GUIDELINES FOR THE ACTIVE RESTORATION OF POSIDONIA OCEANICA

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GUIDELINES FOR THE ACTIVE RESTORATION OF *POSIDONIA OCEANICA*

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1. CONTEXT, DEFINITIONS AND AIM OF THE GUIDELINES

1.A. General context

Posidonia oceanica (L.) Delile is an endemic seagrass of the Mediterranean Sea which forms vast meadows (>2 million hectares) between the surface and 40 meters depth. This engineer species is the keystone of a major ecosystem, which offers several ecosystem services, due to its high primary production (a planetary scale), its biodiversity (1) and its ability to store and sequester carbon for millennia (2). Consistently with their crucial role, these meadows are legally protected or subject to specific regulations in all the Mediterranean countries where the species is present (3), which unfortunately does not guarantee an absence of regression. Due to the expansion of coastal human populations, seagrass loss, over relatively short time scales, has been reported worldwide, including in the Mediterranean Sea (4, 5). Even if a decrease in these regressions is documented in Europe, with a rate of area loss of the seagrass meadows of 27%. decade^{-1} observed in the 1980s, and only 8.3%. decade^{-1} in the 2000s (6), extensive localized regressions can be observed particularly concerning Posidonia meadows (7, 8).

The main causes of the regression of the Posidonia meadows, priority habitat 1120* *sensu* Habitat Directive (9), are associated with water quality, construction of coastal infrastructures, laying of underwater pipelines and cables, anchoring, aquaculture facilities and trawling. The species is relatively resistant to temperature variations and competition with alien species, whereas it is reported as to suffer from even slight fluctuations of salinity and turbidity, as well as the increase of sedimentation rate (10). In addition, *Posidonia oceanica* has a slow growth rate (on average between 100 and 1 000 cm per century) that makes recovery difficult when impacted (1). In fact, because of its intrinsic features and ecological needs, Posidonia meadows are exposed to multiple threats due to the strong and global anthropic pressure that characterizes the Mediterranean as a whole. As the degradation of the Posidonia meadows concerns many Mediterranean countries, a regional approach is required to better protect the Posidonia meadows. This approach has been implemented through the Specially Protected Areas Regional Activity Centre (SPA/RAC) of the Mediterranean Action Plan (UNEP/MAP), which assists Contracting Parties to the Barcelona Convention to fulfill their obligations regarding the SPA/BD Protocol and the regional action plans dedicated to the endangered habitats and species. This approach was recently reinforced with the Mediterranean Posidonia Network (MPN) initiative. The MPN has been initiated after the Natura 2000 Biogeographical Process Networking Event "Anchors Away: Mitigating Direct Anthropogenic Impacts on Posidonia beds" (11), organized by the Hellenic Centre for Marine Research and supported by the European Commission in 2019. The objective is to protect 100 % of Posidonia threatened by 2030. The network gathers 10 countries having Posidonia meadows in their national water. The MPN aims notably to prevent anchoring by vessels above Posidonia meadows, share joint solutions at Mediterranean scale and promote actions to safeguard the meadows.

Among these solutions, the first option must be to follow a hierarchy of mitigation, which means avoiding causing damage, minimizing any damage caused and applying protective measures (12). Nevertheless, to reverse strong negative trends, there is an additional need to implement ecological restorations, even if it may not be the first or the best options for conservation management. As affirmed by Gann *et al*. (13), ecological restoration, when implemented effectively and sustainably, contributes to i) protecting biodiversity, ii) improving human health and wellbeing, iii) increasing food and water security, iv) delivering goods, services, and economic prosperity, and v) supporting climate change mitigation, resilience, and adaptation. It is a solutions-based approach that engages communities, scientists, policymakers, and land managers to repair ecological damage and rebuild a healthier relationship between people and the rest of nature. When combined with conservation and sustainable use, ecological restoration is the link needed to move local, regional, and global environmental conditions from a state of continued degradation, to one of net positive improvement.

1.B. Definitions

“**Restoration”** is the process of assisting the **recovery** of an ecosystem that has been degraded, damaged, or destroyed (14). In the context of the European Union restoration strategy (15), this is the process of **actively or passively** assisting the recovery of i) an ecosystem towards good condition, ii) a habitat type up to the highest level of condition attainable and its favorable reference zone, iii) a habitat or species to a level of sufficient quality and quantity, or populations to satisfactory levels, with the aims of conserving or enhancing biodiversity and ecosystem resilience. The term “**recovery**” refers to the objective of ecological restoration interventions. Their aim is to achieve conditions like those of the original state or like those of a reference ecosystem, in terms of the specific composition, structure, and functionality. “**Passive restoration”** can be defined as mitigating human threats to favorize natural regeneration when natural recovery potential is high, making it the most cost-effective approach. “**Active restoration”** is the process of actively assisting the reestablishment or increase of organisms or depleted populations through assisted regeneration or reconstruction (13).

In general terms, "**transplantation**" refers to the action of relocating plants, such as Posidonia cutting, from one location to another; in particular Calumpong & Fonseca (16) describe "transplants" as the operations of transferring plant material from a donor meadow to a new host site.

In this document, the term “**operational**” refers to the restoration techniques that are currently considered as being the most effective. Although there are still some limitations, the feedback gained is adequate to validate the methodology and assess its overall efficiency. It does not mean that these techniques enable compensation and/or large-scale transplantation measures. On the contrary, “**Research and Development**” (R&D) involves carrying out additional experiments to expand knowledge base, aiming to devise new techniques or discover innovative ways to improve the existing ones.

The term "**restoration success**" lacks a universally agreed-upon definition. Fraschetti *et al*. (17) propose a highly successful ecological restoration project/study as one when the achieved restoration goals lead to ≥ 50 % survival of restored organisms for the entire intervention area. Conversely, a restoration failure is characterized by an outcome of ≤ 10 % survival of restored organisms. In the case of engineer species with slow growth such as *Posidonia oceanica*, it might be useful to distinguish between “**transplantation success**” which would relate to a specific survival rate value, to be reached within a predefined time interval after planting, and a “**restoration success**” which would imply the recovery of habitat structure, species composition, ecological functioning or ecosystem services that had been lost at a specific site, with the establishment of different targets, depending on the variable or process focused on. Even if these limits can be useful, it seems that a “**reference trend**”, observed at the level of the transplanted meadow, and compared with those of a native healthy one, could be more appropriate to define whether a transplantation is successful or functional (18). The choice of this “reference trend” will depend on whether the focus is on “transplantation” or “restoration”, i.e., focusing on the survival rate or performance of the transplanted material or any aspect of the ecological functioning of the transplanted area.

The plant material suitable for Posidonia planting includes cuttings, clods, or seedlings. "**Cuttings**" can be obtained by cutting from a donor meadow (19, 20, 21) or collected from drifting material present on seafloor (22, 23, 24) or beach cast, after storms (25) or damaging anthropic activities (e.g. anchoring; 3, 7). In this last case, they can be designated as "fragments" instead of cuttings. These are both parts of orthotropic or plagiotropic rhizomes with one, two or more "**shoots**" (*sensu* 22, 23, 24), "**leaf bundles**" (*sensu* 19, 20), or "**foliar bundles**" (*sensu* 26). "**Clods**" (27, 28) or "**sods**" (*sensu* 29) are portions of meadow including sediment extracted from the donor site and transported to the receiver site. "**Seedlings**" may be obtained from seeds grown in aquaria and acquired from fruits washed up on the beaches (30).

1.C. Purpose of the guidelines

As initiated by other international or regional agreements (e.g. OSPAR, UNEP-MAP), seagrass restoration operations require an analysis of existing practices in order to identify what is "working" (operational techniques) and what is still considered as Research and Development (R&D).

This initiative aims to guide public policies, Marine Protected Areas (MPA) managers, decision-makers, associations and scientists. The decision-making process, regulations and source of funding will be different if the restoration project is a working or R&D operation.

The main objectives of this restoration guidance are as follows:

- Present and describe case studies showcasing successful Posidonia restoration measures, particularly those employing nature-based solutions,
- Analyze previous unsuccessful experiments in order to identify the causes of failure,
- Investigate main topics R&D would necessary,
- and by doing this, develop structured Posidonia restoration guidelines involving the successive steps from the need to restore, the planning, the site selection, the actual restoration measures, the monitoring and the assessment.

The report does not discuss the different European / national regulations that can be required when transplanting Posidonia.

2. OPERATIONAL TRANSPLANTATION AND RESTORATION

Recent research programs have been implemented to identify operational restoration methods and to propose guidelines to guarantee the success of these restorations, as detailed in the results of the LIFE SEPOSSO European Program (31, 32). According to these publications, several operational transplantation techniques are available, based on cuttings, clods or seedlings. Nevertheless, at present, clods need further R&D, due to the few experiments carried out, the high level of mortality and the insufficient monitoring. Even if seeds plantation can be considered as operational, its utility is constrained by the irregular availability of seeds, only possible following a natural flowering event.

Based on cuttings, only a limited number of experiments, carried out before the 2000s, have been recently monitored (Table 1). Recent experiments, benefiting from a sufficiently long monitoring period to assess their performance, remain rather scarce (33, Table 1). According to available data[1], a minimum period of four years is deemed necessary, starting from the establishment of the transplants, to evaluate the success of these experiments (more details can be found in appendix 1 to 7).

The main criteria, commonly used to assess the transplantation success, are the survival rate of cuttings or shoots, and the increase of the density of shoots per m^2 or of the surface of the bottom occupied by the Posidonia shoots. The criteria used to assess the restoration success are rather the comparison between the criteria measured in a healthy meadow, in the vicinity, and those observed at the site of transplantation. For instance, in the Port-Cros experiment (Table 1; Appendix 1) the density of shoots per m^2 in 2023 (mean value: 515) is equivalent to that observed in natural Posidonia patches, both within and surrounding the transplant, at the same depth (mean value: 500; min.: 480; Max.: 517). Similarly, in the Palermo Gulf, the total primary production agrees with the estimates reported for several Mediterranean meadows.

The analysis of these criteria (Table 1) shows that the results are as different as the diversity of the experimental conditions:

(i) sites corresponding to MPAs, such as Port-Cros (Appendix 1) and Capo Carbonara (Appendix 4) and anthropized sites, such as Rapallo (Appendix 2), Palermo (Appendix 3) and Golfe Juan[2] (Appendix 7),

(ii) transplantations carried out with cuttings issued from a donor meadow (e.g. Port-Cros, Rapallo, Palermo), from drifting materials, uprooted naturally by storm surges (Pollenca bay; Appendix 5) and/or matte landslides (Capo Carbonara) or rhizomes uprooted (Golfe Juan) or clods broken up by boat anchoring (Giglio island; Appendix 6),

(iii) transplantations carried out in shallow water (Pollenca bay, Rapallo) down to more than 20 m depth (Giglio island).

Significant successes have been documented on sites subject to considerable disturbances in the past (e.g. Palermo), even though the most noteworthy results are observed within an MPA. Transplantations using cuttings from a donor meadow, at an intermediate depth, show the best outcomes (Table 1).

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[1] : These data are not exhaustive and are derived from the discussions of the MPN working group on restoration.

[2] Golfe Juan is a Natura 2000 site, considered as MPA in France.

Table 1: Main results of the case studies based on cuttings, with **a minimum of four years of monitoring**. Y0: year of the implementation of the experiments; Ym: last year of the monitoring; Multiplier factor = Ym / Y0; *: cuttings issued from donor meadows; **: drifting material; nb.: number; n.d.: not determined due to the impossibility of differentiating the cuttings from each other; -: no data; sd: standard deviation.

Experimental site	Parameters	Y0	Ym	Multiplier factor
Port-Cros*	Total nb. of cuttings	1988 - 1995 : 301	2023 : n.d.	
	Density of cuttings.m^{-2}	1988 - 1995 : 141 (min. 28 – Max. 849)	2023 : n.d.	
	Total nb. of shoots	1988 - 1995 : 613	2023 : 53 400	87.1
	Density of shoots.m^{-2}	1988 - 1995 : 241 (min. 99 – Max. 915)	2023 : 515 (min. 389 - Max 725)	2.1
	Surface area	1988 - 1995 : 3 m^2	2023 : 105 m^2	35.0
Rapallo*	Total nb. of cuttings	1996 - 1997 : 500 (200+300)	2019 : n.d.	
	Density of cuttings.m^{-2}	25	2019 : n.d.	
	Total nb. of shoots	1996 - 1997: 618 (measured from the 200 cuttings) + 600 (estimated for 300 cuttings) = 1 218	2019 : 4 567	3.8
	Density of shoots.m^{-2}	1996 - 1997 : 61,8 (on 200 cuttings)	2019 : 195	3.2
	Surface area	1996 - 1997 : 20 m^2	2019 : 24 m^2	1.2
Palermo*	Total nb. of cuttings	2008 – n 400 (= 20 cutting.m^{-2} *20 m$^{2)}$	2022 : n.d.	
	Density of cuttings.m^{-2}	2008 : 20	-	-
	Total nb. of shoots	2008 : 1 313	2022 : 6 300	4.8
	Density of shoots.m^{-2}	2008 : 66	2022 : 331.6	5.0
	Surface area	20 m^2	19 m^2	1.0
Capo Carbonara MPA – Villasimius**	Total nb. of cuttings	2017 : 15 000	2023 : 6 500	0.4
	Density of cuttings.m^{-2}	2017 : 30	2023 : 13	0.4
	Total nb. of shoots	-	-	
	Density of shoots.m^{-2}	-	-	
	Surface area	1 000 m^2	928 m^2	0.9
Pollença bay**	Total nb. of cuttings	2018 - 2019 : 12 800	2023 : 12 010	0.9
	Density of cuttings.m^{-2}	2018 -2019 : 16	2023 :14 – 15	0.9
	Total nb. of shoots	2018 - 2019 : 59 680	2023 : 42 902	0.7
	Density of shoots.m^{-2}	2018 - 2019 : 69 - 82	2023 : 43 – 63	0.6 – 0.9
	Surface area	2018 - 2019 : 800 m^2 in 2 ha	800 m^2 in 2 ha	1
Giglio island**	Total nb. of cuttings	2019 - 2022 : 13 095	2023 : 11 431	0.9
	Density of cuttings.m^{-2}	2019 - 2022 : 6 ± 1 (sd)	2023 : 5 ± 1 (sd)	0.8
	Total nb. of shoots	2019 - 2022 : 63 835	2023 : 75 612	1.2
	Density of shoots.m^{-2}	2019 - 2022 : 30 ± 3 (sd)	2023 : 35 ± 2 (sd)	1.2
	Surface area	-	-	
Golfe Juan**	Total nb. of cuttings	2019 : 5 262	2023 : 2 263	0.4
	Density of cuttings.m^{-2}	2019 : 71.9	2023 : 32.5	0.4
	Total nb. of shoots	2019 : 16 923	2023 : 7 277	0.4
	Density of shoots.m^{-2}	2019 : 225.3	2023 : 104.4	0.5
	Surface area	2019 : 77.6 m^2	2023 : 69.7	0.9

Therefore, operational technical solutions are available to achieve “successful” transplants (in terms of survival rate of cuttings after three years or survival rates of shoots after five years) in a wide range of situations. The biggest limit to consider if a transplantation is a success is the lack of information related to the natural trend. In France, as an example, experts consider than a survival rate of cuttings, issued from a donor meadow, of 75 % after three years, and a number of shoots after five years higher to the number of shoots after three years can be considered as a transplant success. However, restoration success (which would imply the recovery of habitat structure, species composition, ecological functioning or ecosystem services) has never been assessed therefore cannot be demonstrated so far and has to be seen as a R&D project.

Likewise, although the examples provided concern limited surface areas, in several cases the techniques used were then applied to larger areas (e.g. Palermo, Giglio island and other sites in the vicinity of Golfe-Juan).

