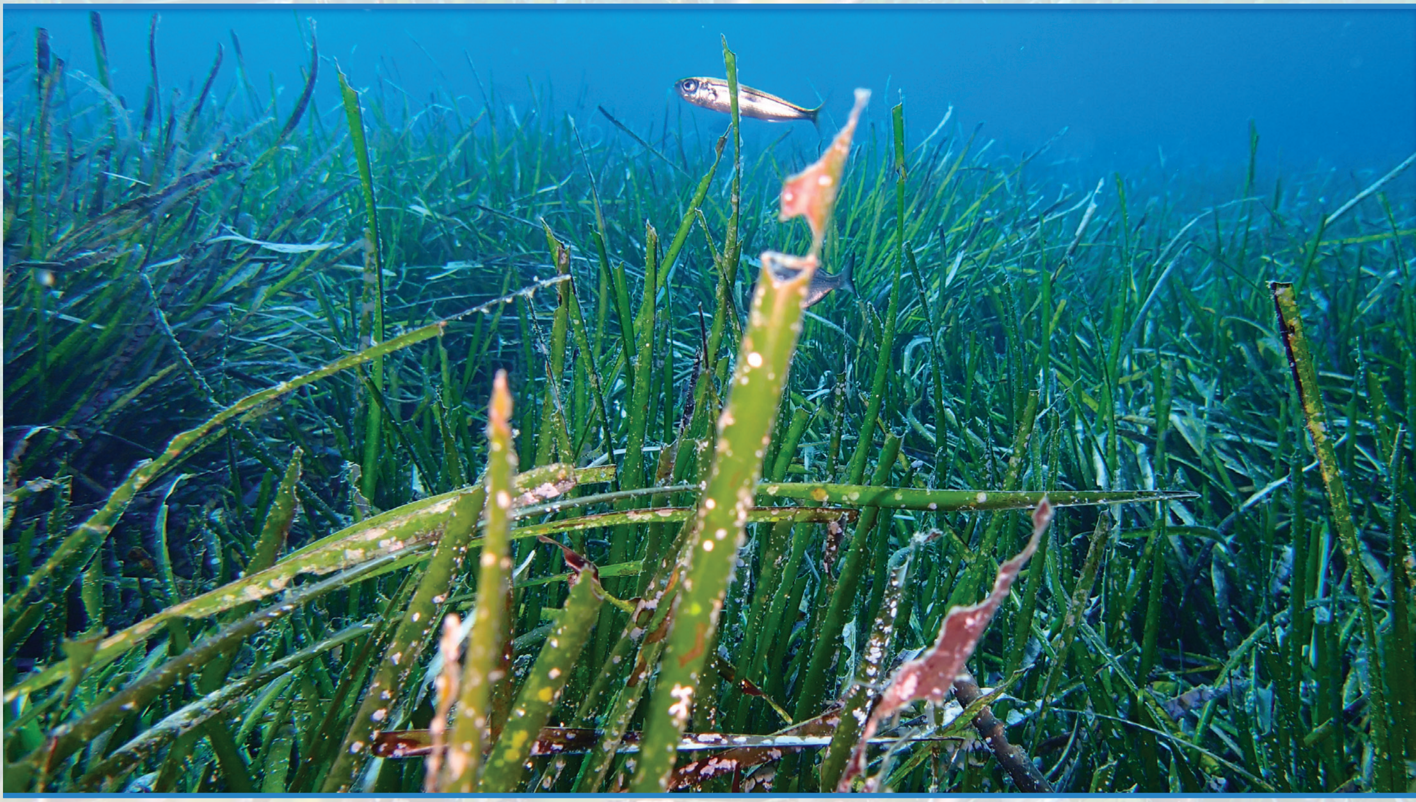
S.E.POS.S.O. life project

Supporting Environmental governance for the POSidonia oceanica Sustainable transplanting Operations

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

MANUAL

for the planning, implementation and monitoring of 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

EDITED BY: La Porta Barbara, Bacci Tiziano
Italian Institute for Environmental Protection and Research- ISPRA,
Via Vitaliano Brancati 48, 00144 Roma (Italy)

AUTHORS
Bertasi Fabio[1],Targusi Monica[1], Badalamenti Fabio[2], Scardi Michele[3], Calvo Sebastiano[4], Tomasello Agostino[4], Zenone Arturo[2,5], D'Anna Giovanni[6], Pipitone Carlo[2], Piazzi Luigi[7], Mancusi Cecilia[8], Cozzolino Giacomo[9], Piazzi Alessandro[9], Cacciuni Anna[1], Lucia Viviana[1], Bulleri Claudia[10], Conconi Stefano[11].

BOX
Importance of genetic components in marine phanerogams restoration.
Procaccini Gabriele[5], Pazzaglia Jessica[12].

[1]Italian Institute for Environmental Protection and Research - ISPRA, Via Vitaliano Brancati 48, 00144 Roma (Italy).
[2]CNR-IAS, Lungomare Cristoforo Colombo 4521, 90149, Palermo (Italy).
[3]Department of Ecology, University of Rome "Torvergata", Via Cracovia 1, 00133, Roma (Italy).
[4]Department of Earth and Sea Sciences (DiSTeM), University of Palermo, Viale delle Scienze, Edificio 16, 90128 Palermo (Italy).
[5]Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Lungomare Cristoforo Colombo 4521, 90149, Palermo (Italy).
[6]CNR-IAS, Via G. Da Verrazzano 17, 91014, Castellammare del Golfo (Italy).
[7]Department of chemistry e pharmacy, University of Sassari, Piazza Università 21, 07100, Sassari (Italy).
[8]Environmental Protection Agency of Tuscany Region - ARPAT, Via G. Marradi 114, 57126 Livorno (Italy).
[9]SETIN, Via Guido d'Arezzo 16, 00198, Roma (Italy).
[10]Port System Authority of the Northern Tyrrhenian Sea, Piazz.le Premula 6/a, 57025, Piombino (Italy).
[11]VESENDA, Via Cuzio Polo Tecnologico 42, 27100 Pavia (Italy).
[12]Department of Life Science, University of Trieste, Via Licio Giorgieri 5, 34127, Trieste (Italy).

CITATION
La Porta B., Bacci T. (2022). Manual for the planning, implementation and monitoring of transplantation of *Posidonia oceanica*. LIFE SEPOSSO (LIFE16 GIE/IT/000761), Rome.

Edition: March 2022

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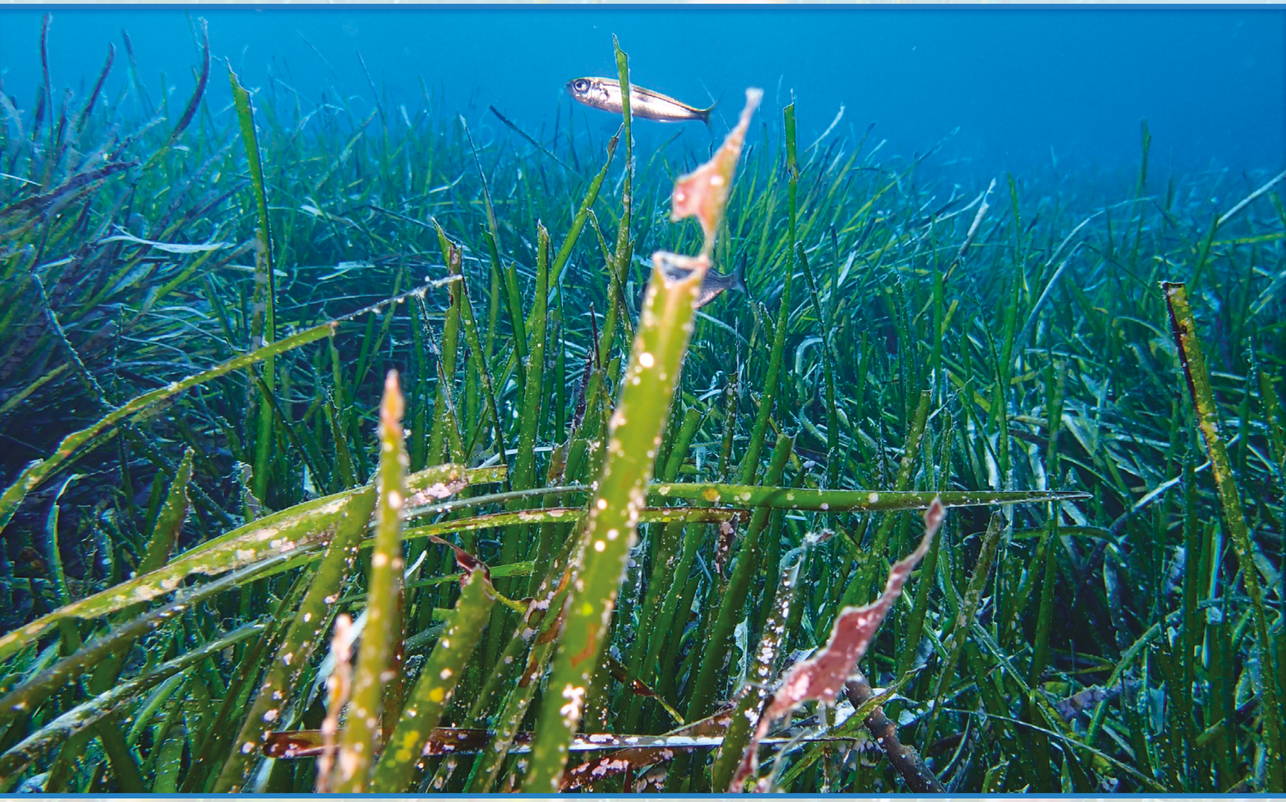

S.E.POS.S.O.
life project

Supporting Environmental governance for the POSidonia oceanica Sustainable transplanting Operations

NATURA 2000

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INDEX

PREFACE

The Italian Constitution, as of February 2022, has included among the fundamental principles, the protection of the environment, biodiversity, and ecosystems. In addition to defining the environment in its broadest sense and from a systemic perspective, it is considered as a 'value' to protect also, "*in the interest of future generations*", for which the exercise of economic activity cannot be carried out to its detriment. At present, 19.1 percent of Italian waters is subject to some conservation measure, however, in order to reach the objectives of the UE Strategy on biodiversity by 2030, this percentage must increase significantly. To this end, the National Recovery and Resilience Plan (NRRP), for Italy, calls for large scale interventions to restore and protect seabeds and marine habitats in national waters, so as to contribute to reversing the trend in Mediterranean ecosystem degradation and increase their resilience to climate change.

Posidonia oceanica meadows, a habitat that is endemic to the Mediterranean and protected (Habitat Directive 1992/43/CEE), even if they only occupy 1% of Mediterranean seabeds, play a vital role in the equilibrium of the marine ecosystem. This marine plant, in fact, produces approximately 20 l/m^2 of oxygen per day and subtracts carbon dioxide from the environment counteracting climate change, is home to about 25% of the Mediterranean marine biodiversity, helps to fight coastal erosion thanks to its dense foliar canopy, and stabilises sandy seabeds with its typical terraced structure called *matte*. Unfortunately, *P. oceanica* meadows are experiencing regression in various areas of the Mediterranean basin: it is estimated that in the last 50 years, their surface has declined of over 30%. The different causes of regression, together with the slowness of the natural recolonisation processes of the plant, have promoted the development of techniques of *P. oceanica* transplantation-over time, as a tool to support or hasten natural regeneration processes.

The LIFE SEPOSSO Project (*Supporting Environmental governance for the POSidonia oceanica Sustainable transplanting Operations* LIFE16 GIE/IT/000761), coordinated by the ISPRA, Italian Institute for Environmental Protection and Research, together with the partners and numerous stakeholders involved, has verified the success of the transplants carried out in Italy and has provided specific tools for their improvement. The "Manual for the planning, implementation, and monitoring of transplantation of *Posidonia oceanica*", represents one of these tools, and provides specialists and the different stakeholders with detailed information and guidelines for planning, implementation, monitoring and management of *P. oceanica* transplants. The process described is applicable to any transplant, aside from its objective, such as the restoration of degraded meadows, the 'mending' of sections of damaged meadows, the compensation of areas of meadows following complete loss due to the execution of marine and coastal projects. It is the very existence of economic activities and coastal infrastructures, potentially damaging for *Posidonia oceanica* meadows, that raises important questions, connected for example, to public information concerning the meadows, to the usefulness and success of transplantation, to the level of participation of the different subjects involved, to sharing and accessing data. The achievement of good governance for restoration activities for *P. oceanica* meadows, destined to a wide and diversified public, will soundly contribute to the transformational change required for the implementation of increasingly effective measures for the protection, conservation, and restoration of this precious and fragile Mediterranean habitat. This will not only play a part in reaching national and European objectives for biodiversity and climate change, but will also favour the maintenance and sustainability of activities that are essential for the coastal areas, such as fishing, tourism, and 'blue' growth, in accordance with European environmental regulations (*i.e. 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).

Alessandro Bratti
GENERAL DIRECTOR OF ISPRA

CHAPTER 1

POSIDONIA OCEANICA MEADOWS

1.1 | BIOLOGICAL CHARACTERISTICS

Posidonia oceanica is an endemic species of the Mediterranean Sea. Whilst often confused with algae, it is a marine phanerogam, a plant organised into roots, stem, called rhizome due to its hypogeal *habitus* and leaves (*fig.* 1.1).

