

COLONIZATION OF TRANSPLANTED *POSIDONIA OCEANICA*: UNDERSTANDING THE SPATIAL DYNAMICS THROUGH HIGH-SPATIAL RESOLUTION UNDERWATER PHOTOMOSAICS

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Abstract – Following the restoration of a *Posidonia oceanica* meadow impacted by the Concordia shipwreck, we investigated the spatial dynamic of the most important and protected Mediterranean endemic seagrass over a two-year period applying three spatial metrics: number of patches, mean patch size and total cover. By means of underwater photomosaics, we noticed a diminution in the number of patches in favour of the mean size and total cover. The outcomes showed that, under suitable environmental conditions, *P. oceanica* colonizes rapidly the dead *matte* substrate. This study underlines the importance of considering the spatial dynamic of transplanted seagrasses in monitoring programmes and gives new insights on the progression rate of transplanted *P. oceanica*.

Introduction

The coastal zone constitutes an interface ecosystem between the land and sea and represents one of the most important contexts in which human activity, economy, ecology, and geomorphology interact. Seagrasses are a mixed group of flowering plants living in shallow coastal marine and estuarine environments worldwide, thriving both on soft and rocky bottoms [1].

P. oceanica is the most important and widespread endemic seagrass in the Mediterranean Sea capable of forming extensive meadows from the sea surface up to 45 meters depth. Due to its sensitivity to human-induced alterations and thanks to its ecological, physical, economic, and bio-indicator roles [2], is protected at both species and habitat level by national and international directives and legislations. Despite the legal framework and protection measures, since the end of last century *P. oceanica* meadows are rapidly declining mainly due to human activities and climate changes [3].

Due to the slow growth rate and the rare sexual reproduction, the damaged *P. oceanica* meadows are unlikely to recolonize naturally. Hence, when the regression factors are removed, and the pre-disturbance environmental features are re-established, active transplantation represents an appropriate intervention to restore the seagrass beds and speed up the recovery process.

Monitoring of transplanted *P. oceanica* has been so far relying on the structural and functional features of the phanerogam, whereas to date the spatial dynamic is not considered.

Indeed, despite the spatial dynamic of natural *P. oceanica* meadows are well described [4], the expansion rate of the transplants is still lacking.

High-resolution underwater photomosaics have been increasingly employed in marine research programmes and monitoring activities thanks to the imagery's quality, low operational costs, fast application, and repeatability. Photomosaics have been used to track the spatial dynamic of natural meadows [5-6] and coralliferous reefs [7-8], to map and classify ecologically sensitive habitats [9-11], nowadays, they are frequently applied in studying the restored seagrass beds [12-15]. Hence, photomosaics represents an important monitoring tool that can be rapidly and frequently applied for sequential surveys over vast areas of restored seagrass to study the spatial dynamic of the transplants and to detect at fine scale both increases and declines in the seagrass cover.

The present study reports the spatial dynamic of *P. oceanica* transplanted during a large-scale seagrass restoration in a previously disturbed area of the Tyrrhenian Sea. This area is located on the site of the Concordia shipwreck and has been subjected to multiple disturbances resulting from the 2012 shipwreck and its removal, which have led to the loss of a well-preserved *P. oceanica* meadow [16-18].

After the wreck removal and three years of remediation activities, any disturbances that caused *P. oceanica* loss were removed, and the phanerogam regression ceased. Since the regression factors were removed and the environmental features were re-established, a pilot study focused on a *P. oceanica* experimental transplantation was carried out in 2016 for designing a specific protocol to be applied at a broader scale [19]. This study laid the basis for a large-scale *P. oceanica* transplantation planned to speed up the recovery of the meadow impacted by the Concordia shipwreck and its removal [20].

Hence, following the large-scale transplantation, we evaluated over 1149 m² and during a 2-year period, by means of high-spatial resolution underwater photomosaics, the spatial dynamic of transplanted *P. oceanica* assessing the number of patches, mean patch size and total cover.

