S.E.POS.S.O.
life project

Supporting Environmental governance
for the POSidonia oceanica Sustainable
transplanting Operations

Life

NATURA 2000

Produced with the contribution of the LIFE Programme of the European Union project LIFE 16 GIE/IT/000761

MANUAL
of techniques and procedures for the transplantation of *Posidonia oceanica*

ISPRA
Istituto Superiore per la Protezione
e la Ricerca Ambientale

Sistema Nazionale
per la Protezione
dell'Ambiente

Consiglio Nazionale
delle Ricerche

UNIVERSITÀ
DEGLI STUDI
DI PALERMO

ARPAT
Agenzia regionale
per la protezione ambientale
della Toscana

SETIN srl
Servizi Tecnici Infrastrutture

vesenda

Autorità di Sistema Portuale
del Mar Tirreno Settentrionale
Porti di Livorno, Piombino,
Capraia Isola, Portoferraio, Rio Marina, Cavo

Università di Roma
Tor Vergata

Produced with the contribution of the LIFE Programme
of the European Union project LIFE 16 GIE/IT/000761

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CITATION

Bacci T., La Porta B. (2021). Manual of techniques and procedures for the transplantation of *Posidonia oceanica*. LIFE SEPOSSO (LIFE 16 GIE/IT/000761), Rome.

Edition: May 2022

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PREFACE

19.1% of Italian waters are now subject to conservation measures, however, in order to reach the objectives of the EU Biodiversity Strategy by 2030, this percentage must increase significantly. To this end, the italian National Recovery and Resilience Plan (PNRR) envisages large-scale interventions to restore and protect the seabed and marine habitats in national waters, so as to reverse the trend in degradation of Mediterranean ecosystems and enhance their resilience to climate change.

The meadows of *Posidonia oceanica* are an endemic habitat of the Mediterranean and are protected under the Habitat Directive 1992/43/EEC. Although *P. oceanica* occupies 1% of the Mediterranean seabed, the plant plays an essential role in the balance of the marine ecosystem, producing about 20 l oxygen/m^2, removing carbon dioxide from the environment, countering climate change, hosting about 25% of the marine biodiversity of the Mediterranean and helping to counteract coastal erosion thanks to its dense leaf cover and stabilising sandy seabeds by trapping sediments in the typical terrace structure called *matte*. Unfortunately, the meadows of *P. oceanica* are in regression in various parts of the Mediterranean basin and it is estimated that their surface area has decreased by more than 30% over the last 50 years. Human activities and related forms of pollution are among the main threats to this ecosystem.

The restoration of this precious habitat through sustainable and effective transplantation activities, in synergy with the many protection and conservation actions, will not only contribute to the achievement of national and European objectives for biodiversity and climate change but will also favour the maintenance and sustainability of fundamental activities for the coastal areas of Italy, such as fishing, tourism and blue growth, in compliance with European environmental directives (*e.g. Habitat Directive 1992/43/CEE, Marine Strategy Framework Directive 2006/56/EC, Maritime Spatial Planning Directive 2014/89/EU, Water Framework Directive 2000/60/EC,* *Environmental Impact Assessment Directive 2014/52/EC, Aarhus Convention, 25 June 1998*).

The LIFE SEPOSSO (*Supporting Environmental governance for the POSidonia oceanica Sustainable transplanting Operations* - LIFE16 GIE/IT/000761) project coordinated by the Italian Institute for Environmental Protection and Research (ISPRA), together with the partners and numerous actors involved, verified the outcome of transplantation carried out in Italy to date and provided specific instruments to improve them.

The "Manual of techniques and procedures for the transplantation of *Posidonia oceanica*", constitutes one of these tools and provides experts and the various stakeholders involved with detailed information and executive methods on the main transplantation techniques used along the coast of Italy and in the Mediterranean.

The accuracy of the information provided is based on the fact that, to date, Italy has invested the most in research and testing of *P. oceanica* transplantation in the Mediterranean basin. Since the 1980s numerous experiments and, since the 2000s, extensive transplantation have been carried out in Italy, some of which are in progress, both for the recovery of degraded meadows and to compensate for the damage caused by coastal works and infrastructure. A number of Italy's leading experts in the transplantation of *P. oceanica*, both LIFE SEPOSSO partners and those external to the project, have contributed to this Manual with the shared purpose of henance the knowledge on transplants and contributing to the common project of restoring this invaluable and delicate Mediterranean habitat.

National Center for the Laboratories Network
Head of Department
Dott. Damiano Centioli

CHAPTER 1

POSIDONIA OCEANICA TRANSPLANTATION TECHNIQUES AND THEIR APPLICATION

1.1 | THE TRANSPLANTATION OF POSIDONIA OCEANICA CUTTINGS

Early experimental transplantation of *Posidonia oceanica* using techniques generally employed to strengthen and improve the conservation status of meadows of other marine phanerogams did not have a positive outcome. Further experiments, starting from those conducted by the French school of Georges Cooper and the *Jardiniers de la Mer* (Cooper, 1982; Augier *et al.*, 1996) and subsequent experiments carried out mainly in Italy, revealed more encouraging results in terms of the survival of the *P. oceanica* transplants over time. The experience gained from previous failures, new discoveries in the biology of the plant, the use of new technologies, as well as the availability of transplant monitoring data over long periods has made it possible to perfect and devise increasingly effective and sustainable transplant techniques (https://lifeseposso.eu; Bacci *et al.*, 2019; Badalamenti *et al.*, 2015; Bouderesque *et al.*, 2021; Calvo *et al.*, 2021; Piazzi *et al.*, 2021).

The two main factors on which the success of *P. oceanica* transplantation depends are the choice of the recipient site and the adoption of the most suitable technique for the type of substratum of the site. Success also depends on the specific methods of execution of the technique, which divers must scrupulously perform, and certain precautions that are sometimes crucial for effective and long-lasting transplantation. (https://lifeseposso.eu; AA.VV., 2020).

A number of anchoring modules, such as concrete frames with metal mesh, different types of metal grids and stakes are, to date, among the most frequently used techniques for fixing *P. oceanica* cuttings to the substratum. Some transplantation sites where these techniques were deployed in the past have now become unique sources of valuable insight essential to the analysis of the long-term evolution of transplanted meadows and the effectiveness of the technique. Over time, additional anchoring methods, such as geomats and biomats, mattresses of various types and anchoring modules in bioplastic, with increasing focus on the environmental sustainability of the technique, have been devised and tested.

The main techniques applied in Italy for transplanting cuttings of *P. oceanica* in the context of environmental restoring or compensation for damage caused to the meadows by marine-coastal works subjected to Environmental Impact Assessment are described below. Together with the transplantation of cuttings, additional methods of meadow reforestation have been developed. These include the transfer of *P. oceanica* clods comprising both plants and the underlying *matte*, the creation of consolidated substrata to accelerate the natural *Posidonia* recolonisation process and the use of seeds and seedlings as transplant material. Experience related to these methods is described in the chapters below.

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1.1.1 | Reinforced concrete frames with wire mesh

Michele Scardi[1]*, Maria Luigi Valiante*[2]

History of the technique

The *Posidonia oceanica* transplanting technique based on concrete frames reinforced with wire mesh was introduced to Italy by Eugenio Fresi at the beginning of the 90s, based on the experiment conducted in France in the 70s by Georges Cooper and the *Jardiniers de la Mer* group (Augier *et al.*, 1996; Cooper 1982). The transplants in France had been carried out on limited surface areas of several hundred square metres and the early experiments in Italy, which took place in the harbour of Porto Conte (Sardinia) and on the seabed in front of the beach, Le Bombarde, just west of Alghero, covered an even smaller area measured in tens of square metres. The results in both countries were positive; the frames used in Italy, however, which had been designed to be less bulky by using a lighter mesh, were arranged in single layers only, in order to improve degradability. It was precisely the aim of minimising the impact on the seabed that lead to experimenting with increasingly lighter and degradable modules, with the aim of favouring the natural disintegration first of the metal mesh and then of the concrete frame, for example, by the abrasive action of sediment.

Brief description of the technique

The quadrilateral concrete frames used as anchoring supports for the cuttings measure 50 cm on each side (*fig. 1*). The internal lumen measures 40 cm on each side, with a surface area of 1600 cm^2, and the thickness of the frame is about 5 cm. The frames are reinforced with polygonal galvanised wire mesh, the mesh opening size of which

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(circa 1.5-2 cm) is adequate for holding the cuttings. The wire mesh must be sufficiently ductile to allow the meshes to be squeezed to secure the cuttings to the frame more effectively.

The cement content of the frames should be as low as possible to ensure a sufficiently rapid degradation whilst not rendering the frames excessively fragile. To prevent the frames from fracturing, it is good practice to place them on pallets as they are made and then to lower them onto the seabed on the pallets and thereafter handle the frames manually.

The frames are covered by italian patent no. 0001360267 ("Anchoring structure for plant organisms").

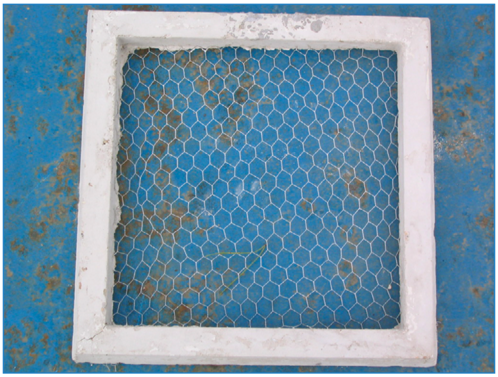

Figure 1 | Anchoring module of cuttings.

Anchoring substratum

Soft seabeds are suitable for laying the concrete frames, as long as the granulometry of the sediment is not so coarse as to suggest the existence of conditions of strong hydrodynamic stress. The latter could displace the frames or submerge them with sediment thereby damaging the transplants. A substratum with coarse sand is obviously not an obstacle, but its dynamism must be carefully evaluated. From this point of view, finer psammitic granulometries may be ideal, as long as they are not combined with an excessively abundant pelitic fraction.

The ideal conditions, for intensity of descending irradiance and for attenuated hydrodynamics, are encountered between 10 and 15 m depth, with any exceptions related to the specific characteristics of the area. Given the ideal texture of the sediment, as specified above, the slope of the seabed should not be particularly pronounced.

Transplantation method

In the first place, when identifying areas suitable for explantation, both the quality of the shoots and their density are taken into consideration, trying to favour explantation sites that are no shallower than those for reimplantation, as it was observed that this condition does not aid the probability of success for the transplantation. In the areas considered optimal for explantation, the density of the shoots and the size of the areas themselves are determined, in order to estimate the total number of cuttings that can be taken.

To date, removal has always been carried out in meadow areas destined for certain destruction due to dredging or the excavation of trenches for pipeline laying. However, even in the context of marked orientation towards conservation (Diaz-Almela and Duarte, 2008), it was deemed possible to carry out the low-density removal of shoots from meadows that are in good condition and capable of quickly offsetting the removal of a small number of shoots to be used for transplantation.

For the identification of suitable sites for transplantation, preference is given to clearings that are located at a depth of approximately 12 m. In the candidate clearings, the apparent health status of the surrounding meadow is estimated, with an expeditious investigation into the lithology of the area and the existence of organic debris, ripple marks, dead *matte* macroalgae and, if at the edge of the meadow, the type of boundary. Furthermore, the presence of anthropogenic pressure “markers” such as abandoned nets, signs of repeated anchoring, mooring post, debris and waste are also detected. Finally, the dimensions of the clearings considered optimal are estimated or, in the case of sites not entirely surrounded by meadows, the surface area deemed useful is evaluated.

In selecting the areas in which to carry out transplantation, those favoured are of intermediate size (e.g. 100-200 m^2), protected by surrounding meadows and free from evident erosive phenomena and sediment mobility. On the contrary, it may not be advisable to consider small surface areas (e.g. 10-20 m^2), to avoid excessive fragmentation of the intervention and therefore making effective monitoring impossible. To optimise operations and minimise downtime, it is advisable to lay the transplantation modules (*fig. 2a*) before proceeding with the explantation of the shoots from which the cuttings for transplantation can be obtained (*fig. 2b*).

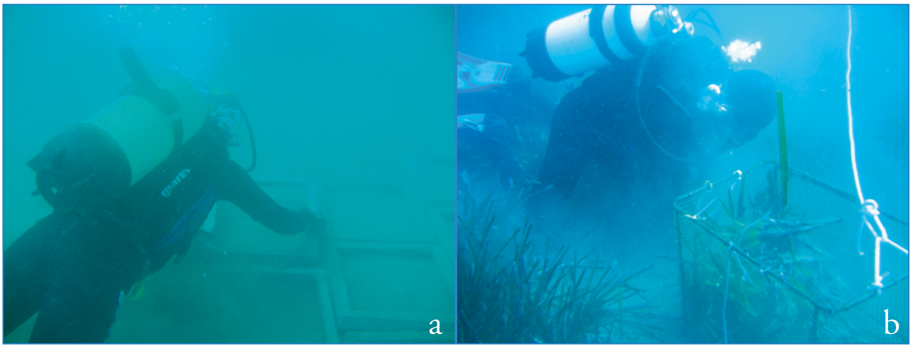

Figure 2 | Laying reimplantation modules (a); explantation of shoots from which to obtain individual cuttings (b).

The shoots from which individual cuttings will be obtained must be explanted with care to avoid damaging them during harvesting. "Clods" (multiple foliar shoots with their respective rhizomes and roots, together with trapped sediment), of different sizes, can also be taken, which can then be broken up to produce single cuttings. When taken from non-margin portions of the meadow, the rhizomes from which the cuttings will be obtained will be predominantly orthotropic. The selection of the cuttings must consider numerous factors, which concern not only the general state of each shoot, but also the state of the leaves, the presence of roots and the thickness of the rhizome. Apart from very brief spells during transport and handling, the cuttings are carried in special rigid mesh containers, taking care that they are constantly immersed in seawater at temperatures as close as possible to those of the depth of removal and in any case not lower than 15 °C and not higher than 25 °C.

The cuttings are planted by hand by divers (*fig. 3*), who insert the rhizomes into the polygonal meshes so that the rhizome or the roots are in contact with the sediment. The wire mesh is then squeezed so that the cuttings are firmly secured to the anchoring module. The modules do not need to be fixed to the substratum or joined to each other.

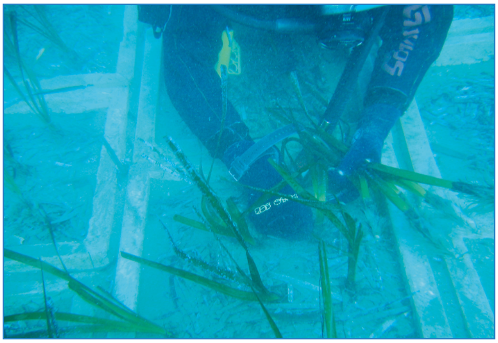

Figure 3 | Transplanting the cuttings.

Executive measures

Experience has shown that the use of unskilled personnel is one of the main reasons for transplantation failure. Therefore, the involvement of persons with specific expertise is recommended. The installation of the modules and the handling of removed plant material can be carried out by Commercial Divers. Removal and planting of the cuttings and the management of the site must, on the other hand, be carried out by Commercial Divers who are professionally recognised graduates in biological disciplines and have experience in handling *Posidonia* cuttings for transplantation. A similar background in biology, but not necessarily with underwater experience, is essential for those who prepare the cuttings at the surface, when the utmost care must be taken during handling, in maintaining the temperature and in minimising exposure to air.

After 10 years, it has been observed that where the transplantation had been carried out methodically, the meadow established is structurally and functionally comparable to a natural meadow, apart from the vertical development of the matte, which, over this period of time nevertheless begins to form.

Figure 4 shows the evolution of an area from the end of transplantation to the moment in which the modules disappear in the sediment, after 1 year, and then again after 5 and 10 years, as the density of the shoots progressively increases.

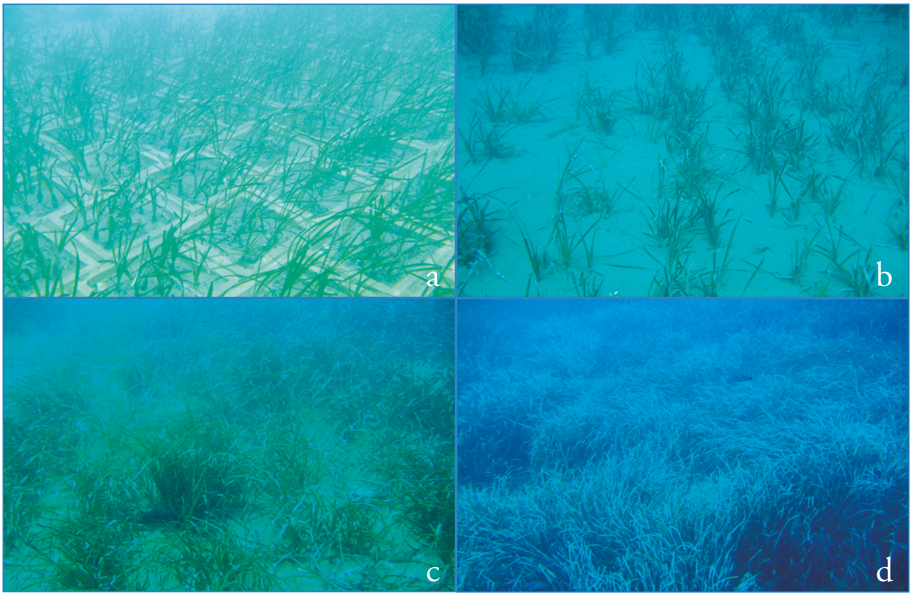

Figure 4 | A transplanted area, immediately after transplantation (a), after one year, with the disappearance of the modules in the sediment (b) and then after 5 years (c) and 10 years(d).