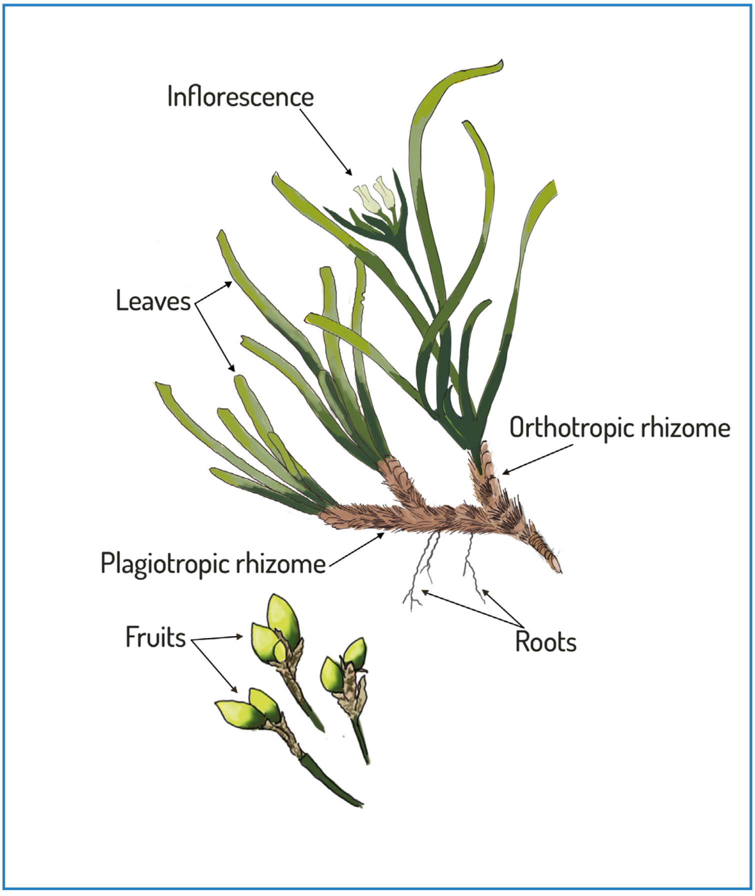

Figure 1.1 | Posidonia oceanica *plant (drawings by Sonia Poponessi, ISPRA - Life SEPOSSO).*

The rhizomes are modified stems, which have the characteristic of growing both horizontally (plagiotropic rhizome) and vertically (orthotropic rhizome). The plagiotropic rhizomes have the function of anchoring the plant to the substratum, thanks to roots on the underside, and of enabling the colonisation of new areas. The orthotropic rhizomes, on the other hand, counter progressive silting from continuous sedimentation and thus take advantage of the available space and light as they grow in height. This vertical development brings about a progressive rising of the sea floor and the formation of what is known by its French term of *matte* (Pérès and Picard, 1964). The overall growth of the *matte*, due to sedimentation and erosive action from wave motion and currents, was estimated at approximately 1 m per century by Molinier and Picard (1953); other studies have estimated much lower average growths of about 10 cm per century (Boudouresque and Jeudy De Grissac, 1983). Regardless of the original substratum of the plant (sand or rock), *matte* consists of several layers of the intertwined rhizomes and roots of old plants and the sediment trapped between these elements; only the top of the *matte* is covered with live plants (*fig.* 1.2). The leaves, which come from orthotropic rhizomes, are ribbon-like with rounded tips and are intense green in colour; they have an average width of one centimeter and can reach one meter in length (Boudouresque and Jeudy De Grissac, 1983); they are differentiated into a photosynthesizing limb and, in leaves that have reached a certain stage of development, a base that is lignified, depending on age. The limit between the limb and the base is formed by a concave line, the ligule, in correspondence with which the leaves detach, leaving the bases on the rhizome. Over time, these are reduced to thin flakes that endure, forming a "sleeve" that envelops the rhizome.

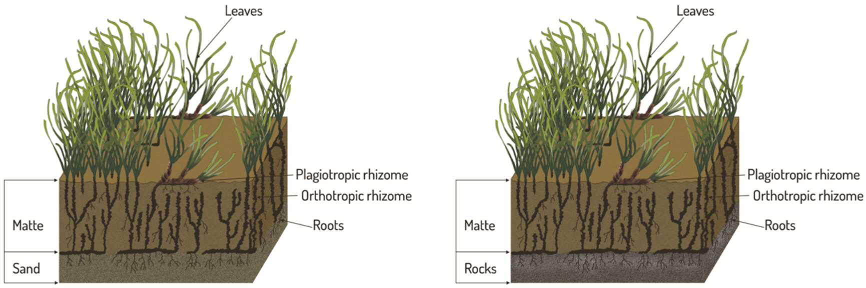

Figure 1.2 | Structure of Posidonia oceanica *on* matte *(from Boudouresque and Meinesz, 1982, modified - drawings by Sonia Poponessi, ISPRA - Life SEPOSSO).*

The leaves are arranged in bundles, each of which contains an average of six or seven leaves distributed in a fan shape: the oldest, of greater length, are found on the exterior of the bundle and younger ones, of smaller size, in the interior. The growth of the leaves, which originate from a basal rather than apical meristem, is specific and this adaptation permits the growth of the leaf blade even when the apex, which becomes the oldest part, first encounters degenerative phenomena.

As far as reproductive features are concerned, *P. oceanica* has both asexual and sexual methods of reproduction. The main method of reproduction of *P. oceanica* is asexual or vegetative by means of stolons; it occurs through the multiplication and growth of plagiotropic and orthotropic rhizomes. This process is especially slow, with the elongation of the rhizomes reaching an average of 2 centimeters per year (Marbà and Duarte, 1997); plagiotropic rhizomes grow faster than orthotropic rhizomes. Furthermore, several rhizomes with both horizontal and vertical development can originate from plagiotropic rhizomes. Sexual reproduction rarely occurs and takes place by means of the production of inflorescences, generally bearing 3-5 hermaphroditic flowers (*fig.* 1.3) (Buia and Giaccone, 2008). The fruits ripen from the flowers (*fig.* 1.4) and, once detached from the plant, float until the pericarp breaks, releasing the seed from which a new plant will develop. This method of reproduction enables the plant to colonise new areas, but at the same time it can determine the beaching of considerable quantities of fruits and seeds, thereby reducing the reproductive success of this species (Molinier and Picard, 1953; Buia and Mazzella, 1991).

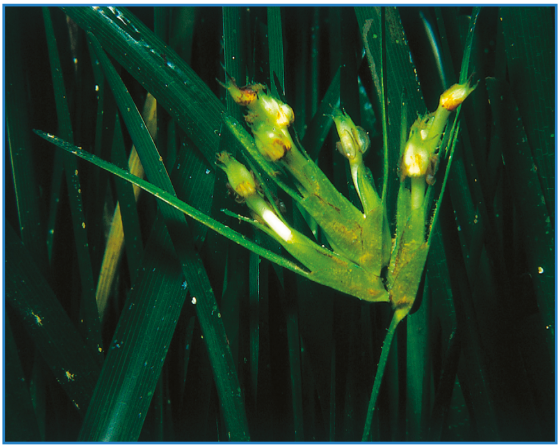

Figure 1.3 | Inflorescence of Posidonia oceanica *(photography: Agostino Tomasello).*

Figure 1.4 | Meadow of Posidonia oceanica *with fruits (photography: Agostino Tomasello).*

1.2 | PHYSIOGRAPHIC FEATURES AND STRUCTURE OF THE MEADOW

Posidonia oceanica needs bright illumination and, therefore, both the clarity of the water and depth are determining factors for its growth.

When *P. oceanica* encounters favourable environmental conditions, it colonises large areas of the seabed, forming vast meadows (*fig.* 1.5). *P. oceanica* meadows extend from the surface to depths of approximately 30-35 meters, greater than 40 meters in particularly clear waters, and cover an estimated area of approximately 12,000 km^2 in the Mediterranean (Telesca *et al.*, 2015).

Figure 1.5 | Posidonia oceanica *meadow (photography: Agostino Tomasello).*

P. oceanica settles more commonly on soft substrata such as sand of varying degrees of coarseness, sometimes mixed with mud, but also on detrital and rocky seabeds. Depending on the composition, "pure" or mono-species meadows (characterised by the *P. oceanica* species alone) and "mixed" or multi-species meadows (characterised by the coexistence of several species of phanerogams) can be found; on the basis of the distribution of the plant on the seabed, on the other hand, the meadows are defined as "homogeneous" (uniform distribution over the entire extension) or "heterogeneous" (irregular distribution) (Buia *et al.*, 2003).

Regardless of the distribution of the meadow, the foliar density decreases as the depth increases or as a function of the transparency of the water, with values in excess of 700 shoots m^{-2} (Pergent *et al.*, 1995) and an average distance between the shoots of about 2 cm (Bacci *et al.*, 2017).

In any case, wherever the plant settles, it considerably modifies the original implant substratum because the foliar layer of the phanerogam forms a trap for the particles suspended in the water column facilitating their sedimentation (Dauby *et al.*, 1995).

The extension of a *P. oceanica* meadow is defined by its "upper limit" and a "lower limit". The upper limit corresponds to the shallowest bathymetry at which the meadow begins and is always very clear cut, whereas the lower limit, the deeper bathymetry at which the meadow ends, can have several conformations, including a progressive or shaded limit, a sharp limit, an erosive limit and a regressive limit (Meinesz and Laurent, 1978; Pergent *et al.*, 1995) (*fig.* 1.6). For further information, see Montefalcone (2009).

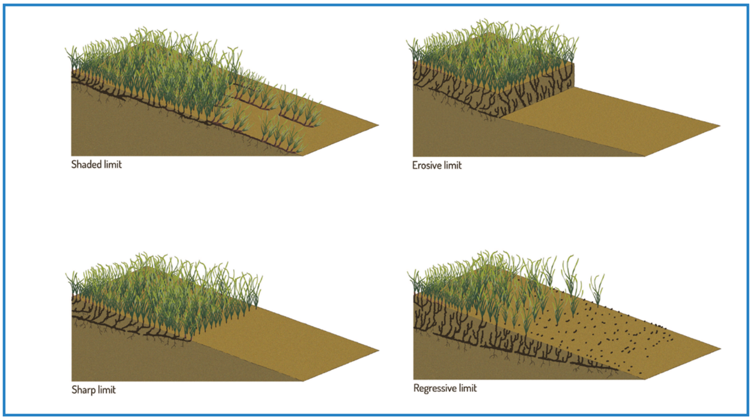

Figure 1.6 | Main types of lower limit of Posidonia oceanica *meadows (shaded limit; sharp limit; erosive limit; regressive limit (from Pergent* et al.,*1995, modified -drawings by Sonia Poponessi, ISPRA - Life SEPOSSO).*

1.3 | ECOLOGICAL CHARACTERISTICS

Posidonia oceanica meadows represent very complex and well-structured biocoenoses, characterised by great biological variability of their plant and animal communities (Buia *et al.*, 2000). The biocoenosis is constituted by the superimposition of photophilic populations, associated with the foliar layer, and sciaphilic populations, associated with the rhizomes and the *matte* (Mazzella *et al.*, 1989; Gambi *et al.*, 1992). The species associated with the foliar layer are often exclusive to the leaves of *P oceanica*; the species associated with the rhizomes, on the other hand, do not present such distinctive exclusive elements and characteristics, as they are similar to the sciaphilic species of the infralittoral or circalittoral coralligenous zones, depending on the corresponding depth and quantity of light (*fig.* 1.7) (Boudouresque, 1968; Piazzi *et al.*, 2002).

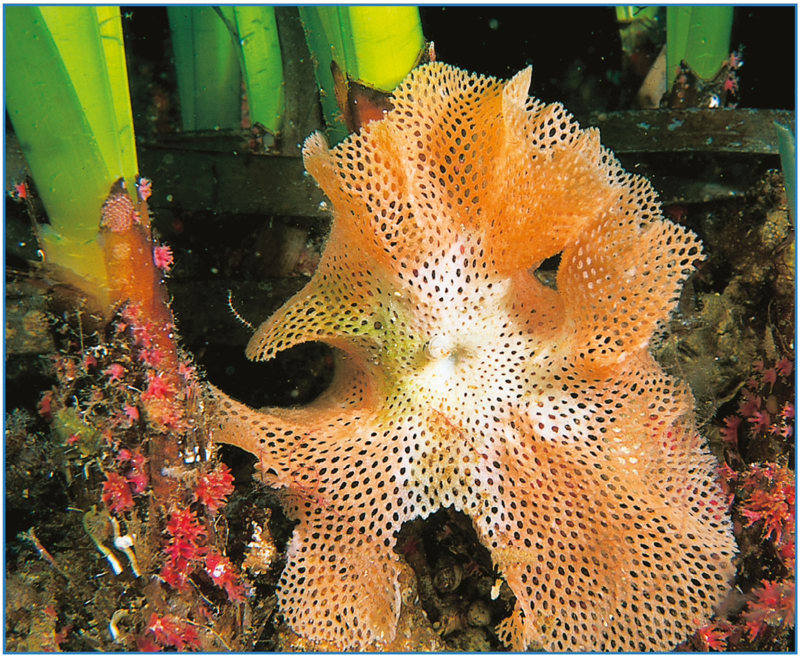

Figure 1.7 | Epiphytic rhizomes of Posidonia oceanica *(photography: Agostino Tomasello).*

The species within the meadow include resident and migratory species: the former spend their entire life cycle inside the meadow, whereas the latter arrive from surrounding environments for the time necessary to search for food or shelter or to reproduce (Buia *et al.*, 2000).

The *P. oceanica* ecosystem also provides nursery areas for juvenile fish and refuge for a large number of organisms, including numerous species of fish, cephalopods and crustaceans, also of considerable economic importance (Francour, 1997). The meadow therefore plays an extremely important role as a hub of biodiversity, hosting about 25% of all species present in the Mediterranean Sea (Boudouresque *et al.*, 2012).

The meadows of *P oceanica* are recognised as one of the fundamental components of the balance and richness of the Mediterranean coastal environment. They are characterised by high levels in the production of oxygen (1 m^2 of meadow can produce 4 to 20 litres of oxygen daily; Bay, 1978) and of organic matter (1 hectare of meadow produces about 20 tons in one year; Boudouresque and Meinesz, 1982) and one of the highest in primary production for the marine environment in the world (Pergent *et al.*, 1994; Pergent-Martini *et al.*, 1994). A considerable part of this primary production (from 25% to 85%, as reported in Boudouresque *et al.*, 2006) is exported in the form of dead leaves to other types of seabed, where it represents a highly significant source of nourishment. The organic matter produced constitutes a direct and indirect food source for numerous organisms, as well as the basis of a complex trophic network (Mazzella *et al.*, 1992). Furthermore, this characteristic of high primary production enables the ecosystem of the *P. oceanica* meadows to sequester large quantities of carbon called "*blue carbon*" and large quantities of carbon dioxide from the atmosphere (Duarte *et al.*, 2005; Kennedy and Björk, 2009; Mcleod *et al.*, 2011), thus helping to reduce the risks due to climate change, with multiple co-benefits (Bindoff *et al.*, 2019). The importance of the *P. oceanica* meadows, which play a fundamental role in the general economy of coastal areas, therefore far exceeds the surface area they occupy of less than 1% of the Mediterranean seabed (Boudouresque *et al.*, 2006)

1.4 | THE ROLE OF POSIDONIA OCEANICA MEADOWS IN COASTAL DEFENCE

Posidonia oceanica meadows are recognised for the fundamental role they play in coastal dynamics by acting on sedimentation, at least on a local scale, and modifying the original sediment of the site (Dauby *et al.*, 1995). This phenomenon is due to the dual action that living leaves exert both on the fine particulate, which is captured and harnessed between the rhizomes, and on the waves and currents whose intensity is considerably reduced; the *matte* is both elastic and rigid and can absorb some of the wave energy (Fonseca *et al.*, 2007; Boudouresque, 2013). Lastly, dead leaves, carried ashore by the currents and mixed with sand, create masses, at times exceeding one meter in height (*banquettes*), which protect the beaches and alleviate the damage caused by storms (Jeudy de Grissac, 1984) and thereby form a significant natural belt for the containment and protection of the coasts from the erosive action of wave motion.

In order to safeguard the *P. oceanica* meadows as natural barriers against coastal erosion, it is of fundamental importance to identify the areas they occupy and study their structural, functional and ecological features by means of specific field surveys that yield up-to-date and detailed maps and reliable information on the health of the meadows and their associated populations.

