

## Stitching up *Posidonia oceanica* (L.) Delile anchorage scars using beach-cast seeds: Results of a six-year study

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### ARTICLE INFO

#### Keywords:

Seagrass  
*Posidonia oceanica*  
Transplantation  
Habitat restoration  
Seed  
Seedlings

### ABSTRACT

Seagrass meadows are experiencing a worldwide decline, driven by human activities and natural disturbances. The degradation of these meadows raises significant concerns regarding the loss of essential ecosystem services. *Posidonia oceanica*, a Mediterranean endemic seagrass, plays a crucial role in coastal ecosystems, contributing to biodiversity, carbon storage, water purification, and shoreline protection. This study reports the outcomes of a six-year transplantation experiment aimed at restoring small damaged patches in a *Posidonia oceanica* meadow in the southwestern Tyrrhenian Sea, within the Egadi Marine Protected Area (MPA). The damage, likely caused by boat anchoring, was addressed using seedlings grown in the laboratory from beach-cast seeds. The experiment evaluated the survival and growth of seedlings planted at three different densities in two sites. After six years, the intermediate planting density (100 seedlings per m<sup>2</sup>) yielded the highest survival rate (76.9%), while the lowest density (40 seedlings per m<sup>2</sup>) resulted in the lowest survival rate (5.1%). Seedlings showed significantly different growth rates and biomass accumulation in the two sites, likely due to variability in sediment accumulation, seabed slope and hydrodynamic conditions. This research highlights the potential of seed-based techniques for restoring seagrass meadows, emphasizing that long-term monitoring and careful selection of transplant sites are essential for the success of such restoration efforts.

### 1. Introduction

The decline of seagrass meadows worldwide, driven by human activities and natural disturbances, has become a matter of significant concern. Seagrasses play a crucial role in supporting biodiversity, promoting carbon sequestration, maintaining water quality, and protecting coastlines (Hemminga and Duarte, 2000). Despite their ecological importance, seagrasses have been adversely affected by anthropogenic impacts such as coastal development, pollution, boat anchoring and trawling (Boudouresque et al., 2009) resulting in severe regression and degradation (McKenzie et al., 2020).

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica*

(L.) Delile forms extensive meadows that play an important role in coastal areas by providing numerous ecosystem functions and services (Campagne et al., 2015). *P. oceanica* meadows provide habitat for many vagile and sessile species (Barbier et al., 2011), limit the spread of invasive species (Pergent et al., 2008), promote water oxygenation (Boudouresque et al., 2012), attenuates wave energy (Infantes et al., 2012) and contribute to nutrient cycling (Barbier et al., 2011). In addition, *P. oceanica* meadows represent one of the largest carbon sinks in coastal areas due to the construction of the mat, a long-lasting belowground biogenic structure composed of intertwined rhizomes, roots and leaf sheaths embedded in the sediment (Monnier et al., 2022; Monnier et al., 2021). Despite their importance, *P. oceanica* meadows

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have declined by 5–20 % over the past century due to anthropogenic impacts (Telesca et al., 2015).

As with other seagrasses worldwide (Collins et al., 2010; Sagerman et al., 2020), in coastal regions exposed to significant recreational activities, such as those in the Mediterranean (Francour et al., 1999; Milazzo et al., 2002), *P. oceanica* is vulnerable to mechanical damage resulting from boat anchoring (Abadie et al., 2016; Carreño and Lloret, 2021; Ceccherelli et al., 2007; Deter et al., 2017; Milazzo et al., 2004; Montefalcone et al., 2008; Pergent-Martini et al., 2022). The direct impact of anchors, including the uprooting of *P. oceanica* shoots or of portions of mat, leads to a reduction in shoot density and meadow coverage, fragmentation and promotes the formation of erosive “inter-mattes”. These intermattes can expand and coalesce, exacerbated by hydrodynamic forces, thereby leading to fragmentation of the meadow (Francour, 1997; Pasqualini et al., 2000). The consequences of boat anchoring on seagrass extend beyond immediate habitat loss, encompassing long-term ecological impacts on species diversity, sediment stability, and nutrient cycling (Abadie et al., 2016; Vega Fernández et al., 2005). Understanding and mitigating the effects of boat anchoring on seagrass is key for the conservation and sustainable management of this critical marine habitat (Milazzo et al., 2002).

Active restoration measures are essential for countering habitat loss and ensuring sustainable coastal ecosystems, particularly for seagrasses. Natural rehabilitation of seagrasses, especially *P. oceanica*, is a slow process, often taking decades for successful recolonization (Badalamenti et al., 2006; Kendrick et al., 2005; Marbà and Duarte, 1998; Vaudrey et al., 2010). Due to its slow growth rate, *P. oceanica* natural recovery after impact is limited. Consequently, active restoration measures are required to support the seagrass regeneration in the immediate aftermath of human-induced impacts, as well as to facilitate the recolonization of impacted areas once environmental conditions have been restored (Paling et al., 2009; Possingham et al., 2015).

The urgency to develop sustainable and scalable restoration solutions for seagrasses has never been greater, as coastal degradation continues to escalate. However, several challenges must be overcome to achieve successful restoration, such as scalability, cost, and limited understanding of restoration dynamics (see Boudouresque et al., 2021 and literature therein), including the role of positive species interactions (Balestri et al., 2021; Valdez et al., 2020). The field of coastal restoration ecology requires innovative methods and strategies tailored to the dynamics of the marine environment. The use of sexual propagules offers promising pathways towards more efficient and effective restoration strategies (Infantes et al., 2011; Pereda-Briones et al., 2018), but further research and large-scale testing are needed to determine its viability.

Among the different restoration techniques proposed (see Boudouresque et al., 2021 and Pansini et al., 2022 and literature therein for an updated review), those involving the use of seeds and seedlings have been applied successfully in several rehabilitation projects and are considered among the most ecologically friendly and low-cost techniques (Gräfnings et al., 2023; Marion and Orth, 2010; Maulidiyah et al., 2024; Unsworth et al., 2019). Seed-based restoration methods ensure the maintenance of genetic variability of transplanted beds, providing evolutionary potential to the restored populations in the face of future environmental challenges (Escandell-Westcott et al., 2023; Hughes and Stachowicz, 2004; Reusch et al., 2005). Furthermore, the ability to propagate plants from seeds in culturing systems potentially boosts the yield of the biological material collected from donor beds. Disentangling the scale of restoration interventions from the amount of biological material obtained from existing populations will leave room for restoration upscaling in the near future (van Katwijk et al., 2016). *P. oceanica* beach-cast fruits and seeds can be found in spring and their appearance could easily be reported and collected through citizen science (Zenone et al., 2024).