The multiplier factor, used to assess the degree of success of the experiments over time, is higher when employing cuttings from a donor meadow, probably due to the better health conditions of the cuttings (34). However, with regard to cuttings from shipwreck materials, even though this factor is lower as it sustains shoots that are destined to die, it still constitutes a form of success, especially since the harvesting of living shoots is prohibited or heavily regulated in some countries (3).

3. LESSONS LEARNT FROM PREVIOUS EXPERIMENTS

Experiences published on transplanting with cuttings or seedlings highlight several aspects that can be considered as valuable lessons before undertaking such endeavors. In order to allow in-depth analysis, the publications are, as usual, numbered and compiled in the bibliography and the main data are summarized in appendices (Appendix 8 for cuttings; Appendix 9 for seeds).

3.A. Cuttings (Appendix 8)

The following factors may be noted:

- Type and origin of material
  - Transplants of fragments of plagiotropic rhizome (20, 35), and particularly those with at least three shoots (one plagiotropic and at least two orthotropic), exhibit higher survival rate (19, 35, 36, 37), ramification rate (38) and root formation (19, 36) than solely orthotropic fragments.
  - Within orthotropic rhizomes, the best survival rate occurs when the fragment has at least two shoots (19, 20, 38) or a long rhizome section (>10 cm; 39, 40).
  - Storm-generated fragments of rhizome are found to be suitable as transplants, either on the beach if collected just after the storm event (minimizing dryness time) (25) or drifting underwater (22). Fragments extracted from drifting blocks of meadow originated by vessel anchoring or other mechanical impacts can also be used (23, 41).

- Conditions of the site
  - Apparently, survival rate increases when fragments are transplanted at shallower depths than the original (collection) depth (36, 42), but Piazzi *et al*. (20) do not demonstrate an effect according to the original depth of fragments. These results seem to be supported by the recovery of nutrient and carbohydrate content in the transplants (42).
  - The substrate that maximizes survival rate of transplants is dead matte, followed by sandy bottoms colonized by *Cymodocea nodosa* (Ucria) Ascherson and/or macroalgae, regardless of the type of fixings used for the transplants.
  - Transplants done on unvegetated sand had very low survival rate after a few months, regardless of the fixings employed. This type of substrate is advisable to be avoided (22, 37, 39).

- Conditions of the transplantation
  - We have to consider that the durability of a fixing system is that of the weakest part of the system, particularly when working with natural fibers (22,35).
  - Various fixing systems, such as different types of grids (plastic, coated wire, or natural fibers), fixed by a heavy frame, or unframed but secured by pickets or similar, have in general positive results (19, 20, 24, 26, 36, 38) with some proving optimal for long-term (21). The use of non-covered metallic grid is discouraged (39).
  - The use of individual fixing methods, including natural or metallic staples or pegs, gives good results (22, 23, 35, 37, 42), with some also proving optimal for the long-term (41).

  - The lodging of fragments among rocks or rubbles, without additional anchoring, has a high percentage of failure and loss of transplants (25, 40).
  - Transplantations carried out during the phase of high metabolic activity show a higher percentage of success compared to those done later (e.g. august; 43).
  - Regarding positioning, there is little evidence about this parameter, but cuttings transplanted up to 5 -10 cm apart exhibit better survival rates (37).

- Monitoring
  - Short-term results may not accurately reflect the long-term transplants performance (21, 22, 23, 41).
  - None of the receiving sites, reviewed by Pancini *et al.* (33), in which the previous presence of an anthropogenic stressor was mitigated, reported a failure of the transplantation operation.
  - The most commonly used response variable to monitor the transplantation success is the survival rate (i.e., the percentage of surviving individuals from the initial plantings; 33).

3.B. Seedlings (Appendix 9)

The following factors may be noted:

- Type and origin of material
  - Fruits collected at the beach are suitable for planting after germination in aquaria (30, 44).
  - Seedlings grown for two months in laboratory generally reach a sufficient size to be successfully planted (30, 44, 45, 46).

- Conditions of the site
  - The substratum constitutes the most important parameters regarding seedling anchorage (see details in 47). Substratum complexity and roughness favors seedling retention and anchorage (46, 48). Thus, vegetated stable substrata (i.e. rock and dead matte) maximize the survival and development of planted seedlings (30, 44, 45, 49, 50) and natural recruits (50, 51, 52) survival and development. Poor or null survival of planted seedlings or natural recruits are observed on sand (22, 53, 54) and gravel, recommending the avoidance of such substrate (30, 44, 50, 51). Using sand as the growing substratum for seedlings before planting promotes seedlings growth (four times more) than maintaining them with no substratum (aquarium glass), but does not affect the survival rate (55).
  - The presence of crustose (i.e. Peyssonellia sp. and Lithophyllum sp.) (50) and turf algae (Halopteris sp. and Dilophus sp) (52) on rocky or dead matte substrata seems to benefit natural recruitment. The presence of the invasive algae *Caulerpa cylindracea* (Sonder) on dead matte increases short-term survival of planted seedlings (46).
  - Seedling experiments show higher survival rates in moderate depths (10 m) than at shallower depth (2 m) on dead matte (51), and exhibit similar results between -12 m and -18 m within Posidonia meadows with sand gaps (54)
  - Seedlings show a better survival rate when they are less exposed to hydrodynamic forces (50) and low exposure areas are suitable for seedling culture (50, 54, 56).

  - High temperatures (>25 ◦C) and salinities (>39 PSU) reduce seedlings’ success rate (see details in 47)

- Conditions of the plantation
  - Seedlings do not benefit from artificial fixing (30). Association with macroalgae such as *Caulerpa cylindracea* or with the seagrass *Cymodocea nodosa* enhances seedling fastening by increasing the substratum roughness and complexity (50)
  - Seedlings benefit from grid/cage protection from predation and grazing especially for the first months after the transplantation. Herbivores may constitute a problem, but seedlings protection, with cages or nets, does not seem to influence survival rates and development (45).
  - Planting level (above or below ground) does not influence growth of seeds and leaves (30, 44, 55).

  - There is no evidence regarding the best positioning for seedling planting.

  - Thermal-printing (priming) proved that seedlings exposed to high temperatures in aquaria are more resistant to extreme temperatures in natural conditions and exhibit higher growth rates (57).

- Monitoring
  - A significant drop-off of survival or density rates occurs during the first year both for planted seedlings (30, 45, 46, 49) and for natural recruits (51, 52). The mortality or density reduction slow down during the second and third year (30, 45, 49, 50, 51). The maximum monitoring period published is 36 months.

4. GUIDELINES FOR DECISION-MAKERS

The main phases of the process of Posidonia restorations concern planning, implementation, monitoring and management of transplants and have been the subject of in-depth analysis (see more details in 32). The key-points according to the available scientific knowledge are summarized below.

There is consensus that although there are many factors on which the success of transplantation depends, the main one is the choice of transplantation areas, so a certain number of prerequisites must be verified before considering a restoration.

Elements to focus on when carrying out an opportunity study for a site of transplantation:

- Before transplantation, it is mandatory to ensure that Posidonia seagrass occurred at the site, in the past.
- It is useless to seek to restore a meadow if the cause of its disappearance is unknown.
- The supposed causes of the disappearance of the meadow ceased to operate or at least is under control to allow the maintenance of the Posidonia in good health.
- The site's suitability to Posidonia restoration is attested by evidence of recolonization (natural cuttings) or the presence of plagiotropic rhizomes in patches of nearest meadows.
- The restoration is not intended to compensate an avoidable impact on the site related to a project: Transplants must not become a way to justify an impact that leads to the destruction of a natural meadow
- An adequate and effective level of protection is required before the beginning of the restoration operation and must be maintained until the end of the monitoring period.
- Regulations and controls (against illegal activities, lack of respect of the existing regulations) must be reinforced before initiating restoration.
- Ensure that the regulations for the conservation of the meadows are applied and/or upgrade them to make them more effective (sanctions, fines) before restore.
- In order to facilitate the adaptation of the transplants the environmental and ecological conditions between the donor site and the receiver site must be similar.
- Donor and receiver sites must be connected by a minimum amount of gene flow, ensuring their belonging to the same genetic cluster.
- Consultation meetings with the decision makers, local authorities, funders, MPA managers are carried out for the design, execution, long term monitoring and communication of the project
- A large-scale operation on one site can only be realized after an experimental transplantation of several hundred cuttings with a scientific monitoring for at least three years, to confirm the suitability of the site for the transplantation (five years is the time required to assess the success).

Transplanting material:

- Cuttings issued from a donor-meadow give better results than fragments issued from drifting material, but the latter have no impact on the ecosystem.
- When available, seeds are suitable material to transplant.
- Evidence suggests that plagiotropic cuttings are more successfully transplanted than orthotropic ones.
- The most suitable cuttings should have at least three shoots (1 plagiotropic and at least 2 orthotropic) or a long rhizome section (>10 cm).

- Cuttings harvested at the same depth or slightly deeper than the receiving meadow are more effective.
- Most of the successful restorations have been carried out at depths between 10 m and 18 m depth, which is considered the optimal depth range where a meadow can thrive. For cuttings as for seedlings, the best option is a good compromise between enough light intensity and low hydrodynamics.
- The restoration success increases as the proximity to the donor site diminishes.
- The substratum complexity and roughness favors seedling retention and fixation of both cuttings and seedlings.
- Dead matte and sand colonized by *Cymodocea nodosa* appear to be the most suitable substrates for cuttings, while rock and dead matte maximize the natural recruitment, the growth and the survival of seedlings.
- In order to avoid damage to the donor meadow, and to ensure a large representation of genotypes, only two cuttings per square meter must be harvested.
- Spring appears to be the most favorable season for transplants.
- Most transplantation failures are attributable to the detachment of the transplantation modules and/or cuttings, so this point need a particular attention. In contrast, artificial anchoring is not as crucial for seedlings.
- Concerning the fixation, the use of biodegradable structures or structure that can be easily removed after the time required to allow anchoring (more or less 3 years) is recommended.
- A distance of 5 to 10 cm between the cuttings seems the most appropriate.
- Seedlings protection with cages or nets can be effective against herbivores.

Monitoring:

Transplantation operation can only be considered successful once cuttings or seedlings are stabilized, show persistent growth, and show an active recolonization process; thus, a monitoring period is required to assess this success.

- « Long term » monitoring must be planned at the beginning of the process.
- It is important to take into account a reference site to compare with the natural trend of colonization.
- According to the experiments, a monitoring period of at least three years for seeds and five years for cuttings is necessary, with at least an annual periodicity.
- Monitoring must be based on functional, easy to measure descriptors, such as: rate of the survival transplanted units or of seeds turning to seedlings, number of shoots per cuttings or of seedlings branching and turning into small clones, shoot density per square meter, maximum length of the foliar bundles, etc...
- Monitoring must be done on statistically significant samples of transplanted material, with standardized techniques and non-destructive methods must be used in preference.
- Underwater photogrammetric technologies may be implemented to acquire high-resolution information and to elaborate micro-cartographies that are useful to monitor both the progress of transplanting operations and Posidonia recolonization dynamics.

In case of transplantation failure during the monitoring phase, the structures installed on the seabed for this purpose must be removed and the site rehabilitated.

5. RESEARCH & DEVELOPMENT: WHAT MUST BE TESTED NOW?

Over the past decade, there has been an increase in experiments concerning Posidonia transplantation, and the following section cannot be considered as complete.

5.A. Availability and origin of material for planting

A main constraint of Posidonia restoration is the availability of material for planting. If this is not a big issue as concerns the "repair" of small sections of damaged meadows, it will certainly be a limitation for full-scale cases of meadow restoration, except if transplantation follows the removal of plants (e.g. submarine cables, pipelines).

- Donor meadow versus drifting-in-seafloor fragments

The latter transplantations have been done using fragments of Posidonia rhizomes either extracted from meadows or collected from the populations of fragments of unknown origin that are found drifting at the seafloor and usually accumulated at meadow edges or in meadow gaps. The advantage of using drifting fragments is that no damage to a donor meadow is incurred. However, as the origin and history of those fragments is not known, it is uncertain if the performance of those drifting fragments is similar to those extracted directly from the meadow. Hence **research is needed to assess i) the performance (survival rate, vegetative development) of drifting-in-seafloor versus extracted-from-meadow fragments, and ii) the effects of the extraction of fragments from donor meadows on the extant plants**. While ongoing experiments are in progress concerning the difference of performance, with preliminary results which exhibit a small decrease in the rate of survival of cuttings after one year and in the mean number of shoots by cutting (34), they must be confirmed. Regarding an allowable threshold for the extraction of plants from a donor meadow, current recommendations concerning extraction intensity (< 1 per m^2; 58) are based on some unpublished experiments (Molenaar H., personal communication). In the 1990s, in Galéria, a 20 m linear length of border of a Posidonia meadow at 12m depth was marked and around sixty plagiotropic rhizomes were taken along this border. After two years, all the rhizomes removed had grown back and there were around 80 new plagiotropic rhizomes which had branched from the orthotropic ones, located behind the initial sample. More recently, in Monaco, an estimate based on the collection of 1 450 cuttings from the living meadow was carried out. Considering the average density (300 shoots.m^{-2}) and the surface area occupied by the meadow (30 000 m^2) in the sampling area, the number of available shoots is 9 000 000. The collection of 1 450 cuttings, with an average of 3.8 foliar shoots each, represents less than 5 600 shoots, i.e. a removal of 0.06 % of the shoots, a quantity that can be easily regenerated in one year under conditions of a normal growth (Molenaar H., personal communication).

- Long-term maintenance of planting units

An important goal, when thinking of large-scale restoration, would be to not depend on Nature to obtain this material but to be able produce it in designated facilities, which is standard in land-plants used in reforestation or restoration projects. **A major line of research for the future would be to develop the plant biotechnological knowledge required to produce viable Posidonia units** that perform in Nature, similar to those used for the current planting operations and to scale up this procedure for mass-production. The first preliminary step could be to obtain viable material for further growth in controlled conditions - mesocosm (45, 59), but also to be able to keep these planting-units, in situ, in specific areas (with optimal conditions of conservation), in the case of management projects of established public interest (for example, installation of cables or pipelines), before their future transplantation. This calls for the **elucidation of the environmental needs for the long-term maintenance of planting units**, either seeds, seedlings or cuttings fragments, in order to produce a stock of planting material collected in Nature whenever is available and to safeguard in good condition for planting whenever required.

- Increase in genetic diversity and more efficient genotypes

The seeds or seedlings obtained from Posidonia fruits collected in beach-cast are a source of material for planting (30). Their use provides a priori a higher genetic diversity of the material compared to rhizomes although this is something that requires quantitative assessment. Marine plants can reproduce both vegetatively and sexually. Clonal propagation enables populations to extend themselves spatially, potentially forming monoclonal populations with low genetic diversity (60). In contrast, sexual reproduction allows an increase in genetic diversity (61) and higher genetic diversity of the transplanted material might favor better performance (62, 63) and allow the emergence of more resistant and resilient populations (57). In terms of restoration, a major challenge will be to choose between transplanting local individuals, traditionally thought to be the best adapted to current conditions, or transplanting climate-adjusted or admixture genotypes which might provide more sustainable options to secure the survival of restored meadows, but will have perhaps initially a lower capacity for adaptation to the local environmental conditions (64). Nevertheless, importing foreign genotypes in transplantation sites can foster genetic pollution, and assessment of population realized-connectivity and main patterns of gene flow, allowing the identification of genetic substructure, should be taken into consideration (64).

Moreover, the flowering of Posidonia meadows is irregular in space and time (65, 66) and essentially unpredictable. Understanding what determines Posidonia flowering is essential for predicting when fruits would be available for restoration **A relevant restoration research topic is to elucidate the flowering mechanism, the physiological status required for flowering to occur and the environmental signals that control it** (see 61). It seems that high seawater temperatures during summer and/or solar activity induce flowering (67, 68, 69), possibly a heat-stress response, which suggests that flowering might be more frequent in the next decades in the scenario of global warming we embarked on (70).