1.5 | THE CAUSES OF REGRESSION OF POSIDONIA OCEANICA MEADOWS

Posidonia oceanica is particularly affected by variations in the environmental quality and disappears when pollution, in a broad sense, is marked; for this reason, *P. oceanica* is considered an excellent indicator of the quality of the environment (Pergent *et al.*, 1995; Montefalcone, 2009).

Despite the importance of this habitat, we have been witnessing a progressive regression and degradation of the meadows, often due to synergism in human activity, since the 1950s (Boudouresque *et al.*, 2012; Giakoumi *et al.*, 2015). In the Mediterranean, between 13% and 50% of the *P. oceanica* meadows have regressed considerably in terms of size, and even been completely lost from some areas, and the remaining meadows have suffered a reduction in density and coverage (Marbà *et al.*, 2014; Telesca *et al.*, 2015). Therefore, Marbà *et al.*, (2014) estimate that the loss of *P. oceanica* meadows has reduced the carbon absorption capacity of the entire Mediterranean basin by between 11% and 52%. Unfortunately, there is a lack of detailed data on the distribution and quality of *P. oceanica* for most of the Mediterranean Sea (Boudouresque *et al.*, 2009), and it is possible that the regression of phanerogam meadows has been overestimated (González-Correa *et al.*, 2007; Boudouresque *et al.*, 2009; Bonacorsi *et al.*, 2013).

A number of authors assert that *P. oceanica* is showing a progressive maladjustment to the Mediterranean environment, leading to a natural rarefaction of the meadows, mainly along the northern coasts (Blanc and Jeudy de Grissac, 1989). The poor success of sexual reproduction seems to have led to a decrease over time in genetic variability within populations, which may have made the species more vulnerable to changes in environmental conditions (Procaccini *et al.*, 1996). However, the main causes of regression in the meadow are to be linked to increasing anthropic pressure on the coastal environment, which determines effects on the meadow essentially attributable to variations in the turbidity of the water column and to variations in sedimentation rates, as well as to the direct effects attributable to the damage caused by trawling and anchorage (Boudouresque *et al.*, 2006).

The high concentration of organic pollutants, due, for example, to urban or industrial wastewater discharge, cause excessive algal development and can cause both an increase in the turbidity of the water and an excessive development of epiphytes on *P. oceanica* leaves (Boudouresque *et al.*, 2009). In both cases, the intensity of light that can reach the plant is reduced, with negative consequences for its survival. Chemical substances (*e.g.* surfactants, heavy metals, etc.) can also cause tissue necrosis, morphological alteration and generally disrupt the normal development processes of the plant (Capiomont *et al.*, 2000).

Another very important aspect that can affect the health of the *P. oceanica* meadows is linked to the variation in sedimentary rates along the coast, brought about by coastal construction projects. The construction of ports and port works in general, as well as the construction of rigid defence works, can drastically interfere with the normal hydrodynamic regime and cause significant alteration in sedimentary dynamics, especially at a local level (Boudouresque *et al.*, 2009). Increases and reductions in sedimentary supplies are recognised as sources of serious problems for the survival of the meadows; the former encourages silting up and consequent suffocation (Marbà and Duarte, 1997; Manzanera *et al.*, 1998), and the latter promoting the undermining of rhizomes, thus making the meadow more sensitive to erosive phenomena (Jeudy de Grissac, 1979; Astier, 1984) (*fig.* 1.8).

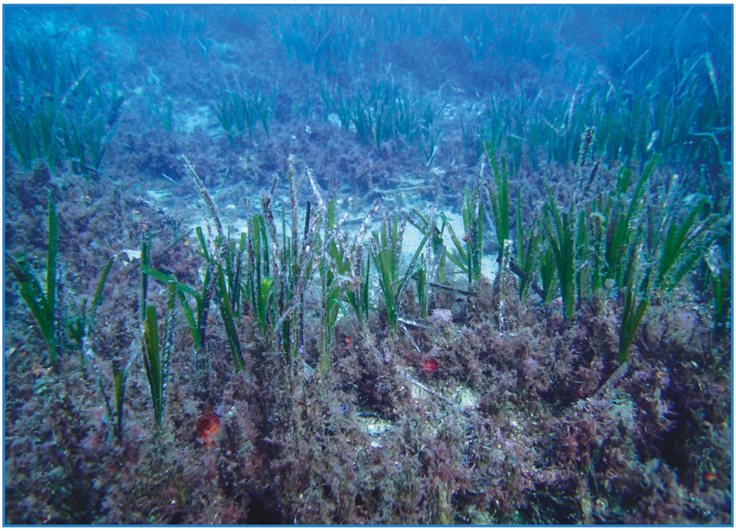

Figure 1.8 | Degraded Posidonia oceanica *meadow (photography: Tiziano Bacci).*

Among the projects that affect the marine and coastal environment and that can have direct or indirect effects on *Posidonia oceanica* meadows, the more common ones are commercial and tourist ports, breakwaters, piers and other coastal protection projects, submarine pipelines (waterworks, oil and gas pipelines), electricity transmission cables, *off-shore* production facilities.

Furthermore, where the meadow already shows significant signs of distress, its health can undergo further deterioration from the introduction of alien species that begin to compete with *P. oceanica*, hindering the re-colonisation process (Montefalcone *et al.*, 2010), as in the case of the expansion of two species of green algae of tropical origin belonging to the genus *Caulerpa* (de Villèle and Verlaque, 1995).

In general, the loss of the vegetation cover of the seabed leads to the erosion of the exposed surface layers (Marbà *et al.*, 2015), which can lead to the release of stored carbon (blue carbon), an increase in greenhouse gas emissions and the acceleration of global climate change (Atwood *et al.*, 2017).

Lastly, additional potential causes of regression of the meadows could be attributable in the future to the effects of climate change, the latter still relatively less known than those related to anthropic pressure. The warming of the Mediterranean Sea and the increase in extreme meteorological phenomena could have consequences for *P. oceanica*, both due to the increase in the average temperature of the water and due to its progressive acidification (Short and Neckles, 1999; Caldeira and Wickett, 2003; IPCC, 2019).

CHAPTER 2

REGULATORY FRAMEWORK

2.1 | POSIDONIA OCEANICA: EUROPEAN DIRECTIVES, INTERNATIONAL CONVENTIONS, NATIONAL LAWS

The conservation and valorisation of *Posidonia oceanica* meadows is established by international conventions, European directives, and national laws.

As a meadow, *P. oceanica* is protected as laid down in the Habitat Directive 1992/43/CEE (incorporated into Italian law by Presidential Decree 357/1997 and subsequent modifications and integrations), as a priority habitat type, whose conservation requires the designation of special areas of conservation. The presence of *P. oceanica* meadows entails the creation of Sites of Community Importance (SCI) and Special Areas of Conservation (SACs) that represent, together with the Special Protection Areas (SPAs) (Birds Directive), the Sites of the Natura 2000, a network of protected natural areas, designated by the European Community, whose purpose is to safeguard the state of conservation of species of flora and fauna, and of types of habitats that require targeted protection measures. In accordance with what is prescribed in the Habitat Directive, any activity that can interfere with the conservation status of a site, even in the case that such activity is carried out outside of the site itself, shall be subjected to a specific procedure, called "Appropriate Assessment" (AA). The AA is aimed at verifying, preemptively, to what extent the expected activities affect the state of conservation of the species and/or habitat for which the site was created, and the failure to observe the specified procedure entails the initiation of infringement procedures by the European Community (AA.VV., 2019a).

Mention is also made of Angiosperms, to which the species *Posidonia oceanica* belongs to, in the Water Framework Directive, WFD 2000/60/CE, incorporated into Italian law by the Legislative Decree 152/2006 and subsequent modifications and integrations, that regulates the ecological status and chemical quality of water bodies. This directive assigns, for the first time, a key role to biological indicators in the assessment of the ecological status of surface water bodies, leaving a supporting role to chemical, physical and morphological indicators. In this context, Italy has identified among Angiosperms the species *P. oceanica* as indicator of ecological status, to be used for the purposes of assessing the ecological status of water bodies.

National implementation of the Marine Strategy Framework Directive, MSFD – 2008/56/CE, incorporated into Italian law by the Legislative Decree 190/2010, lastly, regards among the habitats being evaluated for ecological status the biocenosis *P. oceanica*. This assessment is made by defining a Good Environmental Status - GES, reached through environmental targets with a view to a sustainable management of the marine environment.

At species level, *P. oceanica* is protected under the Bern Convention (Appendix 1) and the Barcelona Convention (SPA/BIO protocol).

The Bern Convention, on the conservation of European wildlife and natural habitats, approved by the European Council with Decision 82/72/CE of 3 December 1981 and ratified by Italy with Law 503 of 5 August 1981, identifies, in particular, the species of flora and fauna strictly protected (that is species and habitats of vulnerable species, at risk of extinction and/or endemic), specifying also the respective protection rules.

Furthermore, it is important to emphasise that the degradation caused to the species and habitats protected under the Bern Convention and the Habitat Directive (and therefore to the *P. oceanica* meadows) constitutes an objective environmental damage, as defined under Art. 300 of the Legislative Decree 152/2006.

The Barcelona Convention (16/02/1976), that originated as a "Convention for the protection of the Mediterranean Sea from pollution" under the auspices of the UNEP (United Nations Environment Programme) was amended in 1995, becoming a "Convention for the protection of the marine environment and the coastal region of the Mediterranean". In this form it was ratified by Italy with the Law n. 175 of 27 May 1999, and it entered into force in 2004. In order to reach the intended purposes, the Convention made use of seven protocols, and in particular regarding the identification of endangered habitats and/or species to protect, the default agreement is represented by the Protocol regarding the Specially Protected Areas and the Biodiversity in the Mediterranean (SPA/BIO Protocol, http://www.rac-spa.org/dl/protocol_eng.pdf).

2.2 | REGULATIONS FOR MARINE AND COASTAL PROJECTS THAT POTENTIALLY AFFECT POSIDONIA OCEANICA MEADOWS

Among the projects that affect the marine and coastal environment and that can have direct or indirect effects on *Posidonia oceanica* meadows, the more common ones, as was already mentioned, are commercial and tourist ports, breakwaters, piers and other coastal protection projects, submarine pipelines (waterworks, oil and gas pipelines), electricity transmission cables, off-shore production facilities.

Some of these projects are subjected to an Environmental Impact Assessment (EIA), and have been grouped in two lists included in the Environmental Act Legislative Decree 152/06 Part II and subsequent modifications and integrations. The lists are: a) Annex II and b) Annex III, incorporating the indications of the Legislative Decree 104/2017, which establishes that the projects in Annex II fall under State competence, and those in Annex III fall under regional competence and of the autonomous provinces of Trento and Bolzano. Projects listed in Annex II-bis in the Legislative Decree 152/2006 Part II and subsequent modifications and integrations, are instead, subjected to an Environmental Impact Assessment (EIA), that falls under State responsibility.

For projects subjected to an EIA that falls under State competence, the authority in charge is the Ministry for Ecological Transition - Directorate General for Environmental Assessments and Authorisations, that is responsible for issuing the EIA measure, whose outcome can be positive, positive with environmental conditions, or negative, for the execution, implementation or dismissal of the submitted project. An environmental condition of the EIA procedure is, "*A binding provision that defines the requirements for the execution of the project or the implementation of the activities arising from it, that is the measures envisaged to avoid, reduce and, if possible, compensate any significant and negative environmental impacts, as well as the measures of environmental monitoring*" (Art. 5 c. 1 o quater). The due process is similar for projects subjected to an EIA that falls under regional competence, for which the authority in charge is the public administration, whose tasks are the safeguarding, protection, and valorisation of the environment, identified in accordance with the provisions of regional laws or of the Autonomous Provinces of Trento and Bolzano.

The projects that can have an impact on a *Posidonia* meadow that is included in the Natura 2000 network, require an Appropriate Assessment (AA) that was introduced by Article 6, comma 3, of the 92/43/CEE "Habitat Directive" and incorporated into Italian law by the Presidential Decree 357/97 (Art. 5), as amended and supplemented by the Presidential Decree 120/03 (Art. 6). These latter identify in the preparation of a specific study for the AA, the tool to determine and assess the effects of projects on protected habitats or species.

The installation of submarine cables and pipelines causes an unsettling of the seabed, and is therefore included in the projects that potentially generate direct or indirect impacts on *Posidonia oceanica* meadows, and subjected to different authorisation procedures, depending on the project's characteristics, such as an EIA or the granting of an authorisation pursuant to the Ministerial Decree of 24 January 1996 and of the Art. 109 co. 5 of the Legislative Decree 152/06.

Both in the context of the EIA and in that of the AA, as it is also for Art. 109 of the Legislative Decree 152/06, *Posidonia* transplantation is commonly indicated as a compensation measure for the damage suffered by the meadows due to the execution or discontinuation of the above-mentioned projects. As with all environmental conditions, also those concerning *Posidonia* transplantation are subjected to an assessment of compliance by the Ministry for Ecological Transition, or by the regional authority in charge, or by the Autonomous Provinces, and therefore detailed planning of transplantation and monitoring activities, renders the assessment of the compensation measure more effective and efficient for the protection of the restored *Posidonia oceanica* meadow.

For a detailed examination of regulatory and technical aspects connected to the EIA and AA procedures, please refer to AA.VV. (2019a, 2022).

2.3 | EUROPEAN AND NATIONAL ENVIRONMENTAL LEGISLATION FOR THE SUSTAINABLE MANAGEMENT OF MARINE AND COASTAL ENVIRONMENTS

Posidonia oceanica meadows are experiencing regression in various areas of the Mediterranean basin: it is estimated that in the last 50 years their surface has decreased of over 30% (Telesca *et al.*, 2015). Human activities and their related forms of pollution are among the main threats for this ecosystem. Therefore, identifying effective tools for an efficient synergy among European environmental legislations represents the best approach for a sustainable management of marine and coastal environments and of this precious habitat. The United Nations have suggested, in March 2019, the restoration of ecosystems (https://www.decadeonrestoration.org), in the decade 2021-2030, with the aim of stepping up existing efforts to restore 350 million hectares of degraded ecosystems at a global level by 2030. The European Union, in May 2020, in the framework of the EU Strategy for Biodiversity for 2030, entitled "*Bring nature back into our lives*", has drawn up a plan for the restoration of terrestrial and marine ecosystems, and in particular for the ones with a high potential for carbon capture and storage. To this end, the National Recovery and Resilience Plan (NRRP), for Italy, calls for large scale interventions to restore and protect seabeds and marine habitats in national waters, so as to reverse the trend in Mediterranean ecosystem degradation and increase their resilience to climate change. In February 2022, in addition, the protection of the environment, of biodiversity, and of ecosystems was added to the fundamental principles of the Italian Constitution. In particular, the amendments concerned Art. 9 of the Constitution, in which, besides defining the environment in its broadest sense and from a systemic perspective, it is considered as a 'value' to protect also, "*in the interest of future generations*", expression which introduces and affirms the principle of intergenerational equity. The second amendment concerned Art. 41 of the Constitution relating to the exercise of economic initiative, adding that private economic initiative cannot be carried out to the detriment of health or of the environment.

