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Transplantation assessment of degraded *Posidonia oceanica* habitats: site selection and long-term monitoring

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Abstract

A model developed for *Zostera marina* was adapted and used to select suitable areas for *Posidonia oceanica* transplantation in the Gulf of Palermo, where recent rehabilitation programmes have reduced human pressure. This model consists of three steps: (1) habitat selection, by calculation of the Preliminary Transplant Suitability Index (PTSI); (2) field assessments and test-transplanting, to evaluate site suitability and to estimate the effects of tearing on transplant units (about 50%); (3) identification of suitable restoration sites, by calculation of the Transplant Suitability Index (TSI). A new parameter was added to the literature model: the number of grids detached, which is linked to factors (hydrodynamic regime, anchoring, fishing) that have a potentially significant effect on the final outcome of the transplant. Only one site (TSI = 16) in the Gulf of Palermo was indicated as potentially suitable for restoration with *P. oceanica*. At this site, a transplant of 40 m^2 was implemented. From 2008 to 2014, transplant effectiveness was evaluated in terms of establishment, detachment and mortality of cuttings and shoot density. Long-term monitoring (6 years) allowed us to detect changes in the structural conditions of the transplanted meadow and to identify the possible turning point in *P. oceanica* recovery (2 years after transplanting). Moreover, 6 years after transplantation, the transplant shoot density of the *P. oceanica* meadow is about 16% greater, with a mean and a maximum value of 11.6 and 17 shoots per cutting, respectively.

Keywords: Seagrass, restoration, *Posidonia oceanica*, site selection, transplant, Mediterranean Sea.

Introduction

Seagrasses are a crucial element of coastal ecosystems due to their different roles, namely, primary producers, substrata for many species, water quality indicators, shoreline erosion protectors (Hemminga & Duarte, 2000) and long-term carbon store (Kennedy & Bjork, 2009). Since 1990, a global seagrass loss of 7% yr^{-1} has been estimated (Waycott *et al*., 2009), compared to 0.5% yr^{-1} for tropical forests (Achard *et al*., 2002; Duarte, 2008), and this represents a major loss of natural carbon sinks for the biosphere (Duarte *et al*., 2010). This seagrass decline is imputable to both natural disturbances and human activities (Boudouresque *et al*., 2009). However, human population expansion is currently the most serious cause of seagrass habitat loss, as it has led to an increase in anthropogenic input to coastal waters (Short & Wyllie-Echeverria, 1996).

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica* (L.) Delile forms dense, wide meadows from the surface down to 45 m and covers 2-4% of the seabed in the basin (approximately 38,000 km^2; Pergent *et al*., 1997). This species is a valuable biotic component of the littoral zone and contributes significantly to primary production, with about 3,500 million tons of carbon produced annually (Pergent *et al*., 1997). *P. oceanica* is the only marine phanerogam able to form *matte*, a biogenic structure resulting from the growth of plagiotropic and orthotropic rhizomes intertwined with roots and autochthonous and allochthonous detritus (Boudouresque & Meinesz, 1982). Moreover, *P. oceanica* beds are considered to be good indicators of water quality (Pergent-Martini *et al*., 2005).

Despite their importance, over recent decades, many *P. oceanica* meadows have disappeared or have been altered (Boudouresque *et al*., 2009). Due to coastal human activities, between 13% and 50% of the beds have markedly regressed in terms of areal extent or have disappeared since the seventies, and the remaining ones have suffered density and coverage reductions in the last 20 years (Marbà *et al*., 2014). Thus, recovery planning is required to arrest and reverse the predicted decline of the species by the middle of this century (year 2049 ± 10, Jordà *et al*., 2012). Seagrass habitat recovery involves identifying and limiting and/or eliminating the causes of degradation (Hobbs & Norton, 1996). The natural recovery of seagrass can lead to restoration of the original environmental conditions of a seabed. However, seagrasses such as *P. oceanica* are so notoriously slow-growing that natural recovery may take anywhere from dozens to hundreds of years (González-Correa *et al*., 2005). Moreover, low flowering (Díaz-Almela *et al*., 2006) and high rates of fruit abortion and predation (Balestri & Cinelli, 2003) further limit the resilience of this species.

Transplantation is considered a possible option for speeding up seagrass habitat restoration (Fonseca *et al.*, 1994). Since the 1970s, restoration with *P. oceanica* in the Mediterranean Sea has been carried out to assess various transplanting and seedling techniques. Transplant success was found to be influenced by the nature of the substratum, with dead *matte* habitat showing higher survival and growth rates than sand, pebble and rock (Terrados *et al.*, 2013). Also, horizontal rhizomes showed higher survival and growth rates than their vertical counterparts (Molenaar *et al.*, 1993; Piazzi *et al.*, 2000). The use of vegetative fragments as planting units has proved more effective than seeds, which are less available (Balestri & Cinelli, 2003; Díaz-Almela *et al.*, 2006; Terrados *et al.*, 2013). Transplant donor populations of *P. oceanica* with the highest genetic variability showed the best growth performance (Procaccini & Piazzi, 2001).