Another **research line relevant to restoration and currently under development is to identify Posidonia populations and genotypes that perform better under different environmental conditions**, for example, heatwaves, high salinity, lower light availability, etc. This would open up the possibility of choosing the genetic material /populations most appropriate for a restoration project considering the local conditions, present or predicted in the future (57). Steps already taken along these lines are the studies of Marín-Guirao *et al.* (71, 72), Bennet *et al.* (73) and Stipcich *et al.* (74) concerning the contrasting heat tolerances of different *P. oceanica* populations, and those of Dattolo *et al.* (75) and Pazzaglia *et al.* (57, 76) on the possibilities for acclimation to heat stress of this species.

The integration of the techniques of assisted evolution (e.g. priming, 57, 77, 78), and epigenetic knowledge in restoration practices of marine phanerogams could become, in the coming years, an important aspect to enhance the success rate and to strengthen the resilience capacities of transplants (79; Fig. 1).

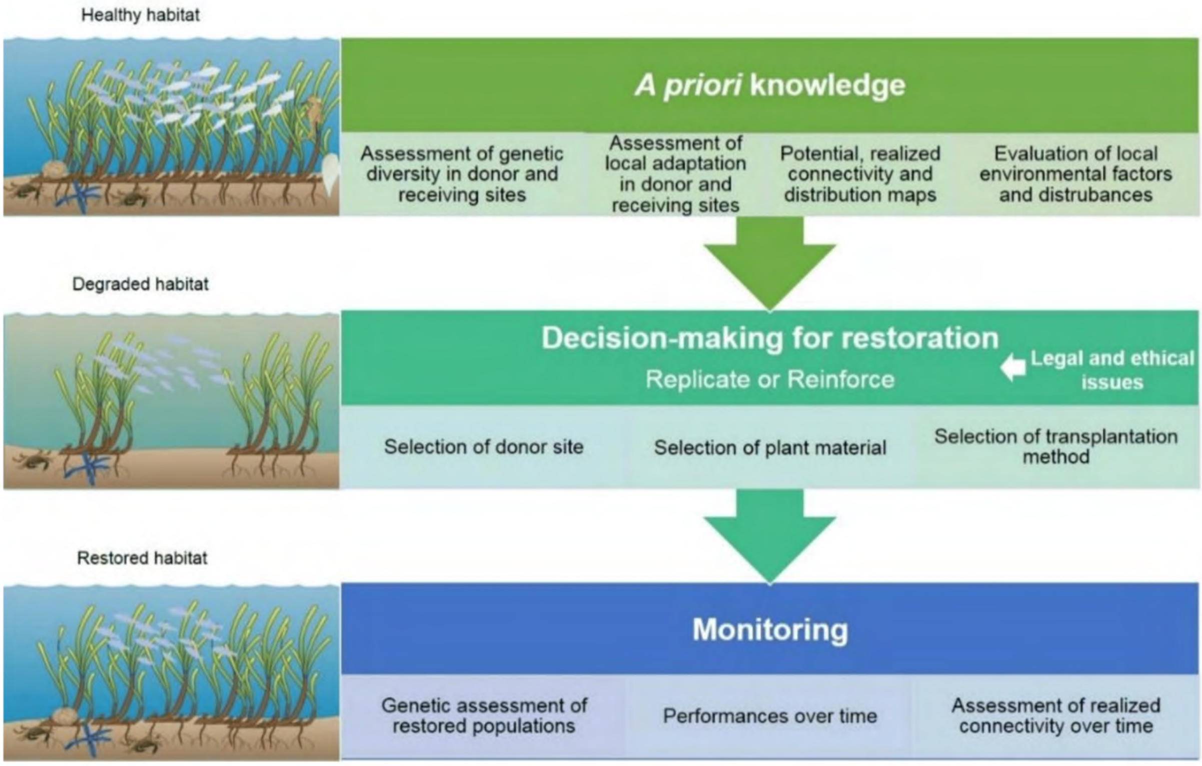

Fig 1.: Diagram showing different aspects of seagrass restoration, with focus on the integration of the analysis of genetic diversity in the different steps (from 64; https://www.mdpi.com/water/water-13-00829/article_deploy/html/images/water-13-00829-g001.png)

5.B. Optimization of transplantation conditions

- Plant-sediment relation

If dead matte is considered one of the optimal substrates for the Posidonia growth, several studies underline significant spatial variations during both cutting transplants and natural meadow recolonization (80, Molenaar H., personal communication). Thus, hydrogen sulfide concentrations persist for an extended period following meadow degradation (80) potentially limiting cutting and/or natural plant growth (81). The plant/sediment interaction deserves particular attention in order to optimize transplantation success; work is underway integrating this aspect but also the characterization of nitrogen-fixing bacterial communities which could be involved in Posidonia growth (82, Boulenger A., Personal communication). The study of seagrass association with bivalves carrying nitrogen-fixing bacterial communities is also showing the potential of improving transplant performance implementing beneficial associations (83).

- Enhancement of transplant fixation

As stated earlier, the fixation of the transplant on the seafloor is a key issue in the implementation of transplantations. Various methods have been tested (31) and are functional, but only some of them can be considered sustainable (58) and easy to remove when natural fixation systems have grown and became operational. In addition, several aspects still need to be tested or are in progress to improve these processes for both cuttings and seeds. The use of biodegradable fiber nets/mats to facilitate the anchoring of transplanting material is one of them (24; RenforC 2023 program – Pergent G., Personal communication). This technique is inspired by the evolutionary series of the meadow where pioneer species build a network of roots and runners to trap drifting cuttings and facilitate their attachment to the substrate (84). Comparison under the same experimental conditions (site, nature and origin of the transplants) is ongoing to assess the interest of this approach (e.g. RenforC 2023 program – Pergent G., Personal communication).

In the same way, other experiments are in progress to identify the best fibers/structure for nets/mats or to test the use of biodegradable establishment structures (e.g. 85, REPOSEED program – 82, REPAIR Project – Boulenger A., Personal communication).

Posidonia seedlings are able to establish in rocky substratum (50, 52, 86) and shallow (depth< 10 m) meadows of Posidonia are common on rocky coasts. The studies on root hairs morphology and the force of seedling attachment to the substratum (48, 87, 88) are preliminary steps to devise a methodology for transplanting in rocky substrates. Balestri *et al* (86) and Guerrero-Meseguer *et al.*, (55) have shown that seedling roots show morphological plasticity, depending on the substratum on which they grow, that could be exploited to this end. Experiments on rocky bottoms are ongoing both for seeds (86) and for cuttings (see also complements in 31, 40, 91). Nevertheless, an improvement in these techniques is essential to carry out transplants on rocky substrates, both concerning the anchoring element, made with a reinforced concrete radial structure with five arms (Calvo *et al.* in 31), and with the wire mesh gabions, filled with suitably sized crushed stone (40).

- Transplantation design

Transitioning from research aimed at producing knowledge necessary to address the different limitations associated to planting a main area, future research should focus i) on the assessment of the recovery of ecological function in the planted areas and ii) the time required for that, that is the actual success of the restoration. More research needs to be done to quantify the mid- and long-term development of the planted units and how planting design may improve this long-term development. The geometric arrangement of the transplants during the positioning phase can be of crucial importance. This geometry can be specific for each technique, but must generally allow the creation of recolonization nuclei that are able to join together and form a continuous meadow over time. Concerning this aspect, seminal studies are those of Molenaar and Meinesz (37) on the spatial arrangement and distance between planted fragments. Doing this requires long-term experiments and hence adds a component of long-term monitoring to the plantings done. Still to be developed is the assessment of the recovery of ecological functioning, the time and the meadow internal structure (shoot density and size) and meadow extent required for it, that will vary depending on the function studied (i.e. comparison of the recovery of habitat for epifauna with the attenuation of waves or the long-term storage of carbon in the sediment). Research that includes space-by-time substitutions are needed for this goal considering the slow growth rate of Posidonia. A recent experiment (e.g. RenforC 2023 program – Pergent G., Personal communication) has been carried out in 2023 to compare several planting designs to optimize this aspect (density, distance or spacing between cuttings)

- Protection against herbivores

Protecting transplants from herbivore pressure also constitutes a particularly interesting axe of research. Indeed, herbivore impact can lead to overgrazing of cuttings and especially seedlings potentially resulting in experiment failure (86). The establishment of specific structures must ensure effective protection of transplants from herbivores but also allow good water renewal and more generally ensure maintenance of environmental conditions (light, nutrient content, sedimentation, etc.). Seedling protection trials have been initiated, following the massive flowering of 2022, and the preliminary results are encouraging (REPOSEED and RenforC programs - 86).

5.C. Monitoring tools

Today, monitoring of transplantation operations require new tools able to irrefutably certify the extent of the interventions and their position in space, so that anyone (scientists, technicians, etc.) can verify the effectivity and the efficacy of these operations at any time. Underwater photogrammetric technologies offer tools to acquire and return information in an extremely accurate manner to monitor the dynamics of Posidonia restoration, contextualizing it in a cartographic representation. Ultra–very high-resolution and accurate mapping technologies are required (92). The integration of high spatial resolution underwater imagery with object-based image classification (OBIA) technique provide the opportunity to count transplanted Posidonia fragments and estimate the bottom coverage expressed as a percentage of seabed covered by such fragments (92, 93). However, the total time required for data processing, which depends on the resolution, quality, and number of images acquired, stay very high, and for large areas photogrammetry requires a significant amount of computing power to generate high-resolution products (92). These existing limitations will have to be solved in order to propose efficient standardized monitoring protocols for future seagrass restoration actions.

6. GENERAL RECOMMENDATIONS & PERSPECTIVES

In view of the experience acquired (parts 2 & 3), some guidelines have been formulated (part 4) and several R&D projects suggested (part 5). It is nevertheless worth recalling a certain number of general recommendations which should be systematically taken into consideration, before considering or carrying out Posidonia transplants (Fig. 2):

- Priority should always be given to the conservation and protection of existing Posidonia meadows, as these actions are often more cost-effective and efficient than restoration.
- Restoration cannot replace conservation, and must be considered as a complementary action and must be envisaged in synergy with it.
- Restoration efforts should not only involve active planting, but also address in priority the removal or reduction of pressures and stressors placed on seagrass beds. This approach, also called passive restoration or assisted restoration, is the first step of a restoration project and an integral part of a successful recovery.
- Operational technical solutions are available to achieve "successful" transplants (in terms of survival rate of cuttings after 5 years) in a wide range of situations. The biggest limit to consider if a transplantation is a success is the lack of information related to the natural trend. *In France, as an example, experts consider than a survival rate of cuttings, issued from a donor meadow, of 75 % after three years, and a number of shoots after five years higher than the number of shoots after three years can be considered as a transplant success.*
- However, restoration success (which would imply the recovery of habitat structure, species composition, ecological functioning or ecosystem services) has never been assessed, therefore cannot be demonstrated so far and has to be seen as a R&D project.
- A large-scale operation can be envisaged only after an experimental transplantation of several hundred cuttings on the receiving site with a scientific monitoring for at least three years demonstrating the suitability of the site for transplant.
- A successful active restoration must necessarily integrate during the planning phase the implementation of continuous protection measures and associated control. This control / surveillance must be done, not only during the implementation phase but also afterwards, so that these restored areas are not exposed to new threats or degradations.
- Assessing the cost of active restoration is complex, due to the lack of standard: the density of shoots per m^2 is not always the same, the depth is different, etc. The cost needs to encompass the cost of passive restoration and associated surveillance / monitoring.
- Posidonia active restoration should not be viewed as a compensatory measure for projects that have a negative impact on seagrass ecosystems. Compensation assumes, at least, being able to balance the losses due to the degradation or destruction of a meadow, in terms of biological diversity, functionality or ecosystem services, by the gains achieved by the transplanted new one. This assumes a true "restoration success" with a complete short-term success of the transplants, followed by the recovery of all ecological functions and services, which, so far, has never been observed, even considering the longest experiments. This being so, at present, it is therefore essential to avoid as far as possible this kind of projects and to prevent their negative impacts through responsible planning and the implementation of mitigation actions.

- Restoration projects owners should consult the responsible environmental authorities before starting projects to know the potential authorizations required in the country's site.
- The involvement of all stakeholders (decision makers, users, funds) is essential, not only for the design and execution of the project but also for long-term monitoring and the eventual success of it.
- In the context of public utility development projects, restorations can be considered, on a case-by-case basis, as an additional measure following an "Avoid, Reduce, Compensate" procedure and accompanied by a mandatory follow-up of at least 10 years or/and as long as last the impacts of the project on Posidonia.
- In countries where the destruction of Posidonia seagrass is prohibited by law or other regulations, based on the model of "the polluter pays" principle, restorations should be considered by the court as a reparation measure ("puller-planter") with a priority given to passive restoration and the associated surveillance of the site (as the recovery of all ecological functions and services by active restoration is still seen as R&D).
- It is important to encourage consideration of habitat connectivity in restoration planning. Healthy, well-connected Posidonia meadows can improve biodiversity and enhance ecosystem resilience.
- Likewise, when planning restoration and conservation strategies, a holistic approach to seagrass management, which integrates water quality, sedimentation and adjacent habitats, must be favored.
- Ongoing research and monitoring efforts are necessary to improve our understanding of Posidonia ecosystems, including their dynamics, weaknesses, and recovery processes. This knowledge can inform better decision-making.
- Considering the fact that Posidonia is a long lifespan species, the restoration of the ecosystem can only be considered through a long-term commitment. Restoration projects can take years to produce significant results, and ongoing maintenance is often required.
- Appropriate financial means and sufficient resources to carry out conservation and restoration actions must be allocated with regard to the ecosystem services provided.

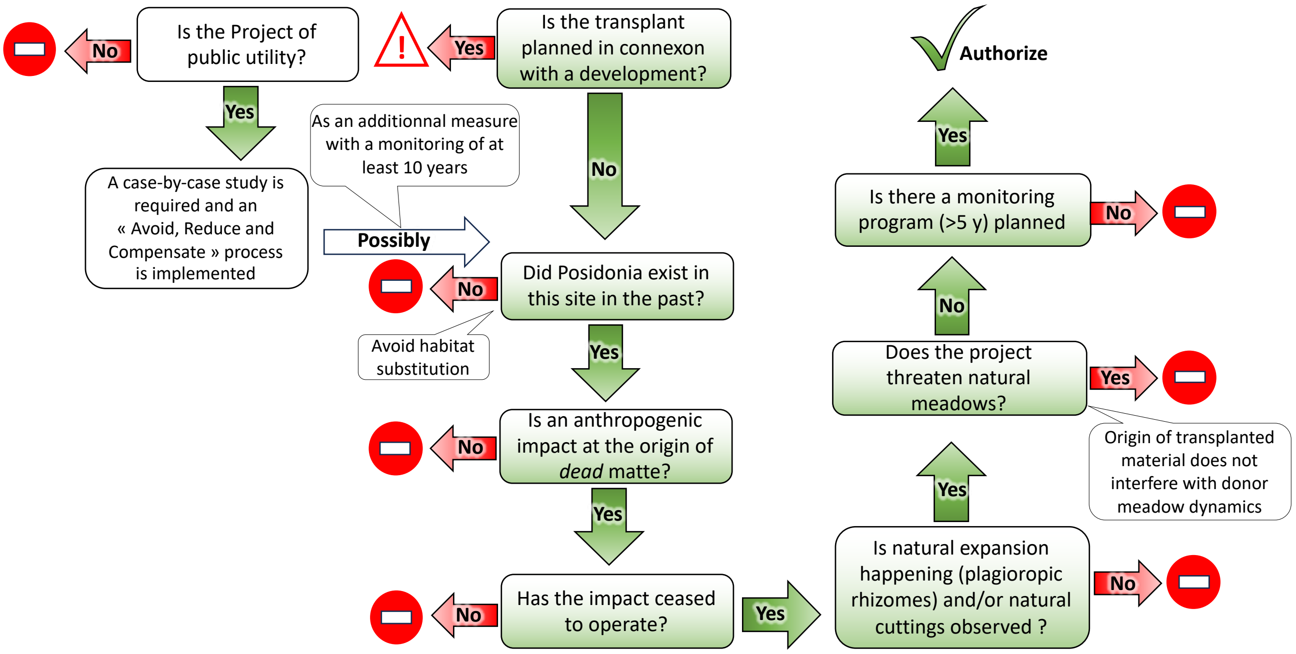

Fig 2.: Diagram showing the main considerations that must be fulfilled to authorize a transplantation of Posidonia.