For several years, the Integrated Maritime Policy (IMP) of the European Union has tried to provide a more coherent approach to maritime issues, with a greater coordination among the various sectors and the different stakeholders involved. The implementation of the IMP shows that a dynamic and coordinated approach in maritime affairs helps the development of the EU's "blue economy", and at the same time ensures the good ecological status of seas and oceans.

The Marine Spatial Planning Directive MSP – 2014/89/CE) is one of the major actions carried out by the EU in the framework of its own IMP. This directive is aimed at promoting a management and exploitation of marine resources that relies on an ever-increasing knowledge of the processes, functions, and services provided by ecosystems, as well as on the importance of their conservation. Planning when and where to carry out human activities at sea, in order for the pressures generated to be as sustainable as possible, will require close integration of ecological, social, and economic assessments, as well as the involvement of the different stakeholders concerned and the policymakers. The Legislative Decree 201/2016 incorporated into Italian law the MSP European Directive, that established to have in place, by March 2021, the marine spatial plans for all surface waters and seabeds over which Italy has jurisdiction. In this context, the implementation of the MSP Directive needs strong interaction with the directives regarding Environmental Impact Assessments, EIA – 2014/52/EC and Strategic Environmental Assessments, SEA – 2001/42/CE), but also with the directives concerned with the quality of marine environments, such as the Water Framework Directive 2000/60/CE and the Marine Strategy Framework Directive (2008/56/CE).

As already mentioned, *Posidonia oceanica* meadows are also considered in environmental law, in the context of authorisation procedures concerned with the execution of marine and coastal projects.

In fact, plans or projects regarding coastal works and infrastructures that could potentially affect *P. oceanica* meadows are subjected to the Environmental Impact Assessment (EIA) authorisation procedure, including those that require an Appropriate Assessment (AA) (art. 6 (3) and (4) the Habitat Directive, 1992/43/CEE). The Directive 2014/52/UE (incorporated into Italian law with the Legislative Decree 104/2017) has introduced new rules that make both the procedures to verify if an EIA is required and the assessment procedures themselves more efficient, increase the levels of environmental protection, and contribute to environmental sustainability (AA.VV., 2019a). In these areas, the damage suffered by the meadows due to the execution of coastal works and infrastructures subjected to EIA procedures is, usually, compensated via *Posidonia* transplantation.

Furthermore, in the context of the Water Framework Directive, WFD 2000/60/CE, Italy has identified among angiosperms the species P. oceanica as an ecological indicator, to be used for the purposes of assessing the ecological status of surface water bodies.

Finally, the national implementation of the Marine Strategy Framework Directive, MSFD – 2008/56/CE) includes biocenosis P. oceanica among the habitats being assessed for ecological quality, and in particular, falls within Target 1.2 "Increase of the number of marine habitats listed in the Habitat Directive and related to the SPA/BD protocol of the Barcelona Convention that preserves or achieves a satisfactory state of conservation".

Lastly, it is important to emphasise the relevance of public involvement and awareness in environmental issues, which in Europe is regulated by Directive 2014/52/UE and by the Aarhus Convention. The principles of this Convention represent a key element in achieving good environmental governance (Bennet, 2016; Madden and McQuinn, 2014; Redpath *et al.*, 2013), a founding essential element of the synergy of European environmental legislation for a sustainable management of marine and coastal environments, including the *Posidonia oceanica* meadows habitat.

CHAPTER 3

TRANSPLANTATION OF POSIDONIA OCEANICA MEADOWS AND ITS GOVERNANCE

3.1 | THE TRASPLANTATION AS A RECOVERY AND MANAGEMENT TOOL OF THE HABITAT POSIDONIA OCEANICA MEADOWS

Although their ecological and economic importance is recognised at the international level, and they are consequently protected by various rules, both at the national and European level, *Posidonia oceanica* meadows are at present experiencing strong regression in different areas of the Mediterranean (Marbà *et al.*, 2014; Telesca *et al.*, 2015). The diverse causes of regression, both natural and anthropic, which were described in the previous paragraphs, together with the slowness of the natural recolonisation processes, promoted over time, the gradually accepted conclusion that it could be necessary to develop transplantation techniques, as a tool to support and/or speed up the natural regeneration processes (Meinesz *et al.*, 1991a). However, considering that *P. oceanica* is one of the Magnoliophyta with the slowest growth rate in the world, its transplantation is also a slow process of recolonisation, in which the selected cuttings must take root and expand until they rebuild, only after many years, a meadow whose characteristics can be assimilated to a natural condition. Transplantation, therefore, must not be conceived as swift greening, as is the case with a lawn in a terrestrial environment, but a tool to promote the resilience of this habitat.

The first *P. oceanica* transplantation experiences, carried out using techniques usually employed to strengthen and improve the conservation state of meadows of other marine phanerogams, did not have a successful outcome. Further experiences, starting from those carried out by Georges Cooper's French school and by the group of Jardiniers de la Mer (Augier *et al.*, 1996; Cooper, 1982) and later experiments, carried out mainly in Italy, over time have shown more encouraging results in terms of *P. oceanica* transplantation (Scardi *et al.*, in print; Calvo *et al.*, 2021 AA.VV., 2020a). The experience gained due to the previous shortcomings, the new discoveries on the biology of the plant, the use of new technologies, as well as the availability of transplantation monitoring data over long periods of time has, in fact, lead to achieve *Posidonia* transplants that are increasingly effective and sustainable (Bacci and La Porta, 2022; Boudouresque *et al.*, 2021; Piazzi *et al.*, 2021; Bacci *et al.*, 2019; Badalamenti *et al.*, 2015).

Alongside the idea of replanting meadows that were destroyed, damaged or suffering due to the alterations caused to the marine and coastal environments (for example changes in coastal dynamics caused by the execution of coastal projects or by changes in climate dynamics), in recent years, the transplantation of sectors of *P. oceanica* meadows, has been identifying, in the context of EIA procedures concerning the execution of coastal projects in Italy, as the form of compensation for the associated impacts.

Aside from the objective for which a transplantation is carried out, there are many factors on which the success of the transplantation depends. The main ones are the choice of the transplantation areas and of the most suitable technique for the type of selected substrate. However, to increase the chances of success of a transplantation intervention, it is necessary to follow a specific process, that enables to manage correctly each of the phases in which the transplantation is structured, and to acquire all types of required data for the different areas of interest. In addition, the specific technical and scientific skills of different operators that are in charge of the various transplantation phases are crucial for the execution of an effective and long-lasting transplantation (https://lifeseposso.eu; Bacci and La Porta, 2022; AA.VV., 2020a).

The main phases of the process, illustrated in this manual, concern: planning, implementation, monitoring, and management of a transplant. Each of these phases includes additional key steps that take into account, for example, accurate environmental characterisations for the selection of donor and receiver meadows, pilot studies for the choice of suitable areas for transplantation and of the most adequate techniques, the choice of the biological material for transplantation, appropriate and detailed monitoring plans to assess the result of the transplantation over time. In addition, in order for a transplant to have greater chances of success, even if carried out following each of the good practices described, it is necessary to manage it over time, and to protect it from those anthropic actions that usually damage natural meadows, such as small-scale fishing, illegal trawling, and moorings of leisure or commercial boats.

The process described is applicable to any transplant, even if carried out with different objectives, such as the restoration of degraded meadows (*e.g.* dead *matte*), the 'mending' of sections of damaged meadows (*e.g.* moorings, trawling), the compensation of areas of meadows following complete loss due to dredging activities or seafloor sealing where they were present caused by the execution of marine and coastal projects (*e.g.* pipelines) (*fig.* 3.1.1). In addition, it is the very existence of economic activities that have generated and still generate impacts on this precious habitat, that raises important questions, connected to public information concerning transplantation, to the level of participation of the various subjects involved in different capacities with transplantation, to sharing and accessing collected data and the availability of results on the efficacy of transplantation. Here too, it is therefore necessary to strive towards the achievement of good environmental governance to support the different phases of the process proposed for transplantation activities of *P. oceanica*.

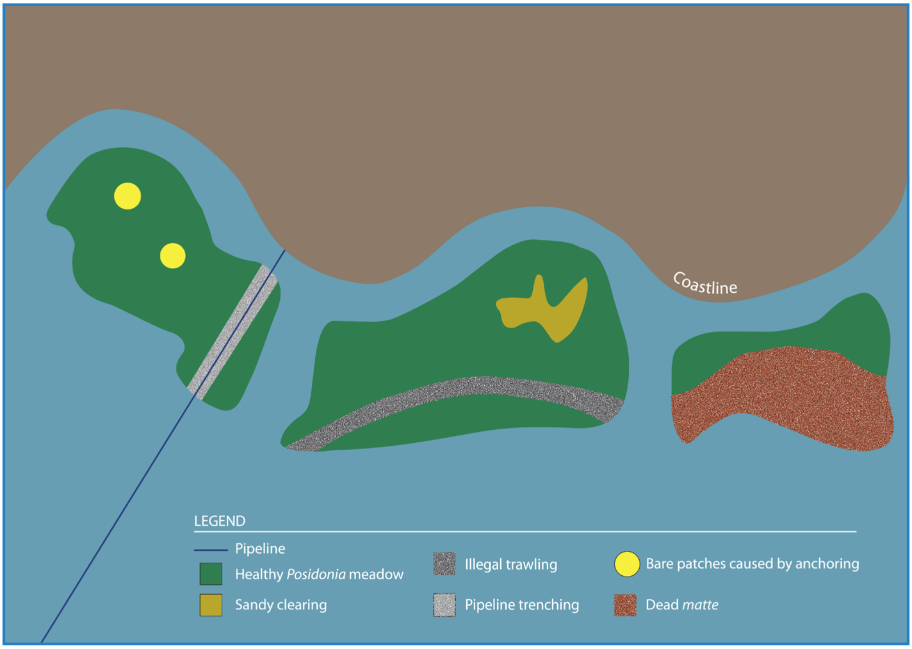

Figure 3.1.1 | Examples of degraded or damaged Posidonia oceanica *meadows (drawings by Sonia Poponessi, ISPRA - Life SEPOSSO).*

3.2 | GOVERNANCE OF POSIDONIA OCEANICA TRANSPLANTATION

Governance in *Posidonia oceanica* transplantation: principles of "good" governance

The existence of economic activities that have an impact on an ecosystem of crucial importance such as *Posidonia oceanica* meadows, raises important issues, all the more so since phanerogam restoration is a much debated topic at the European level (Cunha *et al.*, 2012). The issues raised concern a number of related problems, such as the selection of the most appropriate transplantation technique and of suitable receiver sites, the availability of the biological material for transplantation, the importance of monitoring activities, the sharing of and access to collected data, and the availability of results on the efficacy of transplantation. The issue of public involvement and awareness in environmental issues in Europe, as already mentioned, is regulated by Directive 2014/52/UE and by the Aarhus Convention, and it is a key element in the achievement of good environmental governance (Bennet, 2016; Madden and McQuinn, 2014; Redpath *et al.*, 2013). There are numerous benefits to involving stakeholders in environmental decisions, and they include the possibility of preventing or resolving conflicts, and of enhancing social acceptance of the initiatives (Gall and Rodwell, 2016; Hagan and Williams, 2016). In addition, public participation can also lead to a general improvement of the entire governance process, by means of the contribution of local knowledge. These are the reasons why the governance process in environmental issues should always be set up properly, and should provide the framework for an adequate involvement of all directly or indirectly interested parties.

Governance is usually defined as the combination of institutions, structures, and processes that determine "who" takes decisions, "how and for whom" they are taken, "if, how, and which" actions are undertaken, and "for what purpose" (Lockwood *et al.*, 2010; Graham *et al.*, 2003). In short, governance is the combination of rules, strategies, and processes that govern the activities of a public or private institution. The primary aim of governance is to ensure good results and the achievement of objectives, integrating the actions of citizens with government action, supporting them and in turn being supported.

The different models of governance entail a combination of approaches focused on three components: State, people (civil society), and market, whose equilibrium varies in different contexts. Governance analysis must examine these three components and the way in which they are related to one another. A single integrated "good" governance method does not exist, and the priority of any particular analysis is to consider the combination of principles that will guide it. For this reason it is important to characterise the key aspects of a governance through the definition of the objectives and the related attributes.

The term environmental governance is used in the case of decision-making processes that are at the basis of the control and management of the environment and of natural resources. A recent review (Bennett and Satterfield, 2018) on environmental governance summarises in a framework the way in which primary objectives and attributes combine with governance elements (*fig.* 3.2.1). This framework identifies four objectives of good governance (Lockwood *et al.*, 2010; Bennett and Satterfield, 2018) which should be: (i) Effective, (ii) Equitable, (iii) Responsive and (iv) Robust. The attributes, as a whole, represent a useful reference for the choice of adequate indicators, necessary for the analysis and the quality of environmental governance.

"Good" governance means a governance that pursues the above said objectives. For each objective there are various attributes, for which it is necessary to have qualitative indicators able to document what has been done to reach a specific objective (*fig.* 3.2.1). It is important to emphasise how the "good" governance principles are inspired by the Aarhus Convention (Decision 2005/370/CE) on the right of citizens to participate in public debates, and to have access to information and to justice in environmental issues.

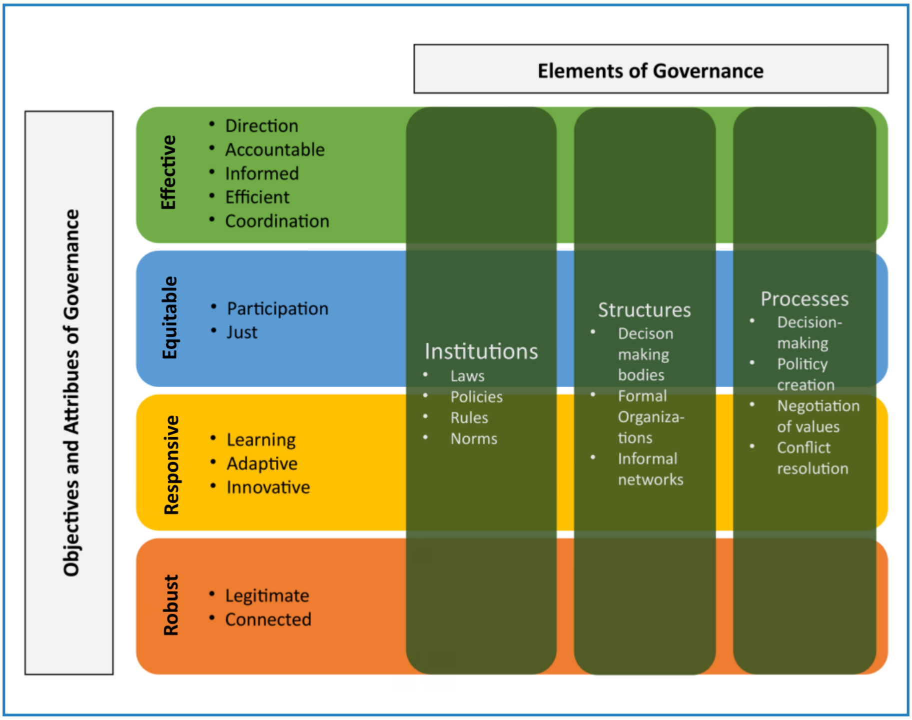

Figure 3.2.1 | Summary overview that defines objectives, attributes and elements of good environmental governance (modified from Bennett and Satterfield, 2018).