Environmental sustainability of the technique

The concrete frames are built in such a way as to facilitate slow disintegration, but clearly this process takes place over a long period of time and above all depends on the extent to which the frames are exposed to hydrodynamic action. Obviously, once they have been incorporated into the *matte* that forms when the transplantation assumes densities equal to or greater than the natural meadow (AA. VV., 2020), the process of disintegration of the frames could be halted.

Whilst the seascape is evidently modified by the installation, as the frames tend to sink quickly into the sediment, they may no longer be visible within a matter of months. The complete integration with the marine landscape begins after about 5 years, when the increased density of the shoots begins to be appreciable; ten years after the transplantation the frames have virtually disappeared (AA. VV., 2020). Until this happens, plant and animal organisms that are normally found on small rocky outcrops are attracted to the solid substratum formed.

With the beginning of the formation of the *matte*, which is evident approximately 10 years after transplantation, carbon sequestration values may begin to liken those typical of natural *Posidonia* meadows.

Period of intervention

Explantation and reimplantation have always been carried out in the autumn and winter, on the assumption that this is the phase of the plant's life cycle in which vegetative activity is less intense. This choice was based on the precautionary principle, but currently there is no counter evidence on a sufficiently wide spatial scale.

Case studies

Summary table of the case studies in Italy where the transplantation technique and operative procedures as described were used, all at depths between 7 and 13 m.

Table 1.1.1 | Transplants made in Italy with concrete frames with wire mesh

Transplantation site	Transplantation substratum/ Depth	Transplantation start (year)	Transplantation surface area (m^2)	Transplanted shoots (~ No.)	Monitoring time span (years)	Monitoring context/references
Porto Conte and Alghero (Sardegna)	sand/10 m	1989	40 m^2	1.280	2 years (1989 - 1991)	EU-ENEL-Italian Ministry for Merchant Shipping Project
S. Marinella (Lazio)	sand/7-13 m	2004	10000 m^2	306.592	16 years (2005-2010; 2015-2021)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2020, Bacci *et al.*, 2019, Scardi *et al.*, in press)
Pioppi Pollica (Campania)	sand/10m	2005	10 m^2	320	5 years (2005-2010)	Environmental Impact Assessment (control area for S. Marinella)
La Maddalena (Sardegna)	sand/7,5 m	2005	10 m^2	320	1 year (2005-2006)	Environmental Impact Assessment (control area for S. Marinella)
Ischia Island of Ischia (Campania)	sand/7-9 m	2009	1600 m^2	50.032	12 years (2009-2021)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2020, Bacci *et al.*, 2019, Scardi et al., in press)
Meloria Shallows Livorno (Liguria)	sand/7 m	2011	2 m^2	64	8 years (2011-2016; 2019)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2021)
Pioppi Pollica (Campania)	sand/10m	2013	3 m^2	96	-	Demonstration activity - Tree Festival (Legambiente)
Acciaroli Pollica (Campania)	sand/12-15m	2014	5 m^2	160	-	Experimental activity

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1.1.2 | Metal grids

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History of the technique

Grids of various types were used in the transplantation of *Posidonia oceanica* in the late 1980s in France (Meinesz *et al.*, 1992; Meinesz *et al.*, 1993; Molenaar and Meinesz, 1992; Molenaar *et al.*, 1993) and were subsequently used in different transplantations in the Mediterranean Sea, especially in Italy, both for experimental purposes and in the context of environmental assessments (e.g.: Piazzi *et al.*,1998; Lepoint *et al.*, 2004; Pereda-Briones *et al.*, 2018; Calvo *et al.*, 2021).

Regarding environmental assessments, the *Posidonia oceanica* transplantation technique using electro-welded metal grids was applied in 2008 in Italy (Pirrotta *et al.*, 2015) along the north-western coast of Sicily, in a degraded coastal marine area of the Gulf of Palermo, as a compensatory measure for the environmental impact due to the construction of the underwater protection barrier, aimed at counteracting the erosion of the beach in Arenella, within the same geographical area. The site of the intervention, selected using the multi-criteria model (Pirrotta *et al.*, 2015), is located on a seabed characterised by dead *matte* structures. Transplantation using metal grids, with specific reference to the experience gained in the Gulf of Palermo case study, is described below.

Brief description of the technique

The anchoring support consists of a 1 x 1 m electro-welded metal

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grid, with Ø 2 mm, 50 x 50 mm mesh, weighing 1.3 kg; this can be anchored to the *matte* by means of ~70 cm long iron hooked starters or fixed to hard substrata using expansion bolts and screws. About twenty *P. oceanica* cuttings carrying at least three shoots, with a final density of ~ 60 shoots/m^2, can be fixed to each metal grid by means of biodegradable tear strips or metal wires. The anchoring support can also be conveniently adapted to other plant organisms.

The simplicity of the structure and the ease of sourcing and handling the components mean that the metal grid can be easily and economically used in seabed restoration using *P. oceanica*.

Anchoring substratum

The metal grid anchoring support can be used effectively in transplantation on dead *matte*. Sandy substrata do not seem to be suitable for the use of metal grids (Scardi *et al.*, 2014), and the literature is scarce regarding rocky substrata.

Transplantation method

The donor meadow should preferably be located near the recipient site. The removal of cuttings/rhizomes will be limited to the plants that colonise the edges of the meadow and will be performed according to sustainability criteria, with a pressure not exceeding 1% shoots/m^2 (Díaz-Almela and Duarte, 2008). Alternatively, to reduce and/or cancel the impact of removal on the donor meadow, rhizomes and cuttings that have become detached and been transported over the seabed as a result of hydrodynamic action can be collected easily and in large numbers in coastal marine areas, where they accumulate to a greater degree during the autumn – winter period (Balestri *et al.*, 2011). The recovery of naturally detached rhizomes or cuttings, however, presupposes planning so that they can be used within a few days to avoid further stress to the plant and increase the chances of success of the transplantation.

Qualified underwater scientific operators with specific experience in handling *Posidonia*, will collect the plant material, mainly consisting of cuttings at least 15 cm long and carrying at least 3 shoots (*fig. 1*). Removal will be carried out where possible near to the recipient site, taking care, in the case of compensation for marine construction projects, to remove plant material from the areas that will be directly affected by the works or excavation, thus minimising the impact of removal. The plant material collected at the donor site will be stored in jute bags and transported to land immediately, immersed in water, for subsequent processing.

Figure 1 | Photographic representation of the main implementation phases of reforestation by means of metal grids, from the preparation of cuttings (a, b), to the placement and anchoring of grids on the bottom (c, d).

The plant material will be selected and subsequently fixed to the ground in the electro-welded metal grid by means of metal wires and/or tear strips, preferably in biodegradable material, taking care that the shoots face upwards from the support (*fig. 2*). Placing the cuttings/rhizomes in this way simulates the position adopted by most plants as they rest on and then anchor to the seabed, after becoming detached from their meadow of origin (Meinesz *et al.*, 1992).

Each metal grid can accommodate up to 20 cuttings/rhizomes for a total of 60 shoots in the case of cuttings. For the entire duration of fixing plants to the metal grid, the plant material will be kept constantly submerged to avoid dehydration.

In order to emulate the *Posidonia* colonisation pattern and to maintain the natural conditions of the marine phanerogam meadows (Olesen *et al.*, 2004; Sintes *et al.*, 2006), the anchoring supports can be arranged in rows, in quincunx and/or in patches formed by one or more grids placed a few meters apart in order to emulate the natural colonisation mechanism and the natural restoration potential of the meadows (Cunha *et al.*, 2012; Calvo *et al.*, 2021).

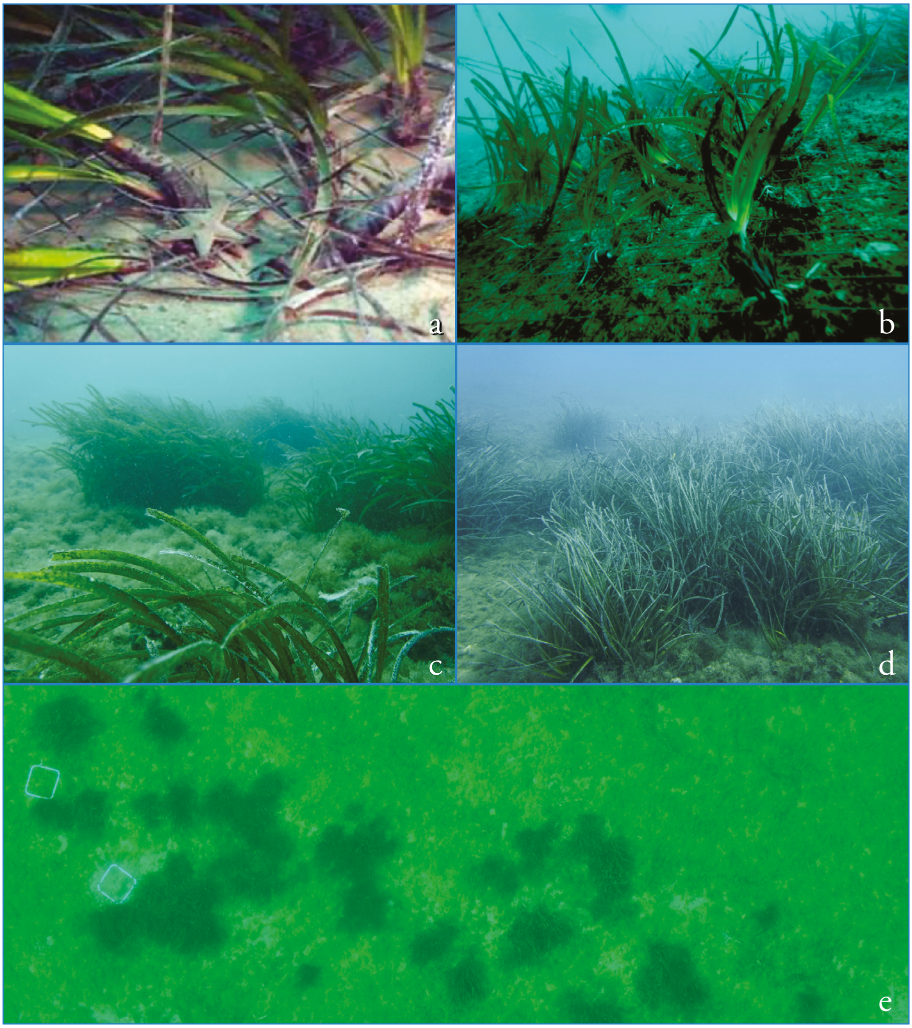

Figure 2 | Structural and functional evolution of the transplantation from 2008 (a), to 2012 (b) to 2020 (c,d); photo-mosaic of the reforestation pilot installation performed in May 2020 (e) (Calvo et al.*, 2021).*

Executive measures

- Before carrying out any large-scale transplantation, check the suitability of the site to receive the transplantation by means of a pilot transplant (Pirrotta *et al.*, 2015).
- The collection area of *P. oceanica* cuttings/rhizomes in the donor

meadow must be situated at a similar depth ≥ to that of the recipient site (Molenar and Meinesz, 1992).
- The plant material (cuttings and rhizomes) taken from the donor meadow must be transplanted within a few hours of explantation.
- The entire procedure on land of fixing cuttings/rhizomes to the electro-welded metal grid must be carried out in suitably sized tanks, keeping the plant material constantly submerged.
- Avoid excessive changes in temperature during transportation and fixing of the cuttings to the support.
- The choice of the donor site must take into account the distance from the recipient site and the quality of the donor meadow.
- Prohibit fishing and anchorage in the area of the transplantation.

Environmental sustainability of the technique

The size and weight of the structure that makes up the electro-welded metal grid makes the anchoring support light, easy to transport and handle and adaptable to substrata with varied morphologies. Furthermore, the small size favors the complete disintegration of the structure over a period of about 4-5 years, which is more than sufficient for the task of anchoring cuttings and rhizomes to the substratum and to enable rooting to take place. The use of tear strips in biodegradable plastic further favors the environmental sustainability of the technique.

Period of intervention

The favourable season for transplantation is late autumn – early winter. In fact, it has been found that mortality is highest for transplantation carried out in early summer, when temperatures exceed 20°C, and lowest for those carried out in autumn, with survival rates ranging between 92% and 97% (Meinesz *et al.*, 1992). Therefore, the actions of collecting the cuttings, setting up the metal grids and their implantation in the recipient site should preferably take place during the vegetative rest period of the plant. This is when the foliar apparatus is shorter, thus facilitating transplantation in all its phases, from collection to implantation.

Case studies

Summary table of the case study of the Gulf of Palermo and of the other case studies in Italy where the grid technique was used.

Table 1.1.2 | Transplants made in Italy using metal grids

Transplantation site	**Transplantation substratum/depth**	**Transplantation start (year)**	**Transplantation surface area (m^2)**	**Transplanted shoots (~ No.)**	**Monitoring time span (years)**	**Monitoring context/references**
Palermo (Sicily)	sand and dead *matte* 13-15 m	2007	15 m^2	900	1 year (2007-2008)	Environmental Impact Assessment (Pirrotta *et al.*, 2015)
Palermo (Sicily)	dead *matte* 14 m	2008	20 m^2	1.200	12 years (2008-2014; 2020)	Environmental Impact Assessment; LIFE SEPOSSO Project (Pirrotta et al., 2015; Calvo *et al.*, 2021)
Vada Rosignano (Tuscany)	dead *matte* 10 m	1994	400 m^2	3000	25 years (1994-1997; 2019)	Experimental transplantation; LIFE SEPOSSO Project (Piazzi *et al.*, 1998; (AA.VV., 2021)
Ischia Island of Ischia (Campania)	sand 7-9 m	2009	30 m^2	968	1 year (2009)	Environmental Impact Assessment (AA.VV., 2014);
Rapallo (Liguria)	dead *matte* 5 m	1996	10 m^2	618	23 years (1996; 2019)	Experimental transplantation (Robello, 2019)

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1.1.3 | Stakes

Giandomenico Ardizzone[1], *Gianluca Mancini*[1], *Andrea Belluscio*[1]

History of the technique

The technique involving the use of stakes of various kinds, sizes and shapes was first showed in Fonseca *et al.* (1982) for the transplantation of *Zoostera marina* Linnaeus 1753. Subsequently, stakes were also used for the transplantation of other phanerogams, such as *Posidonia oceanica,* both in experiments aimed at verifying the effectiveness of the stake as an anchoring system and/or analysing the survival rates of cuttings in different conditions of depth, substratum and spacing patterns (Genot *et al.*, 1994; Meinesz *et al.,* 1993; Molenaar and Meinesz, 1995; Robello, 2019) and in large-scale transplantation aimed at environmental restoration (Castejón-Silvo *et al.*, 2021).

In the case of the latter, the technique was recently used for transplanting *P. oceanica* on the Island of Giglio as part of an environmental restoration plan for the area impacted by the sinking of the Costa Concordia in 2012 (Bacci *et al.*, 2016; Mancini *et al.*, 2019). Transplantation using stakes is described below, with specific reference to the case study of the Island of Giglio.

Brief description of the technique

The stake used on the Island of Giglio is made up of two 0.6 cm diameter iron rods welded together in one or more points and each curved at one end to form two crescents, which have the purpose of holding the *Posidonia* rhizomes. The other end of the stake is pointed to facilitate penetration into the substratum. The tensile strength of the stakes, measured under water using a spring dynamometer, is 6 kg (59.5 N) on *matte*, more than enough to ensure

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that the rhizome is firmly held. The dimensions of the stake: length 30 cm, width 10 cm, each crescent is 5 cm wide and 8 cm long (*fig. 1*, *fig. 2*).

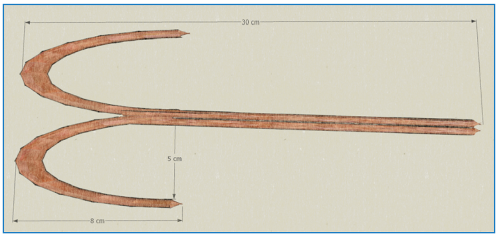

Figure 1 | The stake used for transplantation.

Figure 2 | Stake before anchoring to the bottom (a); stakes ready to be positioned to fix the rhizomes (b).

From this 30 cm long model, two variants have been developed. The first type, to be used on *matte*, is thinner and shorter (20 cm) and the second is longer (50 cm) and suitable for use on soft seabeds as necessary.

Anchoring substratum

The substratum on which the *Posidonia* transplantation was carried out on the Island of Giglio is made up of dead *matte*. This had been an area of *Posidonia* meadow, which died because of the Concordia shipwreck and the work carried out for its removal. After the removal of the ship and the shadow it created and eliminating the materials that covered the *matte* (sediment, cement, anthropogenic waste), the substratum was once again a suitable habitat for *Posidonia*.

The transplantation was carried out on the gently sloping seabed between 8 and 23 m; a limited experimental transplantation was carried out at greater depths, up to 30 m.

