scale, ranging from regional to global levels, providing valuable information for decision-makers (Ribic *et al.*, 2010). In addition to the physical removal of marine litter, some interventions also concentrated on the proper management of the collected litter promoting their recycling and environmentally sustainable disposal. Given the global nature of the marine litter issue, it will be essential to deploy collaborative efforts among the Mediterranean countries and the international organizations to address the problem in a coordinated manner.

3 Conclusions

These guidelines provide an inventory of the most significant active restoration projects developed in the last decades in the Mediterranean Sea on marine habitats and on threatened species, also including examples of artificial reef installation aimed to enhance biodiversity and recent experiences in the assessment and removal of marine debris and litter, such as abandoned fishing gears.

The frame of each section (for species and habitat) includes: i) description of the overall ecological status of species/habitat in the Mediterranean Sea and the context for its restoration; ii) main causes of degradation; iii) examples of ecological restoration available in literature (from grey literature and scientific papers); iv) international, national, and local pilot projects related to species/habitats restoration; and iv) subjects involved in the restoration.

For each restoration intervention reported, the following details have been described (when available): i) sites (donor and receiving) of the intervention; ii) environmental context of the donor and the receiving sites; iii) methods and materials used; iv) numbers of individual transplanted, surfaces restored; v) indicators and time used in the monitoring; vi) success rate; vii) strengths and limits of each methodology adopted for restoration.

The guidelines distinguish the most successful experiences (i.e., those reaching the highest survival rates after a predefined time interval), which can be considered as the "best" restoration technics so far, from those attempts and experiments that are still considered as research and development. A restoration success, although not commonly agreed, is defined as a highly successful ecological restoration project/study where the restoration goals achieved ≥ 50% survival of restored organisms for the entire intervention area, whilst a restoration failure has an outcome of ≤ 10% survival of restored organisms (Fraschetti *et al.*, 2021). It must be, anyway, taken in mind that the transplanting success, which relates to a specific target survival value to be reached within a predefined time interval after transplantation or restocking, is different from a true "restoration success", which would imply the recovery of habitat structure, species composition, ecological functioning, and ecosystem services that have been lost in a specific site (Pergent-Martini *et al.*, 2023).

From each species/habitat restoration intervention described in this manual, some lessons and best practices can be learnt, especially considering the successful examples (i.e., those that achieved ≥ 50%

survival of restored organisms for the entire intervention area). Measures and lessons can also be learnt from previous non-successful experiments. Suggestions on the way forward (e.g., environmental context of the donor and the receiving sites, methods and materials, proper substrates, anchoring and fixation, monitoring time, and descriptors to be measured) can be drawn from the various examples available in literature. However, the true “restoration success”, which would imply the recovery of the full set of ecological functions and services, has never been evaluated to date, even considering those long-term examples where the restored species/habitat have been surviving for long time (e.g., more than 35 years for the oldest restoration intervention made for *Posidonia oceanica*). It is true that the restoration of ecological functioning and services would require long time, likely decades for slow-growing species such as seagrass and gorgonians. Since most of the restoration interventions have been developed only recently, these unconsidered aspects in ecological restoration will require specific efforts in the future monitoring activities.

Similarly, the role of active restoration to ensure carbon sink optimization and buffering resilience to climate extremes has been widely recognized and discussed, but it has been rarely addressed. The increasing necessity to reduce atmospheric carbon dioxide (CO_2) concentrations pushed to quantifying the capacity of coastal ecosystems to sequester carbon, referred to commonly as ‘Blue Carbon’ (Monnier *et al.*, 2022). Among coastal habitats, seagrass meadows are considered as natural carbon sinks due to their capacity to store large amounts of carbon in their sediments over long periods of time. Seagrass are mainly involved in carbon capture from the atmosphere, carbon fixation in the biomass of the living canopy (i.e., the aboveground living biomass, representing the organic matter of the seagrass meadow resulting from carbon capture/fixation), carbon sequestration (i.e., the part of the aboveground living biomass from canopy going into the belowground biomass of the matte through burial processes), and the carbon stock (the organic carbon accumulated over the last millennia in the long-living tissues of the matte) (Monnier *et al.*, 2022).