The phenomenon of sexual reproduction in *P. oceanica* has been described as infrequent (Balestri and Vallerini, 2003). Recent observations, though, have revealed a significantly higher frequency of

flowering events related to rising sea temperatures during the summer months (Diaz-Almela et al., 2007; Diaz-Almela et al., 2006; Stipich et al., 2024). Despite this, sexual propagation in *P. oceanica* is impaired by various bottlenecks encountered throughout the phases of flowering, seed production, seed recruitment and seedling establishment (Kendrick et al., 2023; Statton et al., 2017; Maulidiyah et al., 2024). Researches conducted in the Ligurian Sea have shown that seedling survival rate can reach levels as high as 66 % within three-year after seed settlement and establishment (Balestri et al., 1998), yet only a small fraction of the total number of seeds released by the mother plant reaches this advanced stage. Following storm events, large quantities of fruits and seeds are often found beach-cast, where many are exposed to the sun and subsequently desiccate (Sutera et al., 2024). This material, if properly and timely collected, could be utilized for transplantation initiatives.

Recent mesocosm studies (Balestri et al., 2021) highlight how intraspecific (i.e. planting density) and interspecific (i.e. proximity to a nurse or a pioneer species) interactions influence the survival and growth dynamics in *P. oceanica* seedlings. High planting densities (hundreds of seeds per m<sup>2</sup>) may promote faster canopy formation, which could increase sediment stability and reduce grazing pressure, while lower densities may reduce competition for resources (e.g. light, nutrients) among seedlings. Furthermore, the presence of surrounding vegetation may promote facilitative mechanisms and enhance seedling performance. However, it remains unclear whether planting density significantly influences biomass allocation to leaves, rhizomes, or roots, as seagrass growth is also strongly modulated by local environmental conditions and by the availability of resources.

The present study reports on the outcomes of an experiment set-up to test a system for establishing seedlings in the seabed conducted in the southwestern Tyrrhenian Sea, within the boundaries of the Egadi marine protected area (MPA). The main aim of the study was to assess the possibility of recovering minor mechanical damage suffered by a *P. oceanica* meadow using seedlings obtained from beach-cast seeds. Specifically, the research was aimed at assessing the feasibility of *P. oceanica* restoration starting from beach-cast seeds grown to the seedling stage in tanks and, primarily, determine the optimal seedling density for possible application in future small scale restoration initiatives. We have (1) compared the survival rates of transplanted seedlings at three densities (40, 100, and 200 seedlings/m<sup>2</sup>), and (2) examined the overall growth and performance of the transplanted seedlings. The main hypothesis was that seedling survival would be influenced by the transplanting density, with higher survival rates observed at higher seedling densities. Furthermore, we hypothesize that at high and intermediate densities the overall plant performance would be better compared to the lowest density due to a positive intraspecific effect, with a greater investment of the plantlet to the root and rhizome compartment, as shown in the mesocosm experiment by Balestri et al. (2021).

## 2. Materials and methods

### 2.1. Seeds and fruits collection

In April 2016, 500 beach-cast fruits were collected along the coast of Marsala (western Sicily). Immediately after collection, the seeds were extracted from the fruits and transported in refrigerated boxes to the IAS-CNR laboratory at Torretta Granitola (southwestern Sicily). The seeds were then rinsed with filtered seawater and placed in 30 l closed-circuit seawater tanks.

### 2.2. Seedlings cultivation

Seeds were checked daily until germination to remove all those that appeared infected with pathogenic microorganisms or no longer viable. Once at an advanced stage of growth (i.e. with the primary root and the first well-developed leaves, approximately three weeks after

germination), 400 seedlings were placed inside perforated polypropylene plastic pots with a diameter and height of 5 cm, containing small calcarenitic pebbles, one seedling per pot. The pots were distributed in four tanks by fitting them into the holes of perforated bricks placed on the bottom of each tank. Seedlings remained in the tanks until November 2016, when 258 of them were randomly and proportionally removed from the four tanks and transferred to Favignana Island (Egadi MPA, 15 km off Marsala) for transplanting (Fig. 1). From the remaining seedlings, 9 from each tank were removed from the pots and measured (see following section for details). The culture conditions throughout the cultivation period were as follows: temperature of 21 °C, salinity of 38 ppt, light irradiance of  $40 \pm 5 \mu\text{moles photons m}^{-2} \text{s}^{-1}$ , photoperiod of 12:12 h light:dark.

### 2.3. Morphological analysis of seedlings before transplanting

Thirty-six seedlings, nine from each of the four tanks were measured for number of leaves (NL), leaf length (LL, cm), leaf width (LW, cm), longest leaf (LstL, cm), leaf surface (LS,  $\text{cm}^2$ ), total root length (TR, m), leaf dry weight (L\_Dw, g), root dry weight (R\_Dw, g), and seed dry weight (Seed\_Dw, g), just before transplantation. A one-way analysis of variance (ANOVA; factor Tanks: 4 levels) was performed on the measured variables.

### 2.4. Description of the transplant sites

Two sites located along the southern coast of Favignana (Fig. 1) were chosen for the transplant of *P. oceanica* seedlings. Both sites were about 200 m off the coast, at 10 m depth and showed clear signs of mechanical impact in the shape of scars, most probably attributable to anchoring. No other anthropogenic activities have been recorded in this area. The scars at both sites were at the same level as the surrounding substrate. At Site 1, a sharp scar (approximately  $2 \times 10 \text{ m}$ ) divided the edge of a seagrass patch in two. A layer of sediment 1 to 5 cm thick, under which a dead