At the time of the transplantation, the dead *matte* was compact and not very damaged; it was covered by a thin layer of coarse sand in parts and had a substantial algal population and was therefore an optimal substratum for the transplanting technique using stakes.

A number of transplantation tests were also carried out on coarse sand (sand between 60 and 98% gravel). The “long” stake seems to keep the rhizome well anchored to the sandy substratum but given their availability, the transplantation was mainly carried out on surfaces covered with dead *matte*.

Transplantation method

The plant material used comprised both orthotropic and plagiotropic shoots, mostly derived from clods, which had become detached naturally due to hydrodynamics or from the anchoring of boats, found on the seabed (*fig. 3*).

Figure 3 | A bay on the Island of Giglio with numerous boats anchored on Posidonia oceanica *in the summer (a); a large clod detached from the meadow (b).*

The *Posidonia* clods are collected by divers and brought to the surface using plastic bags (nets) (*fig, 4*). On board the boat, the nets are immersed in containers of seawater and protected from the sun. For its conservation, the material is then transferred to the transplantation area where it is placed inside a special enclosure built on the seabed for up to a maximum of 3 days to avoid damaging rhizomes, shoots and leaves (*fig. 5*).

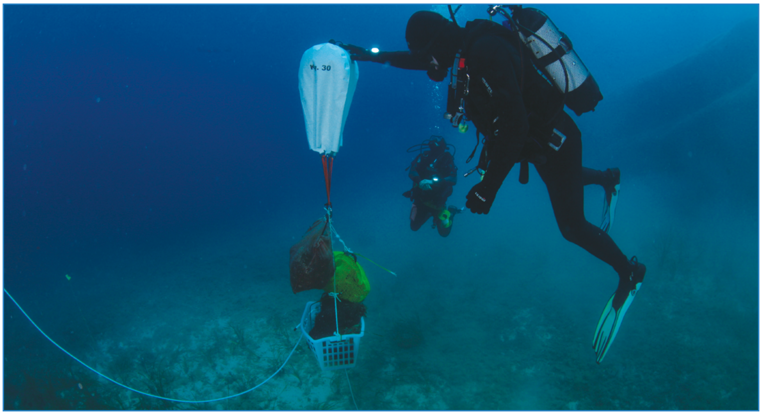

Figure 4| Transfer of biological material.

Figure 5 | Conservation in enclosure built on the seabed of collected Posidonia oceanica *cuttings.*

Before their transplantation, the clods are cleaned, and dead or damaged parts removed under water. The larger cuttings are divided into smaller parts 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. Orthotropic shoots have also been used.

Underwater operators implant the individual cuttings manually, fixing them by means of one or two stakes, depending on their length, to the substratum. The stake is pushed as far as possible by hand, after which a small hammer is used to better secure it in the substratum, if necessary. This operation is done with great care to prevent the stake from breaking the rhizome (*fig. 6*).

Figure 6 | Phases of transplantation when the operator places the cuttings inside the 1 x 1 m aluminium frame (a); detail of transplanted cuttings (b).

The seabed is prepared in advance by dividing the entire transplantation area into 10 x 10 m squares marked off with ropes fixed to the substratum and georeferenced. Inside the square, each operator has a 1 x 1 m portable aluminium *frame* within which transplantation begins. The *frame* is used to ensure 100% coverage of the area to be transplanted (*fig. 7a, b*).

Once the cuttings have been positioned within the metal frame, this is moved to the adjacent position where transplantation continues. This process is repeated until the transplantation of the whole area is completed. The cuttings are placed randomly, trying to space them out evenly. The density of the cuttings and shoots in the area transplanted on Giglio is 5-9 cuttings/m^2, corresponding to an average of 23-28 shoots/m^2.

Figure 7 | Using the 1 x 1 m square method (a), the positioning of the cuttings is carried out homogeneously throughout the area (b).

The advantages of this transplantation method include the ease and speed of execution under water and not having to move heavy materials from one point to another or build structures above or below the water. This means that pontoons on the surface and work sites on shore are not necessary.

Executive measures

Any issues with the transplantation method regard the use of transplanting material that is not in good health, plants left for too long in the enclosure and plants damaged by the excessive force used on the stake during the fixing phase. Therefore, the following are recommended:

- use plagiotropic rhizomes with roots and, preferably, viable-looking separating shoots;
- do not use transplanting material collected more than 3 days previously;
- push the stake into the substratum by hand, without applying too much pressure to avoid breaking the cutting and possible bacterial and fungal infections and consequent death of the plant material.

The tests carried out before the actual transplantation included verifying whether there was a difference in the survival rate of the cuttings/foliar shoots taken at varying depths and planted at different depths. It was observed that the best result was obtained by planting the rhizomes at their original depth; this observation is in accordance with the probabilities of greater success attributed to biological material obtained from depths greater than or equal to those of transplantation observed by Molenaar and Meinesz (1992).

No criticalities were found such as to make transplantation difficult or unsuccessful, although some drawbacks of natural origin were found both on natural and transplanted meadows, represented by i) massive blooms of epiphytic mucilage on the leaves from April to July and ii) high grazing activity on adult and subadult leaves by the sea bream *Sarpa salpa*.

Environmental sustainability of the technique

The ferrous material of which the stake is made is subject to rapid corrosion in seawater. After 4 years, the stakes used had a 20% reduction in thickness, with the most exposed parts loosing 50%. It is therefore estimated that within 8-24 years the stakes will completely disintegrate, thereby limiting the impact on the environment.

The stakes are almost invisible once planted in the *matte*. The rhizomes, together with the algae growing on the *matte*, tend to hide the remaining part of the stake from view.

The stakes do not increase the availability of habitat and do not alter what was the landscape of the original meadow. Furthermore, if the intervention is unsuccessful, the problem of lasting bodies on the seabed, "foreign" to the marine environment, does not arise.

The search for and collection of naturally available plant material detached from the seabed made it possible to avoid taking material from the surrounding natural meadows. Much of this material would have died.

Period of intervention

P. oceanica transplantation around the Island of Giglio was carried out between May and October in 2019, 2020 and 2021. Further interventions are planned for the same period of 2022. Even if the literature available reports late autumn – early winter (period of vegetative stasis of the plant) as the best time for transplanting (Meinesz *et al.*, 1992), in the case of Giglio, the late spring – autumn period was chosen since two considerations. Firstly, during this period there is greater availability of clods (removed from the substratum by the anchors of pleasure boats) and of uprooted material (as a result of winter storms) in good condition. The second is linked to logistical needs, as in summer the number of operating days with good weather conditions is certainly higher than in winter. Because of the evident facilitation offered, the periods of intervention observed in this case study can be advised for future projects of this kind.

Case studies

Summary table of the Giglio case study and other case studies in Italy where the stake technique was used.

Table 1.1.3|Transplants made in Italy with the stakes.

Transplantation site	Transplantation substratum/ depth	Inizio Trapianto (anno)	Transplantation surface area (m^2)	Transplanted shoots (~ No.)	Monitoring time span (years)	Monitoring context/references
Giglio Porto Island of Giglio (Toscana)	*matte* 8-23 m	2019 (in progress)	1.700 m^2 to 30/09/2021 (2.000 m^2 total)	47.900 (over the period 2019-2021)	3 years (2019-2021)	Environmental Restoration[2]; LIFE SEPOSSO (Mancini *et al.*, 2019; Mancini *et al.*, 2021; AA.VV., 2021)
Ischia Island of Ischia (Campania)	sand/ *matte*/ 8 m	2009	20 m^2	600	10 years (2009-2019)	Environmental Impact Assessment (Scardi and Valiante., 2014).
Rapallo (Liguria)	*matte*/5 m	1997	5 m^2	300	23 years (1997;2019)	Experimental transplantation (Robello, 2019).

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1.1.4 | Geomats and biomats

Stefano Acunto[1]*, Luigi Piazzi*[1]*, Francesco Cinelli*[1]

History of the technique

The technique described here stems from the idea of using materials and methods already successfully used on land in Soil and Water Bioengineering works. In these applications, live plants are used for erosion mitigation and consolidation, generally in combination with other materials (wood, earth, rock, geotextiles, galvanised mesh, etc.). Techniques and materials are commonly used in terrestrial and fluvial environments and, starting from early experiments on sand and *matte* (Cinelli *et al.*, 2007a; 2007b; Cinelli *et al.*, 2014) and a subsequent large-scale application carried out on sand as a compensation measure resulting from the Environmental Impact Assessment of works carried out in a port (Acunto *et al.*, 2015), the most recent and satisfactory applications in the marine environment date back to 2016 (Acunto *et al.*, 2017; Frau *et al.*, in press; Piazzi *et al.*, 2021) and to 2019 (Acunto *et al.*, in press; Piazzi *et al.*, 2021).

Brief description of the technique

The technique involves the use of "MacMat®" geomats or biomats in 100% natural coconut fibre as an anchoring support for cuttings, seeds and/or seedlings of *P. oceanica*.

MacMat® geomats are three-dimensional structures in polypropylene filament capable of holding sediment. In the "MacMat® R" range of geocomposites, the geomat is extruded onto an 8 x 10 cm, 2.70 mm diameter double twisted steel woven mesh (*fig. 1*). The geocomposites in the "R" range have tensile strengths of up to 200 kN/m.

In the "R.E.C.S.® - Cocco" (*Reinforced Erosion Control System*) ge-

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ocomposites, a coconut fibre net (biomat) is coupled to the double twist metal mesh described above (*fig. 1*). The latter has a Mass/Unit Area of 700 g/m^2 and has good mechanical resistance to longitudinal traction (= 20 kN/m).

Both geocomposites are normally sold in 50 x 2 m rolls (*fig. 2*), from which mats can be easily cut to the size required. Mats varying in size between 5 m and 12.5 m in length by 2 m in width gave excellent guarantees of stability and capacity (*fig. 3, 4 and 5*).

The plant supporting mats are spread on the seabed and anchored to the substratum by means of simple stakes made from 1.4 cm diameter metal rods at least 120 cm long.

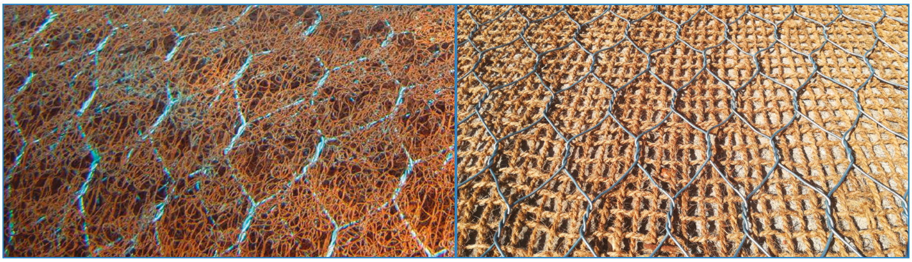

Figure 1 | a) close-up of the "MacMat® R" and b) "R.E.C.S.® - Cocco" geocomposites.

Figure 2 | 50 x 2 m geocomposite rolls.

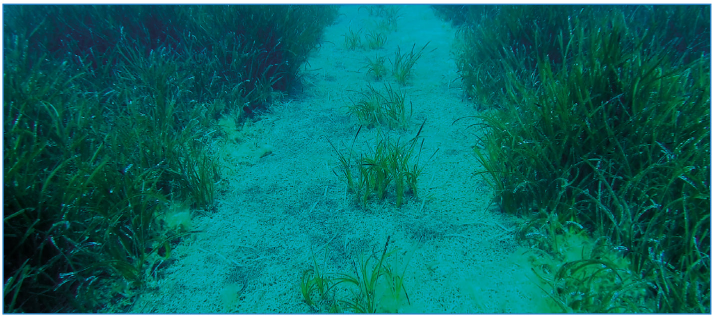

Figure 3 | MacMat® R geomats in situ three years after the transplantation of cuttings at the Capo Carbonara Marine Protected Area (Villasimius, CA).

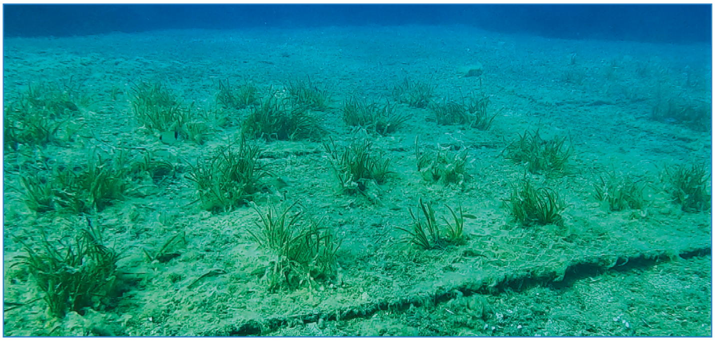

Figure 4 | R.E.C.S.® - Cocco biomats in situ one year after the transplantation of cuttings at Cavo (Island of Elba, municipality of Rio - LI).

Figure 5 | R.E.C.S.® - Cocco biomats in situ with seeds and seedlings of Posidonia oceanica *one year after sowing at Cavo (Island of Elba, municipality of Rio - LI).*

Anchoring substratum

For the technique described, the best substratum proved to be *P. oceanica* dead *matte*. Use on sand is also possible. However, in this case the installation site must not be subject to high levels of hydrodynamic action. It is also deemed that the technique can be used with excellent results on the soft beds of lagoon environments for the transplantation of species of phanerogams other than *P. oceanica* adapted to these particular habitats (e.g. *Zostera* spp., *Cymodocea nodosa* etc.).

The technique can potentially be used at any depth within the survival range of *P. oceanica*, thus permitting the restoration of meadow surfaces that have deteriorated for a variety of reasons. The experiments carried out so far have shown that in oligotrophic waters and coasts exposed to high levels of hydrodynamic action, the ideal depth of use of the technique is between 12 and 20 m. In this bathymetric interval it is possible to obtain the right balance between underwater operativeness, for the installation of the mats and transplanting phases, and optimal conditions of hydrodynamic action and lighting for the survival of the plants.

In the tests using this technique, the inclination of the substratum was almost always subhorizontal or in any case slight (at most about 10°). However, in a small experimental area at Lido di Capoliveri (Island of Elba) (AA.VV. 2021) two, 2 x 2 m MacMat® R geomats were tested on dead *matte* with inclination up to 25° with good results. Therefore, given that geocomposites are used for controlling surface erosion and greening embankments on land with maximum inclination of 65° -70°, there is no reason why the mats should not be installed on steeply inclined seabeds.

Transplantation method

The first stage of the transplantation is the preparation of the geomats in the size required. Once ready, the mats can be handled without the need for special loading equipment and transported, together with the anchoring stakes, on boats that need be no longer than 10 m. Once *in situ*, all the material is lowered to the seabed, where Commercial Divers lay and anchor the mats using the stakes and mallets weighing at least 5 kg. At least 1 stake per m^2 of geomat is necessary.

Installation of the geomats and transplantation are completely distinct phases. This makes it easier to manage the two underwater sites, which have totally different scheduling and safety and authorisation procedures because the professionals involved are different: Commercial Divers for the installation and qualified Scientific Divers with specific experience in handling *Posidonia* cuttings, for the transplantation and subsequent monitoring.

Transplantation material can be obtained from donor meadows or in the form of naturally uprooted plants that are often found in heaps in the meadow to be restored or from other meadows, as necessary.

In the most recent experiments, use has been made of naturally uprooted cuttings recovered in areas immediately adjacent (within a distance of 2 km) to the transplantation sites, but also of drifting or beached seeds and seedlings. Both orthotropic and plagiotropic cuttings were used, with a preference for the latter with 1 to 3 shoots; the rhizomes were cut, using pruning shears, to a length of about 10 cm.

The best results were obtained when the time between harvesting and reimplantation was minimised: between one harvesting session and reimplantation there was an interval of 1-2 hours, during which the best vegetative material available could be picked. All this can be done in 4-5 hours, during which time the cuttings, taken to the surface after collection, are always kept immersed in tanks or hung overboard the support boat in shell nets that are also used during handling underwater. If needs be, the cuttings can be kept immersed for up to 24 hours in open water or even longer in tanks with controlled environmental conditions.

The cuttings are secured to the mats by wedging the rhizome into the weave of the extruded PVC or vegetable fibre net with the aid of a garden dibber. Seeds and seedlings are placed manually without any mechanical aid. The plants are arranged in recolonisation nuclei comprising a minimum of 20 cuttings, making sure that the centre of each nucleus is at least 1 meter away from all adjacent nuclei. A checkerboard arrangement is therefore created of as many nuclei as there are m^2 of the matting previously positioned on the seabed.

Executive measures

The choice of the "R.E.C.S.® – Cocco" geocomposites proposed in this report appears to be the best when considering their complete biodegradability associated with excellent capacity to wear well and maintain their function of holding the plants *in situ* for a period (at the time of writing this report already exceeding 24 months) sufficient to guarantee the stabilisation and radication of cuttings and seedlings in the reimplantation substratum.

In addition to the choice of an appropriate site, the mats should be positioned so that they adhere as much as possible to the seabed and do not lift at the edges or in the centre; this will minimise drag caused by the movement of the water and facilitate the rooting of plants to the primary substratum. Mats should be at least 5 m in length, as shorter lengths will result in less flexibility in the supporting metal mesh.

In the conditions of depth and type of substratum recommended, metal anchoring rods, with diameter 1.4 cm and measuring at least 120 cm, can be used. About 20 cm of each stake must be bent to form an "L" to facilitate insertion and to grip the mats more effectively. In situations of strong hydrodynamic action (e.g. shallow and exposed areas less than 12 m in depth), anchoring systems with greater strength must be evaluated (e.g. Manta Ray Anchors system).