Effective

An effective governance should be: 1) direction, aims and objectives of the transplantation should be communicated in an extremely clear way to all stakeholders; 2) accountable, the decisions taken and the instruments chosen to reach the objectives should be transparent; 3) informed, the decisions pertinent to a transplantation should be taken considering the best available technical and scientific information; 4) efficient, timing and costs of transplantation operations should apply criteria of efficiency and efficacy; 5) coordinated, the actions of the different institutions involved should be coordinated. The first four attributes should be part of the documents provided by those carrying out a transplantation who wish to execute a project that produces negative impacts on *P. oceanica*, while institutional bodies should establish coordination.

Equitable

An equitable governance calls for the possibility of involving stakeholders and of granting them access to justice in the context of initiatives of *P. oceanica* transplantation as a compensation tool for the impacts of marine projects on the ecosystem. The attributes of an equitable governance require for it to be: 1) participative, able to involve stakeholders through specific meeting places, information, and structures to share information and knowledge related to transplantation; 2) just, able to grant access to the mechanisms that ensure recourse to a judicial arbitration in case of an alleged failure of compliance with the rules. The first attribute should be part of the tasks of those who carry out a transplantation, while the second should be ensured by institutional bodies.

Responsive

A governance is responsive when it is: 1) able to create knowledge, the details of the methods used and the results obtained should be made public and easily accessible; 2) adaptive, able to identify and correct shortcomings, limits, or inconsistencies found during ongoing activities through iterative processes; 3) innovative, the decisions taken are the result of experimentation and innovative approaches. All attributes should be provided for both by those carrying out a transplantation and by the institutional bodies.

Robust

A governance is robust when it is able to tackle the problems that can weaken its functioning and efficacy. In the case of *P. oceanica* transplantation, the key attributes of a robust governance are: 1) legitimate, ensured by the authority and representativeness of the institutions involved, and supported by a shared vision, and 2) the ability to create interconnections among the governance players, facilitating collaboration, the exchange of information and knowledge, and the dissemination of acquired innovation. All attributes should be ensured by those carrying out a transplantation.

Table 3.2.1 shows some examples of indicators for the assessment of an effective, equitable, responsive, and robust governance, both for *P. oceanica* transplants aimed at compensating impacts caused by marine projects on *Posidonia* meadows, and for those aimed at restoration activities, such as the restoration of degraded meadows and research activities. In the case of compensatory transplants carried out with an Environmental Impact Assessment procedure, some of the “good” governance objectives will be part of the activities of those who propose the project that damages the meadow.

For a more thorough assessment with a view to the governance of *P. oceanica* transplantation, please refer to Lucia *et al.* (2022), AA.VV. (2020c), and to Zenone *et al.* (2021).

Table 3.2.1 | "Good" governance objectives, attributes and indicators for Posidonia oceanica *transplants aimed at compensating impacts caused by marine projects on Posidonia meadows and aimed at the restoration of degraded meadows and at research activities. SH: stakeholder.*

OBJECTIVE	ATTRIBUTES	INDICATORS	RESPONSIBILITY OF	PROJECT PHASE/ TRANSPLANTATION
EFFECTIVE	Direction	Communication strategies used to inform the public and the SH; Results of SH analysis aimed at investigating their level of interest.	Those who carry out the transplantation	Before the EIA preliminary phase/ transplantation planning phase
	Accountable	Means of communication and elements provided to SH concerning the decisions taken, the chosen instruments, and the financing channels used to carry out the transplantation.	Those who carry out the transplantation	Before the EIA preliminary phase/ transplantation planning phase
	Informed	Adopted selection criteria and information produced to support the proposed transplantation technique/experimentation.	Those who carry out the transplantation	Before the EIA preliminary phase/ transplantation planning phase
	Efficient	Solutions adopted to ensure adequate execution times and costs.	Those who carry out the transplantation	Before the EIA preliminary phase/ transplantation planning phase
	Coordination	Measures taken to ensure coordination, collaboration, and synergy among SH.	Those who carry out the transplantation/ public institutions	During the project's entire execution phase/ before, during, and after transplantation
EQUITABLE	Participative	Documents on the ways to engage and the level of participation of SH (n. of organised events, n. of participants, n. of interventions, creation of a public committee, etc.).	Those who carry out the transplantation	Before the preliminary phase/before transplantation
	Just	Creation of an area to grant easy and affordable access to citizens to a potential judgement in case of possible unlawful conduct, in accordance with the Aarhus Convention.	Public institutions/ Those who carry out the transplantation	During the project's entire execution phase/ before transplantation
RESPONSIVE	Learning	Scientific and technological know-how connected to transplantation is available, easily accessible, and explained in simple words; Summary of monitoring results is available to lay persons.	Those who carry out the transplantation	After transplantation
	Adaptative	Short-term monitoring plan and strategies for ensuring the integrity of transplantation; Assessment plan and chosen variables to verify transplantation performance.	Those who carry out the transplantation	Before transplantation
	Innovative	Presence of scientific hypotheses and/or pilot studies to support technical decisions that were taken; Methods to choose transplantation sites; Opportunities for SH access to the information pertinent to innovative approaches.	Those who carry out the transplantation	Before transplantation
ROBUST	Legitimate	Documented mediation efforts concerning the different visions of the players involved in transplantation procedures; Preparation of a plan to enhance the value of transplantation sites.	Those who carry out the transplantation	Before transplantation
	Connected	Creation of technical discussions and work groups among involved players, aimed at managing acquired knowledge of P. oceanica transplants synergistically.	Those who carry out the transplantation	During the project's entire execution phase/ before, during,and after transplantation

CHAPTER 4

TECHNICAL ASPECTS OF THE TRANSPLANTATION OF POSIDONIA OCEANICA

Procedure for the transplantation of *Posidonia oceanica*

The transplantation of *P. oceanica* is generally a difficult and delicate operation because of the great complexity of the system the meadows themselves represent. The correct management of transplantation requires specific technical-scientific skills, as well as the application of a specific procedure, which allows the correct management of all the various phases of the transplantation and the acquisition of all necessary types of data in the different areas of concern (*fig.* 4.1) (Bacci and La Porta, 2022; AA.VV., 2020a; Van Katwijk *et al.*, 2016, Calumpong and Fonseca, 2001).

The procedure and the various phases can be summarised as follows:

1) Planning of the *Posidonia oceanica* transplantation

- Characterisation and assessment of the donor meadow;
- Characterisation and assessment of the site of the receiver meadow;
- Assessment of the ecosystem services of the donor and receiver meadow;
- Selection of the transplantation technique.

2) Execution of the transplantation of *Posidonia oceanica*

- Selection of biological material for transplantation;
- Positioning of the transplantation modules.

3) Monitoring of the transplantation of *Posidonia oceanica*

- Verification of the success of the transplantation operation;

4) Management of the transplantation of *Posidonia oceanica*

The procedure described in this manual is also applicable to the transplantation of *P. oceanica* prescribed as a compensatory action on the basis of Environmental Impact Assessments, but the following aspects should be specified: i) the donor meadow generally coincides with the meadow damaged by the construction of the works; ii) the explantation of biological material from the donor meadow and subsequent transplantation must be performed before the meadow is damaged by the works; iii) transplantation planning, including the implementation and monitoring of pilot transplants, must be included by the proposer of the works in the Environmental Monitoring Plan, produced as part of the Environmental Impact Statement (EIS). For an in-depth examination of the management of *P. oceanica* transplantation activities within the Environmental Impact Assessment and Appropriate Assessment, please refer to Lucia *et al.* (2022) and Pacione *et al.* (2022).

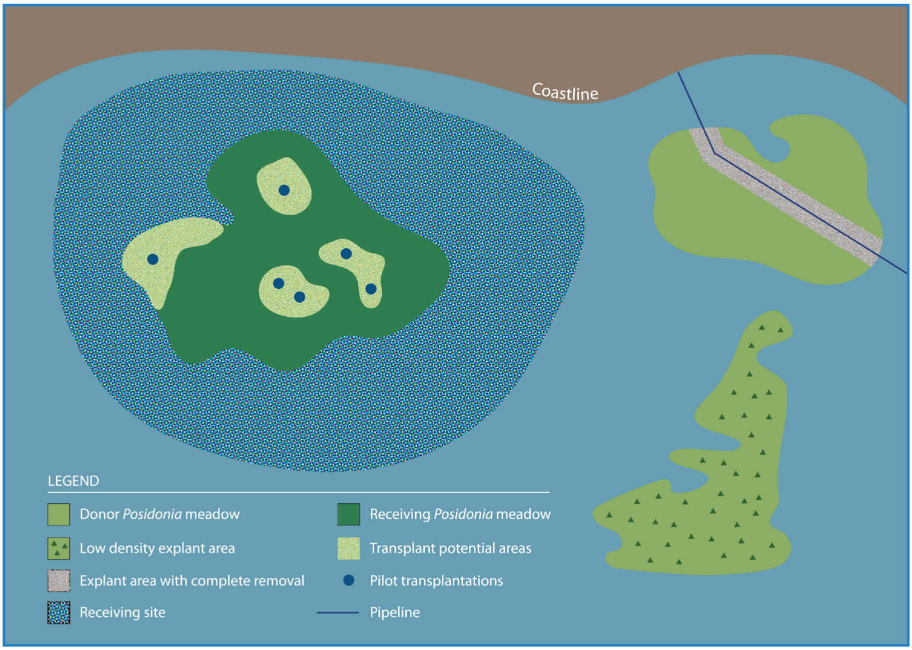

Figure 4.1 | Example diagram of the different areas involved in the planning, implementation and monitoring of Posidonia oceanica *transplantation.*

4.1 | PLANNING OF THE POSIDONIA OCEANICA TRANSPLANTATION

For the development of a site-specific decision-making strategy, to be carried out before starting any *Posidonia oceanica* transplantation operation, it is necessary to take into account various aspects considered essential to the effective planning of the transplantation.

4.1.1 | Characterisation and assessment of the donor meadow

The characterisation and assessment of the donor meadow is a very delicate phase in the transplantation of *P. oceanica*. The health status of the donor meadow and its size, the quality of the cuttings to be used for transplantation and the distance from the receiver site are some of the main factors that can affect the success of transplantation (Tan *et al.*, 2020; Díaz-Almela e Duarte, 2008; Campbell, 2002).

Table 4.1.1.1 shows the activities and their purposes for the characterisation and assessment of the donor meadow in order to select the most suitable areas for the explantation of biological material, to be used for transplantation.

Table 4.1.1.1 | Main activities and their purposes for the characterisation and assessment of the donor meadow in order to select suitable areas for explantation of Posidonia oceanica.

ACTIVITY	PURPOSE
Preliminary screening of available previous data	Orient the subsequent characterisation surveys to assess the most suitable areas for the explantation of biological material for transplantation. If the explantation area coincides with an area subject to the removal of portions of meadows, the screening represents only an environmental contextualisation.
Remote surveys	Provide data for assessing the depth, distribution and relative facies of the donor meadow for identifying the most suitable areas for the explantation of biological material for transplantation.
Surveys *in situ*: structural, functional and ecological descriptors of the donor meadow	Provide data for the assessment of the health status of the donor meadow and the quality of the cuttings to be used for transplantation in order to maximise the chances of rooting.
Assessment of the distance from the transplantation site	Reduce the stress for explanted cuttings during the transfer phase between the areas of explantation and transplantation.

The following are the types of basic data to be acquired in the various activities identified in Table 4.1.1.1 for the characterisation and assessment of the donor meadow (AA.VV., 2020b).

Preliminary screening of available data

The acquisition of data, originating in scientific literature and grey literature and from open-access data sources at European, national and regional level, must concern: historical (> 20 years) and current (< 20 years) distribution of the *Posidonia oceanica* habitat and its relative *facies*, bathymetry of the meadow and the structural, functional and ecological descriptors of the meadow (AA.VV., 2020b).

Information relating to the perimeter of any Natura 2000 network sites and the presence of any Marine Protected Areas (MPAs) must also be acquired.

Additional information to be sourced is the documented presence of recent *Posidonia* transplantation in the donor meadow as this can be an obstacle to identifying areas suitable for explantation within the same meadow.

Remote surveys

The acquisition of morpho-bathymetric data of the meadow is generally carried out by surveys using Side Scan Sonar (SSS), Multi Beam (MB) or video-photographic tools, such as Remotely Operated Vehicle (ROV) (Bosman *et al.*, 2021). Alongside this type of surveillance, new technologies and methodologies provide additional and effective tools for the acquisition of such data, for example satellite remote sensing, aerial remote sensing using Remotely Piloted Aircraft (RPA) and the use of autonomous underwater vehicles (AUV). The integration and combination of different methodological techniques today offer an effective method for the high resolution mapping of the seabed and habitats (Rende *et al.*, 2020; Veettil *et al.*, 2020; Castillon *et al.*, 2019; Gumusay *et al.*, 2019; Mohamed *et al.*, 2018).

Surveys *in situ*: structural, functional and ecological descriptors of the donor meadow

The acquisition of quantitative data, relating to the structural (density of foliar shoots), functional (phenological and lepidochronological parameters) and ecological (abundance and composition of the associated fauna and flora) parameters of the donor meadow, must be acquired by divers experienced in monitoring *P. oceanica* meadows according to specific reference methodologies (Bacci *et al.*, 2020; Buia *et al.*, 2003; Short and Coles, 2001).

Assessment of the distance from the transplantation site.

The distance between the donor meadow and the receiver meadow can have a great influence on the logistical and/or procedural choices of the operation (Díaz-Almela and Duarte, 2008). Furthermore, this distance may also reflect a genetic variability of plants between a receiver meadow and a donor, an important factor to be taken into consideration when planning *Posidonia* transplantation (Pazzaglia *et al.*, 2021; Ehlers *et al.*, 2008; Procaccini and Piazzi, 2001).

The importance of the genetic component in the restoration of marine phanerogams is addressed in the following Box.