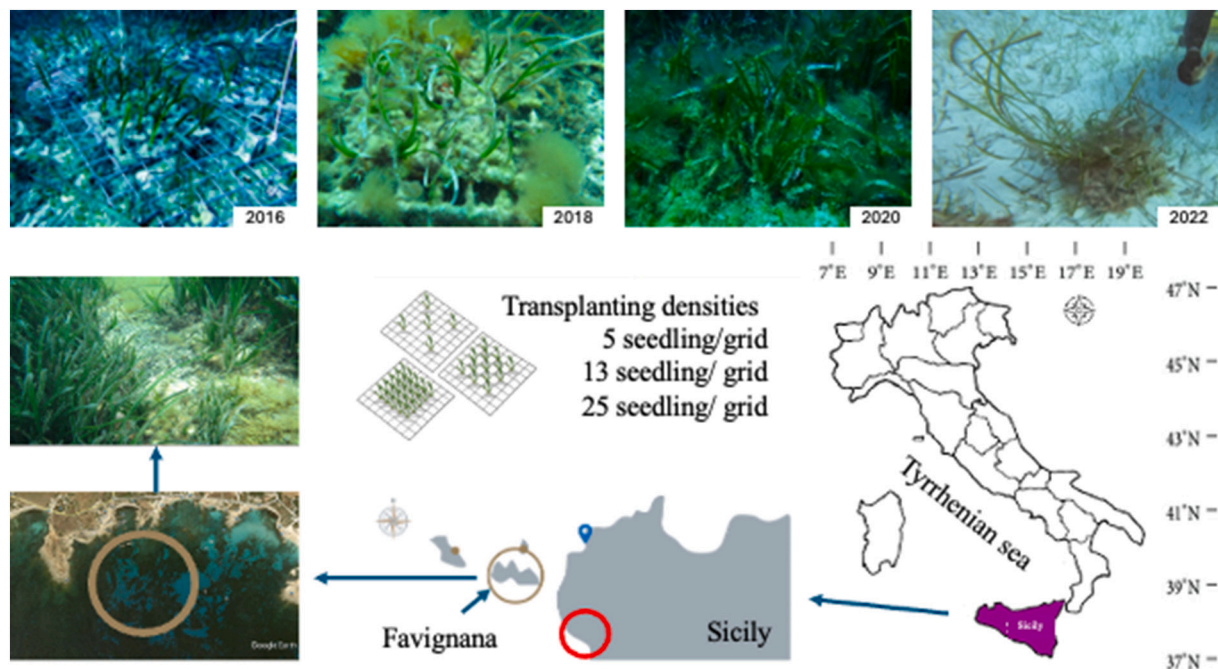
matte was visible, covered the scar. The scar surface appeared flat, with a few shoots of the seagrass *Cymodocea nodosa*. At Site 2, a strip of eroded dead matte approximately  $3 \times 10 \text{ m}$  was evident, with no significant sand cover. The seabed in the scar at this site appeared slightly sloping at an angle of about  $20^\circ$  but remained consistent in elevation with the surrounding substrate.

The tops of the pots were positioned at the same level as the seabed embedding the pots into the dead matte. The embedding process involved inserting the pots approximately 4 cm into the dead matte, which was covered by a more or less thick layer of sediment. This ensured that the pots were in line with the surrounding substrate.

### 2.5. Experimental design and statistical analysis

The experimental design involved the transplant of pots containing seedlings onto the dead *P. oceanica* matte using  $35 \times 35 \text{ cm}$  steel grids with 5 cm mesh. Two hundred and fifty-eight pots were placed by mean of nylon cable ties in 18 grids (6 grids for each of three different densities: 5, 13, and 25 pots per grid, resulting in about 40, 100, and 200/ $\text{m}^2$ , Fig. 1), which were distributed randomly at the two sites. Each site received three grids for each density, secured to the seabed by small steel posts.

The transplantation sites were visually monitored by scuba divers once a year for six years. However, performing non-invasive counts or detailed checks was challenging due to the gradual growth and expansion of the grids with the natural meadow. Only the data gathered during the first 18 month were deemed reliable despite challenges in underwater monitoring. In June 2022, almost six years after the transplantation all the grids were retrieved, and pots were removed and counted. It was observed that all pots had remained in place in their grid. Some of the pots contained no or dead plantlets, but the majority contained alive and healthy plantlets that, once in the laboratory, were rinsed with freshwater and cleaned from all epiphytes or encrusting organisms.



**Fig. 1.** - Seed collection area along the main Sicilian coast and seedling transplanting sites in Favignana Island. The red circle indicates the collection area, while the brown circle indicates the area with the transplanting sites and a detail of the scar. A schematic representation of the three transplanting densities per grid - 5, 13, and 25 seedlings per grid, corresponding to 40, 100, and 200 seedlings per  $\text{m}^2$ , respectively - is shown in the center of the figure. Six grids were placed for each density, three at each site. The first three photos in the sequence at the top of the figure shows a grid shortly after transplantation (2016), 2 years later (2018), and 4 years later (2020). The last photo (2022) depicts a grid that was removed and placed on a sandy seabed 6 years later. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



The surviving plantlets were carefully extracted from the pots, and the number of branches and vertical rhizomes (shoots) produced was counted. Each plantlet was partitioned into leaves, roots and rhizomes for detailed morphological and biometric analysis. Biomasses were measured as dry weight after oven-drying the samples at 60 °C until a constant weight was reached (precision 0.0001 g). The following quantitative morphological variables were measured for each plantlet to evaluate growth performances: number of leaves (NL); leaf length (LL, cm), leaf width (LW, cm), longest leaf length (LstL, cm), total leaf surface (LS, cm<sup>2</sup>), longest root length (LstR, cm), total roots length (TR, m), number of new rhizomes (N\_Riz, sum of orthotropic rhizomes (vertical shoots) that developed from the plagiotropic rhizomes and of plagiotropic rhizomes derived from branching of the plantlets over the six year period), main plagiotropic rhizome length (Riz\_L, cm), leaf dry weight (L\_DW, g), roots dry weight (R\_DW, g), rhizome dry weight (Riz\_DW, g), number of branches (NB). Additionally, the root shoot ratio (RSR) was calculated as the ratio between belowground (root and rhizome) and aboveground (leaves) biomass. The relative growth rate (RGR) index was calculated using the dry weight (Dw) values at transplantation and after six years, as follows:  $RGR = \ln(Dwf) - \ln(Dwi)/t$ , where Dwf and Dwi are the final and initial dry weight of the plantlet and seedling, respectively, and t is the elapsed time, which in this study corresponds to 5 years and 8 months.

A generalized linear model (GLM) was used to test for the effects of pot density on seedlings survival and growing performance. A post-hoc test was performed to determine statistical differences. An ANOVA was performed to detect the effect of Density (3 fixed levels: 40, 100, 200 seedling/m<sup>2</sup>) and Site (2 random levels: Site1 and Site2) on the measured variables and on the belowground (roots and rhizomes) and aboveground (leaves) biomass of the transplanted seedlings. The slopes were compared computing estimated least-squares means for Density and Site factors.