When using naturally uprooted plants, it is necessary to ascertain the viability of the cuttings.

Environmental sustainability of the technique

The use of geocomposites embraces an interesting approach recently developed in Soil and Water Bioengineering. This approach is based on the emergent trait-based mimicry of the plant itself that can generate self-facilitation (Temmink *et al.*, 2020): many marine organisms are able to reduce physical stresses through emergent properties that allow them to survive in otherwise adverse conditions. *Posidonia oceanica* is capable of building a secondary substratum, the *matte*, which allows the plant to modify the physical properties of the surrounding environment and thus colonise otherwise unsuitable substrata. Geomats mimic this emergent property of *P. oceanica*, favouring, in addition to the anchoring of cuttings and seedlings, the entrapment of sediment, the stabilisation of the substratum and the colonisation of an algal population similar to that of the *matte*. This aspect is particularly important as it favours the structural and functional restoration of an ecosystem similar to that of natural meadows and, ultimately, the recovery of degraded meadows (Valdez *et al.,* 2020).

The use of biodegradable mats is a particularly sustainable transplantation method from an ecological point of view and is therefore suitable for large-scale projects; for this reason, the use of the R.E.C.S.® - Cocco system has been proposed and an advanced evaluation is also underway of bioplastics based on hydroxyalkanoates, obtained from bacterial activity on organic substrates, which could replace the polypropylene filament in the MacMat® mats.

The use of naturally uprooted cuttings found *in situ* also constitutes a significant development in terms of the success of the transplantation.

Period of intervention

Posidonia oceanica can theoretically be transplanted at any time of year, although the most favourable periods may be spring and autumn. In fact, the period of maximum growth begins in spring, thus allowing the transplanted cuttings to make the most of this opportunity. In autumn, the plants will have been able to accumulate reserve substances during the favourable season and are thus more resistant to stress, even if they are going towards a period of vegetative slowdown. In the case of the technique proposed here, the transplant can be carried out throughout the favourable period (May – October). However, the spring or early summer period is preferable as the structures and transplanted cuttings are able to stabilise during the summer period, when meteorological conditions are more favourable, and before facing the late autumn and winter period, when the probability increases of storms that could uproot the newly transplanted cuttings or damage the mats. In fact, incipient stabilisation of the structures through sediment entrapment and the growth of benthic organisms are observed within just a few months of planting (Piazzi *et al.*, 2021) and, moreover, the plants have been able to integrate more closely with the mats by generating new roots.

Case Studies

Summary table of the case studies in Italy where the transplantation technique as described was used.

Table 1.1.4 | Transplants made in Italy using geomats and biomats.

Transplantation site	Transplantation substratum/ depth	Transplantation start (year)	Transplantation surface area (m^2)	Transplanted shoots (~ No.)	Monitoring time span (years)	Monitoring context/references
Cavo Island of Elba (Tuscany)	dead *matte* and sand/ 7 m	2006	10 m^2	200	3 years (2006 - 2009)	Experimental activity (Cinelli *et al.*, 2007a; 2007b; Cinelli et al., 2014)
Capo Linaro (Lazio)	sand/7-15 m	2012	10.000 m^2	320.000	3 years (2012 - 2015)	Environmental Impact Assessment (Acunto *et al.*, 2015)
Villasimius (Sardinia)	dead *matte*/ 15-20 m	2016/2017	1.000 m^2	30.000	4 years (2017 - 2021)	LIFE+ RESMARIS Project; LIFE SEPOSSO Project (Acunto et al., 2017, in press, Frau *et al.*, in press, Piazzi *et al.*, 2021, AA.VV, 2021 b)
Cavo Island of Elba (Tuscany)	dead *matte*/ 15 m	2019	100 m^2	2.000	2 years (2019 - 2021)	Experimental activity; LIFE SEPOSSO. (AA.VV.2021 a, Piazzi *et al.*, 2021)

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1.1.5 | Biodegradable modular supports

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History of the technique

The modular anchoring system in bioplastic material "Support system for the plantation of plant organisms in the marine environment" was tested for the first time in 2011-2012 on a sandy seabed in the Capo Gallo – Isola delle Femmine Marine Protected Area at a depth of 12 meters and on a seabed of *Cymodocea nodosa* and dead matte at Mondello (Palermo) at a depth of 6 meters (Calvo *et al.*, 2014).

The idea arose from the need to develop an environmentally friendly and sustainable product that is easy to use and capable of reducing the costs of reforestation and providing an appropriate anchoring system for the cuttings/rhizomes of *P. oceanica* and other plant organisms, especially in the early stages.

The prototype of the anchoring support comprised a modular radial structure (Calvo *et al.*, 2014) that can be anchored to the seabed by means of a quick-fixing stake (*fig. 1*).

The structure had a central hub, fixed to the stake by a nut, and six arms on which there were a variable number of supports (pincers) for the optimal fixing of the cuttings. The pincers were arranged with an inclination of 45° in order to mimic the angle of clonal expansion with which the plant colonises the seabed in nature (Marbà and Duarte, 1998).

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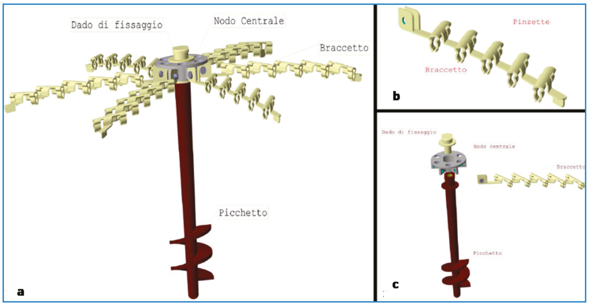

Figure 1 | Biodegradable support in Mater-Bi (Italian Patent no. 0001400800/2013) assembled radially a) and close-up of the individual modules: arm b) and stake with assembly method c). Caption: dado di fissaggio = fixing nut; nodo centrale = central hub; braccetto = arm; picchetto = stake; pinzette = pincers

Brief description of the technique

Subsequent modifications to the prototype have led to the creation of a modular system for anchoring plant organisms to the seabed formed by a radial structure in bioplastic, capable of accommodating plant organisms that are fixed with tear strips to each of the five spokes (arms) and anchored to the seabed by means of a central hub coupled to a fixing stake (*fig. 2*).

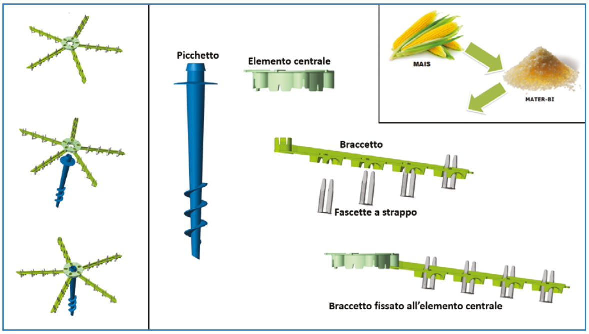

Figure 2 | Modular system for anchoring plant organisms (Italian Patent no. 102015000081824/2018). Caption: picchetto = stake; elemento centrale = central hub; braccetto = arm; fascette a strappo=tear strips; braccetto fissato all'elemento centrale= arm fixed to central hub.

The anchoring system has a diameter of 75 cm and can accommodate from one to four cuttings, each one bearing at least three shoots, on each arm; thanks to this modular approach, it is also applicable to other plant organisms by adapting some of the parts.

The principles on which the patent is based are:

- the extensive use of biodegradable plastic material (Mater-Bi®) to ensure the dissolution of the apparatus that supports and anchors the cuttings at the end of the rooting process;
- application on a variety of substrata that are penetrable by the picket, such as dead *matte* sand and *C. nodosa* meadows. Furthermore, coupled to an anchoring element in reinforced concrete (see “Radial structures in reinforced concrete chapter”) it can be adapted to rocky substrata, artificial reefs (Tomasello *et al.*, 2019) and crushed stone used to cover trenches dug for laying pipelines, cable ducts, gas pipelines, etc.;
- simplification of anchoring techniques and engineering of the process to increase installation efficiency and reduce costs;
- reduction in the need for divers, since most of the activities are carried out ashore;
- module nature of the system, which allows a high degree of flexibility in seabed coverage strategies and application for a variety of plant species;
- use of bio-inspired geometries that emulate the natural colonisation of the seabed by the plant (Marbà and Duarte, 1998);
- possibility of positioning the units on the seabed in patterns; currently the units can be positioned in rows or in patches.

The modular anchoring system is covered by the following patents:

- Italian patent: No. 0001400800/2013
- European design: No. 003000686-0001/2016
- Italian patent: No. 102015000081824/2018

Anchoring substratum

The modular anchoring system in bioplastic material is mainly used in transplantation on dead *matte*; experimentation on soft substrata (sandy seabeds with different granulometric compositions) is still lacking. Appropriately combined with an element in cement, patent pending, it can be used in reforestation even on hard substrata such as artificial reefs (Tomasello *et al.*, 2019), crushed stone and a thin layer of coarse sand on crushed stone (http://bluegrowth-place.eu) or dead *matte*. For further details on its use on hard substrata, see chapter of the *Radial structures in reinforced concrete*.

Transplantation method

The donor meadow should preferably be located near the recipient site. The removal of cuttings will be limited to the plants that colonise the edges of the meadow and will be performed according to sustainability criteria, with a pressure not exceeding 1% shoots/ m^2 (Díaz-Almela and Duarte, 2008). Alternatively, in order to reduce and/or cancel the impact of removal on the donor meadow, rhizomes and cuttings that have become detached and been transported over the seabed as a result of hydrodynamic action, can be collected easily and in large numbers in coastal marine areas, where they accumulate to a greater degree during the autumn/winter period (Balestri *et al.*, 2011). The recovery of naturally detached rhizomes or cuttings, however, presupposes planning so that they can be used within a few days to avoid further stress to the plant and increase the chances of success of the transplantation.

Qualified Scientific Divers with specific experience in handling *Posidonia* cuttings, will collect the plant material, mainly consisting of cuttings at least 15 cm long and carrying at least 3 shoots (*fig. 3*). Removal will be carried out near to the recipient site, taking care, in the case of compensation for marine construction projects, to remove plant material from the areas that will be directly affected by the works or excavation, thus minimising the impact of removal. The plant material collected at the donor site will be stored in jute bags and immediately transported ashore immersed in water for subsequent procedures.

Figure 3 | Brief representation of the phases in reforestation, from the preparation of the cuttings (a, b), to the preparation of the plant (c, d), to the fixing of the cuttings (e, f).

The vegetative material will be appropriately selected and subsequently fixed by means of tear strips to the arms, taking care that the shoots face upwards from the support. Placing the cuttings in this way simulates the position adopted by most plants as they rest on and then anchor to the seabed, after becoming detached from their meadow of origin (Meinesz *et al.*, 1992). The arms carrying the cuttings will be immediately hooked to the central element and temporarily placed in containers with seawater (*fig. 3*).

Each arm can accommodate two to four cuttings for a total of 10/20 plant elements for each anchoring system and at least 30/60 shoots. To avoid dehydration, the plant material being fixed to the arms of the star is kept constantly submerged throughout the procedure.

At the same time, Commercial Divers will prepare the field to be reforested by arranging the poles in the substratum on which, subsequently, the anchoring system with the cuttings will be hooked by means of the central element.

In order to emulate the colonisation mechanism of *Posidonia* and potential for replenishment of meadows of marine phanerogams under natural conditions (Olesen *et al.*, 2004; Sintes *et al.*, 2006), the anchoring supports can be arranged randomly, individually in rows or in modules formed by 6 anchoring systems, in order to emulate a patch containing several hundred shoots. The spatial distribution of the modules, in relation to the morphologies of the seabed present in the recipient site and the possible state of fragmentation of the natural meadow, can also be prefigured as a "mending" approach *sensu* Renzo Piano (2014; 2019) by transplanting patches of *P. oceanica* ("small drops" *sensu* Renzo Piano) within a mosaic of natural meadow and dead matte. In this case, it will also be possible to recognise the dynamics of the patches as natural phenomena and to emulate the natural restoration potential of the meadows (Cunha *et al.*, 2012; Calvo *et al.*, 2021). Below are some images (*fig. 4*) of a recent transplant carried out in Capo Feto (Sicily).

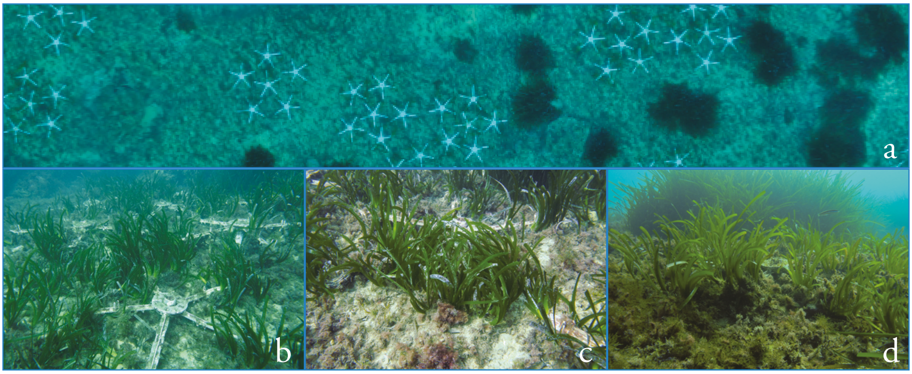

Figure 4 | photo mosaic (a) and close-up views of the transplantation carried out on dead matte at Capo Feto (Sicily) (b, c, d).

Executive measures

- Before carrying out any large-scale transplantation, check the suitability of the site to receive the transplantation by means of a pilot implantation (Pirrotta *et al.*, 2015);
- The collection area of *P. oceanica* cuttings/rhizomes in the donor meadow must be situated at a similar depth ≥ to that of the recipient site (Molenaar and Meinesz, 1992);
- The plant material (cuttings and rhizomes) taken from the donor meadow must be transplanted within a few hours of explantation;
- The entire procedure on land of fixing cuttings/rhizomes to the structure must be carried out in suitably sized tanks, keeping the plant material constantly submerged;
- Avoid excessive changes in temperature during transportation and fixing of the cuttings to the support;
- The choice of the donor site must take into account the distance from the recipient site and the quality of the donor meadow;
- Prohibit fishing and anchorage in the area of the transplantation.

Environmental sustainability of the technique

The bio-plastic material in which the support was made (Mater-Bi®) consists of starch and biodegradable polyesters derived from vegetable oils, with intrinsic biodegradability and absence of toxic effects when exposed to marine microorganisms. Biodegradation times are compatible with those necessary for the cuttings to root and establish a stable attachment to the substratum of the implantation site (Campani *et al.*, 2020). The results of marine biodegradation have been verified by *Certiquality* as part of the *Environmental Technology Verification* (ETV), pilot program, which ascertains the performance of innovative environmental technologies.

Period of intervention

The favourable season for transplantation is late autumn – early winter. In fact, it was found that mortality was highest for transplantation carried out in early summer, when temperatures exceed 20°C, and lowest when carried out in autumn, with survival rates ranging between 92% and 97% (Meinesz *et al.,*1992). Therefore, the actions of collecting the cuttings, setting up the supports and their implantation in the recipient site should preferably take place during the vegetative rest period of the plant. This is when the foliar apparatus is shorter, thus facilitating transplantation in all its phases, from collection to implantation.

Case studies

Summary table of the case studies in Italy where the transplantation technique and operative procedures as described were used.

Table 1.1.5 | Transplantations made in Italy using biodegradable modular supports.

Transplantation site	Transplantation substratum/ depth	Transplantation start (year)	Transplantation surface area (m^2)	Transplanted shoots (~ No.)	Monitoring time span (years)	Monitoring context/ references
Capo Gallo Isola delle Femmine (Sicily)	sand/12 m	2011	5 m^2	300	20 months (2012 - 2013)	Experimental activity (Calvo *et al.*, 2014)
Mondello Palermo (Sicily)	c. nodosa and dead *matte*/ 6 m	2012	10 m^2	600	2 years (2012-2014)	Experimental activity
Priolo Gargallo (Sicily)	dead *matte*/ 13 m	2013	15 m^2	900	1 year (2013-2014)	Progetto PON ReC TETIDE
Priolo Gargallo (Sicily)	dead *matte*/ 13 m	2014	2.000 m^2	60.000	years (2014-2021)	PON R&C TETIDE Project; LIFE SEPOSSO Project (Bacci et al., 2019, AA.VV., 2020)
Priolo Gargallo (Sicily)[4]	artificial reef/ 11 m	2016	12 m^2	360	4 years (2016-2020)	PON R&C TETIDE Project; (Tomasello *et al.*, 2019)
Mondello Palermo (Sicily)	dead *matte*/ 6 m	2015	50 m^2	1.500	6 years (2015-2021)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2021)
Mondello Palermo (Sicily)[4]	Thin layer of dead *matte*/ 6 m	2016	12 m^2	360	5 years (2016-2021)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2021)
Porto Grande Siracusa (Sicily)	dead *matte*/ 6 m	2016	300 m^2	9.000	5 years (2016-2021)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2021)
Isole Incoronate (Croatia)	dead *matte*/ 11 m	2019	100 m^2	2.200	-	Interreg Italy - Croatia - SASPAS Project
Capo Feto Trapani (Sicily)	dead *matte*/ 7 m	2019-21	100 m^2	3.000	20 months (2019 - 2021)	PON - PLaCE Project
Capo Feto Trapani (Sicily)[4]	Pietrame/9 m	2020-21	100 m^2	3.000	18 months (2020 - 2021)	PON - PLaCE Project
Capo Feto Trapani (Sicily)[4]	sand on crushed stone/10 m	2020-21	100 m^2	3.000	18 months (2020 - 2021)	PON - PLaCE Project
Ostuni (Puglia)	dead *matte*/ 8 m	2021	200 m^2	2.500	-	Interreg Italy - Croatia - SASPAS Project
Baia degli Infreschi Camerota (Campania)	dead *matte*/ 2 m	2021	50 m^2	1.260	-	SEA FOREST LIFE Project
Golfo di Santa Manza Bonifacio (Corsica)	dead *matte*	2021	100 m^2	3.000	-	RenforC-– G.I.S. Posidonie Project
Bandita Palermo (Sicily)[5]	dead *matte*/ 13 m	2021	1000 m^2	24.000	-	PON Marine Hazard Project

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[4] Transplantation with additional concrete anchoring support.