A number of studies have revealed the great importance of careful habitat selection for seagrass transplantation (Fonseca *et al.*, 1998; van Katwijk *et al*., 2009). Geomorphological factors such as sediment features, nearshore hydrodynamics, and nature of the substratum are very important for the selection of seagrass transplantation sites. Several studies investigated the complex interaction between these factors and both the architecture and the state of health of *P. oceanica* meadows (De Falco *et al*., 2000; Folkard, 2005; Infantes *et al*., 2009, 2011; Manca *et al*., 2010; Vacchi *et al*., 2012, 2014), but no model has ever been set up taking them into account for the realization of a *P. oceanica* transplant. Recently, qualitative and quantitative models have been developed to assess both the suitability of the area to recover and the potential for successful transplantation with *Posidonia australis* J. D. Hooker (Campbell, 2002) and *Zostera marina* Linnaeus (Short *et al.*, 2002). In particular, the model developed by Short *et al.* (2002) synthesizes the available historic and literature-based information, reference data and simple field measurements to identify and prioritize locations for large-scale *Z. marina* restoration.

Another focal point of marine restoration projects is monitoring of transplant performance. The main objective of many restoration activities is to establish system resilience (Thom *et al.*, 2012). For several authors "a restoration process re-establishes a dynamically stable ecosystem that is fully developed structurally and functionally and is resistant and resilient to disturbances" (Thom *et al.*, 2012 and references therein). However, although establishing a resilient habitat is a goal implicit in many restoration plans, only a few seagrass system restoration projects have considered a long enough monitoring period to assess system recovery after implementation; most published projects had short monitoring periods (<1 year) (Cunha *et al.*, 2012).

In this context, this study had two main aims: i) to select a suitable site for *P. oceanica* transplanting in a previously degraded habitat using the modelling approach; ii) to achieve a medium-scale implant of *P. oceanica* within the selected area and apply a long term monitoring programme to evaluate its performance.

Materials and Methods

Study area

The study area is the Gulf of Palermo, along the north-western coast of Sicily (Fig. 1). Since 1950, the coastal zone near Palermo has been exposed to multiple pollution sources due to chaotic city planning, improper disposal of waste and untreated wastewater. A trophic alteration and high concentrations of P-PO_4 (3-45 mmol·l^{-1}) and chlorophyll *a* (2-35 mgl^{-1}) were recorded during the summer season (Calvo *et al.*, 1994). As a consequence, the *P. oceanica* meadow growing in this area regressed and residual patches were detected from 11 to 21 m depth (Tomasello *et al.*, 2007). In recent years, there has been a marked improvement in water quality in the Gulf of Palermo with TRophic IndeX (TRIX) levels from good to high (ARPA Sicilia & Università degli Studi di Palermo, 2006). This was due to reconstruction work in the entire metropolitan area, focused mainly on purification, proper wastewater disposal, and rehabilitation and stabilization of the rubble landfills along the coast.

Site selection

In order to identify suitable areas for *P. oceanica* seagrass transplants in the Gulf of Palermo, we used the model proposed by Short *et al.* (2002) for *Z. marina*. This model consists of three steps: (1) habitat selection; (2) field assessments and test-transplanting; (3) identification of suitable restoration sites.

Habitat selection

The first step of the site-selection model, namely, identification of a potential seagrass habitat, involves calculating the Preliminary Transplant Suitability Index (PTSI) (Short *et al.*, 2002). This is a multiplicative index based on environmental information from literature sources or, if these are not available, from field observations. In this study, most of the parameters and relative ratings for PTSI calculation were according to Short *et al.* (2002) (Table 1). However, some modifications were made, relating to the characteristics of *P. oceanica* and the Mediterranean habitat in which it grows. In particular, unlike *Z. marina*, *P. oceanica* is able to form *matte* (Boudouresque & Meinesz, 1982), to grow on different types of substrata (Hemminga & Duarte, 2000) and is a slow-growing species (González-Correa *et al.*, 2005).

For historical seagrass distribution, we used available *P. oceanica* and/or dead *matte* distribution maps, as they provide clear evidence of the past presence of meadows (Leriche *et al.*, 2004; Table 1).