Materials and Methods

Study area:

Fieldwork was carried out on the east side of Giglio Island (central Tyrrhenian Sea, Italy), inside the restricted area interested by the Concordia shipwreck (upper panel of Figure 1). The *P. oceanica* meadow settled within the area was mechanically and physically affected by the shipwreck and its removal, leading to the disappearance of 8427 m² of *P. oceanica*, and leaving on the seabed a dead *matte* substratum from 5 to 35 meters depth (olive green tone in the upper panel of Figure 1) [16-18].

Since the regression factors were removed and the physico-chemical parameters of the water column were re-established, a transplantation area of 2048 m² was selected within the 8427 m² of dead *matte*, extended from 10 to 23 meters depth and previously colonized by *P. oceanica*, to perform a large-scale restoration [20] with the methods proposed in [19].

Within the 2048 m², three transplantation areas (red polygons and labels A1 – A3 in the lower panel of Figure 1) were selected, for a total extension of 1149 m², where performing the investigation.

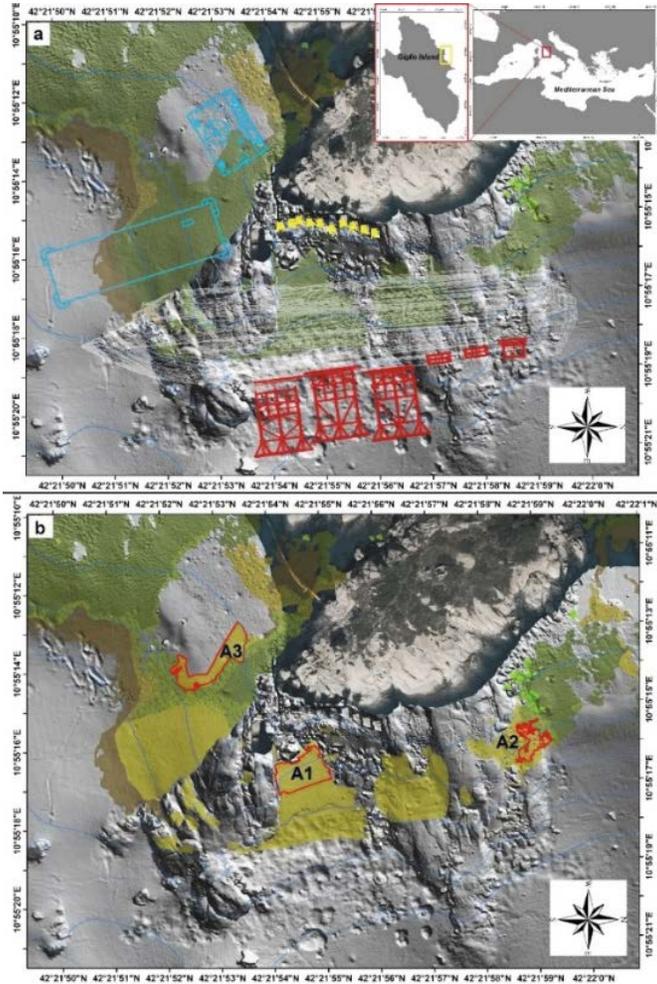


Figure 1 – Study area where the investigation was carried out. Panel a) Transplantation was performed within the Concordia shipwreck area. Both the 2012 shipwreck (grey outline) and its removal operations (supporting vessels in light blue, platforms in red, anchor blocks in yellow) led to the loss of a well preserved *P. oceanica* meadow (green hues). Panel b) The aforesaid events left on the seabed a dead *matte* substratum (olive-green colour). Three transplantation areas (red polygons and labels A1 – A3) were selected to carry out the investigation.

Photogrammetric surveys and spatial dynamic:

High resolutions (centimeter scale) underwater photomosaics were performed to assess the spatial dynamic of the newly transplanted *P. oceanica*. Photomosaics were

performed in 2019, 2020, 2021 every June and October, respectively before and after the beginning of the transplantation activities, according to [14-15].

Next, the high resolution photomosaics were imported and analysed through Geographical Information System (GIS) software. The transplanted *P. oceanica* patches, represented by a single cutting if separated at least 5 cm from the surrounding fragments or aggregation of cuttings instead, were manually outlined by the freehand drawing tool in ArcGIS10.2.2 and the spatial metrics i) number of patches (NumP), ii) mean patch size (MPS) and iii) total cover (TC) were calculated in ESRI ArcMap 10.2.2 by using the Attribute Table and the Calculate area tool included in the Spatial statistic toolbox. NumP and MPS represented respectively the amount of *P. oceanica* patches and their average size (expressed in m²), TC is defined as the sum of all the patches size (in m²). The spatial metrics were calculated every year in all the areas from the photomosaic performed in October.