[5] Transplantation in progress.

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1.1.6 | Radial structures in reinforced concrete

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History of the technique

It is known that in natural conditions *Posidonia oceanica* is able to settle both on soft and hard seabeds, given that the species has a root system that is capable of adapting to different types of substratum, such as sand, *matte*, rock and crushed stone (Calvo *et al.*, 1995; Boudouresque *et al.*, 2009; Hemminga and Duarte, 2000; Procaccini *et al.*, 2003; Di Carlo *et al.*, 2007; Torta *et al.*, 2015; Badalamenti *et al.*, 2015; Balestri *et al.*, 2015; Zenone *et al.*, 2020a,b). However, transplantation of *P. oceanica* cuttings onto cohesive rock seabeds has been scarcely studied. Artificial reefs have been conceived for a variety of purposes (Baine, 2001; Bulleri and Chapman, 2010), including the increase of natural productivity by providing new habitats to aggregate organisms (Thierry, 1988; Paxton *et al.*, 2018), the creation of habitats for desired target species (Sheehy, 1986) and the protection of small/juvenile organisms and nursery areas (Seaman, 2000). However, in this field of research, very little attention has been given to the aesthetical and perceptive integration within the underwater landscape (Inglis *et al.*, 1999; Dinsdale and Fenton, 2006; Needham *et al.*, 2011). Lately, an anchoring support in cement material in the patenting phase is being used for transplanting *P. oceanica* on blocks of artificial rock made up of innovative reefs (Tomasello *et al.*, 2019), designed to host cuttings of marine phanerogams (www.progettotetide.com), and

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on crushed stone covering a trench after the laying of a gas pipeline (http://bluegrowth-place.eu).

Brief description of the technique

The anchoring element comprises a reinforced concrete radial structure with five arms that are capable of anchoring and supporting the growth of cuttings/rhizomes of *P. oceanica* and plant organisms, at the same time promoting resistance to the hydrodynamic actions of waves and currents.

In particular, this technique:

- Allows the fixing of plant organisms to the anchoring structure by means of tear strips in bioplastic material and favours the collection of sediment to facilitate plant growth.
- Provides the "*Support system for the plantation of plant organisms in the marine environment*" (see *"biodegradable modular supports" chapter*) stable anchorage on hard, natural and artificial seabeds.

The system offers a rough contact surface and a low ratio between the surface area exposed to wave motion and weight. The roughness obtained by selecting the coarseness of the aggregates offers a favourable growth substratum for the roots and, by choosing colours similar to those of the site, the aesthetic impact is minimised.

The weight and shape of the reinforced concrete element has been optimised so that it can be handled independently by means of lifting balloons or support from the surface. The hydrodynamics of the shape permit an almost vertical fall for angles of entry into the water of less than 30°, thus making it, in favourable weather and sea conditions, possible to install directly from the surface (http://bluegrowth-place.eu).

Anchoring substratum

The technique was conceived to enable reforestation on hard substrata, whether natural (compact rock, boulders and loose crushed stone of varying granulometry) or artificial (concrete blocks, artificial reefs and quarry debris to protect offshore installations). Under the same conditions it can also be used on dead *matte* and soft substrates (sand, *Cymodocea nodosa* meadows, etc.). Elements of the system can be combined to form a hexagonal grid and dense covering of the seabed; alternatively, they can be applied singly in accordance with the natural progression of the rocky seabed. Tests showed that, through the joint use of bioplastic material and reinforced concrete, it is possible to obtain structures with sufficient durability to ensure that the cuttings achieve stable radication both on soft and hard substrata.

The ease of production, hauling and handling enables the technique to be employed on natural and artificial hard substrata for restoration using marine phanerogams. Moreover, it can be used to create a temporary herbarium that can be moved to the transplanting site at a later date, as is the case during marine construction projects, planning for which must envisage compensation, through transplantation, for the impact.

Transplantation method

The donor meadow should preferably be located near the recipient site. The removal of cuttings/rhizomes will be limited to the plants that colonise the edges of the meadow and will be performed according to sustainability criteria, with a pressure not exceeding 1% shoots/m^2 (Díaz-Almela and Duarte, 2008). Alternatively, in order to reduce and/or cancel the impact of removal on the donor meadow, rhizomes and cuttings that have become detached and been transported over the seabed as a result of hydrodynamic action can be collected easily and in large numbers in coastal marine areas, where they accumulate to a greater degree during the autumn/winter period (Balestri *et al.*, 2011). The recovery of naturally detached rhizomes or cuttings, however, presupposes planning so that they can be used within a few days to avoid further stress to the plant and increase the chances of success of the transplantation.

Qualified Scientific Divers with specific experience in handling *Posidonia*, cuttings, will collect the plant material, mainly consisting of cuttings at least 15 cm long and carrying at least 3 shoots. Removal will be carried out near to the recipient site, taking care, in the case of compensation for marine construction projects, to remove plant material from the areas that will be directly affected by the works or excavation, thus minimising the impact of removal. The plant material collected at the donor site will be stored in jute bags.

The reinforced concrete structures are positioned on the seabed by Commercial Divers by means of lifting balloons or directly from the surface (*fig. 1*), according to a spatial distribution previously decided on the basis of the morphology of the substratum.

The plant material is fixed to the reinforced concrete structure as follows:

- directly underwater by means of tear strips in biodegradable material to fix each plant organism to the surface of the structure through cavities or holes.
- the "*Support system for the plantation of plant organisms in the marine environment*", prepared with plant organisms fixed with tear strips (see "*Biodegradable modular supports" chapter*), fixed to the reinforced concrete structure with a locking bolt in biodegradable material.

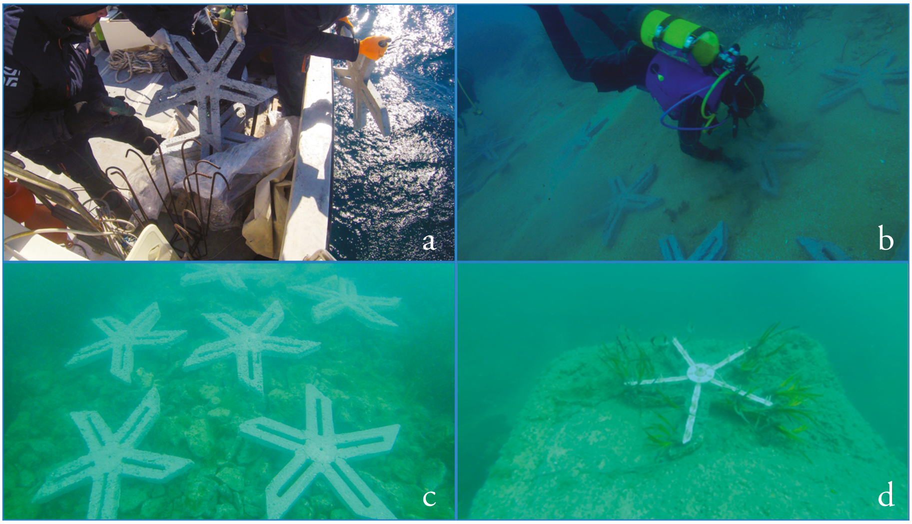

Figure 1 | Positioning of reinforced concrete structures on the seabed (a, b); reinforced concrete structures, with cuttings fixed to biodegradable supports, placed on crushed stone (c) and artificial reef (d).

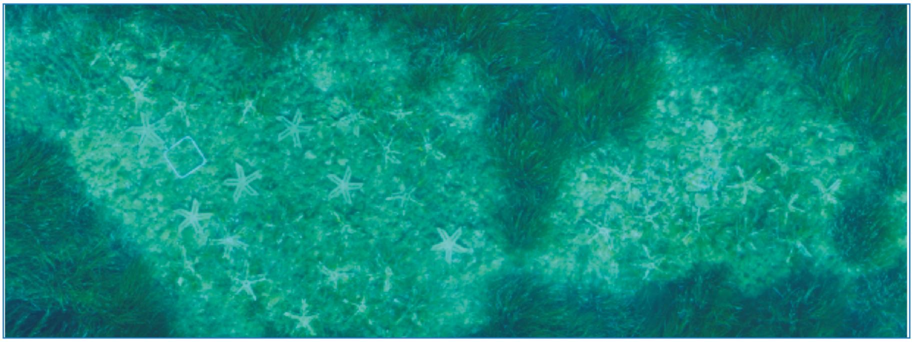

Figure 2 | Photomosaics of transplantation on crushed stone carried out in Capo Feto.

Executive measures

- Before carrying out any large-scale transplantation, check the suitability of the site to receive the transplantation by means of a pilot implantation (Pirrotta *et al.*, 2015).
- The collection area of *P. oceanica* cuttings/rhizomes in the donor meadow must be situated at a similar depth ≥ to that of the recipient site (Molenaar and Meinesz, 1992).
- The plant material (cuttings and rhizomes) taken from the donor meadow must be transplanted within a few hours of explantation.
- Avoid excessive changes in temperature during transportation and fixing of the cuttings to the support.
- The choice of the donor site must take into account the distance from the recipient site and the quality of the donor meadow.
- Prohibit fishing and anchorage in the area of the transplantation.

Environmental sustainability of the technique

The reinforced concrete element is made of sand, concrete and gravel with a 6 mm diameter ribbed iron rod reinforcement. With a small footprint and a weight of 13-15 kg, it is easy to transport and manoeuvre, as well as being adaptable to substrata with varied morphologies. Furthermore, it represents the suitable colonisation substratum for numerous organisms whilst carrying out the task of anchoring cuttings and rhizomes to substrata that are not easily penetrated. Use in combination with the biodegradable modular support and, as an alternative, with tear strips in bioplastic, further favours the environmental sustainability of the technique.

Period of intervention

The favourable season for transplantation is late autumn – early winter. In fact, it has been found that mortality is highest for transplantation carried out in early summer, when temperatures exceed 20°C, and lowest when carried out in autumn, with survival rates ranging between 92% and 97% (Meinesz *et al.*, 1992). Therefore, the actions of collecting the cuttings, setting up the supports and their implantation in the recipient site should preferably take place during the vegetative rest period of the plant. This is when the foliar apparatus is shorter, thus facilitating transplantation in all its phases, from collection to implantation.

Case studies

Summary table of the case studies in Italy where the transplantation technique as described was used.

Table 1.1.6 | Transplantations made in Italy using radial structures in reinforced concrete.

Transplantation site	**Implantation substratum/ depth**	**Transplantation start (year)**	**Transplantation surface area (m^2)**	**Transplantation surface area (~ No.)**	**Monitoring time span (years)**	**Monitoring context/ references**
Priolo Gargallo (Sicily)	artificial reef/11 m	2016	12 m^2	360	4 years (2016-2020)	PON R&C TETIDE Project; LIFE SEPOSSO Project (Tomasello *et al.*, 2019)
Mondello Palermo (Sicily)	Thin layer of sand on dead *matte*/ 6 m	2016	12 m^2	360	4 years (2016-2020)	Environmental Impact Assessment; LIFE SEPOSSO Project (AA.VV., 2021)
Capo Feto Trapani (Sicily)	Crushed stone/9 m	2020-21	100 m^2	3.000	18 months (2020-2021)	PON PLaCE Project
Capo Feto Trapani (Sicily)	Thin layer of sand on crushed stone/10 m	2020-21	100 m^2	3.000	18 months (2020-2021)	PON - PLaCE Project

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1.1.7 Crushed stone-filled gabions

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Origins of the technique

The technique using gabions filled with crushed stone is inspired by observations first made in 1993 in Capo Feto (Mazara del Vallo, Sicily) (Di Carlo *et al.*, 2005; 2007), where, without human intervention, *Posidonia oceanica* propagules colonised many of the mounds of limestone rocks used to cover a previously excavated area (Badalamenti *et al.*, 2006; 2011) (see chapter 2 for further details).

The technique was developed to verify, validate and improve on the natural recolonisation results obtained in the Capo Feto area of Sicily and possibly extend it to other sites that risk suffering the impact from the excavation of trenches for the installation of offshore pipelines and cables in meadows of *Posidonia oceanica.* The technique is mainly to be used for pilot transplantation projects aimed at verifying the feasibility of using crushed stone to cover areas affected by mechanical impact from the removal of the plant and the substratum on which it rests. Furthermore, it lends itself to experimentation into the most effective supports to which the rhizomes can be fixed to facilitate their stability and growth.

Brief description of the technique

The method involves the use of wire mesh gabions (sometimes called mattresses), filled with suitably sized crushed stone to allow the penetration of the roots, and the consequent anchoring and rad-

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ication of the *P. oceanica* rhizomes. The gabions vary in size, with a length of up to 2 m and a height of up to 50 cm (*fig. 1*). They have a lid that can be opened to allow the positioning of the rhizomes inside the structure.

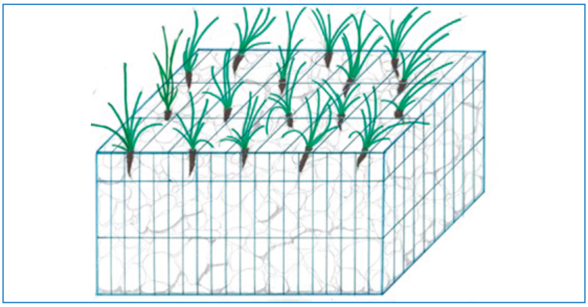

Figure 1 | Gabion filled with crushed stone.

The wire mesh is made of stainless steel, with a section of about 4 mm. The grid mesh dimension is 5 x 20 cm, whereas the mesh dimension of the lid is 15 x 10 cm. To fill the gabions, calcareous crushed stone was used as follows: 40% rocks of average size 19 x 12 x 10 cm and 60% rocks of average size 15 x 10 x 7 cm (Alagna *et al.*, 2019).

Alagna *et al.* (2019) have verified the possibility of growing *P. oceanica* rhizomes in stone-filled gabions by testing 5 rhizome anchoring methods (*fig. 2*):

A) placed freely under the rocks;

B) placed under the rocks and fixed with ties to the top of the gabion;

C) fixed to a pebble by means of an elastic net and a tie;

D) placed inside rigid mesh pockets;

E) placed inside rigid mesh boxes filled with small pebbles.

Methods D and E, both using 1 x 1 cm mesh and each placed amongst the stones in the gabions, were the best in terms of the rate of rhizome survival (93% and 67% respectively after 30 months)

A patent application has been submitted for this methodology (European Patent 2548435A1).

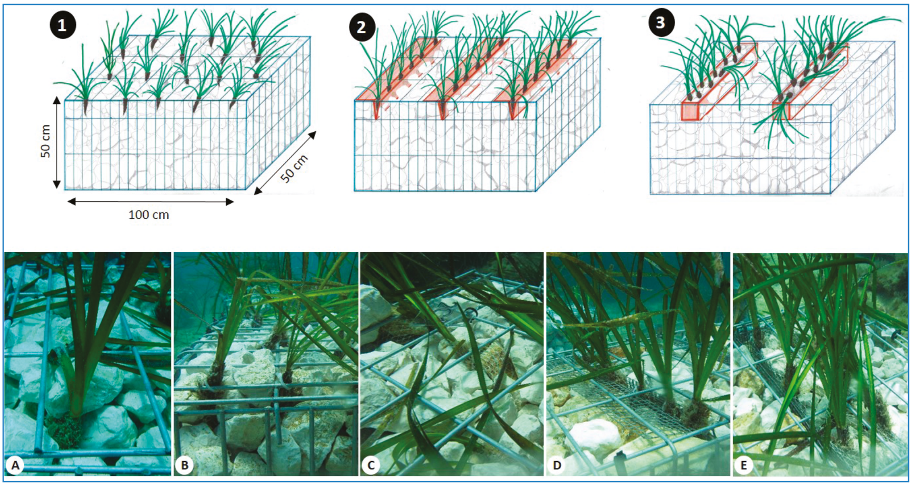

Figure 2 | Summary of transplant techniques and anchoring devices tested in the study. The upper part of the figure shows a sketch of the gabions used. 1) simple without supporting structures, for the transplantation of free rhizomes, held by ties to pebbles; 2) with metal mesh pockets inserted in the upper layer of the gabion used in the "pockets" technique; 3) gabions with mesh boxes inserted in the upper layer, used in the "box" technique. The lower part of the figure shows close-ups of the transplant techniques. A) rhizomes placed freely under the rocks; B) rhizomes placed under the rocks and fixed with ties to the top of the gabion; C) rhizomes fixed to a pebble by means of an elastic net and a tie; D) rhizomes placed inside rigid mesh pockets; E) rhizomes placed inside rigid mesh boxes filled with small pebbles (from Alagna et al., 2019).