It is therefore important to keep in mind that restoration of Posidonia meadows is necessarily a long-term activity, which can be costly and must be included within an overall management approach. In addition, several elements must be investigated in future R&D activities to enhance: site selection, performance of the planting material, availability in the framework of a large-scale project, efficiency of the techniques of transplants, monitoring tools, etc., or to determine the prerequisites necessary to achieve a true restoration of the ecosystem (period of time, evidence, criteria to monitor). The main challenge will be to identify, then to solve all these issues in order to make operational the restoration of the Posidonia ecosystem, in a context of climate change.

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APPENDIX 1

Summary of *Posidonia oceanica* cuttings transplanted 28 to 35 years ago in the Posidonium of the Port-Cros National Park

Heike Molenaar

Context

From 1988 to 1995, cuttings and plantlets (germinated seeds) of *Posidonia oceanica* (L.) Delile, taken from various sites in the Mediterranean basin, were transplanted within the Port-Cros National Park. This collection of strains was named "Posidonium" by Professor Alexandre Meinesz in 1993.

The successive contributions of various strains of Posidonia transplanted in a single location protected from any anthropogenic disturbance today constitute a unique biological capital through the diversity of plant origins. Indeed, throughout the Mediterranean, Posidonia meadows are made up of large monoclonal tufts (vegetative multiplication of the rhizomes in place), flowering is rare and therefore genetic enrichment within a meadow is very rare.

Transplanting operations and applied techniques:

The Posidonium cuttings and seedlings were planted on dead mat between 13 and 15m deep near 5 natural tufts of expanding Posidonia, the whole forming an area of 625 m². The cuttings come from 13 different origins around the Mediterranean and the plantlets from germinated seeds come from 6 different sites in France.

Some cuttings have been thriving there for 35 years, others for 34 years, 33 years, 32 years, 31 years and 28 years. The seedlings, depending on the batch, have been developing there for 35 years, 33 years, 31 years and 28 years. The Port-Cros Posidonium is therefore a unique experience due to the very long-term monitoring of cuttings and seedlings. This report only concerns transplants of cuttings.

The year of transplantation, the number of cuttings and the provenances are distributed as follows: - In 1988, transplantation of 122 cuttings from Athens (Greece), Golfe Juan (Alpes Maritimes, France), Izmir (Turkey), San Bainsu (South Corsica, France) and Port-Cros (Var, France), control batch. - In 1989, transplantation of 105 cuttings from Algiers (Algeria), Banyuls-sur-Mer (Pyrénées-Orientales, France) and Piantarella (South Corsica, France). - In 1990, 16 cuttings from Taranto (Southern Italy). - In 1991, 3 cuttings from Majorca (Balearic Islands, Spain). - In 1992, 51 cuttings from two sites on the island of Ischia in Italy (Castello and Lacco Ameno). - In 1995, 4 cuttings from Malta.

Table 1 indicates for each origin: - the year of transplantation in Posidonium, - the number of cuttings transplanted, - the total number of leaf bundles at the time of transplantation, - the morphology of the cuttings at the time of transplantation, - the number of leaf bundles carried by each cutting at the time of transplantation, - the position in which the cuttings were transplanted (horizontally with the main rhizome lying on the substrate or vertically with the rhizome planted in the substrate), - the fixing method (in batches on mesh, individually on a plastic-coated vertical stake or individually on a galvanized steel stake, curved at one end to insert the rhizome in a horizontal position with the stem of the stake pressed into the substrate).

Thus, 301 cuttings were transplanted into Posidonium between 1988 and 1995, which represents at time T0 for each provenance, very variable batches both in number of cuttings and in their morphology (from 2 to 56 cuttings per batch, some orthotropic with 1 to 3 leaf bundles, other plagiotropic with 2 to 6 leaf bundles).

It was only after 1990 that we gathered the first results which allowed us to define which morphological type of cuttings was most appropriate to obtain the best survival results (plagiotropic with 3 to 6 leaf bundles).

Other experiments carried out between 1989 and 1992 in Cannes (Alpes Maritimes, France), in Nice (Alpes Maritimes, France) and in Corsica (in Galéria, Haute Corse and in Lavezzi, South Corsica, France), on nearly 4 000 experimental cuttings made it possible to define, in addition to morphology, a certain number of criteria making it possible to obtain cutting survival rates greater than 80 %. These criteria are in particular: the harvest depth depending on the depth at which we want to carry out transplants, the transplanting season, the spacing between the cuttings, the arrangement of the cuttings, the substrate, the method of fixing the cuttings to the bottom.

Table 1: Origin and status of the different batches of *Posidonia oceanica* cuttings at time T0 of their planting in the Posidonium of the Port-Cros National Park. Nb: number; Plagio: plagiotropic rhizome; Ortho: orthotropic rhizome; GSS: Galvanized steel stake.

Origin of batches	T0: year of transpl.	Nb of batches	Nb of bundles	Type of batches	Nb of bundles /batch	Position	Fixing technique
Athens	1988	2	6	Plagio.	3	Horizontal	GSS
Golfe Juan	1988	56	56	Ortho.	1	Horizontal & vertical	Mesh plastic stake
Izmir	1988	5	7	Ortho.	1 - 2	Horizontal	GSS
San Baïnzu	1988	7	28	Plagio.	3 - 5	Horizontal	GSS
Port-Cros	1988	52	52	Ortho.	1	Horizontal & vertical	Mesh & plastic stake
Alger	1989	8	12	Ortho.	1 - 3	Horizontal	GSS
Banyuls	1989	60	65	Ortho.	1 - 2	Horizontal	GSS
Piantarella	1989	37	37	Ortho.	1	Horizontal	GSS
Tarento	1990	16	80	Plagio.	3 - 6	Horizontal	GSS
Majorca	1991	3	7	Plagio.	2 - 3	Horizontal	GSS
Ischia Castello	1992	11	46	Plagio.	3 - 5	Horizontal	Mesh
Ischia Lacco Ameno	1992	40	205	Plagio.	3 - 6	Horizontal	Mesh
Malta	1995	4	12	Plagio.	3	Horizontal	Mesh

Monitoring activities:

Each year, from 1989 to 1995, at the time of new transplants, monitoring of the cuttings transplanted in previous years was carried out to evaluate either the total number of leaf bundles in the different batches, or the areas occupied by the different batches. Four follow-ups were carried out over the following 28 years:

In 1997, the number of leaf bundles was counted in each batch of the 13 provenances.

In 2006, the number of leaf bundles and the surface area of the tufts formed by the different batches were measured.

In 2012, the surface area occupied by each batch from each provenance was measured.

In 2023, each batch had its surface area measured, its density measured (in number of leaf bundles per square meter), which made it possible to estimate the total number of leaf bundles present in each lot of provenance difference.

Results:

The table 2 shows part of the results collected by the measurements carried out in February 2023.

Table 2: Evolution of the total number of leaf bundles of the batches, of the surface occupied by the batches at time T0 of their planting and in February 2023, i.e. 28 to 35 years later.

	T0: Planting from 1988 to 1995	T+28 to +35 February 2023	Multiplication factor
Total number of foliar shoots transplanted	613	53 400	X 87
Total area transplanted	3 m^2	105 m^2	X 35
Variation of transplanted surfaces	0.07 m^2 to 0.38 m^2	2.9 m^2 to 15.5 m^2	X 10 to X 116

The number of leaf bundles carried by the 301 cuttings transplanted between 1988 and 1995 was 613 at time T0, or on average two leaf bundles per cutting. This number increased to 53,400 leaf bundles in 2023, approximately 87 times more, which represents on average 177 leaf bundles per cutting.

Of course, each cutting has not evolved exactly like its neighbor. Some cuttings formed more new bundles than others depending on their origin, their initial morphology, the space they had available and their proximity to other clumps in the neighboring herbarium. Overall, they formed 2 to 3 new leaf bundles per year of transplantation, which corresponds to normal development of plagiotropic rhizomes.

The areas occupied by the different batches at time T0 varied greatly and depended on the number of cuttings transplanted (number varying from 2 to 56 cuttings depending on the origins). The initial clumps were all less than 0.5 m^2 and some are now up to 15.5 m^2.

The total surface area occupied by cuttings from the 13 provenances added together was 3 m^2 at time T0 and this surface area increased to 105 m^2, an area 35 times larger after 35 years of growth.

Concerning the number of leaf bundles, 3 large groups have been distinguished:

- Cuttings whose multiplication factor of leaf bundles in 35 years is low, between X 27 and X 69 with the lowest factor for those from Taranto, then Ischia Lacco Ameno, then Golfe Juan and finally those from Banyuls.
- Cuttings whose leaf bundle multiplication factor varies from X 91 to X 155 with in ascending order those of Piantarella, San Bainsu, Malta, Port-Cros then Ischia Castello.
- Cuttings whose leaf bundle multiplication factor is very high, between X 301 and X 524 with in ascending order those from Algiers, then Izmir and those from Majorca which present the highest factor of all the origins.

APPENDIX 2

Effectiveness of a *Posidonia oceanica* transplantation in the gulf of Rapallo 23 years later

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Context

The site selected for transplanting *Posidonia oceanica* is located within the marina of Rapallo (Genoa), in the Tigullio Gulf, North-Western Ligurian Sea (Fig. 1). Regression of *P. oceanica* meadows in Liguria has become particularly acute since the 1970s, because of the huge coastal development that characterized this region. During this period, the *P. oceanica* meadow developing within the marina of Rapallo has been impacted by the extension of the dam of the touristic harbour, which caused the disappearance of large areas of the meadow within the marina. In addition, two significant events took place in the same period: i) the construction of the Carlo Riva Port; and ii) the huge urbanization along the coastline near the Rio Tuja. Both events caused, for several years, significant water turbidity in the whole Tigullio Gulf. The narrow entrance of the Rapallo harbour and the consequent diminished water exchange with the open sea enhanced the turbidity of the waters within the marina, further damaging the meadow because of the continuous fine sediment inputs. A healthy meadow remained only in the eastern side of the Tigullio Gulf.

Figure 1: Selected area (yellow star) for transplanting *Posidonia oceanica* within the marina of Rapallo (Genoa).

Transplanting operations and applied techniques:

About two decades later, a pioneering intervention of meadow restoration was planned to reconstruct a small portion of the meadow within the marina of Rapallo (Bavestrello & Cattaneo-Vietti, 1997). A first transplanting was carried out in November 1996 in the shallow waters in front of the Avenaggi Street. A total of 200 cuttings were collected from the nearby San Michele di Pagana meadow at a depth of 12 m and transported within tanks to the Rapallo harbour. Five metallic grids, each covering a surface of 2 m², were fixed at the bottom characterized by dead matte, at a depth of 5 m. Cuttings were fixed at the grids by means of plastic bands.

A second transplanting was conducted, in the same area, in March 1997. A total of 300 cuttings were collected in the nearby Prelo cove, at a depth of 5 m. Each cutting was then secured to a metallic stake and the stakes were planted at the bottom along six parallel transects, each 10 m long, and at 20 cm intervals. After transplanting, the ropes used to visualize the transects on the bottom were removed. At the end of the intervention, 500 cuttings were transplanted over a surface of about 20 m², resulting in a density of 25 cuttings per m².

Results

After one year from the transplantation, both techniques showed positive results in terms of shoot survival and rhizome length. Cuttings over the grids recorded a loss of about 15%, while those fixed by stakes had a loss of about 50% (Bavestrello & Cattaneo-Vietti, 1997). Three major storms occurred during the winter period; cuttings on the grids did not appear to have suffered any damage but the cuttings fixed by stakes were largely damaged, despite they experienced only one storm. This notwithstanding, the cuttings transplanted with stakes showed, after only two months, a better stability of the rhizomes in the substrate than those attached to the grids, as the metallic grid is likely to slow down the rooting process. Following the first year after the intervention, no other monitoring activities have been conducted on this transplanted meadow of *P. oceanica*.

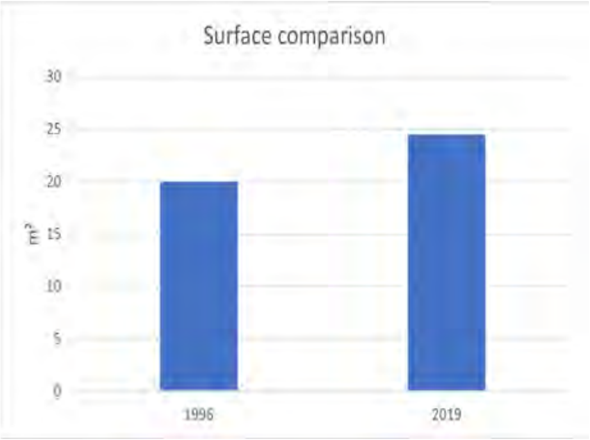
In 2019, a new monitoring was conducted on this site to verify the existence, after 23 years, of the transplanted patch of *P. oceanica* in the marina of Rapallo. The transplanted meadow was still there, and its surface appeared increased. The metallic grids used in the first transplanting were still visible on the bottom (Fig. 2), whilst the stakes used in the second transplanting were not found.

Figure 2: Grids used during the first transplanting intervention are still visible on the bottom 23 years later.

Using the closed polygon technique, the transplanted meadow was mapped with detail (Fig. 3), and the total area covered was measured on a GIS platform. The shoot density was also measured with a 20 cm x 20 cm square.

Figure 3: Surface covered by the transplanted meadow in the marina of Rapallo in 2019.

The total area covered by the meadow slightly increased in the last 23 years, from 20 m² to 24 m² (Fig. 4). The most significant result was found in the shoot density: the estimated number of shoots on the total area covered by the meadow in 2019 is about 4767, with an average value of 195 ± 8 shoots per m², compared to the 25 shoots per m² in 1996 (Fig. 5). Although the meadow area increased by "only" 17 %, the success of transplanting is most evident looking at the shoot density, which increased approximately eight times.



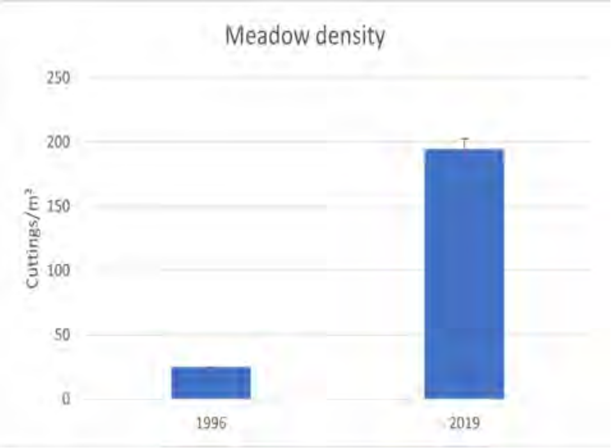


Figure 4: Total area (in m^2) covered by the transplanted meadow in 1996 and in 2019.

Figure 5: Meadow shoot density (cuttings m^{-2}) of the transplanted meadow in 1996 and in 2019.

The location of the transplanted meadow within a touristic marina must be taken into account when discussing the success of this pioneering intervention. Since we are in a heavily anthropized area that is often exposed to high water turbidity and intense hydrodynamics, the success of this transplanting is even more remarkable.