The parameter of proximity to a natural seagrass bed

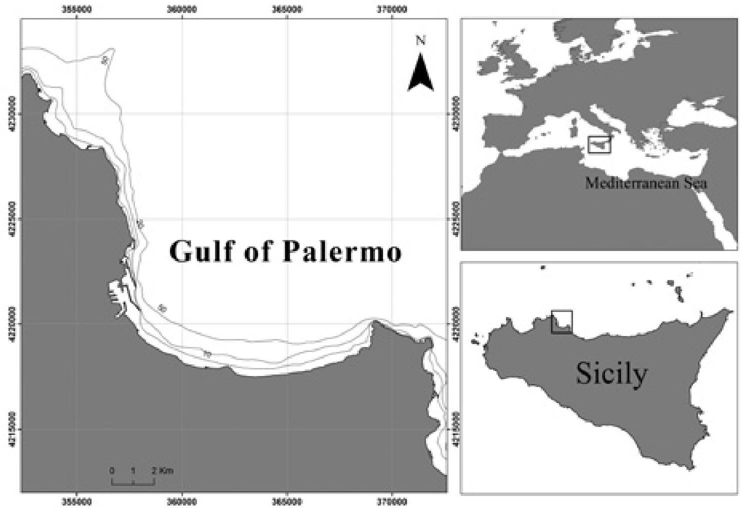

Fig. 1: The Gulf of Palermo.

takes into account the maximum distance at which the plant could naturally recolonize the area (Short *et al*., 2002). The distance estimated for *P. oceanica* is 70 m (Migliaccio *et al*., 2005). Thus, only potential transplant sites located more than 70 m from an existing meadow were considered.

The highest PTSI score for the sediment parameter was assigned to "sand with *Cymodocea nodosa*", this being described as a pioneer species, which creates consolidated organic matter on the sediment for later colonization by *P. oceanica* (Molinier & Picard, 1952). In contrast, seagrasses grow with difficulty on silt because of poor anchoring and possible increase in water turbidity. Although *P. oceanica* is also able to grow on rock (Di Maida *et al*., 2013), rock was excluded from the model because it is very difficult to anchor cuttings on this type of substratum.

The water depth parameter was rated by identifying local upper and lower depth limits for seagrass beds in the Gulf of Palermo (ARPA Sicilia & Università degli Studi di Palermo, 2006). Average bed depth in the area was estimated at 14 m.

We characterized water quality by detecting TRIX

Table 1. Parameters and corresponding ratings used for calculation of PTSI. Data sources are in brackets.

Parameter	**PTSI rating**	**References of general model**	**References of present study**
Historical *P. oceanica* distribution (distribution maps)	1 = previously unvegetated 2 = previously vegetated or dead *matte* presence	Short *et al*., 2002; Leriche *et al*., 2004	Ministero dell'Ambiente, 2002
Current *P. oceanica* distribution (distribution maps)	0 = currently vegetated 1 = currently unvegetated	Short *et al*., 2002	Ministero dell'Ambiente, 2002
Proximity to natural *P. oceanica* bed (map and GIS calculation)	0 for < 70 meters 1 for > 70 meters	Migliaccio *et al*., 2005	
Sediment (distribution map)	0 = rock and silt 1 = sand 2 = sand with *Cymodocea nodosa*	Molinier & Picard, 1952	Ministero dell'Ambiente, 2002
Water depth (map and GIS calculation)	0 = deeper than lower limit or shallower than upper limit of local beds[a] 1 = between the upper limit and the average depth – s.d. of local beds[a] 2 = average depth ± s.d. of local beds[a] 1 = between the lower limit and the average depth + s.d. beds[a]		ARPA Sicilia & Università degli Studi di Palermo, 2006
Water quality (distribution map or GIS calculation)	0 = poor 1 = average 2 = high	Vollenweider *et al*., 1998; D. Lgs 152/99	ARPA Sicilia & Università degli Studi di Palermo, 2006

[a] Measurements at local natural *P. oceanica* beds

(ARPA Sicilia & Università degli Studi di Palermo, 2006), according to the criteria of Vollenweider *et al.* (1998) for Mediterranean waters, which are used by the Italian authorities to monitor the trophic state of sea water and provide uniform criteria for the classification of coastal waters (D. Lgs 152/99). Although the TRIX scale of coastal water classification was separated into four quality classes describing different levels of eutrophication (D. Lgs 152/99), the PTSI scores for water quality were categorised into three levels, aggregating the central classes of the TRIX scale.

The PTSI parameters were rasterized using the GIS platform into different layers with 30x30 meter cells, each of which showed values corresponding to the assigned rating. The product of the scores obtained by overlapping the layers allowed us to calculate the PTSI index for each cell, subsequently converted to suitability ratings. PTSI can assume values such as 0, 1, 2, 4, 8 and 16, with the following ratings: PTSI < 4, unsuitable for trial; 4 ≤ PTSI ≤ 8, suitable for trial; PTSI = 16, highly suitable for trial, with greater possibility of success. Consequently, a map ranking all areas of potential *P. oceanica* habitat in the Gulf of Palermo was generated.