Anchoring substratum

The use of a consolidated substratum (in this case, stones blocked inside a gabion) for transplantation was developed with the specific aim of restoring *P. oceanica* meadows damaged by dredging of the substrate on which plants had settled, uprooting plants and *matte*. The technique could be extended to areas where the meadows have been completely submerged by inconsistent substrates (fine and coarse sand). Without prior testing, use on dead *matte* is not recommended, as the weight of the gabions and alteration of small-scale hydrodynamics, resulting from their placement, could compromise the stability of the *matte*.

The technique described was used at depths greater than 10 meters. Its use at lower depths should first be tested.

The proposed technique aims to verify whether stones of various sizes and types offer suitable habitats for the transplantation of *P. oceanica* rhizomes. The actual transplant should be carried out on beds of crushed stone, after having carried out a test with the gabions. The beds of crushed stone can have dimensions and disposition that will vary according to the sites for transplantion.

Transplantation method

The procedures for carrying out the transplantation are as follows:

- Selection of the donor meadow and suitable site for transplantation.
- Sourcing of orthotropic rhizomes from those that have become naturally detached from the donor meadow or by means of manual explantation from the donor site. The rhizomes should be between 10 and 15 cm in length.
- Storage for limited periods of time (<3 days) of rhizomes in the sea in containers anchored to the seabed pending transplantation. Storage for longer periods (1-2 months) can also be carried out in tanks, both outdoors and indoors (for more information, see Balestri *et al.*, 2011 and Marín-Guirao *et al.*, 2011, respectively).
- Transplantation of rhizomes in gabions and final assembly of the latter. This operation is preferably carried out on board a suitable boat (pontoon with crane), using tanks capable of containing the gabions and guaranteeing the continuous immersion of the plant material during fastening to the supports (*fig. 3*).
- The positioning of the gabions on the seabed takes place using the pontoon crane and with the help of Commercial Divers.
- It is advisable to place the gabions next to each other. Positioning will depend on the area of the test site.

- The method tested in the Capo Feto area proved to be very effective and had a high survival rate among the transplanted rhizomes after 30 months. Furthermore, the ramification of the transplanted rhizomes caused the initial implantation density to be exceeded, with an overall survival rate of the seedlings varying between 274.72% and 422.22% after 30 months (Alagna *et al.*, 2019).

Figure 3 | gabion arrangement on the pontoon (a); tanks used for the gabion set-up phases (b); preparation of a gabion (c); use of the crane and Commercial Divers) to position the gabions with plants in the sea (d).

Executive measures

Particular attention should be paid to the size of the rocks used for filling the gabions; rocks should be mixed and vary between a maximum of 13 x 9 x 6 cm (width, height and depth) and a minimum of 6 x 4 x 3 cm, with a prevalence of larger-sized crushed stone, approximately 70% (for more information see Alagna *et al.*, 2019). Beds of crushed stone placed to cover trenches dug for pipe laying may use stones of greater maximum dimensions (for further information see Di Carlo *et al.*, 2005).

Environmental sustainability of the technique

This technique is based on the idea of restoring the ideal natural habitat for the implantation and growth of *P. oceanica*. This plant often settles on consolidated substrata and then propagates towards other types of substratum with plagiotropic rhizomes and grows vertically through orthotropic rhizomes forming, if the conditions are suitable, the *matte*. As mentioned in the previous paragraphs, this method aims to verify, even on sites other than the one where it has already been tested, the feasibility of rehabilitating portions of meadow destroyed by dredging by recreating substratum conditions similar to those in which the plant was originally permanently established and grew as part of a meadow. Once the ramification and growth of the rhizomes have settled and started, if the gabions, or any other solution used to contain the stones, are not too high, the structure will fit into the surrounding environment, offering a new habitat for colonisation by other species as well. In fact, the steel, or other material used to make the gabions, will oxidise and disappear after a few years and the stones, if chosen from quarries in situ, will integrate with the natural rocky substrates of the area, making this technique highly sustainable. The possibility of restoring the ecosystem services of the pre-existing meadow, on the other hand, will depend on the success of the transplantation and the development of the *matte*, a process that will take many years.

Period of intervention

The removal and planting of the cuttings should preferably take place during the vegetative rest period of the plant. However, the experiments carried out in Capo Feto began in May and led to satisfactory results (Alagna *et al.*, 2019).

Case studies

Summary table of the case studies in Italy where the transplantation technique as described was used.

Table 1.1.7 | Transplantations made in Italy using crushed stone-filled gabions.

Transplantation site	**Transplantation substratum/ depth**	**Transplantation start (year)**	**Transplantation surface area (m^2)**	**Transplanted shoots (~No.)**	**Monitoring time span (years)**	**Monitoring context/ references**
Capo Feto (Mazara del Vallo)	Crushed stone/12 m	2012	15 m^2	480	2,5 years (2012- 2015)	Experimental activity (Alagna *et al.*, 2019)
Bagnoli (Napoli)	Crushed stone/ 11-12 m	2018	12 m^2	288	3 years (2018-2021)	Experimental activity

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1.2 | TRANSFER OF POSIDONIA OCEANICA CLODS

The transplantation of marine phanerogam clods, portions of meadow including sediment, has been used in the reforestation of the seabed.

The transfer of clods rather than that of individual cuttings in many cases calls for the use of mechanical means, which thus limit work time and the need for personnel. Furthermore, the substratum in which the plant lives is an integral part of the clod and could facilitate its survival in the transplantation phase.

Clod transplantation has been used especially in the oceanic environment (e.g. Paling *et al.*, 2003; Fishman *et al.*, 2004; Uhrin *et al.*, 2009; Matheson *et al.*, 2017). In such environments, phanerogam transplantation generally involves species characterised by rapid growth and which live on soft seabeds, generally at shallow depths. In these conditions such transplantation methods can take place using mechanical means and with high speeds of execution.

In the Mediterranean, however, clod transplantation has so far been little used (Deschamp *et al.,* 2017; Sánchez-Lizaso *et al.,* 2009; AA.VV. 2020), probably because of the technical problems associated with the work carried out at the depths where phanerogam meadows are distributed. Except for lagoon species, to which similar methods can be more easily applied, the transplantation of marine species, such as *Posidonia oceanica*, involves working at greater depths. Furthermore, additional difficulties are also attributable to the characteristic of the *matte* of *P. oceanica*, which is generally taller and more solid than that of other species, as well as to the nature of the Mediterranean seabed, which is often uneven.

In the Mediterranean, the transfer of clods of *P. oceanica* by mechanical means was carried out as a compensatory measure in the light of Environmental Impact Assessments (EIA). Firstly, in 2005 in Spain as part of the expansion project for the port of Luis Campomanes (Altea, Alicante), when 200 m^2 of meadow in 40 cm high clods measuring approximately 1 m^2 were transferred into specially dredged holes (Sánchez-Lizaso *et al.,* 2009). The most recent transfer of clods was in 2017 as part of the project for the seaboard extension of the Principality of Monaco, where the compensatory measures using advanced technologies provided for transplantation of an area of *P. oceanica* of approximately 500 m^2 in 60 cm high clods measuring 0,8 m^2 (Deschamp *et al.,* 2017). In Italy, the only transplantation using *P. oceanica* clods dates back to 2014 in the area of the Gulf of Follonica and is the subject of further study in this Manual.

To date, the different methods used for the transfer of *P. oceanica* clods are still being tested. The main causes of death of the clods were attributed to the system for removal and/or to anchoring to unsuitable substrata, to the inappropriate choice of the transplant site, as well as to the consistency of the *matte* and the distance between clods and/or from natural meadows (Deschamp *et al.*, 2017; Sánchez-Lizaso *et al.*, 2009; Bedini *et al.*, 2020; AA.VV., 2020). Furthermore, it is emphasised that data on the performance of transplantation using clods of *P. oceanica* is scarce and never long-term. Therefore, further evaluations of trends in the parameters investigated in the existing case studies will have to be postponed to future monitoring campaigns.

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1.2.1 | Transfer of *Posidonia oceanica* clods: the Piombino case study

Claudia Bulleri[1], Francesco Sozzi[1], Michele Magri[2], Roberto Bedini[3]

History of the technique

Following the awareness that higher survival rates of transplantation using clods could be achieved by increasing the size of the units transplanted (Walker, 1994), thanks to the previous experiment in an oceanic environment and after the first experiment into transferring *Posidonia oceanica* clods by mechanical means carried out in Spain (Sánchez-Lizaso *et al.*, 2009), in Italy the first transfer of large clods of *Posidonia oceanica* was carried out in 2014 (AA.VV. 2019; Bedini *et al.*, 2020). The transfer was carried out using appropriate mechanical equipment for the size of the clods and for the donor site meadow, characterised by tall, solid *matte*. The transplant was carried out as part of the Environmental Impact Assessment of the actions envisaged by the new Port Development Plan for the Port of Piombino (LI) in 2014. The necessity to intervene in an area of intense in- and out-bound maritime traffic to the Port of Piombino and to implement the new port infrastructures quickly, including the dredging approximately 3,000,000 m^3 of sediment, lead the Port Authority of Piombino (today Port System Authority of the Northern Tyrrhenian Sea) to propose to the then Ministry for the Environment and the Protection of the Land and Sea (today Ministry for Ecological Transition) the technique of transferring “Clods” of *P. oceanica,* from the area affected by the works to the Gulf of Follonica, neighbouring the Port of Piombino. The following describes the transplantation of *P. oceanica* in clods, with specific reference to the experience gained in the case study of Piombino.

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Brief description of the technique

The technique involves the transfer of clods of *P. oceanica* measuring 4 m^2 (*fig. 1*). In order to avoid splitting during the removal of these 1-metre-high clods of solid *matte* from the seabed and their subsequent positioning, a hydraulic clamshell bucket (*fig. 2*) was used, rather than a grapple bucket. The transport vessel used was a split barge, which can take water on board, thus favouring the survival of the marine organisms living amongst the *matte,* rhizomes and leaves of *P. oceanica* until the clods are positioned on the seabed (*fig. 3*). The clods were placed one at a time in the centre of the hull and then placed on the seabed.

Figure 1 | Posidonia oceanica *clod (a) and close-up of* Posidonia oceanica *clod (b).*

Figure 2 | *Hydraulic clamshell bucket with explanted* Posidonia oceanica *clod.*

Figure 3 | Posidonia oceanica *immersed in seawater and inside the hull of the barge.*

Anchoring substratum

The *Posidonia oceanica* clods were placed in six different areas near the upper border of the recipient meadow (*fig. 4*); a number of modalities were envisaged for the positioning, without embedding, of the clods in these areas, as follows:

- on sandy seabed outside the meadow, close to the upper border;
- on sandy seabed outside the meadow, away from the upper border; with the aim of creating new meadow nuclei;
- on sandy clearings within the existing meadow.

The areas where the clods were positioned are at a depth of between 11 and 13 meters. The total surface area of the transplantation was 1362 m^2.

Transplantation method

As a result of the surveys undertaken using ROV images and transepts performed by underwater scientific operators during the months prior to the transfer of the clods, explantation within the area authorised could be carried out directly by the dredger. Immediately after their explantation from the seabed, the clods were placed in a basin of seawater. The exact point where the clod should be sunk for positioning on the seabed was indicated by the divers by means of buoys. Each clod was given a numbered identification tag and then anchored to the seabed using 4 x 1.50 m long stakes. Before the transfer of the *Posidonia oceanica* clods, some of the vagile organisms (crustaceans, molluscs, fish, echinoderms) found on and among the leaves in the donor area had been captured in plankton nets by scuba divers and thereafter transferred to the recipient area.

The operation involved the transfer of 340 clods, containing a total of about 380,000 shoots, to areas identified as suitable in the Perelli area of the Gulf of Follonica.

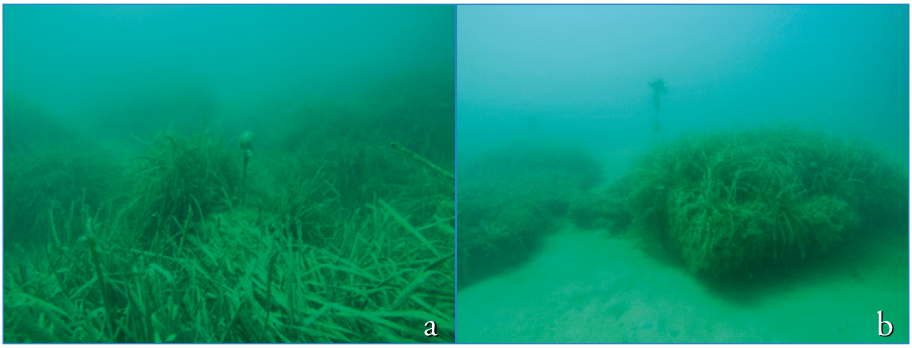

Figure 4 | Transferred clods near the Posidonia oceanica *meadow.*

Executive measures

Based on the experience gained, the following measures are recommended:

- Label the clods immediately after laying them. A clod with a central stake fitted with a white buoy and label is easily distinguishable; without such labelling, it becomes very difficult to identify the transferred clods in poor visibility.
- By logging the survey areas and mapping the clods by means of standard environmental monitoring methodologies, the clods can be identified using three complementary methods:
  - geographic coordinates that identify the start and end of the areas where the clods have been positioned;
  - clod-labelling by area and by progressive number;
  - underwater mapping of the clods in relation to the boundaries of the meadow.
- Place the clods in specially prepared holes and/or in areas adjacent

to the natural meadows in order to minimise their erosion by local hydrodynamic activity.

- Fixing stakes are not necessary because the clods are very large. The 1.5 m stakes initially used for fixing the clods were found to be too short and were subsequently used for the sole purpose of labelling the clods.

Environmental sustainability of the technique

The decision to remove plants together with the matte substratum in which they had lived for years was guided by the intent to minimise damage and increase the chance of survival of transplanted plants.

One of the most significant features of *Posidonia oceanica* rhizomes is their ability to grow both vertically (orthotropic) and horizontally (plagiotropic). These qualities enable the plant to contrast silting and propagate in nearby areas. Clod transfer provides that between each clod a passage is left for new plants to “colonise” over time so that old and new rhizomes can intertwine, thereby increasing the stability of the seabed.

Period of intervention

The transplantation was carried out in June 2014 and the transfer of the clods lasted approximately 20 days. Even though the vegetative stasis period of the plant (end autumn – early winter) is generally used for transplanting *P. oceanica* using other techniques, no data is available regarding the impact of the transfer period on the conservation and vitality of the plants of the clod and therefore it is not currently possible to indicate the best period of intervention for this type of technique.

Case studies

Summary table of the case studies in Italy where the transplantation technique as described was used.

Table 1.2.1 | Transplantations made in Italy using Posidonia oceanica *clods.*

Transplantation site	**Transplantation substratum/ depth**	**Transplantation start (year)**	**Transplantation surface area (m^2)**	**Clods transferred (~ No.) Transplanted shoots (~ No.)**	**Monitoring time span (years)**	**Monitoring context/ references**
Località Perelli Piombino (Tuscany)	sand/11-13 m	2014	1360 m^2	340 clods 380.000 shoots	5 years (2014-2019)	Environmental Impact Assessment; LIFE Project SEPOSSO (AA.VV., 2020)

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CHAPTER 2

NATURAL RE-COLONISATION OF POSIDONIA OCEANICA MEADOWS ON CONSOLIDATED SUBSTRATA

2.1 | THE NATURAL RE-COLONISATION OF POSIDONIA OCEANICA IN CAPO FETO (SICILY)

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Context

The laying of the TRANSMED gas pipeline between Capo Bon (Tunisia NE) and Capo Feto (Sicily SO, Italy) involved the dredging of two trenches through a pristine meadow of *Posidonia oceanica* off the coast at Capo Feto. The first excavation, 1980-1981, caused the loss of over 50 ha of meadows at depths between 0 and 30 m (Badalamenti *et al.*, 2011). The trench was filled with residual materials from the excavation and calcarenite rocks. A second excavation took place in 1992-1993 and on this occasion the trench was covered with limestone rocks in order to cover the excavation and stabilise the seabed.

As a result of these two excavations, the meadow suffered direct impact from the removal and burying of plants and indirect impact from the suspension for many months of a "*plume*" of fine sediment and by the movement of coarse sediment on the seabed. Overall, the two interventions caused meadow loss of 81.20 ha (Badalamenti *et al.*, 2011). In 1993 the area affected by the excavations was a mosaic of heterogeneous substrata: sand, gravel, calcarenite rocks, dead *matte* and mounds of limestone rocks (*fig. 1*); on the latter, vegetative fragments documented natural recovery of *P. oceanica* (Badalamenti *et al.*, 2011).