Recovery of those ecosystems that act as carbon sink, such as seagrass meadows and algal forests, will help mitigating the effects of climate change. For instance, the carbon stocks in the first 2.5 m of matte in *Posidonia oceanica* meadows have been estimated ranging between 5.6-36.9 million t C_{org}, corresponding to 11.6-76.8 years of CO_2 emissions from the population of Corsica (Monnier *et al.*, 2022). This sounds particularly attractive for private companies wishing to develop approaches on the potential generation of carbon credits from ecosystem restoration. However, development of the exceptionally high matte structures in *P. oceanica* (reaching several meters in height) requires millennia (Monnier *et al.*, 2022), thus shifting the issue of Blue Carbon sink optimization following active restoration interventions to a temporal scale that is far away from the current human interest. Considering that the matte of *P. oceanica* grows in height for about less than 1 cm by year at the Mediterranean level (Pergent *et al.*, 2001), it is difficult to quantify the carbon stock, and thus the carbon credits, resulting from a newly settled restoration intervention at the pilot scales reached to date.

The restoration interventions made on target species focused only on threatened or protected species. Active restocking thus represents the necessary solution to ensure the persistence of these populations severely affected by human exploitation (such as *Patella ferruginea* and *Corallium rubrum*) or by climate change effects (such as *Pinna nobilis* and gorgonians). Effective and successful restoration methods have been developed for some species (e.g., *Patella ferruginea* and gorgonians) but not yet for others (e.g., *Pinna nobilis*), thus requiring further efforts in the coming years to obtain successful results and protocols to up scale the procedures. The EC Life Pinna project, aimed at restoking *Pinna nobilis* in areas where it is locally extinct, is really challenging as the adult survivors are continuously declining from the few donor areas that remain in the Mediterranean Sea.

The restoration interventions carried out on marine habitats are designed to support their natural recovery. Active restoration projects are thus implemented where the habitat showed alarming signs of decline due to past human pressures, providing the full recovery of the environmental condition regime. This is especially the case of algal forests and seagrass meadows that experienced dramatic reductions in the Mediterranean Sea in the last century. To date, a significant number of successful experiences has been developed for both macroalgae and seagrass active restoration. Restoration of the *Cystoseira* s.l. vegetated habitats has been the target of international projects (one still ongoing) that provided the best practices for the *ex-situ* cultivation of these species and for their subsequent transplantation in nature. Implementation of restoration programs on this habitat in the last years provided insights into the processes followed and the best practices learned from successful interventions, which resulted in recommended protocols that will be used in the next years for scaling up the procedures at all levels and for making them transferable to other areas.

Seagrass meadows active restoration interventions benefit from the longest experience and tradition in the Mediterranean. Meadows of *Zostera* spp. and of *Cymodocea nodosa* have been successfully transplanted in many areas with various methodologies, including both cuttings and seedlings. Experiences on *Posidonia oceanica* restoration are the most widespread today, and the longest carried out, providing a good knowledge of successful procedures that can be translated in lessons and best practices needed to scale up the procedures at all levels. Some general best practices can be recognized for *P. oceanica* transplantation (some of these can also be generalised to other restored/restoked species), which can also be found in the recent synthesis made by the Mediterranean Posidonia Network (MPN) (Pergent-Marini *et al.*, 2023).

Selection of donor and receiving sites:

- A comprehensive environmental and socioeconomic characterization of both the donor and the receiving sites must be carefully defined before planning any ecological restoration project. Receiving sites must display similar environmental and ecological conditions of the donor sites;
- It is mandatory that a suitable receiving restoration site provides historical existence of the restored species;
- The selected receiving site must provide evidence of natural recolonization;
- The receiving site must have a sustainable environmental regime, which means that all the main disturbances that caused meadow regression in the past have been eliminated or significantly reduced and the environmental conditions have been fully restored;
- The restoration offers a chance for the system to recover more quickly, once the protective actions have been implemented and the pressures are no longer acting or have been minimized;
- The receiving site should have an adequate and effective level of protection, especially in the first months after the restoration when the transplanted shoots are more vulnerable to physical damages.
- Transplanting should be carried out within areas where means of surveillance and controls are available, like Marine Protected Areas or areas subjected to any kind of protection (such as SCIs and SCZs), or at least areas regulated through buoy fields on the surface, to avoid any mechanical human disturbances;
- The responsible authorities must be involved in all the phases of the restoration project, also to obtain the required permits to implement them;
- The transplantation intervention must be included in an overall meadow management strategy at large spatial scale, involving stakeholders (promoters, financers, responsible environment authorities, users) and local communities.