A multivariate approach was used utilising eleven of the fourteen above-mentioned quantitative variables (after the exclusion of the highest correlated ones), which were classified in three different groups (representing: leaves, roots and rhizomes). The group of qualitative variables was composed of densities and sites. Multiple factorial analysis (MFA, [Escofier and Pagès, 1994](#)) was applied to (1) coordinate the analysis of each group within a rigorous and reproducible framework, and (2) provide a clear representation of their associations. The process of conducting an MFA involved several steps. Initially, a separate principal component analysis (PCA) was carried out for each group of quantitative variables. Secondly, each group of the entire matrix was weighed by the inverse of the first eigenvalue of its separate analysis to balance inertia between the different groups and thus balance their influences. This method ensures that all groups are considered on an equal basis. The third step involved performing a global PCA, which analyzed the patterns of different groups of variables in a single factorial space. By assessing the correlation between the global (defined by all variables) and the partial scatter (defined by each group), it was possible to determine whether the structure shown by each axis of the MFA was common to several groups or if it was specific to one group ([Hanafi, 1996](#); [Escofier and Pagès, 1994](#); [Pagès, 2004](#)). All statistical analyses were conducted using the software R ([R Core Team, 2024](#)) and the Emmeans ([Lenth, 2024](#)), Factoextra and FactoMiner packages ([Kassambara and Mundt, 2020](#); [Lê et al., 2008](#)).

### 3. Results

Seed germination in the tanks had a success rate of 80 %, counting 400 viable seedlings four weeks after fruit collection. The survival remained high until the transfer of the pots to Favignana, with 90 % viable seedlings after seven months of cultivation ( $n = 360$ ). No significant differences were observed among the seeds grown in four tanks for any of the measured variables (Table S1 b). On average, at the time of transplantation, the seedlings had a total biomass of  $0.263 \pm 0.073$  g.

The leaf system was well-developed, with  $7.31 \pm 1.19$  leaves of an average length of  $9.03 \pm 1.39$  cm and a leaf surface area of  $43.32 \pm 11.88$  cm<sup>2</sup>. The root system was robust with a total length of  $15.64 \pm 5.36$  cm and adhered firmly to the pebbles within the pots (Table S1 a).

Mortality varied among seedling densities during the first 18 months after transplantation. Approximately 23 % of seedlings at a density of 40 seedlings per m<sup>2</sup>, and 68 % and 82 % of those at densities of 100 and 200 seedlings per m<sup>2</sup>, respectively, were still alive 18 months after transplantation (spring 2018). During the underwater monitoring no macroscopic signs of predation by herbivorous fish (in the study area: only *salema*, *Sarpa salpa*), sea urchin or crustaceans were observed.

After six years at sea, no significant differences ( $p > 0.05$ ) in survival rates of transplanted seedlings were observed between the two sites or across the three density levels ([Fig. 2a](#)). However, a significantly lower survival rate (5.1 %) was recorded at the density of 40 seedlings/m<sup>2</sup> at Site 2 ([Fig. 2a](#)). The highest survival rate (76.9 %) was observed at Site 1 at the density of 100 seedlings/m<sup>2</sup>, with an average survival rate of approximately 38.2 % across sites ([Fig. 2a](#)).

The production of new rhizomes did not differ significantly among the three planting densities ( $p > 0.05$ ). However, rhizome production was significantly lower at Site 2 compared to Site 1 ( $p < 0.05$ ) ([Fig. 2b](#), Table S2 a, b). In contrast, significant differences among the three densities were recorded for rhizome length (RizL) and dry weight (Riz\_Dw), with both variables being higher at higher planting densities compared to the lowest density (40 seedlings per m<sup>2</sup>). Seedlings at Site 1 generally outperformed those at Site 2 for most variables, including NL, LstR, LS, TR, L\_Dw, R\_Dw, and Tot\_Dw. Overall, approximately 9 % of the plantlets exhibited branching. None of the seedlings branched at the lowest density, while the highest branching rate (19 %) was recorded at density 100 at site 1. Each seedling showing branching had on average  $1.2 \pm 1.6$  vertical rhizomes. No significant differences were observed for the RSR variable across densities and sites (Table S2 a, b). The RGR, on the other hand, showed significant differences both among the three densities and between the two sites (Table S3). Specifically, the index was lower ( $0.28 \pm 0.24$ ) at the lowest density compared to medium and high densities ( $0.74 \pm 0.13$  and  $0.82 \pm 0.09$ , respectively) and was higher at Site 1 ( $0.73 \pm 0.19$ ) compared to Site 2 ( $0.50 \pm 0.33$ ).

The relationship between aboveground and belowground biomass was positive for all treatments ([Figs. 3, 4](#)). The slopes for the three densities were 0.354, 0.382, and 0.361, respectively, and did not show significant differences ( $p > 0.05$ ). A local effect was evident when the two sites were compared, as slopes (0.304 and 0.196 at Site 1 and Site 2, respectively) resulted significantly different ( $t_{(14)} = 2.24$ ;  $p < 0.05$ ). Biomass production at Site 2 was significantly ( $p < 0.05$ ) lower than at Site 1, especially in the aboveground compartment ([Fig. 4](#)).

The first two dimensions of the MFA analysis on quantitative morphological variables accounted for 81.80 % of the total inertia (MFA.Dim.1: 65.23 % and MFA.Dim.2: 16.57 %; [Fig. 5a](#)). Four groups of variables were distinguished, including those that described Leaves, Roots, and Rhizomes (quantitative variables) and those that described the transplanting conditions (qualitative variables) ([Fig. 5b, d](#)). The Roots and Rhizomes group was highly correlated with MFA.Dim.1 ( $r^2 > 0.90$ ) and well represented on the first dimension ( $\cos^2 > 0.7$ ), while the Leaves group was correlated and represented the second dimension ( $r^2 > 0.7$ ;  $\cos^2 > 0.15$ ).

The variables RizL, L\_DW, Nriz, R\_DW, TR, LstR positively contributed to MFA.Dim.1, while, LstL Riz\_DW, LL contributed to MFA.Dim.2 ([Fig. 5b, c](#)).