In 1992-1993 the pipeline was upgraded and a new trench was excavated. On this occasion the dredged materials were deposited onto an area where the *P. oceanica* meadow was already significantly damaged and calcareous rocks with an average size of 19.2 ± 9.5

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SD cm in length, 15.9 ± 2.3 SD cm wide, 10.6 ± 1.9 SD cm high were unloaded from pontoons to fill the trench. This operation led to the formation of beds of limestone rocks and mounds measuring 1.5 ± 0.4 SD m in height and 5 ± 1.1 SD m in diameter (*fig. 2*) (Di Carlo *et al.*, 2005). The limestone rocks had been unloaded from dump barges onto the trench, forming mounds described. At the end of the dredging operations in 1993, the impacted area comprised a heterogeneous mosaic of materials: sand and gravel derived from the erosion of the calcarenite material, mounds of calcareous rocks used as filling material, large areas of dead *matte* at the edge of the trench, sandy areas where *Cymodocea nodosa* had settled and large blocks of rock remaining in the trench area after the first excavation.

Monitoring of recolonisation of *Posidonia oceanica* along the course

Materials and methods

The study area is located off the south-west coast of Sicily (41° 73' N, 18° 12' E) and extends to the south-west on a large calcareous platform at a depth of approximately 30 m. Between 1981 and 1995, three maps of the area were produced and compared to evaluate the loss of portions of meadow and the variation in the composition and type of substrata following dredging. The mappings were built by compiling information obtained from aerial photographs, Side Scan Sonar (SSS) and Remotely Operated Vehicle (ROV). The entire data set was imported into a GIS environment to show the distribution of substrata before (1979 map) and after the first dredging event (1993 map) and after the second dredging event (1995 map). By means of the 3 mappings, 7 categories of substratum were distinguished within the area affected by the excavations:

1) P. oceanica,

2) Cymodocea nodosa,

3) P. oceanica dead *matte*,

4) fine sand,

5) sand and gravel,
6) calcarenite and calcareous rocks,
7) and mounds of limestone rocks.

The latter have been divided into three parts: crests, sides and valleys between adjacent mounds.

ROV images were used to estimate the percentage coverage of *P. oceanica* within the area dredged between 1993 and 1999 on 5 of the 7 substrates identified, respectively: 1, 3-4, 6-7. The density of the foliar shoots was measured exclusively under water on the substratum "mounds of limestone rocks" in the three different parts previously identified and at control sites at depths of 5, 10 and 15 meters for three consecutive years (2001-2003).

Results

The natural recovery of the meadow through vegetative propagules was monitored between 1993 and 1999 and analysed as a function of time and the substrates present in the study area (Alagna 2010; Badalamenti *et al.*, 2011). This recovery was observed only on the beds of calcareous rocks whereas no significant increase was recorded over the years on dead *matte* sand or large calcarenite boulders.

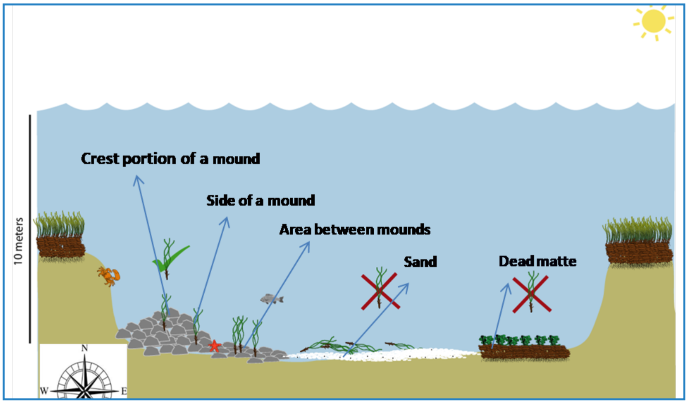

Figure. 1 | Schematic view of the Capo Feto trench filled in the eastern portion with crushed stone in 1993. The substratum deriving from infill comprises mounds of rocks on which rhizomes of Posidonia oceanica *settled naturally.*

The percentage coverage of *P. oceanica* in the valleys between adjacent mounds of calcareous rocks increased continuously from 0.75 ± 0.41 (SD) % in 1993 to 44.38 ± 3.05 (SD) % in 1999 in the shallow meadow (5-15 m), and from 0.75 ± 0.41 (SD) % to 26.88 ± 2.30 (SD) % in the deep meadow (16-25 m) (Badalamenti *et al.*, 2011).

On the basis of the maps made, an overall natural recovery of the meadow of 3.24 hectares in six years was estimated, equal to 4.1% of the damaged meadow.

Figure. 2 | Recolonisation of mounds of calcarenite rocks through vegetative propagules, years 2001-2003.

Monitoring of the natural recovery of the meadow continued from 2001 to 2003. The density of the foliar shoots in correspondence with the beds of crushed stone showed a constant increase over time (Di Carlo 2004; Di Carlo *et al.*, 2005; 2007). In particular, in the valleys between adjacent mounds, the density of foliar shoots went from 170 ± 17 (SE) shoots m^{-2} in 2001 to 342 ± 17 (SE) shoots m^{-2} in 2003 (Di Carlo *et al.*, 2005). Observations recently conducted along the gas pipeline corridor confirm the persistence of patches of *P. oceanica* that are continually expanding to form quite extensive strips of meadow and beneath which calcarenite and calcareous crushed stone are to be found (2010-2013, personal observation A. Alagna, F. Badalamenti) (*fig. 3*).

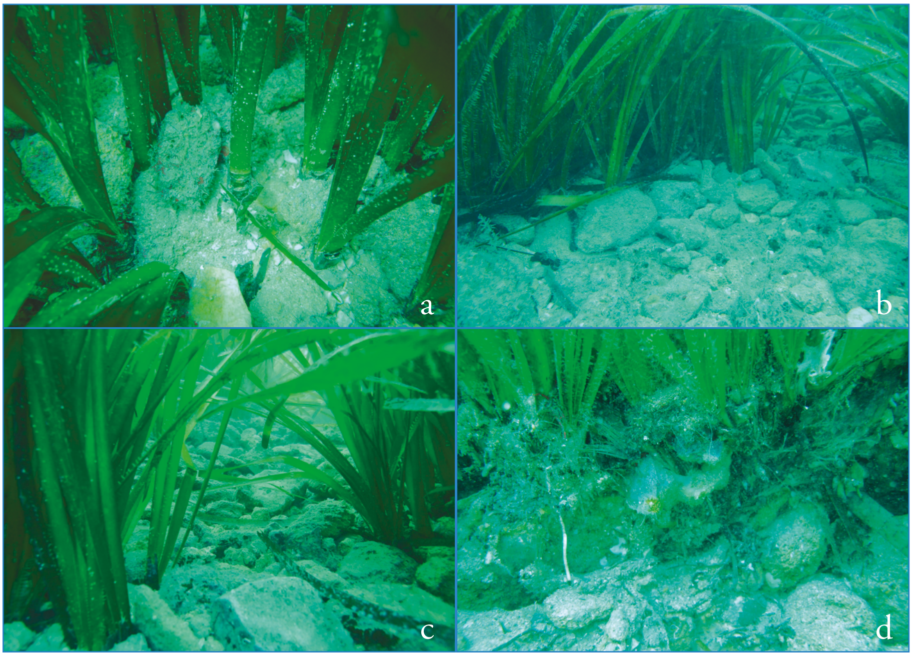

Figure 3 | Persistence and coalescence of patches of Posidonia oceanica *along the trench-laying corridor. The substratum on which the patches have developed is made up of calcarenite and calcareous crushed stone.*

Concluding considerations

The beds of calcareous crushed stone have characteristics of stability (resistance to hydrodynamic action) and complexity (availability of cracks between adjacent rocks) capable of allowing the settlement and recruitment of naturally dispersed rhizome fragments, unlike the other substrata in the area, which lack the necessary complexity or stability (*Cymodocea nodosa*, dead *matte* calcarenite rocks, sand).

The case of Capo Feto is of enormous importance to the ecology of the restoration of *P. oceanica* meadows, with significant implications for the protection and management of this species. This is due to the fact that, without costly human intervention, recovery took place naturally, on a substratum originally devoid of vegetation and at much greater speeds than those reported in the literature (Meinesz and Lefèvre, 1984; González-Correa *et al.*, 2005, 2008); equally significant is that monitoring, albeit using several methodologies, has spanned 20 years.

Should the laying and burying of offshore cables and pipelines necessitate the excavation of meadows, the use of stone infill materials such as those used at Capo Feto, characterised by suitable stability (i.e. low friability and high resistance to erosion) and complexity, is highly recommended as these enable and facilitate the natural recovery process of the meadow by acting as a propagule "catcher". A supply of propagules from adjacent meadows and the absence of sources of disturbance for the plant (excessive turbidity and sedimentary disturbance) are necessary conditions for the process to begin. In the case of Capo Feto, one of the largest meadows in the Mediterranean became a guaranteed and abundant "lateral supply" of propagules. Furthermore, the area where one trench had been dug and refilled with rocks was left a few decimetres lower than the level of the surrounding meadow, thus acting as a veritable sinkhole for propagules. When contemplating ways of achieving natural recolonisation of *P. oceanica* similar to that observed at Capo Feto in degraded sites in other parts of the Mediterranean, thorough consideration of these particular aspects of the use infill rocks should be given.

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2.2 | THE NATURAL RE-COLONISATION OF POSIDONIA OCEANICA ALONG THE EXCAVATION FOR THE ISCHIA GAS PIPELINE (GULF OF NAPLES)

Maria Cristina Buia[1], Marcello Cotugno[1]

Context

This report describes the natural recolonisation of *Posidonia oceanica* observed along the shallow portion of the San Pietro meadow, in front of the port of Ischia (Gulf of Naples), 10 years after its destruction, brought about during laying of the terminal section of the gas pipeline from the mainland, and subsequent covering with rubble. The area falls within the Special Area of Conservation (SAC) “Ischia, Vivara and Procida Seabed” (IT8030010) (Cigliano *et al.*, 2009) and is an integral part of the Regno di Nettuno Marine Protected Area (www.nettuno.amp).

The gas pipeline runs on the seabed to the entrance to the port of Ischia, on the northern side of the island. Over the years, this side, characterised by low-lying and sandy coasts, has undergone major transformation (52%) as a result of the construction of commercial ports and marinas, excavation for road infrastructure between municipalities and the installation coastal defences. These manmade works have had a considerable impact on the coastal vegetation, causing progressive fragmentation and regression in the shallower stands. It should be noted that the area in the immediate vicinity of the port where the pipeline arrives is subject to intense maritime traffic, especially in the summer; hydrofoils and ferries, in particular, passing through the area produce high levels of hydrodynamic action, which increases in intensity as the depth of the water decreases and as the proximity to the coast augments.

The study area is part of the San Pietro meadow, which primarily

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covered approximately 3 km^2 and was planted on *matte* with heights in excess of 1.5 m (as evidenced by the excavation work). Up to a depth of approximately 8 m, the pipeline rests, without being buried, on the meadow, which shows no signs of alteration over time. Between depths of 7.5 m and 4.5 m, the gas pipeline was buried in a trench of approximately 300 x 6 m in width, which was subsequently covered with lumps of igneous rock, essentially trachyte and phonolite lavas, measuring approximately 10 cm in diameter.

Monitoring of recolonisation of *Posidonia oceanica* along the course

Materials and methods

Over the period 2009-2019, the course was inspected regularly and filmed throughout the area of the excavation. In 2019, the density of *Posidonia* shoots in newly recolonised spots was estimated and the evaluation of their spread along the route was mapped. The density of the shoots was measured using a 40 x 40 cm frame.

A technique combining digital photogrammetry and Geographic Information System (GIS) tools was used to map the new colonies.

In particular:

- sub-areas of about 10-20 m in length and 6m in width were identified underwater along the route. In each sub-area, guide ropes (in numbers varying between 5 and 10 depending on the dimension of the sub-area and the presence of new spots) were placed on the seabed at right angles to the axis of the course and parallel to each other, 2-3 m apart. White-black Bakelite targets were placed at the 4 vertices of each sub-area and their position georeferenced on the surface while keeping a rope stretched between target posts (*fig. 1*);
- each sub-area was mapped by photogrammetry (Abadie *et al.*, 2018). By maintaining a fixed distance from the seabed and a slow pace, following the guide ropes, a large number of good quality frames could be recorded using a 4K/25fps video camera. The

distance of the ropes, which varies with the aperture of the filming field and the visibility, must ensure a lateral overlap of the images of at least 70% for subsequent image processing;

- in the laboratory, mapping in raster format was used for the analysis of the percentage coverage of *P. oceanica* throughout the course. The videos obtained were processed using the Free Video to JPG Converter software to extrapolate the frames for analysis. 300-1800 frames were extracted from each film; their subsequent processing with Agisoft Photoscan software also provided automatic calibration to correct focal length and lens aberration. Using structure from movement (SfM) algorithms, 3D models of the seabed were produced and orthophoto mosaics were georeferenced using the open source software, QGIS, thus enabling the surface area of the newly colonised spots to be ascertained and the calculation of the percentage coverage along the entire route.

Results

Favoured by the limited extension of the course and by the presence of *Posidonia oceanica* in adjacent areas, explanted cuttings and/or clods have found, over time, a suitable bed on the rocky substratum for implantation of the phanerogam. Over the entire duration of observation, the radication of cuttings, seen in adjacent areas and in the shallowest part at the upper boundary of the meadow, was never observed on sandy substrata.

An area of 736.82 m^2 was mapped in which 184 newly colonised spots were found. These cover an area of 67.76 m^2, approximately equal to 10% of the area in question (Cotugno *et al.*, 2020). New implantation was greatest in the deepest portion of the route (7.5-6.3 m^2) (*fig. 2*) where, over a length of 128 m, 121 new colonisation areas were counted, covering an area of 40.25 m^2 on a total area of 334 m^2 (approximately 12%). In this section, the level of structural complexity of the new colonies was found to be high, with the density of shoots greater than that recorded at the same depth in control meadows: 139 ± 4 no. shoots in 1600 cm^2 vs. 121 ± 4 and 94 ± 6

(Cotugno *et al.*, 2019). The extent of natural recolonisation decreases towards the shore, as depth decreases. The second section examined, of approximately 403 m^2, appears to have coverage of only 26 m^2, distributed over 63 new spots.

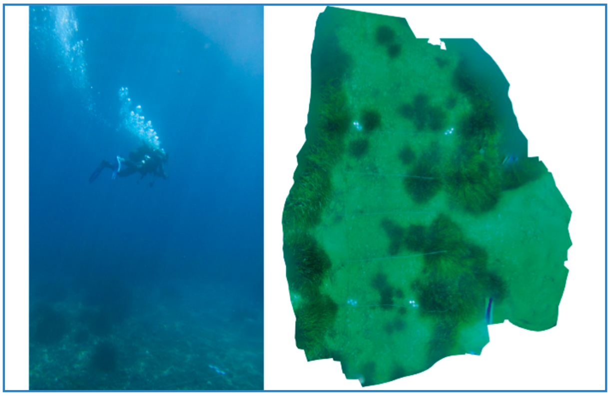

Figure 1 | Filming along ropes positioned on the seabed in a sub-area marked off by 4 white/black Bakelite targets, whose georeferencing was done on the surface.

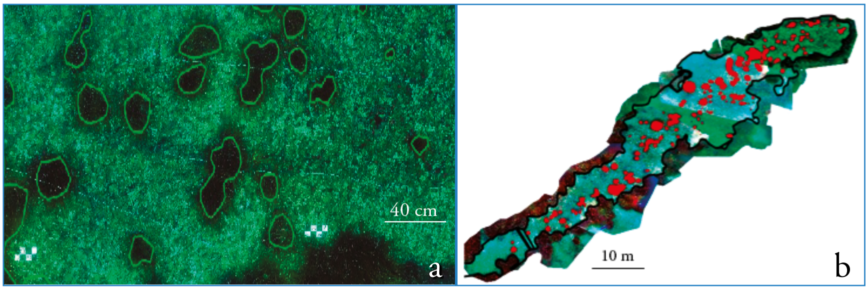

Figure 2 | Close-up of a part of the course: polygon layer for QGIS surface area elaboration (a); final map of the newly colonised spots, in red (b).

Concluding considerations

The natural recovery of the *Posidonia oceanica* meadow after the laying of the gas pipeline ten years ago, represents a significant result both in the light of the scarcity of long-term data on the dynamics of this species (except for the data recorded at Capo Feto - Sicily (Badalamenti *et al.*, 2006) and the particular environmental conditions of the site on the Phlegraean Islands, located at the entrance to a port with very heavy shipping traffic.

The results confirm the success of the natural recruitment of this phanerogam thanks to the cuttings set adrift from nearby populations (Balestri *et al.*, 2011), a phenomenon often observed along the trench in recent years. The role played by rocks in catching and trapping the drifting cuttings testifies to the importance of the type of substratum (nature and size) and the depth of the site (the greater the depth, the greater the probability that transported cuttings will settle) in ensuring success of new plant settlements. Despite guidance given before the works to use calcareous rocks, volcanic stones were used, probably because they were cheaper and more readily available as they are typical to the area, and this slowed down the settlement process of the new seedlings. Volcanic rocks, being more coherent, probably required the growth of an algal biofilm with pioneer species, including calcareous forms, thus slowing down the rooting and adhesion of the cuttings.