Data analysis:

MPS spatial metric, calculated separately for each transplanted area, was tested for the time effect (3 levels for area 1: 2019, 2020, 2021; 2 levels for areas 2 and 3: 2020, 2021) with Generalized Linear Models (GLM) selecting a gaussian distribution. The best models were selected according to the Akaike Information Criterion (AIC) value, and a significance level of 0.05 (P-value < 0.05) was chosen within each regression model. Data were analyzed in the R platform version 4.0.2.

Results

The three spatial metrics showed similar patterns during the study period, that is a diminution in the number of patches (NumP) in favour of their mean patch size (MPS) and total cover (TC) (Figures 2-3).

At the end of the transplantation, in October 2019, the area 1 was characterized by 2347 NumP of *P. oceanica* with a MPS \pm SD of 0.0176 ± 0.0169 m² and a TC of 41.36 m². Over the study period, the NumP decreased whereas both the TC and MPS significantly increased (Table 1) showing in October 2021, after 2 years from the transplantation, 1719 NumP with a MPS of 0.0309 ± 0.0316 m² and a TC of 53.20 m² (Figure 2, panel a). Considering the ratio between TC of patches and transplanted surface, from 2019 to 2020 and from 2020 to 2021 the area 1 showed an increase respectively of +0.6 % and +1.6 % (Figure 2, panel a).

At the end of the transplanting activities, in 2020, the areas 2 and 3 were respectively characterized by 799 and 1883 NumP, 0.0263 ± 0.0185 m² and 0.0158 ± 0.0109 m² MPS, 21.06 m² and 29.76 m² of TC (Figure 2, panels b and c). After one year from the transplantation, the area 2 reduced both the NumP (695) and the total area (19.56 m²) whereas the MPS did not significantly increase its value (0.0281 ± 0.0225 m²) (Figure 2, panel b) (Table 1). Considering the ratio between TC of patches and transplanted surface, from 2020 to 2021 area 2 showed a diminution of -0.7 % (Figure 2, panel b).

The area 3 reduced the NumP (1717) while significantly increased both the MPS (0.0237 ± 0.0206 m²) and the TC (40.69 m²) (Figure 2, panel c) (Table 1). Considering the ratio between TC of patches and transplanted surface, from 2020 to 2021, area 3 showed an increase of +2.5 % (Figure 2, panel c).