Factor Map ([Fig. 5d](#)) identified segregation between samples, with densities of 100 and 200 seedlings/m<sup>2</sup> being very similar and density of 40 seedlings/m<sup>2</sup> distinct from the others due to differences in growth performance. Additionally, the two sites resulted separated in the plot ([Fig. 5d](#)).

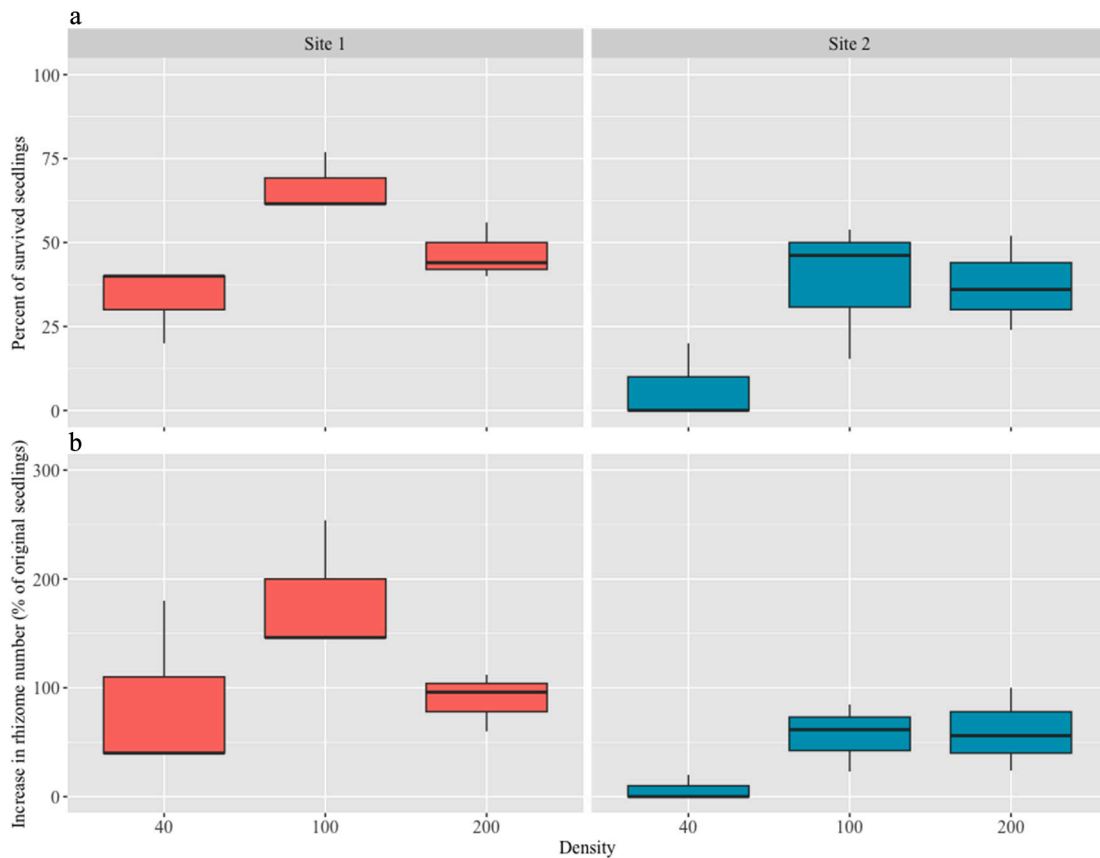


Fig. 2. – a) Percentage of survived seedlings and b) percentage increase in number of both vertical and horizontal rhizomes relative to the original number of seedlings planted at the three densities (about 40, 100, and 200 seedling/m<sup>2</sup>) in the two sites (Site 1, Site 2) after six years.

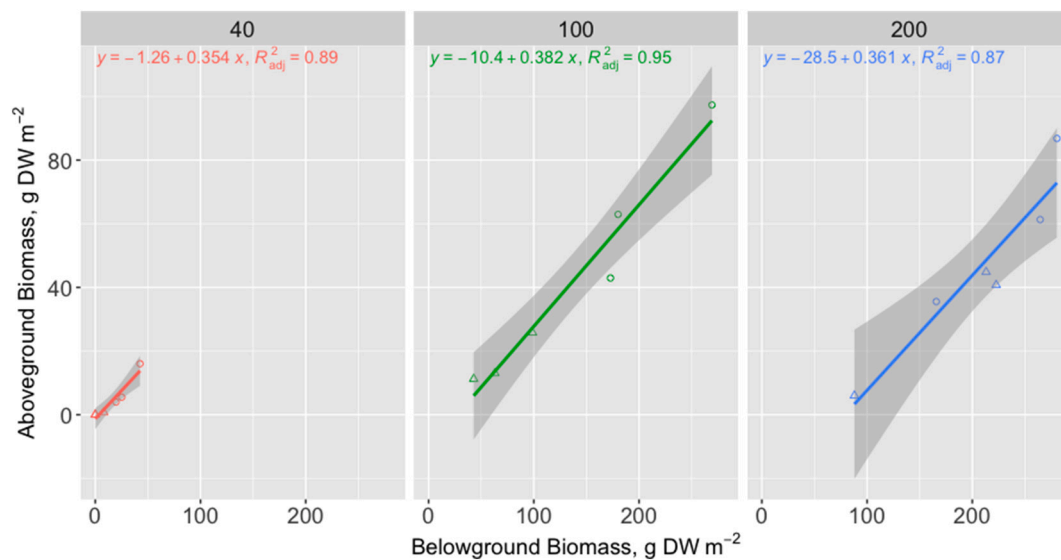


Fig. 3. –Relationship between above- and belowground biomass allocation patterns in seedlings at the three densities. Circles represent seedlings at site 1, Triangles represent seedlings at site 2. Equations are reported in the graphs. 40, 100, 200 correspond to 40, 100, 200 seedlings/m<sup>2</sup>.

#### 4. Discussion

The results from a six year old transplantation of laboratory-grown *Posidonia oceanica* seedlings at sea indicate the feasibility of using the experimental approach adopted in restoring small seagrass patches damaged by boat anchoring.

Our data suggest that an initial density of approximately 100

seedlings/m<sup>2</sup> (i.e., 13 pots per grid) provided the best results with a survival rate of 76.9 %. Although no significant differences attributable to the three densities were detected due to the high variability between grids and sites, lower survival rates were observed at the lower pot density (5 pots per grid, corresponding to ~40 seedlings/m<sup>2</sup>), compared to the higher densities (13 and 25 pots per grid, corresponding to ~100 and ~200 seedlings/m<sup>2</sup>, respectively).