However, the location of the site in the eastern side of the Rapallo gulf shelters the transplanted area from the most severe storm (Oprandi et al., 2020), thus ensuring the survival of the shoot for the last 23 years. This restoration intervention represents a unique case, since there are no other documented examples in the literature of successful transplantations over such a long-time scale.

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APPENDIX 3

A multi-criteria chronological framework for the restoration of degraded *Posidonia oceanica* meadows in the Gulf of Palermo (Italy): a 15-year history, from siting to upscaling.

Sebastiano Calvo, Geraldina Signa, Salvatrice Vizzini, Antonio Mazzola, Agostino Tomasello.

Context:

Following the recovery of environmental conditions through the reduction of human pressures responsible for environmental degradation, reforestation is currently considered a suitable option to accelerate the restoration of *Posidonia oceanica* meadows. The Gulf of Palermo, along the north-western coast of Sicily (Fig. 1), has been exposed to multiple pollution sources for several decades due to chaotic urban expansion, improper waste disposal and untreated wastewater. An increase in trophic status due to high nutrient and chlorophyll-a concentrations in the water column has been recorded in the southern part of the gulf (Calvo *et al*., 1994), as well as changes in the geochemical characteristics of the sediment surface layers, reflecting increased runoff and terrigenous loading during the second half of the last century (Di Leonardo *et al.*, 2012). As a consequence, the *P. oceanica* meadows growing in this area regressed with only remnant patches remaining in the 11 – 21 m depth range (Tomasello *et al*., 2007). In recent decades, there has been a marked improvement in water quality, with TRophic IndeX (TRIX) ranging from good to high (Pirrotta *et al*., 2015).

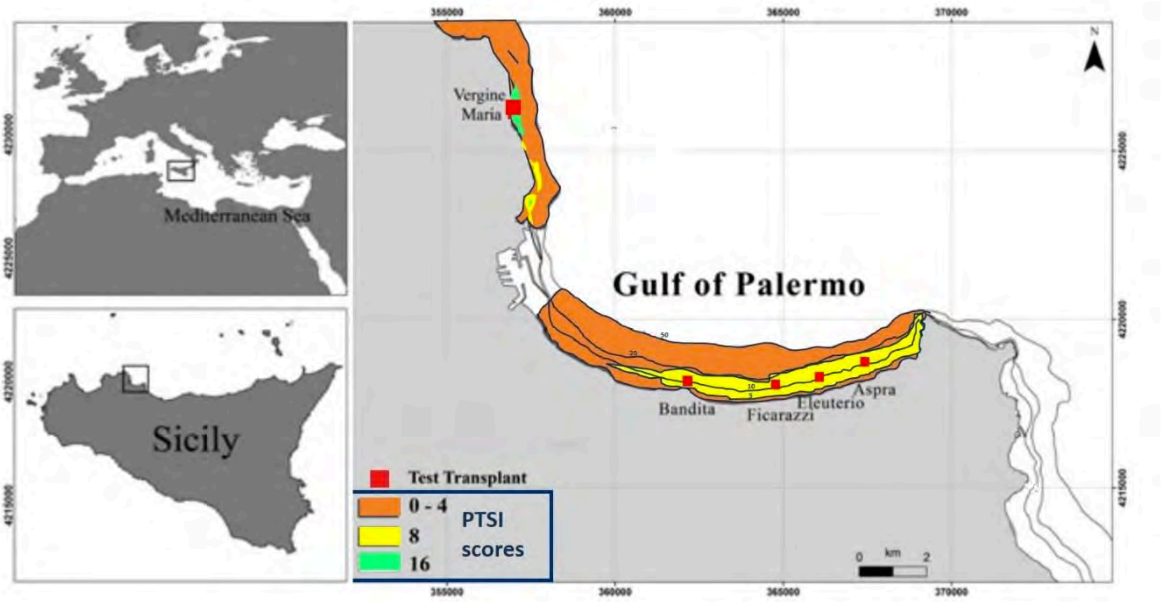

Figure 1**:** Study area (PTSI scores map) and locations of pilot transplantation are shown. From Pirrotta *et al*. (2015).

Transplanting operations and applied techniques:

In this context, a multistep approach for the restoration of *P. oceanica* has been developed, including the following phases: 1) implementation and application of a multi-criteria site selection model, including historical and literature-based information, reference data and pilot test field measurements; 2) small-scale transplant intervention and decadal monitoring programme to test restoration success; 3) transplant up-scaling to implement a large-scale restoration project by using an innovative bio-inspired approach.

1) The site selection model allowed the identification of suitable areas to be prioritized for *P. oceanica* restoration. The model included the integration of the Preliminary Transplant Suitability Index (PTSI), the Transplant Suitability Index (TSI) and multiple transplant pilots at approximately 13 m depth (total

width of 15 m^2) (Pirrotta *et al*., 2015) (Fig. 1). Both indices are based on the calculation of multiple parameters and relative assessment in a GIS environment. Recently, the PTSI has been further implemented with the introduction of parameters obtained from satellite data and the development of a freely downloadable tool (for details see Calvo *et al*., 2021a; 2022a).

2) A monitoring programme carried out at different hierarchical levels twelve years after transplantation, allowed to assess the performance of a 20 m^2 of *P. oceanica* transplanted at the Bandita coastal area (Fig. 1), with a mean shoot density of 66 shoots m^{-2} fixed on dead *matte* by metal grids (Calvo *et al.*, 2021b). Photomosaics revealed 23 transplanted patches of both regular and irregular shape, ranging from 0.1 to 2.7 m^2 with a total are of almost 19 m^2. The density of the meadow was 331.6 ± 17.7 shoots m^{-2} (five times higher than the initial value), which is about the same as the nearest natural meadow (331.2 ± 14.9 shoot m^{-2}). Total primary production, estimated by lepidochronology combined with meadow density and phenological variables, varied between 333.0 and 332.7 g dw m^2/year in the transplanted and natural stands, respectively, in agreement with the estimates reported for several Mediterranean meadows (Fig. 2), demonstrating that some structural and functional traits of the *P. oceanica* meadow have been successfully restored in only 12 years after transplantation.

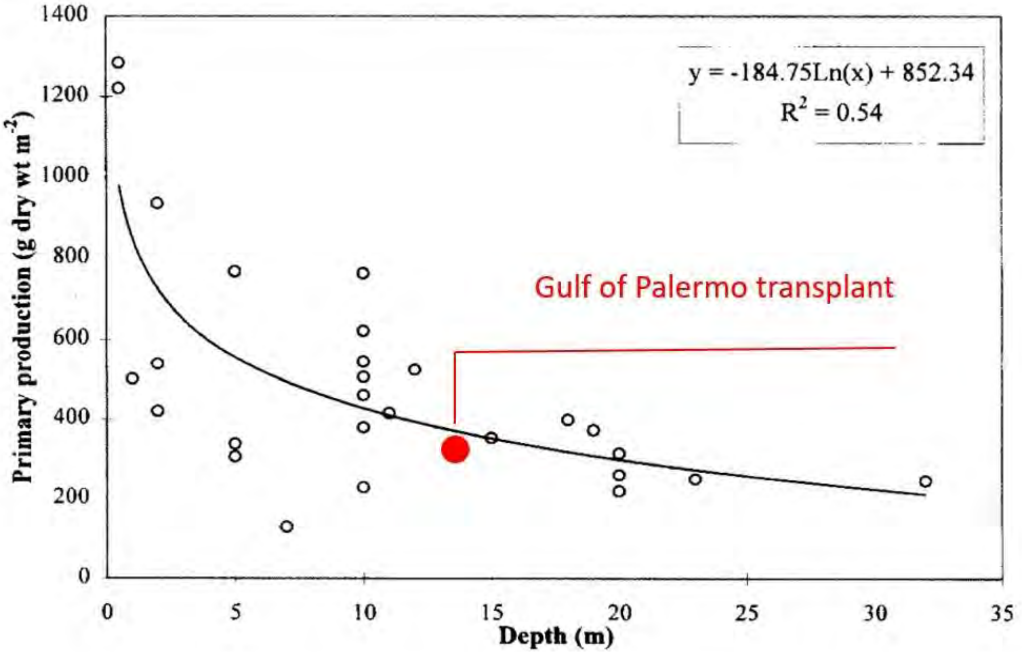

Figure 2: Relationship between primary production and depth estimated in several sites in the Mediterranean Sea (from Pergent *et al*., 1997). The value recorded in the transplanted meadow at the Bandita coastal area is indicated.

3) More than 22 000 *P. oceanica* shoots from a donor meadow were transplanted in winter 2021 on a dead *matte* substrate in the Bandita coastal area, using the biodegradable anchoring modular system (Fig. 3) described by Calvo *et al*., (2022b), covering a total area of 1 200 m^2.

Figure 3: Modular biodegradable (Mater-Bi) anchoring system of cuttings (European Design No.: 003000686-0001/2016 and Italian Patent No. 10201500008182/2018) (Calvo *et al.*, 2022b).

Results

During the first year after transplantation, the plant performance, in terms of cuttings detachment and survival, was better than the previous intervention carried out in the same area with traditional anchoring supports (metal grids), suggesting an improvement due to the new technology employed.

Moreover, underwater visual censuses showed that a few months after transplantation, the fish assemblage was more similar between the transplanted and donor meadows than between them and the nearby dead matte area (Bruno *et al.*, 2023). In addition, the increase in similarity between the two vegetated sites over time was mainly due to the increase in abundance of seagrass-associated fish (*i.e.*, labrids and sparids) in the transplanted meadow. Although long-term monitoring is considered to be of paramount importance for assessing the effects of seagrass transplantation interventions, these new results have shown that the first signs of recovery of transplanted meadows are already evident in the first year after transplantation, demonstrating that some recovery dynamics occur even in the short term.

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Project PON Marine Hazard Cod. PON03PE_00203_1 per il Piano Stralcio «Ricerca e innovazione 2015-2017» PON «Ricerca e Competitività. Project PON01_03112 dal titolo: "Tecnologie avanzate ecoˇsostenibili finalizzate alla bonifica ed al ripristino di aree marine costiere degradate”.

APPENDIX 4

Posidonia oceanica restoration in the Marine Protected Area Capo Carbonara by applying naturalistic engineering techniques

Francesca Frau, Maria-Francesca Cinti, Stefano Acunto, Fabrizio Atzori, Nicoletta Cadoni, Maria Leonor Garcia Gutierrez, Luigi Piazzi

Context

Implemented within the LIFE+ Programme (2007-2013), RES MARIS project (LIFE13 NAT/IT/000433) "**R**ecovering **E**ndangered habitat**S** in the Capo Carbonara **MARI**ne area, **S**ardinia" aimed at the restoration and conservation of marine and terrestrial ecosystems of the emerged and submerged beach system and, among them, of the priority habitat 1120* according to the EU Habitats Directive: Posidonia beds (*Posidonion oceanicae*). The main actions addressed for this habitat were the restoration of damaged areas and the implementation of mooring fields to prevent free anchoring on Posidonia beds.

The restoration action took place in the Capo Carbonara Marine Protected Area, Municipality of Villasimius, Sardinia (Italy), characterized by extensive and well-structured *Posidonia oceanica* meadows where several patches of dead matte have been detected. These patches showed long-standing signs ranging from 15 to 20 meters depth probably due to the mechanical impact of fishing gears or free anchoring caused before the establishment of the MPA (Acunto *et al.,* 2017). A surface of about 1000 m^2 of dead matte of *Posidonia oceanica* has been restored.

Transplanting operations and applied techniques

The applied procedure draws on the naturalistic engineering techniques usually employed in terrestrial systems. Reinforced geomats (Macmat® R) obtained by a three-dimensional polymer matrix extruded onto a double twisted steel woven mesh were employed. The decision to use this specific product was guided by the good grip these mats show on dead matte (Cinelli *et al.,* 2007, 2014). Forty geomats of different sizes were used to cover the entire surface (1000 m^2) and anchored to the substrate using iron stakes about 120 cm long.

For the first time among the *Posidonia oceanica* transplantation experiments to date, fragments of the plant uprooted naturally by storm surges and/or matte landslides collected on the surrounding bottoms were used (Frau *et al.,* 2023). Once collected and appropriately placed in holding tanks, they were processed to make cuttings, i.e. to obtain parts of the plant that could be used as transplanting material in the geomats previously laid on the seabed (Fig. 1).

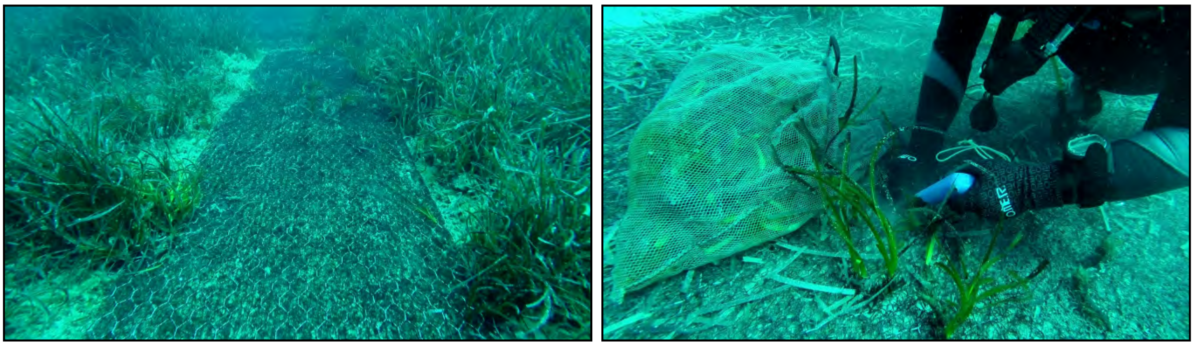

Figure 1: Phases of transplantation.

Each cutting consisted of an approximately 20 cm rhizome fragment with 1 to 3 shoots. About 500 plots formed by 30 cuttings each were transplanted. These operations will be performed by qualified personnel (certified scientific divers).

Monitoring activities

The restoration set up was completed in Spring 2017 (Fig. 2) and monitored every year for six years. During each survey the number of transplanted plots still *in situ* on the whole area was counted, moreover, the mean number of cuttings per plot were estimated on a statistically significant number of plots (at least 3 for each geomats). The number of shoots per plot was also evaluated starting from 2019 (Fig. 2).

Figure 2: Images of the transplants after the set up (left) and monitoring activities (right).

Results

Six years after the set-up, 93% of the transplanted plots were still *in situ*. Between 2018 and 2019, some geomats were lost due to storms. In 2023, the average survival of cuttings in the plots was 43.3% and the number of shoots 19.5 ± 9.0.

Both the survival of cuttings and the number of shoots decreased during the first years but remained fairly stable after 2020 (Fig. 3).

A loss of cuttings in the early stages after transplanting is a common trend for *Posidonia oceanica* (Piazzi *et al.,* 1998). Furthermore, it must be considered that the use of naturally uprooted cuttings precludes the control over the quality of the original transplanted material. Survival can be assessed not negative, as the loss of plots was low, the reduction was interrupted by years and 30% of established plots is considered sufficient to lead to successful recolonisation of the site (Pirrotta *et al.,* 2015).