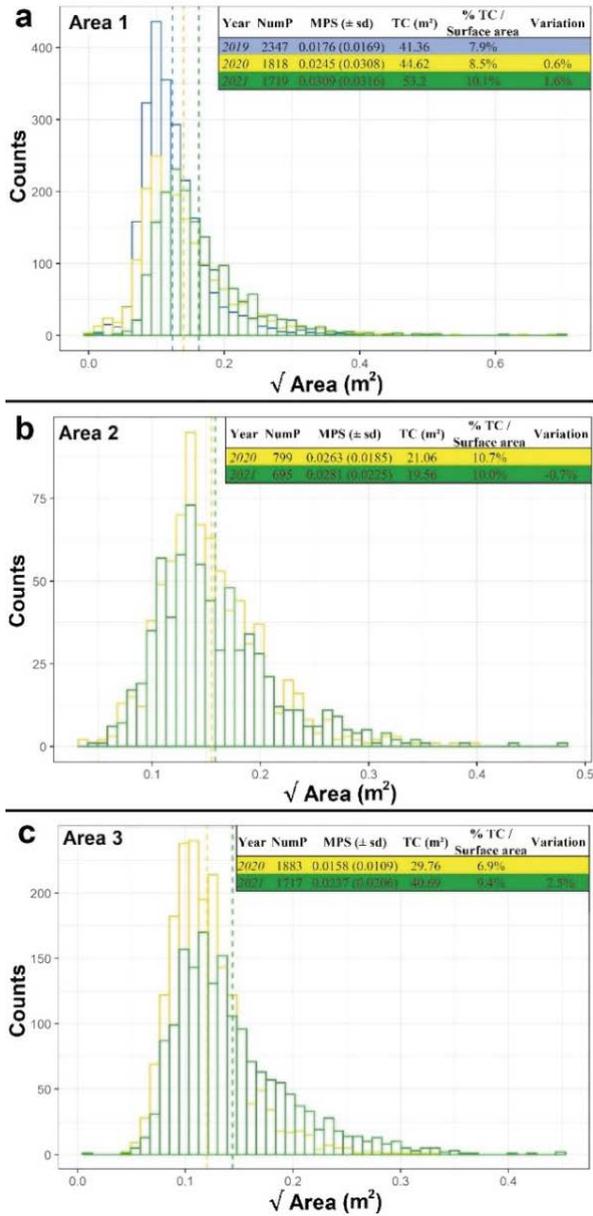


Figure 2 – Size frequency distribution for transplanted *P. oceanica* patches within the 3 areas. Spatial metrics of area 1 are reported in panel **a** (2019 in blue, 2020 yellow, 2021 green), area 2 in **b** (2020 yellow, 2021 green) and area 3 in **c** (2020 yellow, 2021 green). NumP refers to the number of patches, MPS to their mean size, TC to total cover.

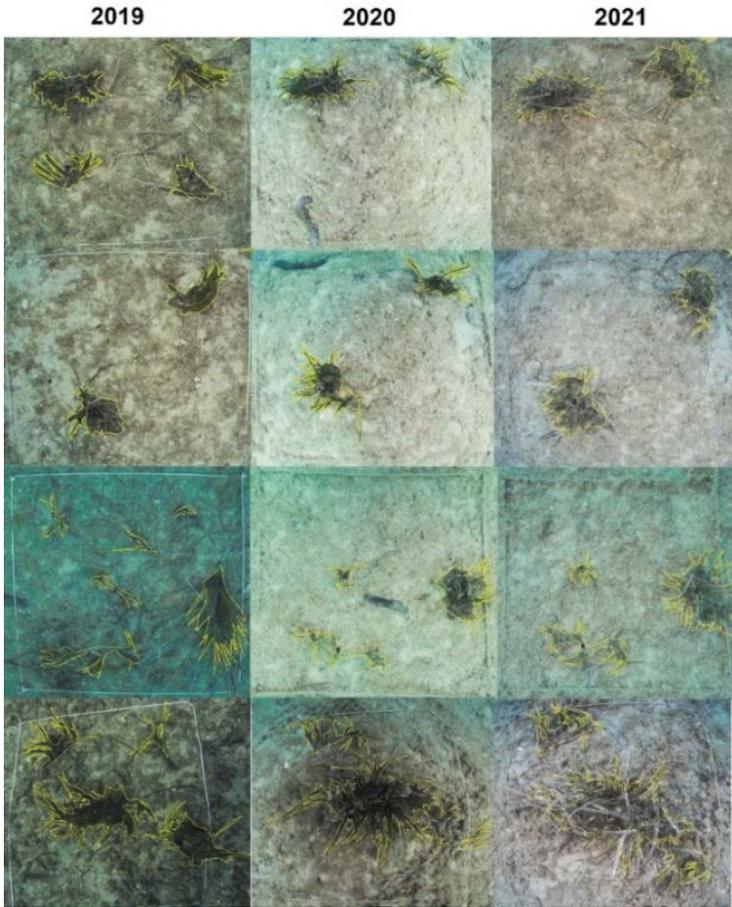


Figure 3 – Temporal evolution of transplanted *P. oceanica* patches within four fixed monitoring squares.

Table 1 – Results from the best GLM fit for mean patch size (MPS) within the 3 areas. The coefficients of effects are reported with Standard Error (SE) in the bracket. The intercept coefficient represents the estimated value in 2019 for area 1 and in 2020 for areas 2 and 3.