Field assessments and test-transplanting

The second step of the model involved obtaining site-specific data. A number of potential seagrass habitat sites in the Gulf of Palermo, determined by PTSI scores in the first step, were evaluated for one year to assess the practicability of restoration with *P. oceanica*. According to Short *et al.* (2002), areas with high boat traffic were identified and excluded as possible transplant sites (e.g. harbours). Eventually, five sites inside the Gulf of Palermo, allocated in areas with PTSI scores of more than 4, were considered. According to the transplanting guidelines of Calumpong & Fonseca (2001) "*donor plants should be recruited from populations in comparable environments*" thus, a *P. oceanica* meadow at Solanto, near the far eastern end of the Gulf of Palermo (Fig. 1), was selected as the donor bed. In July 2007, about 700 shoot cuttings growing on sand, at a depth of 16 m, were collected according to harvesting guidelines introduced by Boudouresque *et al.* (1995) and standardized by Díaz-Almela & Duarte (2008) in order to minimize damage to the donor bed. In particular, material suitable for transplanting, consisting of terminal plagiotropic cuttings with three leaf bundles (Molenaar *et al.*, 1993; Piazzi *et al.*, 1998; Fig. 2), was selected.

The mean leaf length of the shoot cuttings (45.7±10.6 cm) was initially estimated in a sub-sample of 40 shoots.

Cuttings were then stored in large coolers with small amounts of sea water to prevent desiccation during transport to the planting sites. Subsequently, cuttings were fixed on galvanized electro welded iron wire mesh (Molenaar *et al.*, 1993). Grids were 1x1 m with a mesh of 5 cm, and 39 cuttings (≈117 shoots) were positioned per grid. Within 12 h from sampling, a test transplant, consisting of 3 grids, was performed at each of the five sites. In addition, three grids were transplanted at one control site (procedural control) close to the donor bed, in order to evaluate the effect induced by cutting transplantation itself. The grids were anchored on sand, the same substratum as the donor bed, using 70cm long iron spikes and at a lower depth (14m) than the donor bed, as previous studies have shown that plants from deeper meadows exhibit higher survival rates when implanted on shallower sites (Piazzi *et al.*, 1998).

Monitoring of cutting establishment and grid number for the test transplants was carried out bimonthly for one year (July 2007 - July 2008).

In addition, in summer 2007, light measurements (photosynthetically active radiation, PAR) were taken just below the surface and just above the sediment using an underwater spherical (4π) quantum sensor (Li-Cor LI193SA). Collected data were then expressed as a percentage of surface light (Dennison *et al.*, 1993). In July 2008, five *P. oceanica* shoots per grid from each test transplant and from the donor bed were collected for laboratory analyses.

Biometric analysis on shoot leaf bundles was carried out according to Giraud (1977) and Pergent-Martini *et al.* (2005). In particular, lengths and number of all leaves on each shoot were measured. Data from a *P. oceanica* meadow located inside the Gulf (Fig. 3) were used as a reference for the variables measured.

Identification of suitable restoration site

The Transplant Suitability Index (TSI) score was calculated for each site on the basis of the PTSI and the results from field assessments. TSI ratings of PTSI and es-

Fig. 2: Transplant unit consisting of terminal plagiotropic cuttings with three leaf bundles.

tablishment factors were assigned according to Short *et al.* (2002) and, for *P. oceanica* leaf variables and light, the same rating as for *Z. marina* growth variables was adopted but considering the limits in relation to *P. oceanica* (Table 2). In addition, the number of detached grids obtained during monitoring of test transplants was taken into account, as this parameter is linked to other factors (hydrodynamic regime, anchoring, fishing) with potentially important effects on the final outcome of the transplant. TSI was calculated according to the following formula:

TSI = PTSI × Establishment × Leaf length × Leaf number × Light × Detached Grids.

Statistical analysis

In order to verify whether establishment rates varied between sites, the Chi-square test was used with a set at 0.05 (Siegel, 1956). In particular, this test checks the null hypothesis that there is equality in the frequency distribution of a contingency table. In our case, the contingency table was generated by crossing the number of not established with the number of established cuttings one year after transplantation at different sites. Differences in leaf biometry among test transplants were assessed by linear model (LM) (Underwood, 1997). Residual homoscedasticity was assessed using the Levene test (Glaser, 1983). When heteroscedasticity of residuals was present, the response variable was transformed to fit the assumptions of linear models. In the parameterization used for LM, all results referred to the intercept, a baseline conventional category (Tomasello *et al.*, 2009a) that, in this case, was set with the procedural control site. All statistical data analyses were performed using SPSS (Statistical Package for Social Science) ver. 15.0.

Recovery design

In November 2008, a *P. oceanica* transplanting was performed in the Gulf of Palermo in order to assess definitively the suitability of the area and plan restoration at larger scale. The transplanting site was chosen in accordance with the outcomes of the site selection model. At this site, 20 grids of galvanized electro welded iron wire meshes (1x1m) were placed in an area of about 40 m^2 at a depth of 14m. The grids were anchored to the substratum using 70 cm long iron spikes. On the upper part of each grid, about 20 *P. oceanica* cuttings, collected from the same donor bed as that of the model (Solanto) and with the same modality, were fixed, giving a total of 1313 shoots positioned with a mean density of 66 shoots m^{-2}. Transplant monitoring was carried out from 2008 to 2014 to assess establishment, detachment and mortality of cuttings and shoot density.