The importance of the genetic component in the restoration of marine phanerogams

G. Procaccini, J. Pazzaglia

Marine plants can reproduce both vegetatively and sexually, on the basis of the characteristics of each species and of external environmental forces. The genetic structure of phanerogam meadows is strongly influenced by the impact of the two types of reproduction. On one hand, clonal propagation enables populations to extend themselves spatially, forming mostly monoclonal populations with low genetic diversity (Arnaud-Haond *et al.*, 2012). On the other hand, even sporadic events of sexual reproduction support an increase in genetic diversity through the presence of new allelic variants, so that the meadows can be formed by a greater number of genotypes and that each genotype can present greater plasticity (Jahnke *et al.*, 2015). This becomes more relevant in the presence of environmental changes, such as for example an increase in temperature, since populations with a greater genetic and genotypic diversity are more resistant and resilient (Ehlers *et al.*, 2008). This is why a restoration plan for marine phanerogams must also include an accurate analysis of the genetic structure, the plasticity, and the adaptability of donor populations, as well as a careful analysis of the environmental factors that characterise the donor and receiver sites (*fig.* 1).

A key element that affects genetic diversity of phanerogam meadows is the degree of connectivity that exists among populations. The dispersal of pollen or fruits released during sexual reproduction or of vegetative propagules is strongly influenced by the intrinsic characteristics of the species (potential connectivity), namely the potential distance that they can cover based on transport dynamics of marine currents, and on the connectivity that actually takes place, and thus on the actual establishment of new genotypic or allelic variants in different populations (Mari *et al.*, 2020). Maintaining some degree of connectivity among populations is crucial since it supports genetic diversity, and thus prevents genetic drift events that cause the loss of allelic variants and the potential fixation of deleterious alleles, compromising their future survival (Alotaibi *et al.*, 2019).

Marine phanerogams are organisms that present a certain degree of phenotypic plasticity, which has enabled them to colonise very heterogeneous environments. As a result, different populations of the same species have adapted to local environmental conditions through a natural selection process. Several studies have emphasised how the populations distributed along a depth bathymetric gradient or along a geographic gradient are locally adapted with different genetic structures (Marín-Guirao *et al.*, 2017; Jahnke *et al.*, 2019). To cope with this, a widely used approach in restoration practices is to choose a donor site close to the receiver site, since it is believed that the plants will adapt more readily to the conditions of the site that is being restored. This opens a discussion that raises several problems on which is the best approach to use. In fact, if on one hand transplanting locally adapted individuals could be the best practice to tackle environmental conditions, on the other hand transplanting the same genotypes in a meadow that is already experiencing regression due to different environmental disturbance factors, could negatively influence the genetic and genotypic diversity and thus the success of the restoration itself (Coleman *et al.*, 2020). In addition, introducing new genotypes selected by a donor site that is distant from the receiver site, on one hand allows to restore the levels of genetic diversity (genetic restoration), but on the other hand could cause deleterious effects due to the low adaptation capacity to the new environmental conditions, as well as determining genetic pollution that could result in the loss of native genotypes.

Integrating genetic information with environmental ones using connectivity maps could be a fundamental tool for the correct selection of the donor site, and especially for monitoring the restoration phase. The subsequent genetic monitoring, in fact, enables to assess the levels of genetic diversity and the positive effects of the restoration over time. This can moreover emphasise the factors and processes that have greater influence on the success or failure of the restoration, especially where the approach used calls for the use of non-local genotypes. In this case, genetic monitoring provides information on the changes that occur to the structure and genetic diversity of the restored population. Considering that the restoration of a meadow and of the associated ecosystem functions can take a very long time, continuous and appropriate monitoring is essential (Statton *et al.*, 2012).

The enhancement of restoration practices and therefore of their success rate over time continues to grow, and at present new approaches of assisted evolution are emerging and establishing themselves in environmental restoration programs (Aitken e Whitlock, 2013). These approaches aim at strengthening resilience capacities of organisms through active interventions of genetic manipulation and through less invasive approaches but that use the plastic capacities of organisms, such as the "hardening" approach, which is widely used in agriculture (Jones e Monaco, 2009), as well as the selection of more plastic genotypes and that are therefore more tolerant to environmental changes (Filbee-Dexter and Smajdor, 2019). Integration of the techniques of assisted evolution in restoration practices of marine phanerogams could become, in the coming years, an important aspect to enhance success rate.

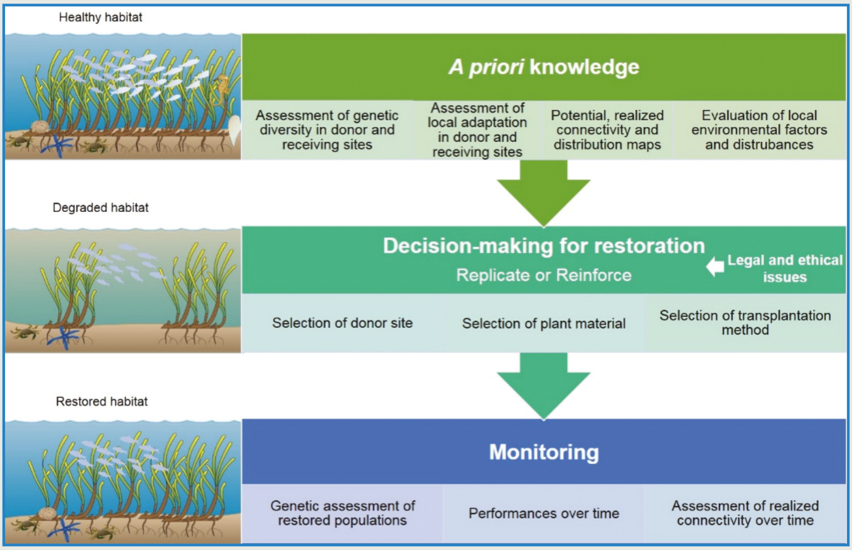

Figure 1 | Conceptual diagram that shows the integration of the analysis of genetic diversity in the different steps that make up a program of environmental restoration of marine phanerogams (from Pazzaglia et al.*, 2021).*

4.1.2 | Characterisation and assessment of the site of the receiver meadow

The selection of the transplantation area is today one of the factors that most conditions the success or failure of reforestation operations in the marine environment (AA.VV., 2021a, 2020a; Boudouresque *et al.*, 2021; Tan *et al.*, 2020; Pirrotta *et al.*, 2015; Van Katwijk *et al.*, 2009; Calumpong and Fonseca, 2001).

In the planning phase of a transplantation operation, it is expected that the characterisation of the receiver site and relative assessments will be carried out both at the level of the areas of the meadow where the transplantation operation is due to be carried out and at a broader level of the receiver *Posidonia* meadow.

Table 4.1.2.1 shows the activities and their purposes for the characterisation of the site and the receiver meadow for the assessment of the most suitable areas for transplantation.

Table 4.1.2.1 | Main activities and their purposes for the characterisation and assessment of the site and the receiver meadow in order to select the most suitable areas for Posidonia oceanica *transplantation.*

ACTIVITY	PURPOSE
Preliminary screening using previous data	Exclude areas not suitable for transplantation and direct subsequent characterisation surveys on potentially more suitable areas.
Remote surveys	Provide data for assessing the depth, distribution and relative facies of the receiver meadow for identifying the most suitable areas for transplantation.
Hydrological and water quality surveys	Provide data for assessing the hydrological dynamics and chemical-physical characteristics of the water column suitable for the rooting and survival of the transplanted cuttings.
In situ observations of environmental criticalities	Provide information on the presence of any critical elements that may compromise the implementation and outcome of the transplantation.
Surveys *in situ*: structural, functional and ecological descriptors of the receiver meadow	Provide data for the assessment of the health status of the receiver meadow to help identify the most suitable areas for transplantation.
Pilot transplantation	Testing both the actual suitability of the identified potential transplantation areas and the effectiveness of the transplantation techniques chosen, through monitoring of pilot transplantation conducted over an adequate period of time.

The types of basic data to be acquired in the various activities identified in Table 4.1.2.1 for the characterisation and assessment of the site and receiver meadow are shown below (AA.VV., 2020b).

Preliminary screening using available previous data

The acquisition of data, originating in scientific literature and grey literature and from open-access data sources at European, national and regional level, must concern: historical (> 20 years) and current (< 20 years) distribution of the *Posidonia oceanica* and its relative *facies*, bathymetry of the meadow and the structural, functional and ecological descriptors of the meadow (AA.VV., 2020b).

Furthermore, the screening must concern the classification of the substratum and the granulometry of seabed sediments, hydrology and water quality. The screening must consider the acquisition of information relating to the existence of any causes of regression of the receiver meadow related to anthropogenic pressure in the area of interest and the natural dynamics of the surrounding coastal environment. Information relating to the uses and constraints of the sea (*e.g.* port areas, collection areas of dredged sediment, offshore cables and pipelines, archaeological areas, aquaculture installations, etc.) are also useful in the transplantation planning phase and selection of receiver meadows.

Information relating to the perimeter of any Natura 2000 network sites and the presence of any Marine Protected Areas (MPAs) must also be acquired.

The documented presence of pre-existing *Posidonia* transplantation in the receiver meadow can represent a favourable or an impeding element to the identification of suitable areas for transplantation within the same meadow on the basis on the outcome of the pre-existing transplantation.

Several qualitative and quantitative models have been developed for the identification of potential areas where marine phanerogam transplantation can be carried out using previous data relating to the area concerned (AA.VV., 2021a; Lanuru *et al.*, 2018; Pirrotta *et al.*, 2015; Campbell, 2002; Short *et al.*, 2002).

Remote surveys

The acquisition of data relating to the depth and distribution of the receiver meadow and its *facies* are generally acquired by means of Side Scan Sonar (SSS), Multi Beam (MB), Sub Bottom Profiler (SBP) or video-photographic tools, such as Remotely Operated Vehicles (ROV) (Bosman *et al.*, 2021). Alongside this type of surveillance and also in this case, new technologies and methodologies provide additional and effective tools for the acquisition of such data, for example satellite remote sensing, aerial remote sensing using Remotely Piloted Aircraft (RPA) and the use of autonomous underwater vehicles (AUV). The integration and combination of different methodological techniques today offers an effective method for the high resolution mapping of the seabed and habitats (Rende *et al.*, 2020; Veettil *et al.*, 2020; Castillon *et al.*, 2019; Gumusay *et al.*, 2019; Mohamed *et al.*, 2018). This type of investigation enables, for example, the measurement of the depth of the receiver meadow so that the site selected for transplantation is at a similar depth and no greater than the donor meadow. These surveys also make it possible to select those areas for transplantation that have a sufficiently large surface area to allow the expansion of the transplanted meadow in the medium and long term (Bacci and La Porta, 2022; AA.VV., 2020a; Boudouresque *et al.*, 2006; Fonseca *et al.*, 1998). Thanks to these surveys, it is also possible to evaluate the type of substratum as well as the presence of elements that indicate strong currents or erosive processes, such as ripple-marks, erosion channels and eroded limits in the receiver meadow, all unfavourable when carrying out transplantation.

Hydrological and water quality surveys

Information on the local water chemistry makes it possible to evaluate the supply of nutrients and inorganic carbon necessary for the photosynthesis of *Posidonia*. At the same time, being able to identify areas with high or low hydrodynamics is essential because they are considered not favourable to transplantation; currents with speeds of between 5 and 100 cm/sec are considered optimal, also from the point of view of the consequent sedimentation dynamics (Koch, 2001).

The use of multi-parametric probes for the acquisition of chemical-physical data of the water column (*e.g.* light intensity, clarity, dissolved oxygen, salinity, temperature, pH, nutrient load and suspended organic and inorganic particles) provides information on the water quality, also a limiting factor for *Posidonia* (Larkum *et al.*, 2006).

In situ observations of environmental criticalities

The possible presence in the potential transplantation areas of excessive organic debris, ripple-marks, invasive and/or alien macro-algae, as well as "markers" of anthropogenic pressure, such as abandoned fishing nets, traces of repeated anchorage, moorings, debris or waste, are a further limiting factor to their suitability. Such data should be collected by divers with experience in monitoring *P. oceanica* meadows using visual surveys.

Surveys _in situ_: structural, functional and ecological descriptors of the receiver meadow

The acquisition of quantitative data relating to the structural (density of foliar shoots), functional (phenological and lepidochronological parameters) and ecological (abundance and composition of the associated fauna and flora) parameters of the donor meadow, must be acquired by divers experienced in monitoring *P. oceanica* meadows according to specific reference methodologies (Bacci *et al.*, 2020; Buia *et al.*, 2003; Short e Coles, 2001). The underwater surveys of potential transplantation areas also enable the acquisition of qualitative information relating, for example, to the apparent health of the meadow, type of limits (progressive, sharp, erosive, regressive), the type of substratum (*e.g.* sand or rock) and the presence of dead *matte* and its consistency.

Pilot transplantation projects

The characterisation of the receiver site, carried out using the various survey methods described above, enables the identification of the areas potentially most suitable for receiving transplantation. The long-term monitoring of a number of sites of transplantation carried out in Italy on a large scale, for which pilot transplantation projects had not previously been carried out, showed a high spatial variability in transplantation outcome (AA.VV., 2021b, 2021c, 2020a). These results confirm the need to carry out specific preliminary pilot projects in the planning phase of a transplantation operation. The pilot projects not only enable the selection of the most suitable areas for transplantation with the most favourable environmental conditions, but also enable testing of different techniques and selection of the most appropriate ones for the chosen area (AA.VV., 2021a; Bacci *et al.*, 2019; Cunha *et al.*, 2012; Pirrotta *et al.*, 2015; Campbell, 2000; Boudouresque *et al.*, 1994).

A pilot transplantation project must be: i) conducted on an adequate number of areas identified as potentially suitable; ii) proportionate to the surface area of transplantation to be carried out; iii) conducted with an adequate but limited number of cuttings for each pilot area; iv) monitored for at least one year in order to comprehend autumn-winter conditions, considered critical during the initial stages of transplantation.

4.1.3 | Assessment of the ecosystem services of the donor and receiver meadows

Ecosystem Services are "the multiple benefits provided by ecosystems to mankind" (Millennium Ecosystem Assessment, 2005). *Posidonia oceanica* meadows, like all other ecosystems, provide these services, favouring human activities and, at the same time, allowing the long-term existence of the habitat and ecosystem components (Scanu *et al.*, 2022; Vassallo *et al.,* 2013).

The assessment of ecosystem services enables, among its various functions, the acquisition of useful information to support the *Posidonia* transplantation decision-making process, with particular reference to:

- the cost-benefit analysis of transplantation, comparing the operating costs with the economic value of the benefits represented by ecosystem services;
- the assessment of alternatives in the choice of various possible donor meadows;
- the assessment of alternatives in the choice of various possible receiver meadows;
- the comparison ante and post-operam of the state of the donor meadows and the transplantation sites, only in the case of Environmental Impact Assessment and Incidence Assessment procedures.

In particular, with regard to the last three points, the assessment of ecosystem services permits the estimation of the economic and environmental benefits of the planned operations, in the potential areas of benefit[1] of both the donor and receiver meadows.

1 Ecosystem services are provided to an area that does not necessarily correspond with the ecosystem itself. "Area of benefit" means the area in which the beneficiaries use the services.