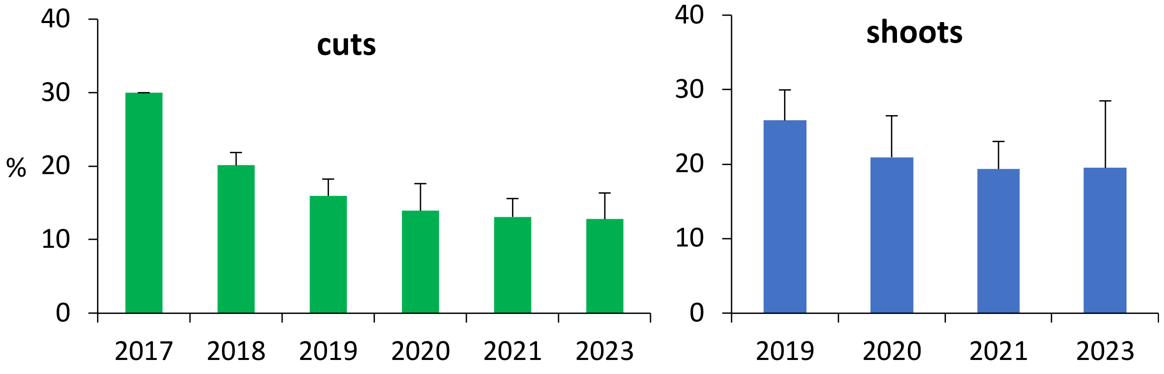

Figure 3: Survival of cutting (mean ± SD, left) and shoot density of transplanting (mean ± SD, right)

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APPENDIX 5

Red Eléctrica de España (REE) Marine Forest, a 2 ha trasplanting of *Posidonia oceanica* in the bay of Pollença, Mallorca, Balearic Islands, Spain

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A restoration project funded by Red Eléctrica de España with the support of the Government of the Balearic Islands and the Ministry of Defense of Spain

Background

Transplanting of *Posidonia oceanica* was done to promote the recovery of lost meadow at the inner part of Pollença Bay, north Mallorca, Balearic Islands, Spain. It is a shallow (depth <5 m), sheltered from waves location. Substratum is dead matte colonized by *Cymodocea nodosa*, *Caulerpa prolifera* and other photophilous macroalgae. Main disturbance in the area is anchoring by recreational boats. The presence of natural recruits (seedlings) in the transplanting area and the observation of active growth of rhizomes at the edge of the extant *P.oceanica* meadow (located at 200 m of the transplanting area) indicate that natural recolonization is taking place. The project is a feasibilty test of the scaling up of a *P. oceanica* planting methodology used in a previous project (Castejón-Silvo and Terrados, 2021).

Transplanting operations and applied techniques

The plant material used for transplanting was plagiotropic rhizome fragments of *P. oceanica* (Molenaar and Meinesz 1995) produced by natural processes (storms) which were collected manually by SCUBA divers from drifting material accumulated in meadow gaps in Pollença bay. The fragments selected for transplanting had a minimum of one apical (plagiotropic, horizontal) and two vertical shoots. The fragments were anchored individually using a staple made of 6 mm in diameter corrugated iron bar with a length of 60 cm and bended in the shape of a "U" (Fig.1).

Figure 1: Fragment tied to staple for individual anchoring

The fragment is tied to the staple with a piece of synthetic fiber cord and two cable ties. This system provides anchoring capacity to the rhizome fragment until it produces roots (Castejón-Silvo and Terrados, 2021).

The fragments were planted manually by SCUBA divers in groups (patches) of 16 (four lines of four fragments) and the distance among fragments was 20 cm (Fig. 2). Patches covered an area of about

$1\ m^2$ and were established following a 5 m by 5 m gridded pattern in the transplanting area. A total of 12800 fragments were transplanted and 800 patches were established. Transplanting was done in four events from March 2018, to November-December 2018, March-April 2019 and, finally, December 2019-February 2020 until the 2 Ha target area was completed. The transplanting area has been delimited with surface buoys to prevent disturbance by anchoring from recreational boats.

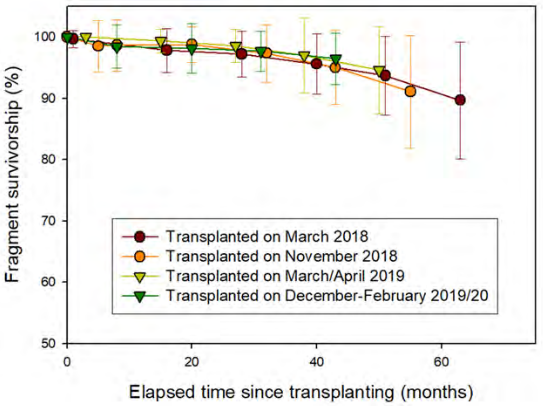
Figure 2: Establishing a patch of 16 transplanted fragments

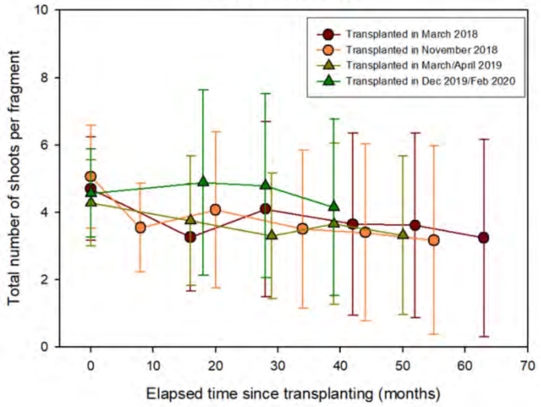
The initial plan to use both *Posidonia oceanica* seedlings and rhizome fragments for transplanting was not possible due to low availability of fruits during the transplanting years (2018 and 2019). However, some fruits were collected in the beach cast and produced enough seedlings to perform two small test plantings. The first one (in September 2018) involved the establishment of four 40 cm x 40 cm plots including 16 seedlings each. Seedlings were planted manually with no anchoring by SCUBA divers (Terrados *et al.,* 2013). The second test (in September 2019) involved the establishment of nine 40 cm x 40 cm plots including 16, 32 or 64 seedlings (n=3 for each planting density level) following the same methodology (Terrados *et al*., 2013).

Monitoring of the survivorship and vegetative development of transplanted fragments is done annually. Fragment characterization is done *in situ* by SCUBA divers and includes the counting of the number of apical (plagiotropic) and vertical shoots of each fragment. This is done to assess changes in size (number of shoots) of the fragments (N = 10 patches of each planting cohort, 40 patches monitored). Additionally, counting the number of living fragments in patches (N = 160 patches monitored) provides an estimation of fragment survivorship (% relative to initial number). Seedling survivorship (% of living seedlings in each plot) and vegetative development (foliar surface calculated from *in situ* measurements of length and width of all the leaves of 10 seedlings in each plot) are monitored annually.

Results

Fragment survivorship (mean ± SD) during the first 3.5-5.5 years after transplanting was higher than 90 % while fragment size, quantified as the total number of shoots in the fragment, did not change during the same monitoring interval although a trend of reduction is suggested (Fig. 3 & 4). The percentage of fragments that have the same or higher number of shoots than at transplanting time varies between 19% and 44%.

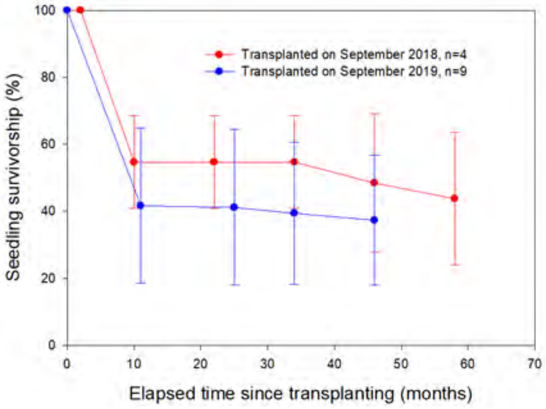


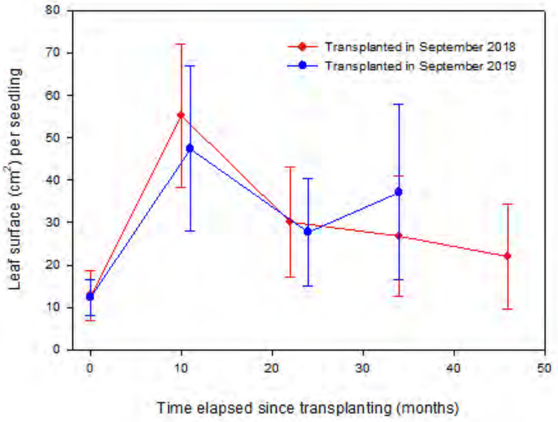


The comparison of results with previous transplantings of plagiotropic rhizomes shows that fragment survivorship in REE Marine Forest is in the upper end of the values obtained previously (Tab. 1):

Survivorship	**Time elapsed**	**Source**
>90 %	3,5 - 5,5 years	Red Eléctrica Marine Forest
90-95%, 70%	1 year, 2 years	Mancini et al 2022 Marine Pollution Bulletin 179, 113683
75%	4.5 years	Mancini et al 2021 *Biological Conservation* 264, 109397
46 % - 55 %	1-3 years	Piazzi et al 2021 *Water* 13, 661
~40 %, ~30 %	3 years, 6 years	Pirrotta et al 2015 *Mediterranean Marine Science* 16 : 591-604
76 %	3 years	Piazzi et al 1998 *Botanica Marina* 41: 593-601
85 %	3 years	Molenaar & Meinesz 1995 *Botanica Marina* 38: 313-322
100 %	1 year	Molenaar et al 1993 *Botanica Marina* 36: 481-488
20 % - 100 %	2-3 years	Meinesz et al 1993 *Botanica Marina* 36: 209-216

The survivorship of the seedlings 4-5 years after transplanting varies between 37% and 44%. Most of the mortality occurs during the first year of seedling life (Fig. 5). The leaf surface of transplanted seedlings increased 5 times during the first year but decreased afterwards reaching values higher than 20 cm^2 per seedling (at least twice the initial size; Fig. 5).





The comparison of results with previous plantings of seedlings shows that seedling survivorship in REE Marine Forest is within the range of values reported previously, including those shown by seedlings established through natural processes (Tab. 2).

Survivorship	Time elapsed	Source
37% - 44 %	4 - 5 years	Red Eléctrica Marine Forest
73 % , 48 %	2 , 3 years	Terrados et al , unpublished results (Mallorca, Alcanada)
62 %	1 year	Piazzi et al 2021 *Water* 13, 661
75 % , 44 %	1, 2 years	Terrados et al 2013 *Botanica Marina* 56: 185-195
70 %	3 years	Balestri et al 1998 *J. Exp. Mar. Biol. Ecol.* 228: 209-225
50% , 45% , 20%	1, 2, 3 years	Meinesz et al 1993 *Botanica Marina* 36: 209-216
70 % - 40 % (natural)	2 years	Piazzi et al 1999 *Aquatic Botany* 63: 103-112

The results obtained until now (September 2023) in the REE Marine Forest project show that it is feasible to transplant plagiotropic fragments and seedlings of *Posidonia oceanica* when the substratum is dead matte and the location is shallow and sheltered from wave action. The survivorship of fragments and seedlings is similar or higher than previous plantings while fragment size (total number of shoots per fragment) remains similar to that at the time of transplanting. These results are hopeful for the success of *P. oceanica* restorations but preliminary because of the slow growth that characterizes the species. Pirrota *et al.* (2015) have shown that a minimum of five years of monitoring after transplanting is needed to assess the actual outcome of the transplanting of plagiotropic fragments of *P. oceanica*. Hence, more than five years of monitoring might be needed to assess the success of *P. oceanica* restoration actions.

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APPENDIX 6

Posidonia oceanica restoration following the Costa Concordia shipwrecking in Giglio Island

Giandomenico Ardizzone, Andrea Belluscio, Gianluca Mancini, Daniele Ventura, Edoardo Casoli, Sara Cardone, Lorenzo Donnini, Fulvia Farina.

Background:

After the collision with the emerging rocks of 'Le Scole', near the coast of Giglio Island (Tuscan Archipelago, Tyrrhenian Sea), the Costa Concordia cruiser ship sunk on 13 January 2012. The impacts on the seabed, due to the physical presence of the wreck and all the following operations needed for its removal, have required complex interventions to facilitate the restoration. Two years were necessary for the re-floating and removal of the wreck (2012-2014), three years for the cleaning operations implying the removal of debris, platforms, cement and fine sediments (2015-2018), and another five years of interventions for the environmental restoration (2019-2024). During the first period, drilling operation to the platform and anchor blocks installation, positioning of mattress filled with cement, and removal of rocks caused a vast production of fine sediments that spread all over the wreckage area. Together with the coralligenous assemblages, a large part of the impacts interested the *Posidonia oceanica* meadow, which in that area died in a short time due to the shadow cast by the wreck's hull and to fine sediment covering the seabed.

Transplanting operations and applied techniques:

At the end of the cleaning operations, any sources of disturbance were removed from the area. Fine-scale cartographic products based on high resolution (20 cm/pixel) acoustic data (Multi-Beam Echosounder, MBES) validated by in situ direct SCUBA sampling and remote (R.O.V.) video observations were used to evaluate the state of the seabed. The only remaining part of the original *Posidonia oceanica* meadow was the biogenic substrate known as 'dead matte', composed of dead rhizomes that could not recover. Based on cartographic analysis, three areas of intervention for the transplantation of *P. oceanica* were identified for approximately 2000 m^2. Although the impact on the meadow affected the seabed up to -30 m depth, we decided to intervene only between -10 and -23 meters, excluding both the shallow (due to the high hydrodynamism) and deep waters (due to the low intensity of the light that reaches the seabed, and which could have created problems to the growth of the transplanted plants). These findings were based on previous published study and also on a study pilot (started in 2016) based on experimental plots (1 x 1 m), covering the depth range of future large-scale transplantation to verify the methodology's effectiveness. Best practices to find and conserve the vegetal material and the clods and rhizomes' fixing methods were tested as well. At the time of the transplantation, the dead *'matte'* was compact and not significantly damaged, partially covered by a thin layer of coarse sand. Because we did not want to alter the seabed by placing artificial support structures, such as iron nets, concrete frames, mattresses, etc., which could change the habitat, we decided to transplant cuttings by attaching them to the seabed with iron stakes capable of degrading in a few years (within 8-24 years) once the complete rooting of the plant has been reached. The stakes were specially designed and built to be easily inserted and hold the rhizomes in the *'matte'* (Fig.1). Once planted in the *'matte'*, the stakes are almost invisible. The plant material comprised both orthotropic and plagiotropic shoots, derived from clods naturally detached due to storms and erosional events along lower limits of meadows.

Most of the material comes from detached clods from boat anchoring, which during the late spring-early fall seasons is very high along the coast of Giglio Island. The *P. oceanica* clods collected by divers around the Island and brought to the surface using plastic bags were rapidly transferred using inflatable boats to the yard area.

Figure 1: Images of the stake of 0.6 cm diameter iron rods welded together in one or more points, each curved at one end to form two curved arms (crescents) holding the *P. oceanica* rhizomes.

Inside the transplanting area, underwater cages were used to store the clods for up to 3 days. Before transplantation, the clods were cleaned, and dead or damaged parts were removed. The larger cuttings were divided into smaller pieces with several foliar shoots and roots. The optimal material preferably comprises 10-30 cm long plagiotropic rhizomes, each with 2-4 foliar shoots and roots in good condition. Underwater operators implanted each cutting manually, fixing them using one or two stakes, depending on their length. This operation was done with great care by SCUBA divers to prevent the cutting of the rhizome. The seabed is prepared in advance by dividing the entire transplantation area into 10 x 10 m squares marked off with ropes fixed to the substratum and georeferenced to facilitate transplanting and monitoring activities. Inside the square, each operator used a 1 x 1 m portable aluminium frame. The frame was used to ensure 100% coverage of the area to be transplanted with a constant density of cuttings. The density of the cuttings and shoots in the area transplanted on Giglio is 5-9 cuttings/m^2, corresponding to 26-33 shoots/m^2.

Monitoring activities:

The Posidonia transplant was monitored constantly with five sampling occasions per year since 2019 with direct SCUBA sampling, counting the number of shoots to estimate the permanence and growth of Posidonia within predetermined monitoring squares that cover 3% of each transplanting area. Part of the monitoring was also carried out with an innovative technique, using underwater photogrammetry to reconstruct accurate photomosaic and 3D models of the areas, useful for large-scale seascape monitoring over time.