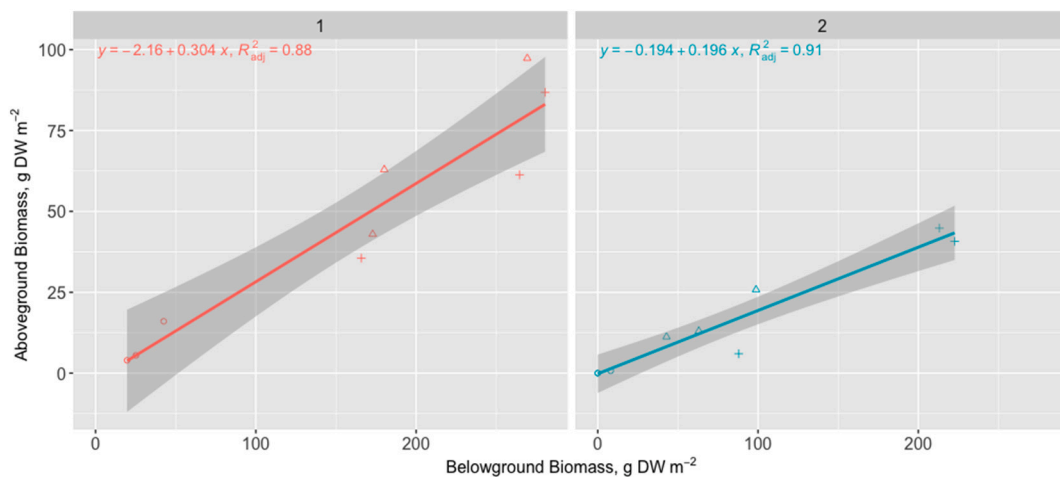


Fig. 4. –Relationship between above- and belowground biomass allocation patterns in seedlings at the two sites (1,2). Circles, triangles and crosses represent seedlings at the three densities of about 40, 100, and 200 seedling/m<sup>2</sup>, respectively. Equations are reported in the graphs.

In grids with the lowest density, pots were arranged far apart and not in contact one another. In contrast, at the highest density, pots were positioned next to each other, whereas at median density the degree of contact among pots was lower and pots were spaced. Our findings suggest a density-dependent facilitation, which seems to be less effective when pots are too closely packed. Very high density might hinder the formation of plagiotropic rhizomes, most likely due to limited space for growth, particularly for the innermost seedlings. On the other hand, positive effects related to plantlet proximity, as hydrodynamic attenuation by shoots, increased nutrient trapping and sediment stabilization, can be lessened at the lowest pot density. However, except for the lowest initial density, the final density of shoots always reached values indicative of a spare meadow, according to the definition by Giraud (1977) (IV type, 150–300 shoots/m<sup>2</sup>).

Current advice in seagrass restoration initiatives suggest the use of early life stages of the plant (Balestri and Bertini, 2003; Bull et al., 2012; Domínguez et al., 2012; Holbrook et al., 2002; Kirkman, 1999; Reed et al., 1998; Ros and Van Katwijk, 2007). Seedlings offer several advantages, including a lower impact on donor meadows, a higher degree of genetic variability, and the potential for preconditioning (priming) to better counter climate change (e.g., Pazzaglia et al., 2022). Additionally, seedling cultivation has significant educational potential, as involving the public and students at the cultivation facilities may raise awareness about the importance of protecting and restoring *P. oceanica* meadows.

Nonetheless, a primary challenge encountered in employing such early life stages for transplantation lies in the limited capacity of propagules to anchor to the substrate during the initial months, often leading to seedling loss (Balestri et al., 1998; Balestri and Bertini, 2003; Cooper and McRoy, 1988). To tackle this issue, various methods have been devised to promote seagrass propagule settlement and plantlet establishment, such as enhancing early root growth (Reed et al., 1998) or employing man-made structures such as nets (Pirrota et al., 2014), iron stakes (Mancini et al., 2022; Mancini et al., 2021), mesh-pots (Terrados et al., 2013; this study), mats (Piazzi et al., 2021) and more recently artificial substrates (Alagna et al., 2020; Zenone et al., 2022).

The mesh-pot method has the advantage of assuring a direct root-sediment connection, thereby mitigating the impact of water currents on belowground structures, as highlighted by Domínguez et al. (2012). In our experiment we filled the pots with pebbles to improve seedling adhesion to the substrate via root hairs (Alagna et al., 2015; Badalamenti et al., 2015; Zenone et al., 2020), increasing the chances for stable settlement before the transplantation phase. The feasibility of the transplanting methodology used in this work is confirmed by the growth performances, with higher RGR values for the higher densities and for site 1, and by a positive relationship between the above- and