Results

Site selection

Habitat selection

PTSI index values calculated for the Gulf of Palermo were only 0, 8 and 16 (Fig. 3). Hence, according to the size of the areas, the five established test transplants were positioned as follows: in the areas with PTSI = 0, being potentially unsuitable because of natural or anthropogenic factors, no test was allocated; instead, four tests were placed inside the suitable area (PTSI = 8) and one within the highly suitable area (PTSI = 16). Finally, the procedural control site was situated close to the donor bed (Solanto) (Fig. 3).

Table 2. Parameters and corresponding ratings used for calculation of TSI. Data sources are in brackets.

Parameter	TSI rating	Reference
PTSI	0 for PTSI = 0–1 1 for PTSI = 2–4 2 for PTSI = 8–16	
Establishment (test transplants)	0 for <20% 1 for 20–40% 2 for >40%	Short *et al.*, 2002
Leaf length (test transplants)	1 for < mean – 1 SD or no data[a] 2 for ≥ mean – 1 SD[a]	
Leaf number (test transplants)	1 for < mean – 1 SD or no data[a] 2 for ≥ mean – 1 SD[a]	
Light (field data)	0 for <10% surface irradiance 1 for ≥10% surface irradiance or no data 2 for irradiance > local *P. oceanica* bed	Lee *et al.*, 2007; Leoni *et al.*, 2008; Ruiz & Romero, 2001
Grids detached (test transplants)	0 for n grids detached > 0 2 for n grids detached = 0	Present study

[a] Measurements at local *P. oceanica* beds

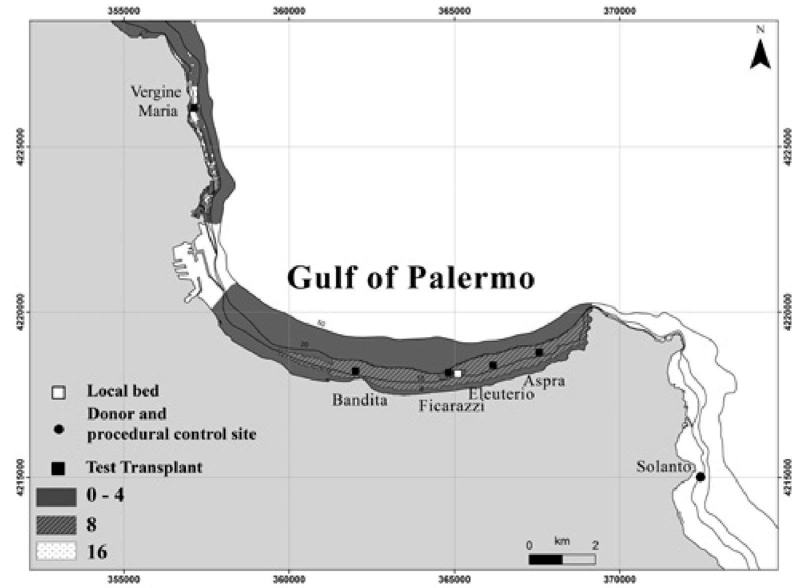

Fig. 3: PTSI map of the Gulf of Palermo and location of sites selected for hosting test transplants.

Field assessments and test-transplanting

At each site, the specific PTSI rating was calculated (Table 3). Thus, at the time of transplant implementation all sites were unvegetated, at a distance greater than 70 m from a natural seagrass bed, at depths of 13 to 15 m and with high water quality. Only at two sites (Ficarazzi and Vergine Maria) the past presence of *P. oceanica* was ascertained and only one (Ficarazzi) was characterised by an exclusively sandy sediment, while *Cymodocea nodosa* was also present at the other sites.

During test transplant observations, the cuttings showed variable establishment patterns among sites (Fig. 4), with significant differences in their frequency distribution at the end of the monitoring year ($\chi^2 = 146.4$ with d.f. = 4, P<0.0001). After any initial mortality, the percentage of established cuttings at the Ficarazzi and Bandita sites (PTSI = 8) remained stable for the rest of the observation period, yielding 52.6±1.3% and 57.2±2.3%, respectively. In contrast, the cuttings planted at the Aspra and Eleuterio sites (PTSI = 8) died progressively because of a burial process during the observation period, until their total death in May at the Eleuterio site, where complete burial of cuttings occurred. The Vergine Maria site (PTSI = 16) showed the highest cutting establishment rate (94.0±0.9% after four months). However, the grids could not to be found after just two months. In the procedural control, an average of 75.7±1.3% of established cuttings were recorded at the end of the monitoring period.