Mean Patch Size (MPS)								
Formula: Patch size (m ²) ~ Time								
AREA 1			AREA 2			AREA 3		
Effect	Coefficient	P	Effect	Coefficient	P	Effect	Coefficient	P
Intercept	0.0176 (0.0005)	***	Intercept	0.0264 (0.0007)	***	Intercept	0.0158 (0.0004)	***
Year (2020)	0.0069 (0.0008)	***	Year (2021)	0.0018 (0.0011)	n.s.	Year (2021)	0.0079 (0.0005)	***
Year (2021)	0.0133 (0.0008)	***						
AIC: -26025			AIC: -7369.5			AIC: -19414		

Significant codes: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. $P > 0.05$

Discussion

The Costa Concordia shipwreck and its salvaging traumatically affected the *P. oceanica* meadow established within the wreck area, leading to its regression [16-18]. After the wreck removal and the cleaning operations, any source of disturbance linked to the phanerogam disappearance was eliminated and the regression ceased. Thus, once re-established the natural environmental features required for the seagrass's survival and growth, an experimental investigation was carried out focused on a *P. oceanica* transplantation and aimed at designing new strategies concerning seagrass restoration. The experimental outcomes proved that the impacted area was suitable for a larger scale restoration and highlighted the feasibility in using planting material produced by boat anchoring or storms (not requiring a donor site and the consequent damage) fixed with chemo-degradable iron stakes [19]. This evidence laid the basis for a restoration upscaling to speed up the recovery of the *P. oceanica* meadow lost after the Concordia shipwrecking [20]. The outcomes confirmed that our protocol could be efficiently applied at larger scales, showing diminutions in cuttings' survival and shoot density over the first year, followed by stability in the number of living cuttings and increases of leaf bundles.

Despite the expansion rates of several transplanted seagrasses are known, such as *Posidonia australis* [21], *Halodule wrightii* [22-23], *Zoostera marina*, *Z. noltii* and *Cymodocea nodosa* [24], quantitative data regarding *P. oceanica* are still lacking.

The present study, although performed over a short-term period, gave new insights on the dynamic of transplanted *P. oceanica*. Despite *P. oceanica* is a slow-growth species, in our study we observed an expansion process occurring in the transplanted patches in the first year. During the study period, the number of patches diminished (especially the smallest ones) in favour of their mean size and total cover. During the first year, we observed an expansion rate ranging from +0.6 % (within area 1) to +2.5 % (in area 3), although a reduction of -0.7 % was also highlighted (in area 2). The natural progression rate of a *P. oceanica* meadow margin owing to plagiotropic rhizomes is estimated to be +2.5 % each year [4]. Hence, the progression rates highlighted in our study were in line with those observed for the re-colonization of natural *P. oceanica*. Under suitable environmental conditions for the survival and growth, especially on dead *matte*, *P. oceanica* (both natural and transplanted) can re-colonize all the bare substratum by the progression of its plagiotropic rhizomes. The colonization-predisposition of transplanted *P. oceanica* was also highlighted by [12] and [19] through the higher primary production and leaves growth of the transplanted plants if compared with the data coming from the natural plants.

Despite this first evidence, a longer monitoring period, at least 5 years, could give more robust and concrete outcomes, also considering the slow rhizome elongation rates of *P. oceanica*. A longer time period may also allow detection of potential scenarios of seagrass dynamics not highlighted in shorter-term studies such as increase or decrease in the transplanted seagrass cover [22]. Furthermore, data on spatial patterns of expansion deriving from lengthened surveys may also be used for developing statistical models useful for predictions of longer-term growth patterns [22].

Regarding the application of non-destructive monitoring methods, the use of high-spatial-resolution underwater photomosaics let the scientist study the structural dynamics of the transplanted *P. oceanica* over vast areas. Applying this rapid, efficient, and low-cost technique to seagrass restoration management could assist during the transplantation

activities and could give, over time, new insights into both the colonization processes and the spatial dynamics of transplants. Hence, considering the knowledge gaps in transplanted seagrass dynamic and that the restoration efforts are growing worldwide, the transplants spatial dynamic merits inclusion in metrics of newly created habitat to be investigated and monitored over time.

Lastly, this study adds a further contribution to seagrass restoration techniques as expected for the “UN Decade on Ecosystem Restoration” and the EU biodiversity Strategy for 2030, aiming at restoring ecosystems across land and sea, especially those with considerable value in terms of goods and services such as seagrasses [25].

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