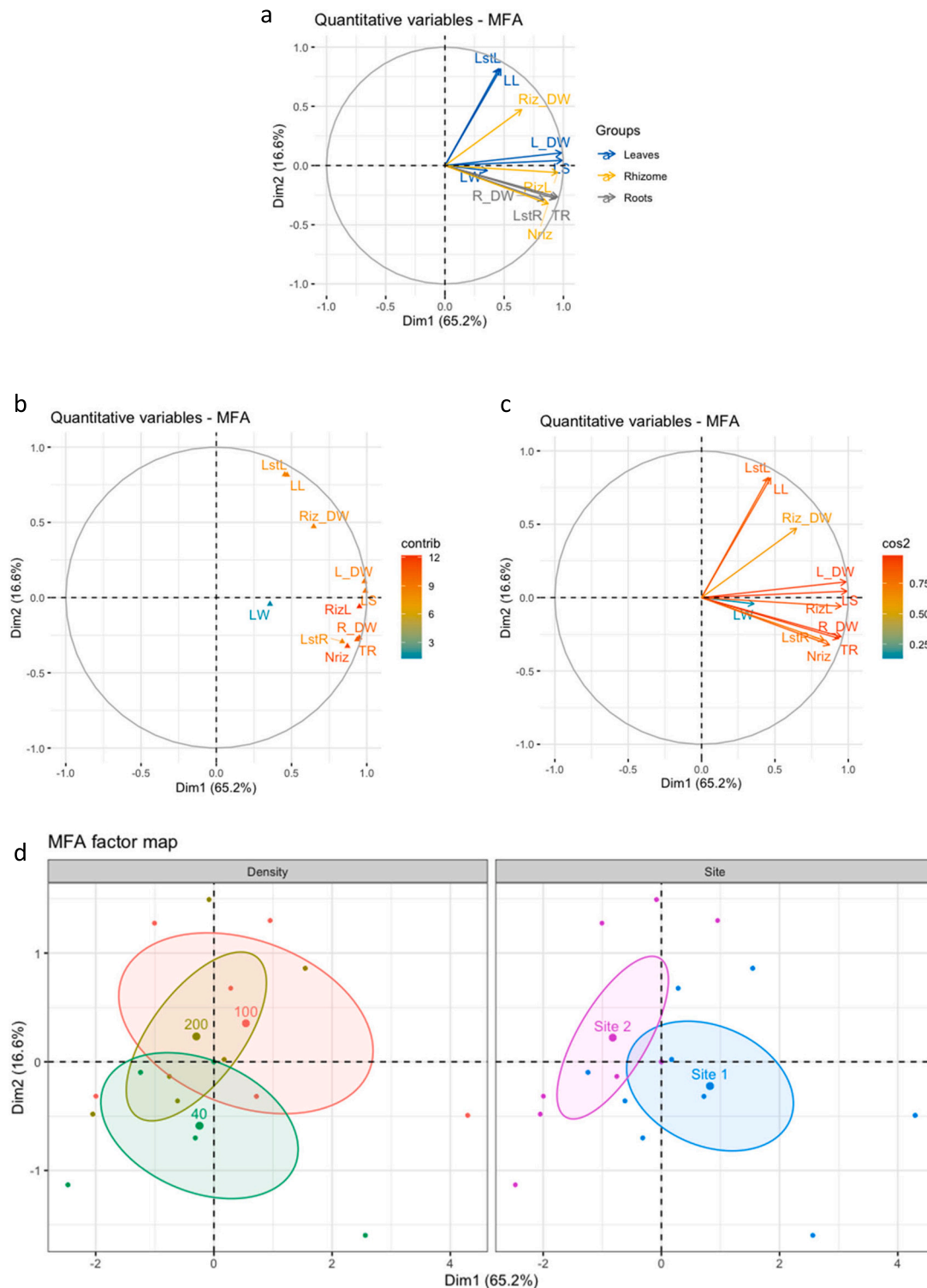
belowground biomass, with no significant differences between the three densities.

Seagrass seed-based restoration techniques show great promise, and it is important to improve our understanding of seedling survival and performance in different environmental contexts for future restoration efforts (Maulidiyah et al., 2024). *P. oceanica* seedlings have been used to study their biology and performance in different environmental contexts (Guerrero-Meseguer et al., 2017). However, few studies have addressed the transplanting of seedlings into the sea and to our knowledge, none have specifically investigated the effect of long-term planting density (Escandell-Westcott et al., 2023). This lack of data makes a direct comparison with our results difficult.

For example, Domínguez et al. (2012) reported a survival rate of 75 % after one year for seedlings planted on dead matte, a value comparable to that observed in our study for seedlings transplanted at medium (68 %) and high (82 %) density after 18 months. Similarly, Terrados et al. (2013) observed an average survival of 44 % for seedlings transplanted at 10 m depth on dead matte three years after transplantation, while none of the seedlings planted on established seagrass survived. At a similar depth, Balestri et al. (1998) and Piazzi et al. (1999) reported survival rates of 69–70 % for seedlings planted on dead matte after 2–3 years. Balestri et al. (2021) also studied the transplantation of seedlings at different densities, with and without the presence of *C. nodosa*, in a mesocosm experiment. They found that all seedlings planted at low density (32 seedlings per m<sup>2</sup>) died within one year. However, survival rates of 63 % and 25 % at medium density (64 seedlings per m<sup>2</sup>), and 92 % and 54 % at high density (128 seedlings per m<sup>2</sup>), were achieved after two years with and without *C. nodosa*, respectively.

Our results agree with those of Balestri et al. (2021), according to whom lower plant density leads to lower survival. Furthermore, the presence of *C. nodosa* positively influenced survival rates. In our study, a density of 100 seedlings per m<sup>2</sup>, comparable to the high-density treatment of Balestri et al. (2021), yielded the best results, confirming that this density appears to be optimal for transplanting *P. oceanica* seedlings. The results of our study confirm previous findings that substrate type, seedling density and the presence of other co-occurring seagrasses can significantly influence survival and growth. Our six-year field evaluation extends these findings and provides long-term insights into factors critical to the success of *P. oceanica* restoration efforts.

The success of an ecological restoration project depends largely on the environmental conditions of the transplant site (Statton et al., 2017). In nature, seedling establishment involves a multi-step process of plant colonization, including seed release, dispersal by various vectors, arrival and retention in a suitable microhabitat, germination, and successful establishment of a new cohort of young plantlets (Culot et al., 2015;



**Fig. 5.** – (a) Correlation between group variables and the factor dimension. (b-c) Contribution of each variable to the dimension and the quality of representation, respectively. The arrows in the graphs indicate the direction of growth of the variables in the factor space, allowing for the identification of which variables have the greatest weight in each component. The centre of the circle of correlations represents the mean of all variables. (d) Contribution of qualitative variables in the segregation of the samples. Abbreviations used are LL = leaf length, LW = leaf width, LstL = longest leaf, LS = leaf surface, LstR = longest root, TR = total root length, Nriz = number of new rhizomes, Riz\_L = rhizome length, L\_DW = leaf dry weight, R\_DW = roots dry weight, Riz\_DW = rhizome dry weight.

Schupp et al., 2010). Among these steps, the anchorage/establishment of seedlings following a dispersal event is increasingly recognized as a crucial stage (Balke et al., 2014; Bouma et al., 2016; Wang and Temmerman, 2013). In a restoration initiative like the small-scale one realized experimentally in this study, most steps are controlled in the

laboratory, with microhabitat selection and establishment - the critical phases of transplantation (Boudouresque et al., 2021; Calvo et al., 2021) - that are human driven.