Linear Model results indicated that the intercept (procedural control) estimated for average leaf length was 26.5±3.4 cm (t−value = 7.8, p <0.001) (Table 4). Only the donor bed showed significant statistical differences from the procedural control, as estimated leaf length was 49.8 cm, with an increment of 23.3±4.9 cm. As regards the number of leaves, estimated as 7±1.8 leaves/shoot in the procedural control, the linear model did not reveal any significant differences from other sites (Table 4). All sites had irradiance values higher than the local seagrass bed (15±0.6% SI), except for the Bandita site (11.2±1.8% SI) (Fig. 5). In particular, all values were above the minimum limit for *P. oceanica* growth survival (Ruiz & Romero, 2001).

Identification of suitable restoration sites

On the basis of PTSI scores and the results obtained from test transplants at the end of the monitoring year (Ta-

Table 3. Parameter ratings of the PTSI and the final PTSI score for the transplant sites.

Parameter	Site				
	Vergine Maria	Bandita	Ficarazzi	Eleuterio	Aspra
Historical seagrass distribution	2	1	2	1	1
Current seagrass distribution	1	1	1	1	1
Proximity to natural seagrass bed	1	1	1	1	1
Sediment	2	2	1	2	2
Water depth	2	2	2	2	2
Water quality	2	2	2	2	2
PTSI	16	8	8	8	8

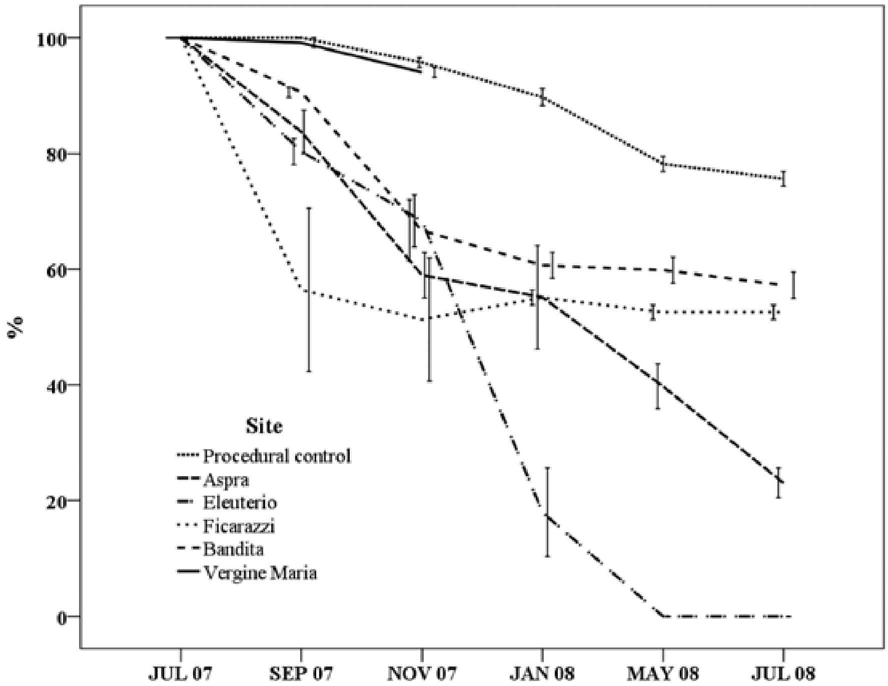

Fig. 4: Establishment percentages (± SE) of *P. oceanica* cuttings in test transplants during the monitoring year.

ble 5), the TSI index was calculated for each site (Table 6). Only the Bandita site produced a score that indicated suitability for *P. oceanica* transplantation in the Gulf of Palermo, with TSI = 16. All the other sites had a TSI = 0, mainly due to the loss of grids in their respective transplant.

Transplantation in the Gulf of Palermo. Monitoring results.

On the basis of the model, the *P. oceanica* implant site in the Gulf of Palermo with the maximum TSI value (TSI = 16) was chosen. The transplant area was located on a seabed dominated by dead *matte* structures (Tomasello *et al.*, 2009b) (Fig. 6), as some studies have shown that *matte* is a particularly suitable substratum for planting seagrass (Di Maida *et al.*, 2013; Terrados *et al.*, 2013)..

Establishment showed a decreasing trend during the first 3 years of monitoring, followed by stationary values of about 32% in the final three years, while cuttings detached from the grids showed the opposite trend, reaching a final value of 61% (Fig. 7A). The increase in the percentage of cuttings found dead was lower, reaching a final value of less than 10%. The average density of the shoots decreased, starting from an initial value of 66 shoots m^{-2} down to 35 shoots m^{-2} (Fig. 7B). Afterwards, mean density of the transplant reached about 76 shoots m^{-2}. The number of shoots per cutting remained almost stable up to 2010, with mean values of 3.5 shoots (Fig. 7C), while it subsequently increased gradually to mean values of 11.6 shoots per cutting and a maximum value of 17 shoots per cutting in 2013.