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CHAPTER 3

THE USE OF POSIDONIA OCEANICA SEEDS AND SEEDLINGS IN TRANSPLANTATION

3.1 | EXPERIMENTS USING SEEDS AND SEEDLINGS OF POSIDONIA OCEANICA IN TRANSPLANTATION PROJECTS IN THE MEDITERRANEAN

Most *Posidonia oceanica* transplantation projects have been planned around the use of vegetative material, mainly cuttings and, in some cases, clods. This choice stems from the fact that the main method of reproduction of this species is vegetative; sexual reproduction is rare and very difficult to predict, with exceptional florescence often in conjunction with high seasonal temperatures recorded in spring and summer. Furthermore, reforestation by means of seedlings from seeds takes much longer and is consequently more costly. Despite this, seeds and seedlings have been used as transplanting material on a number of occasions, especially for small research projects.

With the view of using material of sexual origin for reforestation, three main aspects have been investigated: 1) cultivation of seeds in a controlled environment; 2) natural recruitment at sea; 3) transplantation of seedlings at sea.

Cultivating seeds in a controlled environment

Most of the seedling studies involved cultivating seeds in a controlled environment. The effects of environmental variables (irradiation, nutrients, temperature, salinity, hydrodynamics, substrate), and biotic factors (presence of *Cymodocea nodosa*), as well as the use of growth stimulators, were tested in tanks to highlight the optimal *ranges* for the survival and growth of the seedlings (Caye and Meinesz 1989; Buia and Mazzella, 1991; Bedini 1997; Balestri *et al.,* 1998; Balestri e Bertini, 2003; Badalamenti *et al.*, 2010; Infantes *et al.,* 2011; Fernández-Torquemada *et al.,* 2013; Alagna *et al.*, 2015; Guerrero-Meseguer *et al.*, 2017 a, b; Hernán *et al.,* 2016, 2017; Guerrero-Meseguer *et al.*, 2020; Alagna *et al.,* 2020; Zenone *et al.*, 2020; Balestri *et al.*, 2021).

The maximum germination rate and survival rate of *P. oceanica* seeds, analysed in Fernández-Torquemada *et al.* (2013), were obtained with a salinity of 37 ‰ and development was lower with higher salinity rates.

Similar results in the effect of an increase in temperature on the early stages of development of *P. oceanica* emerged in Hernán *et al.* (2016, 2017) and in Guerrero-Meseguer *et al.* (2017 a). Temperatures above 27°C limited growth by inhibiting the photosynthetic system, highlighting an increase in leaf death and senescence and a decrease in leaf and root growth.

Furthermore, the role of hydrodynamic action on the stabilisation of *P. oceanica* seedlings was studied in Infantes *et al.* (2011), showing high losses with a water mass velocity greater than 18 cm s^{-1}.

The manipulation of a number of variables has shown higher survival and growth rates with greater irradiation and with a lower concentration of nutrients (Caye and Meinesz, 1989). Studies on the effect of nutrient increase were also analysed in Balestri *et al.* (1998), similarly highlighting a reduction in root production.

A number of biotic factors, such as the presence of the phanerogam *Cymodocea nodosa*, also seem to affect the settlement and growth of *P. oceanica* seedlings. In Balestri *et al.* (2021) higher settlement values are highlighted in the presence of this phanerogam, showing the importance of this interaction and also suggesting indications in terms of planning for future restoring projects using seedlings.

The effect of the application of plant growth stimulants was tested on *P. oceanica* seeds grown in tanks in Balestri and Bertini (2003), highlighting the effects on the initiation of development and root growth. Root emergence time was 2-3 times shorter in treated seeds than in controls, whereas no dose-related effects were observed.

Finally, further experiments in Alagna *et al.* (2015) were carried out under controlled conditions in order to test the settlement and growth of seedlings on different types of substrate (rock *vs* sand). The conclusions of the study show that the use of consolidated substrata (rock) is of primary importance for the seedlings to settle. This substratum must also have an appropriate complexity for the seedlings, i.e. centimetres, so that this allows the seedling to slide and settle in the interstitial spaces between adjacent rocks and to survive the early phases of the life cycle in which it lacks a well developed root system. In this regard, further studies concerning the adhesion capacity of *P. oceanica* to the substrate and the possible implications in environmental restoring are examined in Zenone *et al.* (2020) and Alagna *et al.* (2020).

Natural recruitment at sea

Natural recruitment at sea has been investigated in a small number of cases, sometimes showing conflicting results. A bibliographic review by Balestri *et al.* (2017) analysed the frequency and extent of *P. oceanica* sexual recruitment phenomena over a period of 11 years. The results showed that the seedlings settle mainly in sheltered areas at shallow depth, more on rock than on sand, or on *matte*. Survival on rock appears to be twice as high as on sand. In Piazzi *et al.* (1999) sand similarly showed the lowest seedling survival values, whereas *matte* showed higher values than rock.

The influence also appears to be demonstrated of different micro-habitats on the survival and growth of seedlings of *P. oceanica.* In recruitment evaluated in Balestri and Lardicci (2008), the seedlings were similarly distributed on rock and sand; however, the distribution did not appear to be random but rather in patches linked to micro-habitat, probably due to hydrodynamic action and the characteristics of the substratum. In recruitment evaluated in Alagna *et al.* (2013), survival was minimal on sand and pebbles, and highest on rocky substrata colonised by macro-algae, with maximum values for substrata populated by *Cystoseira* spp; on the other hand, growth was greater for substrata populated by *Halopteris* spp. and *Dilophus* spp. compared to *Cystoseira*. The results therefore show that canopy-forming species favour recruitment but interfere with growth, which is greater in populations of less complex and smaller species, probably due to a greater availability of light and nutrients.

Transplantation of seedlings at sea

The transplantation of seedlings at sea has also been used in a limited number of cases and mainly for experimental purposes. The survival rates of seedlings on *matte* generally assessed on a few hundred seedlings after three years, were found to be highly variable across the case studies (Meinesz *et al.*, 1993; Balestri *et al.*, 1998; Domínguez, *et al.*, 2012; Piazzi *et al.*, 2021; Terrados 2017), ranging between encouraging values above 50% and extremely low values close to zero.

In the transplantation projects carried out, some of which continue to be monitored today, seedlings grown from seeds in tanks and seedlings found in the sea were used; several anchoring techniques were also tested (plastic grids, metal grids, plastic pots, hemp containers filled with gravel, elastic nets with pebbles, metal cages filled with stones, biomats, etc.) as well as the anchoring of shoots without the aid of supports (Castejón-Silvo *et al.*, 2018; Terrados *et al.*, 2013). Whereas a number of experiments do not show that the anchoring technique determines the survival rate of seedlings (Terrados *et al.*, 2013), the absence of studies aimed at comparing the different anchoring techniques under the same conditions (e.g. substratum, hydrodynamic action, depth) should nevertheless be highlighted.

As observed in natural recruitment, in transplantation projects carried out with the use of anchoring supports, the nature of the substrate also seems to be decisive in the evolution of the system. In this regard, in Balestri *et al.* (1998), a high survival rate of seedlings transplanted on *matte* was highlighted, whereas on pebbles the seedlings did not survive after the first few months. The movement of the pebbles in the water and continuous rubbing probably causes physical damage to the seedlings, whereas on *matte* the presence of algal vegetation can favour their retention and rooting.

Furthermore, the evaluation of the influence of the substratum on the root development of *P. oceanica* seedlings was investigated in a number of experiments in the natural environment in Balestri *et al.* (2015). In this regard, the seedlings, implanted on two different substrata (sand and rock) showed that the horizontal growth, biomass and total length of the roots were not influenced by the substratum, whereas the structure of the root system changed. On sand, the roots grew vertically to a length of 13 cm, whereas on rock, growth was more horizontal and did not exceed 7 cm. Ramification was greater on sand whereas the diameter was greater on rock. This highlights the plasticity of growth of the roots that enables *P. oceanica* to implant itself on both substrata.

Further studies have evaluated the effect of herbivores, as well as increases in nutrients and the presence of the invasive alga *Caulerpa cylindracea*, on seedlings of *P. oceanica* transplanted *in situ*. An exclusion experiment using cages in Balestri *et al.* (1998) highlighted that the exclusion of herbivores is not relevant to the survival of the seedlings, whereas the short-term results of Pereda-Briones *et al.* (2018) indicate that both nutrients and *C. cylindracea* can have a positive effect on seedling survival and growth.

Whilst *P. oceanica* seeds are currently used mainly for research purposes, the high survival rates obtained both during cultivation and when translocated into the sea mean that, when available, they could provide additional biological material in future transplantation projects at sea. With a variety of possible techniques, the important factor is to ensure the suitability of the site, particularly in relation to the type of substratum, water chemistry and hydrodynamic action. Since several anchoring techniques may be appropriate, the use of natural and/or biodegradable anchors is therefore increasingly desirable.

The next paragraph describes the transplantation of *P. oceanica* seedlings at sea, referring specifically to the experience of the Favignana (Aegadian Islands) case study.

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3.1.1 | Plantation of *Posidonia oceanica* seedlings to re-connect portions of damaged meadows

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Context

Over recent years it has been observed that *Posidonia oceanica* seedlings successfully colonise hard seabeds and that their settlement occurs by means of adhesive root hairs (Badalamenti *et al.*, 2015). This strategy represents an early settlement mechanism and favours the persistence of plants on rocky rather than soft substrata (Alagna *et al.*, 2015; Badalamenti *et al.*, 2015). Root hairs appear soon after germination in the hypocotyl and in the main and adventitious roots. The anchoring system of *P. oceanica* appears to be highly efficient, especially in the early stages of the plant's life, when the seed reaches the recruitment site and settles. One of the main impacts that determine damage and regression of *Posidonia oceanica* meadows is certainly due to mechanical causes, for example from trawling (Telesca *et al.*, 2015). Milder damage, but still capable of causing serious problems to the meadows over time, is that caused by the anchors of small boats (Milazzo *et al.*, 2004). Around the smaller islands of the Mediterranean, now popular destinations for maritime tourists, this type of damage has often been witnessed. Anchors cause injury to the meadow, a scar from which it will struggle to recover and which, in certain conditions, will even enlarge as a result of erosion of the edges (*fig. 1*).

The CNR-IAS of Palermo and Castellammare del Golfo, as part of the Marine Hazard project, PON03PE_00203_1, Italian Minis-

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try of Education, University and Research (MIUR), and in collaboration with the Aegadian Islands Marine Protected Area, has developed an experiment for the transplantation of *Posidonia oceanica* seedlings in an area where there are signs of the mechanical impact from anchors on the *Posidonia* grove in Favignana.

The aim is to carry out experimental transplantation in the seabed of Favignana (Aegadian Islands) starting from beached seeds which are then germinated in pots filled with stones alone and with stones together with rock wool of different densities, as support.

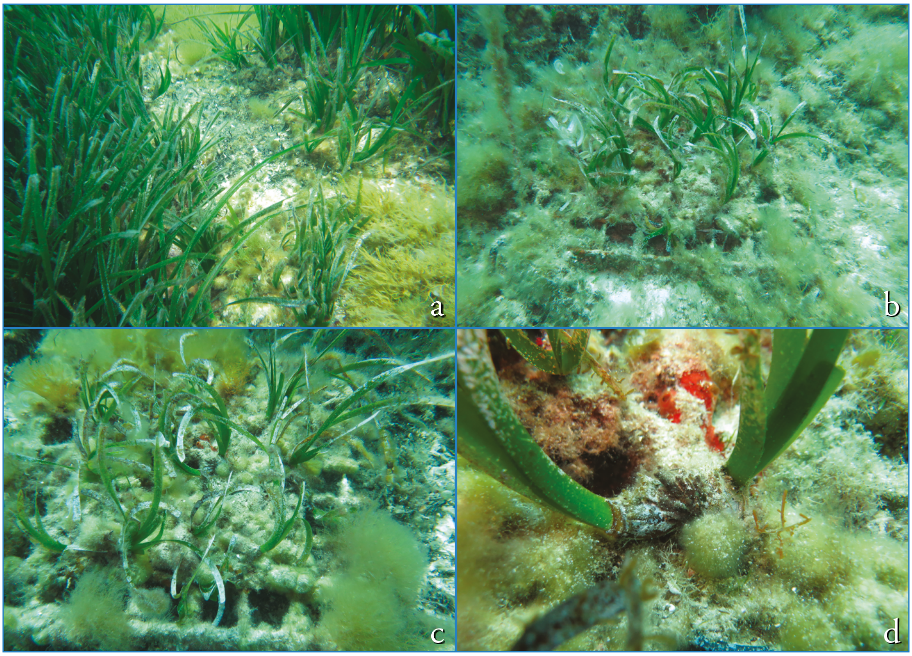

Figure 1 | Phases of monitoring of Posidonia oceanica *seedling transplantation using grids. close-up of an area with a "scar" caused by an anchor (a); close-up of a grid in monitoring in May 2018 and 2019, respectively (b and c); close-up during the 2019 monitoring of a seedling, which is developing a plagiotropic rhizome (d).*

Description of the germination technique

In April 2016, 500 beached seeds were collected along the coasts of the municipality of Marsala (TP). Immediately after collection, the seeds were transported to the laboratory in Torretta Granitola in dedicated tanks and placed in the smaller tanks in apparatus called "Wet Lab" to germinate; prior checks were carried out to identify and remove those seeds that were contaminated by mould or no longer viable. Wet Lab, produced by the French company ECOCEAN, is an insulated shipping container for housing small marine organisms (larvae and juvenile forms). The container measures 6 x 2 m and is equipped with 24 20-litre tanks, 5 300-litre tanks and a water purification system complete with mechanical and biological filters and UV lamps. The artificial lighting comes from neon lamps (natural/warm light colour temperature), and the room temperature is regulated by an air conditioner (which also keeps the water temperature constant) plus an emergency refrigerator, in case of necessity. The water in the tanks is completely filtered 3 times/hour.

At an advanced stage of growth, when, approximately three weeks after harvesting, the primary root and the first well-developed leaves appear, the seedlings were placed inside 400 perforated pots (with lumen and height of 5 cm); 200 of the pots contained pebbles of calcareous material into which the seed was placed and to the remaining pots rock wool was added to cover the opening. The pots were placed into the chambers of the cavity bricks placed on the bottom 4 of the Wet Lab tanks (*fig. 2*). The seedlings remained in the Wet Lab until November 2016, when they were taken to Favignana for transplantation on the seabed.

Figure 2 | Close-up of the Wet Lab tanks with seedlings in pots slotted into cavity bricks.

Description of the transplantation technique

The experimental design involved transplanting the seedlings in their pots onto dead *Posidonia matte* using 35 x 35 cm steel grids with 5 cm mesh (*fig. 1*). The grids were filled with the two types of pots, with stones (CP) and with stones and rock wool (CPLR), in three different densities, 5, 13 and 25 pots per grid, for a total of 18 grids and 258 seedlings. The grids were distributed over an area on the south coast of the island of Favignana where small impressions on the plant had been recorded and which had been caused by the anchors of pleasure boats, leaving scars in the substratum stripped of the rhizomes of the plant (so-called dead *matte*).

In November 2016, the 258 pots containing the approximately 8-month-old seedlings were wrapped in elastic cotton nets and transported, in insulated boxes to avoid thermal stress, from the Wet Lab to the island of Favignana. Once on the island, the seedlings were transferred to a boat made available by the Aegadian Islands Marine Protected Area. The experimental transplantation was carried out in Calamone on 21 November 2016 (*fig. 3*). The divers fixed the grids in the area where the "dead *matte* scars" were to be found. The steel grids were fixed to the seabed at a depth of 10 m using small steel stakes (*fig. 4*).

Figure 3 | filling of the grids with the Posidonia oceanica *seedlings contained in pots protected by elastic cotton nets on board the boat (a) and lowering into the sea (b).*

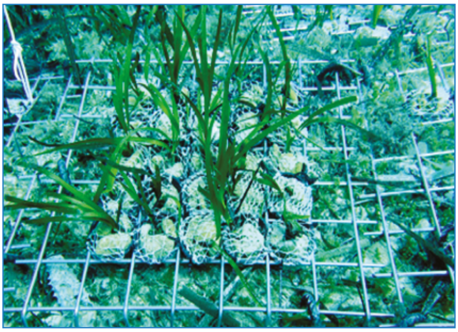

Figure 4 | Close-up of a grid, with a density of 25 pots, placed on the Posidonia oceanica *dead matte.*

Results

The survival rate in the tanks was 80%, corresponding to 360 viable seedlings. The transplantation was periodically checked to verify the status of the grids and, in May 2018, 2019 and 2021, to estimate the survival rate of the seedlings by type of pot and by density. Monitoring found that all the grids were in place and that all the pots remained in place in the grids. Over time, a reduction was observed in the survival rate of the seedlings, varying between a minimum loss of 18% in the grids with a high density of seedlings and a maximum of 100% in several low-density grids. Survival was found to be proportional to the initial density. In both types of pot, the greatest survival rate was recorded where the density was 25 seedlings per grid and the lowest where the density was 5 seedlings per grid. The pots containing stones and rock wool (CPLR) showed relatively higher survival rates than pots containing stones alone (CP).

Environmental sustainability of the technique

The research briefly described in this report is still ongoing. The results, although encouraging, are preliminary. Further experimentation is needed before a hypothesis regarding small-scale reforestation by means of seedling transplatation can be formulated. The frontier topics addressed in the aforementioned Marine Hazard project include the choice of eco-compatible substrates that can be used to support the implantation of seedlings and combat infection derived from pathogens that develop during the germination phases of the seeds and the growth of seedlings.

Period of intervention

Seeds are harvested between April and June and sown preferably in November.

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Produced with the contribution of the LIFE Programme
of the European Union project LIFE 16 GIE/IT/000761

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