*Posidonia oceanica*, as other persistent seagrasses, allocates a substantial portion of its energy resources towards the development and



maintenance of below-ground structures, which gives the plant greater physiological resilience. Nevertheless, future studies should evaluate the effect of artificial structures used to anchor the seedlings to the substrate on their growth performance. Due to the introduction of undesirable material into the natural environment, the plastic mesh pots used here are limited to experimental purposes only. For large-scale initiatives potentially involving thousands of seedlings, alternative methods for transplanting already settled seedlings should be employed. An example is the module proposed by [Alagna et al. \(2020\)](#), which unfortunately was not yet fully developed at the time of our seedling transplantation.

In this study, site selection emerged as a confounding variable. Specifically, the two designated transplant sites exhibited variability in seabed features. Site 2 had a steeper slope and was covered by a lower amount of sediment compared to Site 1, which was almost horizontal, covered by a thicker layer of sediment, and colonised by sporadic clumps of the seagrass *Cymodocea nodosa*. This species might have enhanced seedling viability, as suggested by other authors ([Balestri et al., 2021](#); [Piazzini et al., 2021](#)) who highlighted the role of *C. nodosa* in promoting the survival of *P. oceanica* shoots.

Another facilitating factor suggested by qualitative observations carried out in our study was that plantlet grown close to the natural meadow edge at both sites appeared larger than those located away from the meadow edges suggesting a potentially positive role played by conspecific adults. Furthermore, seedling density may have affected the sediment deposition rate, as denser populations with greater leaf coverage result in a more efficient sediment trap ([Hendriks et al., 2008](#); [Koch et al., 2006](#)). The multiple factor analysis confirmed significant differences in seedling performance between density levels, emphasizing the role of seedling density in modifying sediment dynamics and growth performance.

At the best performing site (Site 1), seedlings exhibited larger leaves and greater root production compared to site 2, while vegetative expansion remained consistent across densities, indicating overall a favourable seedling health status. Disparities in growth performance between sites were highlighted by the multiple factor analysis, as well as by variables such as rhizome length and rhizome dry weight, revealing the adverse impact of sediment scarcity and/or the lack of *C. nodosa* on the seedlings transplanted at Site 2. These seedlings exhibited reduced leaf and root production, along with smaller vegetative expansion compared to Site 1. Furthermore, the seedling biomass allocation was significantly different across sites. At Site 2 the plantlets allocated more biomass in the belowground compartment compared to Site 1. A similar pattern was observed in vegetative propagules colonising an artificial substrate made of rubbles, with biomass skewed from shoots to roots in the rubble field, more exposed to hydrodynamics and sediment erosion, compared to the natural meadow growing on mat ([Di Carlo et al., 2007](#)). It can be hypothesized that hydrodynamic conditions at Site 2 were conducive to sediment erosion and nutrient scarcity compared to Site 1, affecting biomass allocation and overall growth performance of plantlets. Furthermore, as suggested by [Balestri et al. \(2021\)](#) the presence of a pioneer species such as *C. nodosa* may have facilitated the establishment and growth of *P. oceanica* at Site 1. Pioneer species are known to play a crucial role in ecosystem engineering, as they can modify the local environment by stabilizing sediments, reducing hydrodynamic forces, and improving microhabitat conditions. These changes likely created a more favourable environment for the successful colonization and development of *P. oceanica*, particularly in the early stages of transplantation.

The ability of seagrass seedlings to root and access essential nutrients contained in the sediment pore water agrees with previous research ([Fourqurean et al., 1992](#)). Sediments serve as a vital source of nutrients for plant growth ([Alcoverro et al., 1995](#); [Hemminga et al., 1991](#)), with grain size serving as an indicator of physical and geochemical characteristics in seagrass habitats ([Erfteimeijer and Koch, 2001](#)). Nevertheless, some authors reported a negative effect of excessive sedimentation on *P. oceanica* meadows, particularly when sediment rate exceeds rhizome

growth ([Manzanera et al., 2011](#); [Marba and Duarte, 1994](#)). [Ceccherelli et al. \(2018\)](#) showed that a sediment layer exceeding a certain threshold, hence partially burying the plant, affects negatively the survival of the plant itself.

## 5. Conclusion

Mitigating the deleterious effects of boat anchoring on seagrass meadows should be a paramount concern, particularly in light of the recent “Restoration Law” issued by the EU Parliament. Small-scale interventions, such as the one proposed here, may have the potential to curtail the impact of erosive *intermattes*.

In conclusion, based on six years of observation and monitoring of seedlings cultivated in controlled facilities during their early life stages and subsequently transplanted into the natural environment, our medium-term results support the viability of this approach for restoring small portions of damaged *P. oceanica* meadows, suggesting that the density at which seedling are transplanted matters. While this study was not designed for restoration purposes, it offers valuable insights into the conditions under which seedling-based transplantation can be effective, particularly in areas with abundant seed availability, and lays the groundwork for scaling and refining the method.

Our findings suggest that achieving a state akin to that of a healthy meadow may require a long period of time, possibly longer than the 10-year timeframe highlighted by [Bacci et al. \(2024\)](#) for detecting recovery of *P. oceanica* shoot density in large-scale transplantation realized using orthotropic rhizomes as transplant units. This projection underscores the need for continued vigilance and long-term management strategies to ensure the sustained recovery and enhance the resilience of the transplanted meadow. Small-scale transplantations repeated annually for several years, together with improved meadow management and an increased awareness in boaters, may contribute to enhance the resilience of mechanically damaged meadows.

## CRedit authorship contribution statement

**Arturo Zenone:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **V. Maximiliano Giacalone:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Marco Martinez:** Methodology. **Carlo Pipitone:** Writing – review & editing, Conceptualization. **Adriana Alagna:** Writing – review & editing. **Eduardo Infantes:** Writing – review & editing. **Giovanni D’Anna:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Fabio Badalamenti:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

Fabio Badalamenti reports financial support was provided by Government of Italy Ministry of Education, University and Research (MIUR). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We would like to thank Giuseppe Di Stefano for his invaluable assistance in fruit collection. We also thank the staff of the Egadi Islands Marine Protected Area (AMP “Isole Egadi”) for their support during field activities. This research was partially funded by the Marine Hazard project (PON03PE\_00203\_1), supported by the Italian Ministry of Education, University and Research (MIUR). AA and AZ were funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of the Italian Ministry



of University and Research funded by the European Union – NextGenerationEU. Project code CN\_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP C63C22000520001 (SZN) and B83C22002930006 (CNR), Project title “National Biodiversity Future Center– NBFC”.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2025.111032>.

## Data availability

Data will be made available on request.

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