Discussion

The site selection model developed in this study allowed us to identify potentially suitable areas for restoration with *P. oceanica*. Moreover, on the basis of model

Table 4. Results of the linear model for the response variables leaf length (cm) and number of leaves (n. leaves). The intercept refers to the procedural control site (Solanto). The effect is reported as the difference from the intercept. In brackets, the standard error of the estimates. The significance of the effects was tested on the natural logarithm of the leaf length variable (Levene test: df: 4; 119, F = 1.115, p = 0.4) and on the untransformed leaf number variable (Levene test: df: 4; 20, F = 1.211, p = 0.34).

Factor	Effect	t – value	Significance	Effect	t – value	Significance
	cm			n. leaves		
Intercept	26.5 (3.4)	7.8	***	7.0 (1.8)	3.8	**
Donor bed	+23.3 (4.9)	4.8	***	-0.3 (1.9)	-0.2	n.s.
Aspra	-1.1 (5.4)	-0.2	n.s.	-2.2 (2.0)	-1.1	n.s.
Ficarazzi	+8.5 (4.9)	1.7	n.s.	-0.8 (2.0)	-0.4	n.s.
Bandita	+4.4 (5.4)	0.8	n.s.	-2.8 (2.0)	-1.4	n.s.

Significance levels: "***"<0.001 "**"<0.01 "*"<0.05; "n.s.">0.05

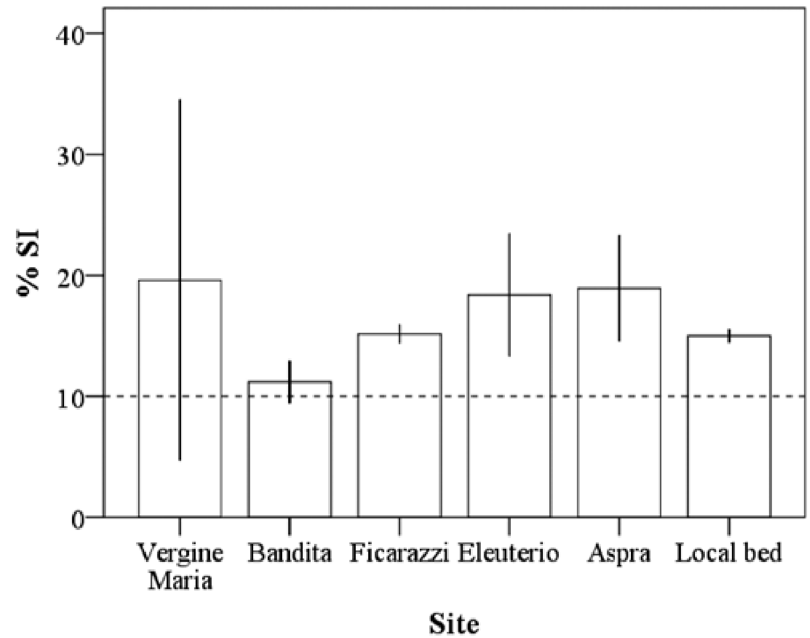

Fig. 5: Average (± SD) sea surface irradiance at each site. Dotted line indicates minimum for *P. oceanica* growth survival.

results, medium-scale seagrass transplantation and its monitoring were implemented for a relatively long time scale (6 years).

Site selection model

Habitat selection and test transplanting

The Preliminary Transplant Suitability Index (PTSI) results were validated through test transplants and monitored for one year at six sites to assess transplantation effects and their variability. Establishment patterns resulting from test transplants were partially in agreement with the pre-selection index prediction. In particular, at some sites the establishment of cuttings was higher than 50%, which fell within the range recorded for other transplantation experiments conducted in the Mediterranean Sea (Meinesz *et al.*, 1992, 1993; Molenaar & Meinesz, 1995; Piazzi *et al.*, 2000). At the end of the monitoring period, establishment of cuttings was nil or below 25% at two sites, due to excessive sedimentation, which buried rhizomes and partially buried leaf bundles. Plants exposed to different sediment rates are able to modulate their

Table 5. PTSI scores and data (±SD) from test transplants for TSI calculation (± SD).

Site	TSI data					
	PTSI	Establishment (%)	Leaf length (cm)	Leaves (N)	Light (% SI)	Grids detached (N)
Vergine Maria	16	-	-	-	19.6±14.9	3
Bandita	8	57.2	30.9±14.2	4.2±1.3	11.2±1.8	0
Ficarazzi	8	52.6	35.0±18.5	6.2±1.9	15.1±0.8	1
Eleuterio	8	0	-	-	18.4±5.1	1
Aspra	8	23.1	25.4±13.3	4.8±1.9	18.9±4.4	1
Local bed	-	-	51.8±23.9	5.5±0.6	15.0±0.6	-

Table 6. TSI rating and scores of the potential restoration sites.