Finally, as part of the monitoring following transplantation, it is necessary to verify that the forecasts of the state and value of ecosystem services made in the planning phase of the transplantation operation are fulfilled, both in the donor meadow and in the transplantation site.

For a more detailed examination of the assessment of ecosystem services, please refer to the "Technical Guide for economic assessment of environmental impact on *Posidonia oceanica* meadows" (Cozzolino *et al.*, 2021), which also gives directions on how to supplement Environmental Impact Assessment and Incidence Assessment procedures with economic-environmental assessments.

4.1.4 | Selection of the transplantation technique

The selection of the transplantation technique during the early planning phase of *Posidonia oceanica* transplantation has proven essential (Bacci and La Porta, 2022; Cunha *et al.*, 2012; Paling *et al.*, 2003). Most transplantation failures are attributable to the detachment of the transplantation modules and cuttings, due mainly to the action of wave motion and seabed currents. These losses are also often linked to anthropogenic activities such as illegal trawling, small-scale fishing and the anchoring of pleasure boats (AA.VV., 2021b, 2021c; 2020a; Van Katwijk *et al.*, 2009; Bull *et al.*, 2004; Meinesz *et al.*, 1993).

Of all the environmental variables influencing the effectiveness of the transplantation technique, the implant substratum is certainly the most important.

Some type 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. Over time, additional anchoring methods have been devised and tested, such as, for example, geomats and biomats, mattresses of various types and anchoring modules in bioplastic; other techniques, with greatest focus on the environmental sustainability of the technique, are also being tested (Bacci and La Porta, 2022; Boudouresque *et al.*, 2021; Balestri *et al.*, 2019; AA.VV., 2014).

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 (Bacci and La Porta, 2022).

All the transplantation techniques exploit the division ability of transplanted shoots and the expansion of recolonisation nuclei. In the long term, these nuclei expand by means of plagiotropic growth to colonise adjacent areas and, where the transplantation has a positive outcome, they restore the meadow, which may reach a similar or comparable appearance to the existing meadow and/or neighbouring natural meadows.

Finally, it should be emphasised that the success of the transplantation technique selected is also greatly conditioned by the technical skill of the divers, who must be adequately trained, in terms both of a background in biology and underwater. This is fundamental for the correct execution of selecting and fixing the biological material for transplantation and appropriate positioning the transplantation modules used.

For a complete examination of transplantation techniques, please refer to the "Manual of techniques and procedures for the transplantation of *Posidonia oceanica*" (Bacci and La Porta, 2022), which describes the main transplantation techniques applied in Italy in the context of environmental restoring or compensation for damage caused to meadows by marine-coastal construction projects subjected to Environmental Impact Assessment.

4.2 | EXECUTION OF THE TRANSPLANTATION OF POSIDONIA OCEANICA

4.2.1 | Selection of biological material for transplantation

The selection of biological material for transplantation is related to the selected transplantation technique and the procedure for planting the cuttings. Furthermore, some modalities of the cuttings management (*e.g.* stocking, manipulation, anchoring) are strictly connected to the planning phase of the transplantation itself.

The main precautions regarding the selection of *P. oceanica* cuttings, to be considered during the transplantation planning stages, are as follows:

- the choice of the type of cuttings (plagiotropic or orthotropic) to be used must first of all take into account the season during which the transplantation operation is planned. The most favourable season for planting plagiotropic (horizontal) rhizomes is spring; when planting orthotropic (vertical) rhizomes autumn should be preferred, as a period of vegetative stasis in which the risk of thermal shock suffered by the plant is lower (Meinesz *et al.*, 1992; Molenaar, 1992; Piazzi e Cinelli, 1995; Piazzi *et al.*, 1998, 2000). The choice of plagiotropic rhizomes appears to guarantee the best results in terms of speed of vegetative growth and survival of the cuttings, thanks to the plant's reserves of nutrients and antibiotic substances, which are essential for sustaining the plant in the critical post-explantation phase (Meinesz *et al.*, 1992; Molenaar *et al.*, 1993). However, the occasionally limited availability of these rhizomes favours the selection of orthotropic rhizomes, which, in adequate environmental conditions, nevertheless achieve good growth speeds (AA.VV., 2020a; Carannante, 2011; Meinesz *et al.*, 1992);

- cuttings should preferably be taken from donor meadows located at a similar depth to that of the receiver meadow (Fonseca *et al.*, 1998). However, it has been observed that when cuttings have been taken from donor meadows at slightly greater depths than those of receiver meadows, they have adapted better during the rooting phase thanks to the new, photosynthetically more effective light conditions (AA. VV., 2020a), reinforcing statements by a number of authors (Piazzi *et al.*, 1998; Chessa and Fresi, 1994, Genot *et al.*, 1994; Molenaar and Meinesz, 1992);

- in order to reduce the pressure and possible negative effects deriving from excessive removal of cuttings, the biological material should preferably be taken from a number of donor meadows, if present in the explantation sites, or, otherwise, from different portions of the same donor meadow (Boudouresque, 2000). The removal of cuttings must therefore be spread over a large area and carried out at a very low density. Boudouresque (2000) indicated a removal density of no more than 2 shoots /m^2, whereas subsequent research showed that the low-density removal of about 1-4 shoots per m^2 is congruent with the conservation of the donor meadow (Valiante *et al.*, 2010).

Finally, clods and cuttings that have become detached naturally due to hydrodynamics or from the anchoring of boats and found on the seabed should be considered as an alternative to material removed from donor meadows (Bacci and La Porta, 2022; Balestri *et al.,* 2011; Boudouresque, 2000).

Where conditions permit, the use of seedlings obtained from seeds might also constitute a potential source of biological material for *P. oceanica* transplantation projects.

Furthermore, the technical skill of the operators is also vitally important in the selection and handling of the biological material to be transplanted. Knowledge of the biological characteristics, structures and functions of *Posidonia* allows the operator to choose the best cuttings for transplantation and reduces the risk of unnecessarily wasting biological material.

For further specifications on the selection of *Posidonia* cuttings according to transplantation technique and the use of seedlings obtained from seeds, please refer to the "Manual of techniques and procedures for the transplantation of *Posidonia oceanica*" (Bacci and La Porta, 2022).

4.2.2 | Preparation and positioning of transplantation modules

As regards the preparation of transplantation modules, it should be noted that the method of anchoring cuttings to supports, carried out on the surface or underwater, depends on the transplantation technique used.

In the literature, it is indicated that in general the cuttings should be placed at a distance of 5-10 cm apart (Molenaar and Meinesz, 1995). However, the density should never prevent sediment retention between cuttings. Various *P. oceanica* transplantation experiments indicate a density of around 30 shoots per m^2 as optimal, as this avoids excessive competition between shoots and promotes rapid plagiotropic growth of rhizomes (Bacci and La Porta, 2022).

In this phase, the involvement of qualified underwater operators with knowledge of marine biological and ecological systems and with specific experience in handling *Posidonia* cuttings for transplantation is essential. This permits better selection of the cuttings destined for transplantation and minimises the stress on the plants during the phase of handling and fixing to supports. The assistance of Underwater Technical Operators (UTO) in this type of activity is essential to the specific technical activities on site.

Furthermore, the geometric arrangement of the transplantation modules carried out by the diver during the positioning phase is also of crucial importance. These geometries, specific for each technique, must generally allow the growing cuttings to create recolonisation nuclei that are able to join together and form a continuous meadow over time. This means that the areas chosen for transplantation should have sufficiently large dimensions to allow the expansion of the transplanted meadow in the medium and long term.

For an examination of the preparation and fixing of cuttings to transplantation modules and their positioning according to the technique, please refer to the "Manual of techniques and procedures for the transplantation of *Posidonia oceanica*" (Bacci and La Porta, 2022).

4.3 | MONITORING OF THE TRANSPLANTATION OPERATION

In order to evaluate the effectiveness of a transplantation operation, it is advisable to devise specific monitoring plans, taking into account whether the transplantation was carried out using cuttings or by the transfer of clods. Regarding the transfer of *P. oceanica* clods, please refer to the Box below.

Monitoring of transplantation carried out using *Posidonia oceanica* cuttings must be carried out at regular, predefined intervals, especially in the period following the conclusion of transplantation, and must cover an adequate time span, evaluated according to the growth rates of the plant. For example, on the basis of the analysis of several transplantation cases carried out in Italy (AA.VV., 2021b, 2021c, 2020a) it emerged that quarterly monitoring is appropriate at least for the first two years after transplantation (short-term monitoring); thereafter, the monitoring becomes six-monthly up to five years after transplantation (medium-term monitoring), and subsequently a monitoring campaign should be planned every year, hopefully up to ten years after transplantation (long-term monitoring). In particular, the parameters to be taken into consideration for monitoring the transplantation operation, especially in the short and medium term, mainly concern functional descriptors (illustrated below), relating to a statistically significant sample of transplanted *P. oceanica* cuttings. The analysis of the aforementioned descriptors must be mainly carried out through non-destructive methods and applying standardised techniques. Furthermore, according to the specific purposes of the study, adequate monitoring should foresee that the analysis of the descriptors (at a site and receiver meadow level), initiated in the phase of characterisation and assessment of the transplantation area, continues throughout the monitoring that follows the transplantation activity. To correctly assess the outcome of the transplantation operation, it is important to monitor a significant number of control areas, identified in portions of the receiver meadow, adjacent to the transplantation area. These control areas enable the reconstruction of the growth dynamic of the natural meadow to be compared with the growth dynamic of transplanted meadow over the entire planned monitoring period.

Table 4.3.1 shows the activities and their purposes in the monitoring of a transplantation operation.

Table 4.3.1 | Main activities and their purposes in the monitoring of a transplantation operation using Posidonia oceanica *cuttings.*

ACTIVITY	PURPOSE
Short-term monitoring (≤ 2 years)	Assess the rooting of the biological material and any non-survival due to natural death of the biological material or mechanical damage (eg. sea storms, fishing, anchors, etc.).
Medium-term monitoring (2 years < x ≤ 5 years)	Assess the growth trend of the transplantation as a balance between any initial losses and the subsequent phase of growth and expansion, in order to achieve the phase of stability.
Long-term monitoring (> 5 years)	Assess the achievement of transplantation maturity and the acquisition of structural and functional characteristics similar to a natural meadow.

Monitoring subsequent to the end of cutting transplantation should therefore include, both for the receiver meadow level and the transplantation area (*fig.* 4.1), the analysis of the following parameters based on the specific purposes of the transplantation carried out:

1) receiver meadow

- functional descriptors: phenological parameters, lepidochronological parameters;
- structural descriptors: shoot density and % coverage;
- ecological descriptors: associated flora and fauna.

2) transplantation area

- functional descriptors: survival rate of cuttings[2], new shoot for-

2 It should be noted that where there is a high rate of progression of rhizomes and formation of new shoots, the estimation of survival of the original cuttings becomes over time increasingly difficult to determine.

mation, rhizome elongation, leaf elongation (Zieman, 1974), root development, phenological parameters (also detectable with non-destructive techniques), lepidochronological parameters;
- structural descriptors: coverage of the area colonised by the cuttings, shoot density[3];
- ecological descriptors: associated flora and fauna.

The descriptors analyzed in the characterisation phase at receiver site can be also monitored in the post-transplantation phases according to the specific aims of the study. In particular, these descriptors refer (paragraph 4.1.2) to the sedimentation rate, granulometry of surface sediments, local hydrodynamic regime and light intensity, water clarity, dissolved oxygen, salinity, temperature, pH, nutrient load and suspended particles (organic and inorganic).

Table 4.3.2 shows the monitoring plan for a transplantation operation using cuttings, which shows both the possible parameters to be investigated and the frequency of surveys (characterisation phase before transplantation and monitoring phase after transplantation).

To verify the success of a transplantation operation using cuttings, it is necessary to quantify the survival rate of the transplanted cuttings, measure their growth and assess the appearance of new foliar shoots in the early years of monitoring (at least five years). Furthermore, the scientific literature available specifically for *P. oceanica* shows that long-term monitoring alone is effective in verifying the performance of a transplantation and assessing the structure and functions of the "new" meadow (Scardi *et al.*, in print; Calvo *et al.*, 2021; Bacci *et al.*, 2019).

As part of long-term monitoring (at least ten years), the duration of which should coincide with the establishment of a consolidated

3 The number of shoots is counted within squares of known size randomly positioned, similar to what was done in the natural meadow; descriptor to be investigated in long-term monitoring.

growth dynamic, the primary production of the meadow, the biocoenoses associated with it, as well as the macrostructure of the meadow itself, which plays a fundamental role in reducing the erosive processes of the coast, should be considered. Long-term monitoring is also the only way to analyse the density achieved by the transplanted meadow compared to natural meadows, without referring to the estimated survival rate of the original cuttings and/or the total number of foliar shoots in the original pilot transplantation units (AA.VV., 2020a).

Furthermore, the acquisition of data relating to the coverage of transplanted meadows is considered a priority. Using new technologies, the acquisition of high-resolution mapping data of the receiver area before and after transplantation, enables better quantification of surviving and lost transplanted surfaces over time (Ventura *et al.*, 2022; Rende *et al.*, 2020; AA.VV., 2020a).

For further information on the parameters and measurement methods of structural and functional descriptors, see Buia *et al.,* (2003).

Table 4.3.2 |Summary diagram for the selection of parameters and sampling frequencies to be adopted to monitor a transplantation operation using Posidonia oceanica *cuttings.*

		Characterisation Monitoring	Short/medium term monitoring		Long-term monitoring
Surveyed area	**Parameter**	**Frequency**	**Frequency Year I and II**	**Frequency Year III - IV - V**	**Frequency from Years VI to X**
Donor meadow	Coverage of the meadow	Once (before ex-plantation)	Based on the purpose of the research	Based on the purpose of the research	based on the purpose of the research
	Shoot density				
	Phenology				
	Lepidochronology				
	Associated flora and fauna				
Site (near the receiver meadow)	Temperature	once (before ex-plantation)	On the basis of the specific site conditions and the purposes of the research	On the basis of the specific site conditions and the purposes of the research	On the basis of the specific site conditions and the purposes of the research
	Salinity				
	Water clarity				
	Light intensity				
	pH				
	Oxygen				
	Nutrient load				
	Particle suspension				
	Sedimentation rate				
	Sediment granulometry				
	Organic substance content				
	Local hydrodynamic regime				
Receiver meadow	Coverage of the meadow	Once (before explantation)	At least once	At least once	At least twice
	Shoot density				
	Phenology				
	Lepidochronology				
	Associated flora and fauna				
Transplantation area (in the receiver meadow)	Survival rate of cuttings	-	every 3 months	-	-
	Root production				
	Rhizome elongation		every 3 months	every 6 months	
	Leaf elongation				
	Coverage of the area colonised by the cuttings		every 3 months	every 6 months	every 12 months
	Formation of new shoots				
	Shoot density		-	-	Based on the purpose of the research
	Phenology				
	Lepidochronology				
	Associated flora and fauna				

Transplantation by transfer of *Posidonia oceanica* clods

Compensatory transplantation using *Posidonia oceanica* clods has been carried out in Italy and other Mediterranean countries as part of Environmental Impact Assessments of projects relating to the expansion of ports or coastal construction projects. This technique, unlike that used for transplanting cuttings, involves the transfer of clods, portions of meadow including the underlying *matte* and trapped sediment, from the meadow area damaged by the construction work to neighbouring areas.