Results

After four years after the beginning of the activities, 2170 m^2 of *P. oceanica* were transplanted, with a density of approximately 26-33 shoots/m^2. The total area was accomplished gradually from 2019 to 2022 by dividing the interventions into five areas with a mean surface of approximately 430 m^2 per year to facilitate the underwater activities (e.g. searching and gathering of vegetal material, mapping and monitoring). In all the areas, an initial loss of shoots during the first year was observed, followed by increased densities. In Area A9 (Fig. 2), the first area to be transplanted, 46 months after transplanting, the mean shoot density (±SD) equals 31 ± 3 shoots/m^2, corresponding to a percentage variation (±SD) of 123 + 1 %.

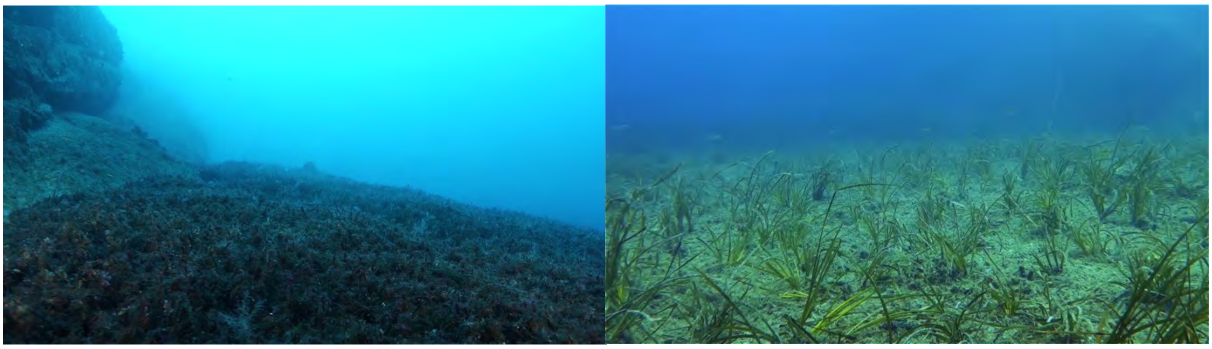

Figure 2: A9 area before transplantation (left) and after (right) indicating the good success of the transplant

The experimental area made in 2017 shows an increase of 250% of the shoot density in 90 months, indicating the good success of the transplant (Fig. 3).

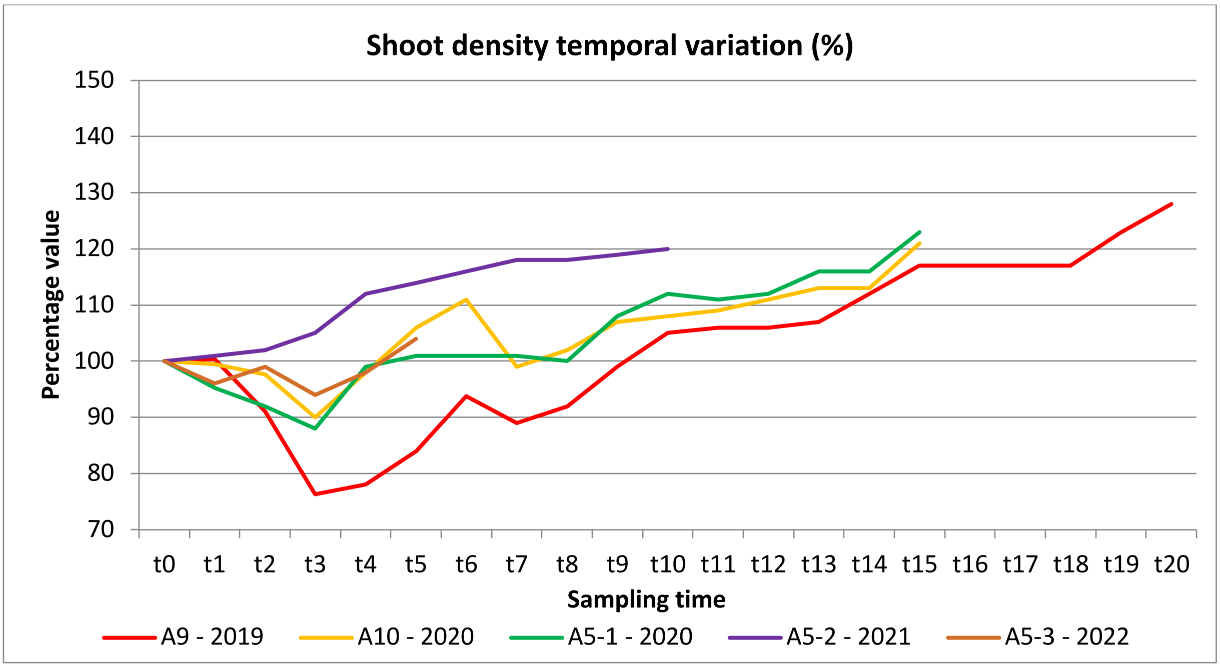

Figure 3: Temporal variation of the *P. oceanica* shoot density (expressed as the percentage of variation from the shoot density at the time of transplanting) transplanted in Giglio Island from 2019. Colours refer to different transplanting areas in different years: 2019 in red (524 m^2), 2020 in yellow (196 m^2) and green (429 m^2), 2021 in violet (594 m^2), and 2022 in brown (427 m^2).

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APPENDIX 7

REPIC program: Restoring **Posidonia oceanica** *meadows impacted by boat anchoring on French Riviera*

Gwénaëlle Delaruelle, Sébastien Personnic, Florian Holon, Pierre Descamp, Jo-Ann Schies.

Context:

A loss of 10 % of Posidonia meadow surfaces has been estimated over 100 years in the Mediterranean basin. In the French Mediterranean, the southern region is the most heavily impacted by anchoring pressure, accounting for 80 % of the AIS anchorages recorded between 2010 and 2020. In the Gulf of Saint Tropez, more than 145 hectares have disappeared since 2010, while more than 225 hectares have been lost in Golfe-Juan since 2006.

Conducted by Andromède Océanologie in partnership with the Agence de l'eau Rhône Méditerranée Corse and NAOS, and with the support of new partners in 2022, namely the Fondation de la mer and the Artelia Foundation, the REPIC (REstaurer la Posidonie Impactée par les anCres) program replants fragments (shoots, rhizomes, roots) of seagrass beds uprooted by anchors during the summer season (or broken naturally).

French authorities adopted a new regulation in 2019 forbidding any anchoring within *P. oceanica* seagrass meadows for boats larger than 24 m. The number of large ships (>24 m) anchoring in *P. oceanica* meadows significantly decreased after the enforcement of the regulation. This reduction has led to a drop in pressure on the meadow and the implementation of restoration measures.

Since 2019, the aim of the REPIC program is therefore to initiate a process of restoration (strengthening populations) of these meadows in several selected areas in order to speed up the recovery of the remaining meadows and reclaim the areas that have been destroyed. It will also provide a better understanding of the complex phenomena involved in the recolonization of Posidonia meadows.

Transplanting operations and applied techniques

Three geographical sites in the Alpes-Maritimes (French Riviera) have been identified for the REPIC program: Golfe-Juan from 2019, Beaulieu-sur-Mer from 2021 and Villefranche-sur-Mer from 2023. These sites were selected according to the presence of dead matte, the depth gradient and the high anchoring pressure close to the location allowing fragments to be easily harvested. Following the past damage to the Posidonia meadow and the new protection measures that have been put in place (notably anchoring regulations), these sites are now ideal areas for testing new methods for restoring the meadows. They are being scientifically monitored for a minimum of five years.

The different restoration techniques tested depend on the morphology, aggregation, arrangement, density and types of preparation of the fragments. Monitoring methods include individual monitoring of fragments in permanent quadrats, visual monitoring of all restored areas using photogrammetry, monitoring of carbohydrate reserves in fragments and monitoring of changes in environmental parameters (temperature, but also other large-scale data available from various sources) in the restoration area. Each year, these restoration experiments have been authorized by prefectorial decrees.

The methodology used in the REPIC program is based on past transplantation experience (see section I. Background) and the optimization of large-scale work.

The restoration methodology is divided into three phases:

- harvesting the fragments: this takes place at a depth of between -8 m and -13 m. The preferred fragments are plagiotropic, 5 to 7 cm long, with several shoots;

- preparation of the fragments: this took place underwater during the first year, then on the boat, where metallic biodegradable staples (complete degradation is estimated at 10 years +- 3 years) are attached to the fragments before they are stored in crates filled with seawater;
- *in situ* restoration of the fragments: The fragments are fixed in the substrate using the staples (Fig. 1). The layout of the fragments varies according to the areas to be restored:
    - squares of 0.25 m² to 1 m² in Golfe-Juan;
    - circles of 0.5 m² in patches of different densities (lines of 5, 10, 20 and 30 shoots by circle) in Beaulieu-sur-Mer and Villefranche-sur-Mer.

Figure 1: A fragment transplanted from the restoration site at Beaulieu-sur-Mer, ©Laurent Ballesta 2023

Monitoring activities:

Different monitoring methods are used each year:

- Monitoring the number of fragments and the density of shoots in permanent quadrats:
    - Monitoring of the number of shoots per fragment identified by a mark (two zones at Golfe-Juan and three zones at Beaulieu-sur-Mer)
    - Monitoring the number of total shoots per quadrat (two zones in Golfe-Juan, almost all zones in Beaulieu-sur-Mer). This method is used mainly for monitoring since 2021 and will be favored for future monitoring in order to establish the growth dynamics of the fragments (regression or progression).
- Visual monitoring of restored areas using photogrammetry (Fig. 2)
- Monitoring of rhizome carbohydrate reserves: The carbohydrate monitoring protocol was carried out at Golfe-Juan in 2020, 2021, 2022 and 2023, and at Beaulieu-sur-Mer in 2022 and 2023 to assess the carbohydrate reserves stored in the rhizomes, at the time of harvesting and then every year (three years) after restoration. These values are compared with a natural seagrass bed at the same depth as the restored seagrass bed. Three samples per site and per year are analyzed.
- Monitoring of environmental conditions to test the site effect (temperature, salinity, currents, chlorophyll a, etc.).

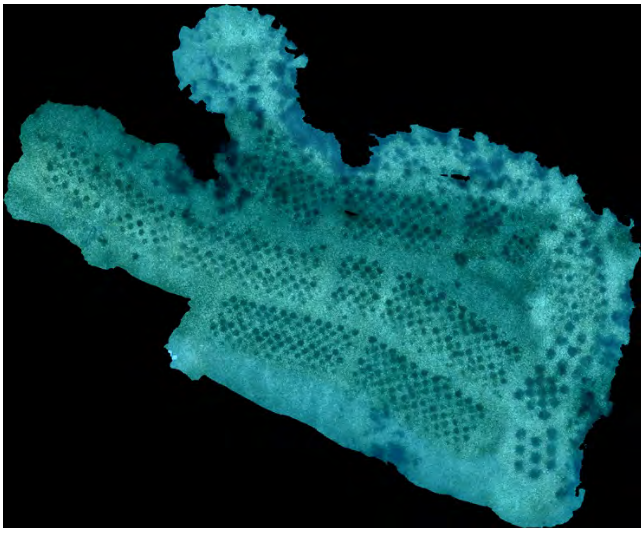

Figure 2: Orthophotography of the Beaulieu-sur-Mer restoration site (1700 m^2) obtained by photogrammetry ©Andromède Océanologie

Results

Since 2019, five to seven divers and biologists spent 358 days on the REPIC operations. In total about 730 hours of diving (266 hours of collection, 320 hours of transplanting and 144 hours of scientific monitoring) were required. The year 2023 results in 5 years of monitoring of the oldest areas transplanted between 2019 and 2022 (94 205 shoots or 28 160 fragments; Fig.3). In the light of the initial results for 2019, it was deliberately decided to prioritise a higher density of bundles, for a more successful operation, rather than a large surface area covered.

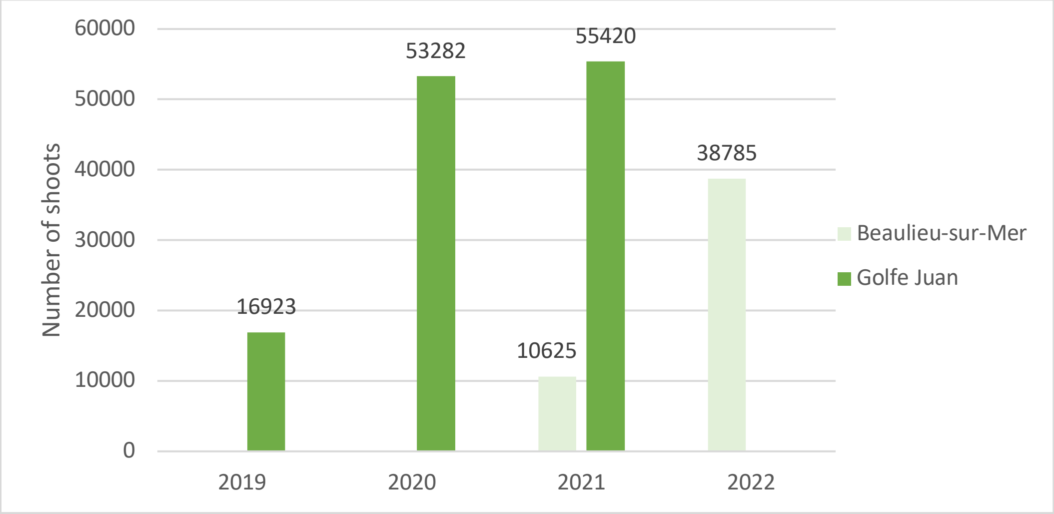

Figure 3: Cumulative number of shoots transplanted per site between 2019 and 2022

The survival rate for the oldest transplanted areas in Golfe-Juan (on 152 m^2 since 2019) was 57 % in 2023 (T+4 years). The highest rate of loss was observed between the first and second years. Thereafter the number of shoots stabilized or even increased.

Over the years, we have refined the methodology based on observations and the first results studied. As far as transplants are concerned, we are continuing to transplant using the circles patches method and are noticing better recovery and survival on circles with a higher density (Fig. 4).

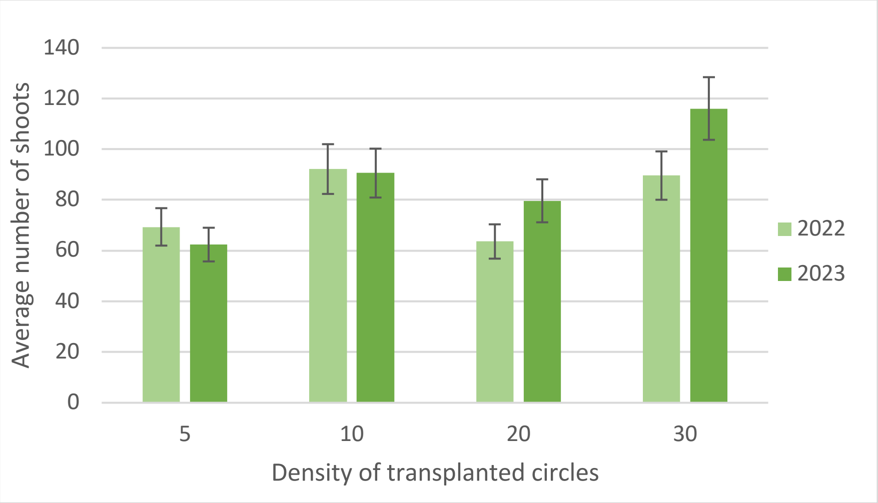

Figure 4: Average number of shoots counted in the permanent quadrats at Beaulieu-sur-Mer at planting year (2022) and at T+1 (2023) Error = inter-counter variability of 10.65 %.