Transplanting with cuttings:

- Cuttings or fragments of rhizome generated by storms, hydrodynamics, and other physical damages (e.g., anchoring) should be a good solution. They can be found in the beach cast after a storm event or drifting underwater. Fragments extracted from drifting blocks of meadow originated by anchoring or other mechanical damages can be used. The use of drifting fragments provides no damage to a donor meadow, although the origin of the fragments may be unknown with uncertain regarding the performance of those drifting fragments;

- When cuttings are harvested from donor meadows, less than 2 cuttings per m² must be collected to preserve existing meadows;
- The depth where cuttings must be harvested at the donor site depends on the depth at which we want to carry out transplants. Most of the successful restorations have been carried out at depths between 10 m and 18 m depth, which is considered the optimal depth range where a meadow can thrive, ensuring a good compromise between enough light intensity and low hydrodynamics;
- Amplified success is reached when the receiving and the donor sites have the same depths. When fragments are transplanted at shallower depths than the donor site (or where they have been collected) the survival also increases;
- The restoration successes increases when the proximity to the donor site diminishes;
- Preparation of cuttings (e.g., cleaning, cutting) before transplantation seems relevant to stimulate roots germination and growth once the cuttings are transplanted on the receiving substrate;
- The most feasible transplanting season is the spring, when the plant starts growing quicklier and have sufficient reserves;
- Plagiotropic cuttings are more successfully transplanted than orthotropic ones;
- Cuttings should have at least 3 shoots (1 plagiotropic and at least 2 orthotropic) or a long rhizome section (>10 cm). Cuttings with only one orthotropic shoot showed lower ramification rate and root formation than plagiotropic fragments;
- The best substrate that maximizes the survival of transplants is dead matte, followed by sandy bottoms colonized by *C. nodosa* and/or macroalgae. Transplants done on unvegetated bare sand had very low survival rates after a few months, regardless of the anchorage;
- The use of biodegradable structures in restoration is recommended, or structure that can be easily removed after the necessary time allowing the cuttings to anchor (more or less 3 years);
- Several transplantation methods have been proposed and tested. The fixing of the cuttings within the bottom is crucial, so the fixing method must be chosen appropriately, and the durability of the anchoring system must be considered. The use of different types of grids, plastic, coated wire, or natural fiber fixed by a heavy frame or unframed but anchored by pickets or similar, showed good results;
- Biomats made by natural coconut fibers fixed by a heavy frame have been widely adopted in recent years; they have a long experience and showed the highest survival rates, also for long-term. Similar successful results have been reached with biobased plastic radial structure made with starch;
- The use of individual anchoring on single cuttings, natural or metallic staples or pegs, had also good results;
- Positioning single cuttings among rocks or rubbles, without extra anchoring, had high percentage of failure and loss of transplants;
- Non-covered metallic grid is discouraged;
- The spacing between the cuttings and the arrangement of the cuttings must reflect the development of the natural meadow and be consistent with the growth rate of *P. oceanica*. Fragments transplanted up to 5-10 cm apart had better survival.

Transplanting with seedlings:

- Seedlings originated from fruits collected at the beach (after mass sexual reproductive events) are suitable for transplant after a period of germination in controlled environment;
- Seedlings grown for at least 2 months in the laboratory have been successfully planted;
- The substratum complexity and roughness favors seedling retention and anchorage. Vegetated stable substrata (i.e., rock and dead matte) maximize the natural recruitment, the growth and the survival of seedlings;

- Transplanting seedlings on living meadows, on sand or on gravel is not feasible, and these substrates should be discarded;
- Seedling experiments on dead matte showed higher survival rates at intermediate depths (10 m) than at shallower depths (2 m);
- Seedlings transplantation in areas with less exposure had a better survival rate;
- Seedlings do not benefit from artificial anchoring;
- Fixing the seedlings on substrates made by natural fibers might increase their stability and survival;
- Seedlings protection with cages or nets might provide protection from herbivores.

Protocol for monitoring:

- Monitoring activities should be planned for at least 5 years after the conclusion of the transplantation activities. A regular monitoring, at least one by year, must be undertaken;
- The classical health descriptors for *P. oceanica* can be measured during monitoring: % survival of the cuttings, shoot density, number of shoots per cuttings, % of seeds turning to seedlings, number of seedlings branching and turning into small clones, etc.;
- Underwater photogrammetric technologies may be implemented to acquire high resolution information and to elaborate micro-cartographies useful to monitor the dynamics of *P. oceanica* restorations;
- The time needed for recovering the different ecosystem functions after transplanting should be investigated in future monitoring activities. Scientific evidence on a proper period of functional recovery monitoring is not yet available;
- Assessments regarding the role of transplanted meadows for Blue Carbon sequestration and stock, in the frame of climate change mitigation, should be enforced.

Restoration activities involving artificial reefs installation to enhance biodiversity and removal of marine debris and litter have been implemented only recently in the frame of the UN Decade on Ecosystem Restoration. Experiences in this context are still limited and did not provide any standardised protocol and recognised best practices.

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ISBN 978-9938-79-373-4