The activities related to the planning of transplantation using clods, such as the characterisation and assessment of the donor meadow, the receiver site and the receiver meadow and the assessment of the ecosystem services of the donor and receiver meadows, are the same as described in paragraph 4.1 "Planning of the *Posidonia oceanica* transplantation". In the case of clod transplantation, however, it should be noted that the selection of the area potentially suitable for receiving the transplantation cannot be identified through pilot transplantation due to the obvious lack of availability of clods to be used in this pilot phase. Mechanical means are used to transfer the clods and differ according to both the size of the clods and the logistical needs in the transfer and positioning of the clods in the receiver areas. In the Mediterranean, in the few cases involving the transfer of clods of *P. oceanica* by mechanical means, the size of the clods varied from a minimum of 0.8 m^2 to a maximum of 2 m^2 and their positioning required the embedding or otherwise of the clod, depending on the case (A.A.VV., 2019b, 2020a; Bedini *et al.*, 2020; Sánchez Lizaso *et al.*, 2009; Descamp *et al.*, 2017). For an examination of transplantation techniques using the transfer of clods of *Posidonia oceanica*, please refer to the "Manual of techniques and procedures for the transplantation of *Posidonia oceanica*" (Bacci and La Porta, 2022).

As regards the monitoring of transferred clods, the parameters to be investigated in the donor meadow and in the receiver site and meadow are the same as those reported in paragraph 4.3 “Monitoring of the transplantation operation”.

Specific considerations regard the methodologies used for the analysis of a number of parameters to be investigated in the monitoring of the transplantation area.

As regards the functional descriptors (propagation of rhizomes in the marginal area of the clod, leaf elongation, phenological parameters, lepidochronological parameters) and ecological descriptors (associated flora and fauna), the same methods for monitoring the *P. oceanica* meadows involving transplantation of cuttings (Buia *et al.*, 2003) are used.

For the structural descriptors (shoot density, coverage of the area colonised by *Posidonia* on clods, size of the clod) it should be specified that: i) the number of foliar shoots is counted within squares of known size and that counting must also be carried out in the centre of the clod; ii) starting from the centre of the clod, in the compass direction North, South, East and West, according to the size of the clod, a number of squares must be arranged at a distance such that they can include the entire area of the clod, including the outer edges of the clod (eg: for clods of size 2 x 2 m place the squares at a distance of about 80 cm); iii) for each zero count, the type, nude or eroded substrate (nude = without *Posidonia* cover, eroded = substratum lost due to erosion) should be noted; iv) the size of the clod is obtained from the North-South length (cm) and the East-West length (cm), which, together with photographs of the clod from above and from the sides, provides information to evaluate the conservation status of the clod.

The metrics for verifying the success of a clod transplantation operation are: i) the count of clods lost (with number of shoots equal to zero) in relation to the total number of clods monitored (expressed as %); ii) the number of shoots per clod in relation to the initial number of shoots (expressed as %), estimated using the methodology described above.

Furthermore, estimates of leaf density (expressed per m^2) and phenological and lepidochronological functional parameters acquired on the clods can be compared with those of the clod donor meadow and also of the neighbouring natural meadow, in order to monitor the dynamics of these descriptors over time.

The management of the site to which *Posidonia* clods have been transferred is an essential phase to guarantee the success of the transfer. The envisaged actions concern specific aspects of transplantation as well as various aspects of the governance of transplantation (Zenone *et al.*, 2021; Ruiz-Frau *et al.*, 2019). In particular, taking into account the nature of this technique, like for transplantation using cuttings it is essential to guarantee the temporary exclusion from transplantation areas of fishing, shipping and anchoring and the protection and development of the transplantation, as specified in paragraph 4.4 "Management of the transplantation of *Posidonia oceanica*".

4.3.1 | Verification of the success of the transplantation operation

A transplantation operation can only be considered successful once the cuttings have stabilised and show good and persistent growth and an active recolonisation process (AA.VV., 2020a; Fonseca *et al.*, 1998). Furthermore, the cuttings can only be considered consolidated once they are able to guarantee the basic functions of the ecosystem: stabilisation of the sediment, supporting the nutrient cycle, biomass production and secondary production. Long-term monitoring of transplantation of cuttings is therefore the best tool for verifying these functions. This is in agreement with observations made during the monitoring of various transplantation operations carried out in Italy (Scardi *et al.*, in press; Calvo *et al.*, 2021; AA.VV., 2020a, Robello., 2019) and recommended by a number of authors (Bacci *et al.*, 2019; Pirrotta *et al.*, 2015; Cunha *et al.*, 2012; Fonseca *et al.*, 1998). Good results in the initial stages of transplantation do not necessarily correspond to real success, just as a low initial performance does not necessarily exclude a positive outcome in the future (Calvo *et al.*, 2020).

In order to be able to verify the outcome of transplantation effectively, it is important to define an appropriate monitoring plan according to the transplantation surface, the technique used and the local environmental conditions. A number of essential aspects to guarantee the effectiveness of monitoring involve:

- the selection of a significant number of sample monitoring areas both in the transplantation area and in the control receiver meadow;
- the selection of a significant number of sample units for the various sample monitoring areas identified;
- the labelling of the sample units, which takes place at the same time as their selection, for which periodic maintenance must be provided.

Metrics and parameters for the verification of a transplantation operation

Table 4.3.1.1 shows the metrics and parameters that best describe the status and growth dynamics of transplantation during the different monitoring phases.

Table 4.3.1.1 | Metrics and parameters for the verification of transplantation using Posidonia oceanica *cuttings.*

	Metrics and parameters for the verification of a transplantation operation	Technical specifications
Short-medium term monitoring	Time progression of the average number of shoots per sample unit in relation to the initial number of shoots (expressed as %).	The total number of shoots per sample unit includes both the transplanted shoots and the new shoots originating from them. The sample unit is the sampling surface in which the total foliar shoots are counted and can be represented by the anchoring module but also by standard surfaces within which the transplanted cuttings are anchored.
	Time progression of the number of sample units lost in relation to the total number of sample units monitored (expressed as %).	The number of sample units lost includes those with zero shoots. The estimate of the transplanted area lost should also be evaluated through analyses of coverage.
	Time progression of the coefficient of variation, calculated on the number of shoots per sample unit.	The coefficient of variation is an effective index of transplantation stability. When its values tends to became constant over time, the coefficient identifies the moment when the mortality rate of the shoots no longer affects growth, which becomes balanced regardless of the density level in the sample unit (AA. VV., 2020a).
	Coverage of the area colonised by transplanted *Posidonia oceanica*.	Once a significant monitoring surface has been defined as representative of the total transplantation area, coverage is expressed as the substratum surface area colonised by the *Posidonia* cuttings in relation to the initial surface area transplanted.
Long-term monitoring	Average absolute density (expressed per m^2) of the transplantation area and the surrounding natural meadow.	The average absolute density, measured according to the methodologies used in natural *P. oceanica* meadows (Bacci *et al.*, 2015; Pergent *et al.*, 1995; Panayotidis *et al.*, 1981; Giraud, 1977), adequately represents the dynamics of transplantation in the long term and allows a comparison with the dynamics of the neighbouring natural meadow (AA.VV., 2020a).
	Coverage of the area colonised by transplanted *Posidonia oceanica*.	Coverage is an indicator of the overall outcome of the transplantation over time. Defined as indicated above, coverage is an effective indicator in the long term to highlight the overall level of development of the transplantation and any surface areas lost.
	Phenology and Lepidochronology.	Phenological and lepidochronological analysis reflects the level of maturity reached over time by the transplantation compared to the adjacent natural meadow. The plant, in fact, initially invests greater resources in the rhizomes, reflecting the need in transplantation areas to expand, than in leaf tissue, required in the natural meadow to increase photosynthetic activity (AA.VV., 2020a).

4.3.2 | Towards open data for the management of *Posidonia oceanica* meadows

As part of the LIFE SEPOSSO project, a survey was carried out to define the state of the art of the availability of environmental data in Italy for use in planning management activities for *Posidonia oceanica* meadows and any transplantation operations (AA. VV., 2020b). The results highlighted various critical issues affecting the objective usability of the data, including: i) availability of data, ii) data quality, iii) data operability, iv) level of detail and spatial coverage, v) access constraints.

From the analysis it emerges that to date, at national and local level (for the project target regions of the Tuscany, Lazio, Campania and Sicily), the effective availability of operational and useful environmental data for the local scale management of *Posidonia oceanica,* meadows is very low.

Data and information relating to the different phases in the procedure for the transplantation of *P. oceanica* should, therefore, be collected in a centralised, standardised and validated manner, in order to provide an open access database to support the planning phases, implementation, monitoring and management of transplantation.

The Box below briefly illustrates the *Posidonia Web Platform* (PWP) created by the LIFE SEPOSSO project for the collection of environmental data related to *Posidonia* meadows and transplantation.

The Posidonia Web Platform - PWP

One of the objectives of the LIFE SEPOSSO project was to create a web platform, the "*Posidonia Web Platform*" (PWP), as a tool for collecting environmental data related to *Posidonia* meadows and the monitoring of transplantation to compensate for damage caused to meadows during marine-coastal construction projects and to restore degraded meadows. In particular, the platform is also a digital instrument for improving the effectiveness and efficiency of the control of EIA procedures, including those relating to works that have impacted on *Posidonia* and, especially, for the phases of verification of compliance with EIA environmental conditions requested, including those relating to the transplantation of *Posidonia* as compensation measure.

Furthermore, in line with the guidelines of the Italian National Recovery and Resilience Plan (NRRP), the "*Posidonia Web Platform*" contributes both to the goal of creating the interoperability of databases and to the acquisition and management of data from *Posidonia* transplantation carried out for the restoration of marine habitats.

The PWP platform contains specific forms that have been created to allow the acquisition and cataloguing of environmental data relating to the sedimentological, hydrological and chemical-physical parameters of the water column, the distribution of *Posidonia* meadows and their structural, functional and ecological descriptors, as well as data deriving from short, medium and long-term monitoring, collected for the verification of transplantation operations and described in this manual. Cataloguing is based on a metadata structure adhering to the INSPIRE Implementing Rules (EC Regulation 1205/2008, Italian Legislative Decree No 32 27 January 2010, Decree 10 November 2011).

The PWP platform provides different levels of access to data to users - public, private, political, inspectors, technicians and citizens - involved in *P. oceanica* management processes and any transplantation. The tool has several modules or "*apps*" that enable the management and centralisation of collaborative processes of data collection, validation, integration and sharing of various types of data and permits the promotion of the transparency requirements of environmental information, in compliance with the Aarhus Convention (25 June 1998).

4.4 | MANAGEMENT OF THE TRANSPLANTATION OF POSIDONIA OCEANICA

The management of a *Posidonia* transplantation site is an essential phase to ensure the success of the transplantation. Management envisages specific aspects to follow up the transplantation as well as its governance (Zenone *et al.*, 2021; Ruiz-Frau *et al.*, 2019).

Table 4.4.1 shows the main activities and their purposes related to the management of transplantation areas.

Table 4.4.1 | Main activities and their purposes related to the management of Posidonia oceanica *transplantation areas.*

ACTIVITY	PURPOSE
Replacement of failures	Compensate, by means of small "repairs", for loss of transplanted cuttings, occurring mainly in the early years.
Temporary exclusion from transplantation areas of fishing, shipping and anchoring	Contribute to ensuring the intactness of the transplantation over time.
Protection of the transplantation	Contribute to ensuring the intactness of the transplantation over time
Appreciation of the transplantation	Raise local public awareness of the existence of the transplantation in the area where it was carried out and manage access by citizens
Decommissioning the transplantation	Clear the area where transplantation has failed of materials used in the various techniques

The following are the main aspects, identified in Table 4.4.1, related to the management of transplantation areas.

Replacement of failures

Small "repairs", performed to compensate for loss of transplanted cuttings, helps to ensure the success of transplantation, especially in the early years. The plan for the replacement non-surviving plants, to be envisaged during the transplantation planning phase, must be implemented as soon as monitoring shows excessive loss of cuttings due to natural death and/or when environmental conditions or any human activity damage the transplantation during the early years. The duration and frequency of the replacement of the non-surviving plants must be defined according to the technique used and it should not be less than two years after the transplantation.

Temporary exclusion from transplantation areas of fishing, shipping and anchoring

In order to protect transplantation sites in areas subject to high anthropogenic pressure, specific ordinances from the competent Authorities should envisage the temporary ban of activities such as recreational and trawl fishing (the latter illegal on *Posidonia* meadows), and the anchoring of pleasure boats and provide for regular surveillance by the Authorities throughout the transplantation process and period of monitoring. In fact, several case studies carried out in Italy (AA.VV., 2021c, 2020a) highlight that the failure to regulate these areas and prevent significant damage to anchoring modules and transplanted plants poses a threat to the success of the transplantation.

Protection of the transplantation

The adoption of measures, such as anti-trawling apparatus and mooring buoys near the transplantation site, serve to protect the transplantation modules over time. When transplanting is carried out as a compensation measure, locating the area of compensation within or near the Natura 2000 site, affected by the impact, where the conditions are suitable for the success of the measure, may be the most preferred option. However, this is not always possible, and a range of priorities should therefore be applied when searching locations that meet the requirements of the Habitats Directive. Compensation outside the Natura 2000 site concerned, both in a common or different topographical or landscape unit, provided the same contribution to the ecological structure and/or network function is feasible. The new location can be in another designated Natura 2000 site or a non-designated location. In the latter case, the location must be designated as a Natura 2000 site and be subject to all the requirements of the Nature Directives, as specified in the guidance document "Managing Natura 2000 sites - Provisions of Article 6 of Directive 92/43/EEC (2019/C 33/01)".

Appreciation of the transplantation

An information and communication plan regarding the *Posidonia* transplantation must be developed in the transplantation planning phases, in synergy with various local entities potentially involved in the transplantation operation. The plan must be operational before, during and after the implementation of the transplantation and ensure transparency and public participation, essential requirements of "good" governance (AA.VV., 2020c).

Decommissioning the transplantation

In the event of a negative outcome for the transplantation, the seabed affected by the operation should be cleared of all materials used in the different techniques, such as transplant modules, fixing pegs, mats, etc. A decommissioning plan must be envisaged in the transplantation planning phase and carried out as soon as monitoring reveals the failure of the operation for adverse weather-marine events or anthropogenic action; this is to avoid the accumulation of alien materials and waste in the sea.

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