We study the effect of depth on the survival rates: they were higher in the -18 m (Beaulieu-sur-Mer; Fig .5) zones than in the -3 m (Golfe-Juan) and -30 m zones (a single zone monitored at Beaulieu-sur-Mer, Fig.6). The survival rate between 2021 and 2023 was 59 % at Golfe-Juan (-3 m; Fig.7) compared with 72 % at Beaulieu-sur-Mer (-18 m; Fig. 8) for the same period.

The remainder of the REPIC program will take these changes into account in order to refine the methodology for re-implanting these transplants.

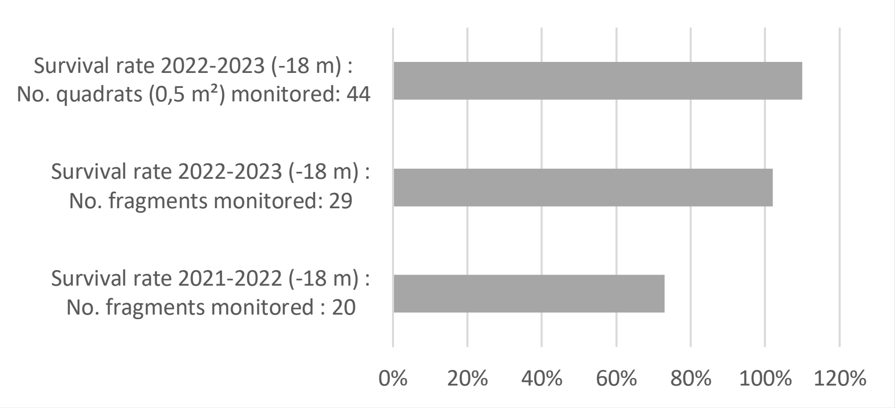

Figure 5: Survival rate of shoots monitored at T+1 year at Beaulieu-sur-Mer, in areas planted in 2021 or 2022.Monitoring was carried out in quadrats or on specifically monitored fragments.

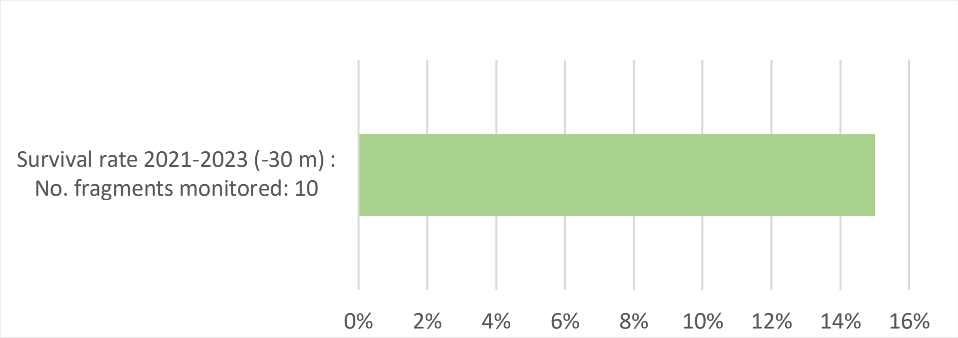

Figure 6: Survival rate of shoots monitored between 2021 and 2023 (T+2 years) in the deepest zone at Beaulieu-sur-Mer

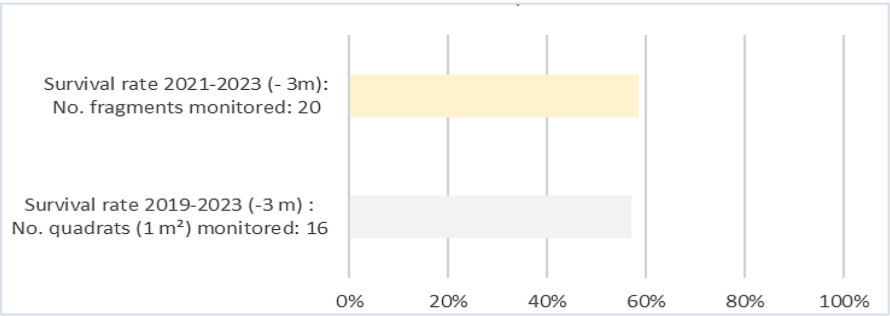

Figure 7: Survival rate of shoots planted in 2021 and monitored in 2023 (T+2 years; shoots counted on monitored fragments) and shoots planted in 2019 and monitored in 2023 (T+4 years; shoots counted in monitored quadrats) at Golfe-Juan

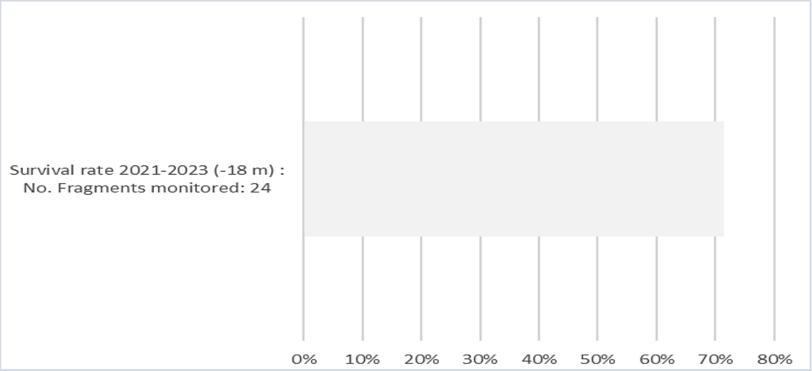

Figure 8: Survival rate of shoots monitored planted in 2021 and monitored in 2023 (T+2 years) at Beaulieu-sur-Mer (-18 m)

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APPENDIX 8

Synthesis of the main publications, sorted by. year, concerning transplantation of cuttings.

N°	Reference	Substrate	Anchorage	Time (in month)	Planting season	Depth	Main results	Relevant information for the future
43	Maggi, 1973	Shell sand	Concretes	12 – 17	March and August	8	For cuttings transplanted in March: percentage coverage of over 60% and for those transplanted in August: percentage coverage of 30 to 40%.	The transplanting trial in March 1971, carried out at the start of the phase of high metabolic activity, gave a much higher percentage of success than the trial of August 1971, carried out during the phase of low metabolic activity.
38	Meinesz *et al.,* 1992	Dead matte	Plastic grid	12	June + Sept. + Dec + March	5.3	Orthotropic (two shoots): Survival 76%	No effect of rhizome length on survival Sept. plantings had better survivals
36	Molenaar & Meinesz, 1992	Dead matte	Plastic grid	12	August	 3 14 20 36	Orthotropic (1 shoot from 30 m): Survival % Root formation % 93 43 96 44 100 28 72 0	Higher survival when depth origin > planting depth
19	Molenaar *et al.,* 1993	Dead matte	Plastic grid	12	August	14	Plagiotropic (1 ap + 2 vert): Survival 100%, root formation 97% Orthotropic (1 and 2 shoots): Survival 31 % and 94%, root formation 14% and 62%	Poorer performance of single shoot orthotropic fragments.
42	Genot *et al.*, 1994		Individual stainless-steel peg	6	January	8 10 15 20	Orthotropic: survival 86% when depth origin > planting depth, 59% when depth origin < planting depth	Higher survival when depth origin > planting depth Higher [Rhl] and carbohydrates in transplants when depth origin > planting depth

37	Molenaar & Meinesz, 1995	*C. nodosa* on sand	Individual wire picket	36	April, June, Oct (time is a misleading factor, not included in the results)	6-8	Plagiotropic (1 ap + 2 vert): Survival 85%, ramification 99% Orthotropic (1 and 2 shoots): Survival 39 % and ramification 96%.	No survivorship on non-vegetated sand. Higher survival of plagiotropic fragments. No clear difference between arrangements Better results at 5 cm than at 10 cm on sand + *C. nodosa*
		C. nodosa on dead matte + Sand			June		Plagiotropic (1 ap + 2 vert): Survival 92%, ramification 99% Orthotropic (1 and 2 shoots): Survival 39 % and ramification 96%.	Better results at 5 cm than at 10, 15, 20 cm on dead matte + *C. nodosa*
26	Augier *et al.,* 1996	Sand/Mud	Concrete frames	115	May	10	Density increases from 120 -> 475 - 873 shoot * m^{-2} Initial surface increase * 7	Two sets of transplants: 1/ Transplanted in May 1983 and followed in January 1992 2/ Transplanted in May 1990 and followed in January 1992
20	Piazzi *et al*., 1998	Dead matte	Plastic coated wire grids	36	June to Sept	10	Plagiotropic (1 shoot): Survival 76%, 5-6 months new shoot production, % rhizome elongation 70% Orthotropic (2 shoots): Survival 59%, >12 months new shoot production, % rhizome elongation 22%	Higher survival, growth and ramification of plagiotropic No effect of depth origin
39	Vangeluwe *et al.,* 2004	Dead matte Sand + *C.nodosa* Sand	Non-covered metallic grid	6	December		Orthotropic (1 shoot, 10 cm rhiz.) After 6 mo leaf nutrient content is not recovered in transplants. Rhizoma and root CNP content is recovered. Root development after 6 mo of transplant	Degradation and failure of anchoring Very low survivorship on non-vegetated sand.

25	Balestri *et al.,* 2011	Artificial calcareous rubble reef	Lodged among rubbles	12	October	1.5	Fragments (plag. + ortho.): Survival 50%, new shoots 40%. The best result of this work regards the culture of fragments: kept for long period (3 yr) during culture the survival and regeneration was better in plag. than in ortho.	Storm generated fragments are suitable for transplanting if collected at the beach just after the storm. Failure of anchoring, 40% losses
21	Pirrota *et al.,* 2015	Dead matte	Galvanized electro welded iron wire mesh	70	July		Plagiotropic (1 ap +2 vert) Survival 32%, decrease of density from 66 to 32 shoot m^2, increase of shoots per fragment from 4 to 11.	Fragment production of shoots started after the fourth year since transplanting Results during 3 yr changed on year 4th
40	Alagna *et al.*, 2019	Gabion mattress filled with rocks	Five different anchoring to the gabions or/and the rocks	30	May	12	Orthotropic (1 shoot, 10 - 15cm, wire-net pockets inserted in the upper layer of the gabion): survival 93%, branching 57%, Increase of reserve structures relative biomass from 19 to 43% (rhiz.), 1 to 11% (root). Density increased by four from 36 to 106 shoot m^2	Better results of anchoring with wire-net pockets inserted in the upper layer of the gabion Lodged fragments among rocks failed as anchoring system, 60% losses
24	Piazzi *et al.,* 2021	Dead matte	Natural fibre coarse net	36	Spring	15 20	Fragment (plag. + ortho.) survival 46%	The anchoring system was successful
35	Ward *et al.*, 2020		Two pieces of bamboo in “V” inverted Coconut fibre pots inverted	15	May	4.5 8	Plagiotropic survival 89% Orthotropic survival 60%	Better results of bamboo anchorage Better results of plagiotropic

22	Castejón-Silvo & Terrados, 2021	Sand Burlap bags filled with gravel	U-shaped iron piece (staple)	48	May - July	15 20 25	Plagiotropic (1 ap + 2 vert) Survival 31% Production of new shoots after 1 year but no development of root system after 2 years	Very low survivorship on non-vegetated sand. Anchoring failure –Better results at higher depths Failure of stabilization measure (burlap gravel bag) for transplant Results after 2 yr changes at year 4
23	Mancini *et al.,* 2021	Dead matte	Iron stakes	52	August - Sept	8-21	Plagiotropic cuttings with a minimum of 4-5 shoots: survival 75%. Increase of density from 30 up to 48 shoots m^2	Vessel anchoring generated fragments are suitable for transplanting Anchoring succeeds
41	Mancini *et al.,* 2022	Dead matte	Iron stakes	24	June Sept	10-23	Survival 79.5% (type of fragment not described) Initial density 100 shoots m^2 up to 105 shoot m^2	Vessel anchoring generated fragments are suitable for transplanting Density results changed between first month and 24 months. Lowest density at 8 months recovered at 24 months.

Appendix 9

Synthesis of the main publications concerning transplantation of seeds

N°	Reference	Substrate	Anchorage	Time (in months)	Planting season	Depth	Main results	Relevant information for the future
49	Meinesz *et al.,* 1993	Dead matte	Metal peg or grid	36	Sept.	11	1st yr 50%, 2nd yr 45%, 3rd yr 20%	Grew in aquaria for 14 months
45	Balestri *et al.,* 1998	Dead matte Gravel	Plastic grid fixed with metal bars	36	July	10	Survival: 1st yr 83%, 2nd yr 80%, 3rd yr 70% Ramification 3rd yr 14% Survival: 1st yr 20%, 3rd yr 0%	Fruits collected at the beach are suitable for transplant Grew in aquaria for 2 months Survival and size of seedlings planted on dead matte was equivalent to natural survival of recruits (66%)
51	Piazzi *et al.,* 1999	Dead matte Rock Gravel	No planting	26		2-10	No manipulative experiment. There is no plantation but the results on natural recruitment can be used for restoring initiatives. Survival: Matte 10m: 1st yr 87%, 2nd yr 70% Matte 2m: 1st yr 87%, 2nd yr 40.5% Rock (10m): 1sr yr 62%, 2nd yr 46.4% Gravel (z=2-10): 2nd yr 0%	Important decrease in seedling density during the first year and stabilized the second year Natural recruitment (proxy of plantation success?), rhizome development enhanced on dead matte and rock No recruit survival (proxy of plantation failure?) in unvegetated gravel and shallow rock bed.
54	Infantes *et al.,* 2011	Sandy area Posidonia meadow with sand gaps	None	7	August	12 & 18	After storms, all Posidonia seedlings disappeared.	Germination in aquarium *P. oceanica* seedlings survived at a higher proportion in the deep than in the shallow sites
44	Dominguez *et al.,* 2012	Dead matte Live meadow	Mesh pot	9	July	8-12	Survival: Matte: 1st yr 75% Live meadow: 1st yr 22%	Grew in aquaria for 2-3 mo Higher survival and size of seedlings planted on dead matte
52	Alagna *et al.,* 2013	Vegetated rock Unvegetated sand	No planting	24		3	No manipulative experiment. There is no plantation but the results about natural recruitment	Null survival of natural recruits on gravel and sand (proxy of plantation failure?)

		Unvegetated gravel					can be used for restoring initiatives.	Natural recruitment (proxy of plantation success?), rhizome and root development enhanced on rocky substrata colonized by turf algae (Halopteris spp. and Dilophus spp) Significant drop-off of seedling density on rock colonized by turf algae was recorded after the first year
30	Terrados *et al.,* 2013	Live meadow	Mesh pot	36	July		No survival of seedlings planted on live meadow	Grew in aquaria for 2-3 mo Seedling survival does not benefit from artificial anchorage
		Dead matte	None				Survival: 75% 1st yr, 44% 3rd yr Ramification: 0% 1st y	No survival of seedlings planted on live meadow
46	Pereda-Briones *et al*., 2018	Dead matte	None	6	June	3	Survival: 50% no *C. cylindracea* 70% with *C. cylindracea*	Grew in aquaria for 2-3 mo Invasive species *Caulerpa cylindracea* does not deter seedling survival. Nutrient addition reduced survival but induced seedling leaf production.
50	Pereda-Briones *et al*., 2020		No planting	19		1.5-4.5	No manipulative experiment. There is no plantation but the results about natural recruitment can be used for restoring initiatives. Survival:	Poor natural recruitment (proxy of plantation failure?) in unvegetated sand or gravel. Natural recruitment and TLA enhanced on shelter areas (<) with rocky substrata and dead matte colonized by macroalgae, particularly crustose (i.e. Peyssionellia and Lithophyllum sp.)
		Rock					Rock: >80% 1st yr, 80% 2nd yr	
		Dead matte					Dead matte: >80% 1st yr, 70% 2nd yr	
		Gravel & Sand					Gravel & Sand: 0% 2nd yr	