Site name	PTSI	Establishment	Leaf length	Leaves	Light	Grids detached	TSI SCORE
Vergine Maria	2	0	-	-	2	0	**0**
Bandita	2	2	2	1	1	2	**16**
Ficarazzi	2	2	2	2	2	0	**0**
Eleuterio	2	0	-	-	2	0	**0**
Aspra	2	1	1	2	2	0	**0**

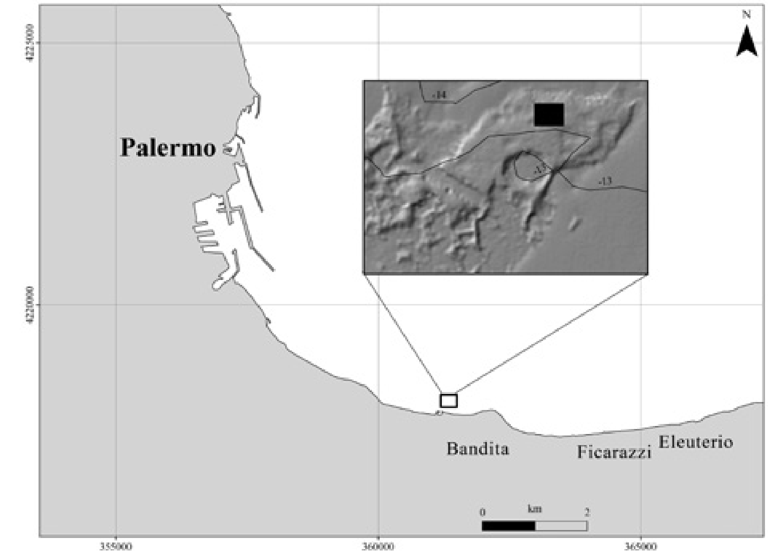

Fig. 6: Area of the Gulf of Palermo chosen for restoration with *P. oceanica*. In relief, dead *matte* formations.

growth to counteract leaf meristem burial (Manzanera *et al.*, 2011). However, these authors indicated burial levels of 4 and 9 cm for partial or total mortality of *P. oceanica* shoots. In our case, plant ability to increase vertical rhizome elongation in response to higher sediment deposition may be reduced by transplantation stress.

Four months after installation, another site showed the highest values of cutting establishment among test transplants in the Gulf of Palermo (94%), in agreement with the PTSI index, which attributed this site with the highest chance of success for marine restoration (PTSI=16). However, no transplanted grids were found during the third inspection survey; thus, complete monitoring was not possible for this site. Grid loss generally affected most of the test transplants as, except in one case, at least one grid was lost. This additional source of disturbance for transplant tests may be due to the effects of local hydrodynamics or, very likely, to anchoring and traditional fishing activities, given that small fishing gear was found attached to remaining grids (Fig. 8).

In addition, data collected on *P. oceanica* bundles highlighted a number of biometric differences among plants transplanted at various sites. The leaves of the donor bed were double the length of the procedural control ones, which exhibited mean values that were statistically equal to the other sites, suggesting that most of the leaf length variability observed (about 50%) could be linked to stress due to tearing of cuttings and sediment removal rather than the different environmental conditions to which test transplants were exposed at the other sites. Cutting tearing inevitably broke clonal interconnections that are essential for supporting seagrass clonal expansion and growth (Marbà *et al.*, 2002). Indeed, clonal interconnections are able to support seagrass growth by transferring nutrients, mainly nitrogen, from older to younger shoots (Marbà *et al.*, 2002).

This resource redistribution between ramets seems to be fundamental for initial nutrient acquisition by shoots and, therefore, the survival of the clone and its expansion. In this study, transplanted shoots, all intentionally selected at a very young stage, could not benefit from the contribution of resources from other ramets and thus obtained them mainly from the leaves. The internal process of nutrient recycling, due to transfer from old leaves to young ones, is also an important process in nutrient absorption in transplanted cuttings (Alcoverro *et al.*, 2000; Lepoint *et al.*, 2002a). However, leaves alone are unable to support the nutrient demand of the transplant when the roots are damaged (Lepoint *et al.*, 2004). Nutrient root uptake sustains about 35% of the annual needs of *P. oceanica* (Lepoint *et al.*, 2002b). Therefore, tearing, which also partially shears off the root system, slows down further recovery of transplanted cuttings, considering that formation of adventitious root in *P. oceanica* requires 3-12 months (Meinesz *et al.*, 1992).

Identification of suitable restoration site

The final step of the model was the calculation of the Transplant Suitability Index (TSI), which indicated that only one site (TSI = 16) was potentially suitable for restoration with *P. oceanica*, whereas 80% of the sites exhibited an index value of zero. Since TSI is a multiplicative composite index, the occurrence of only one parameter equal to zero is sufficient to reset the final score. In our case, grid detachment, added as a new parameter to the model, was responsible for the fact that most of the sites were considered unsuitable for implementation of extensive *P. oceanica* transplantation. It was observed that detachment was mainly due to anchoring and caused especially by artisan fishing. In the Mediterranean Sea, several studies have assessed the impact of anchoring on *P. oceanica* meadows, particularly at sites that are highly

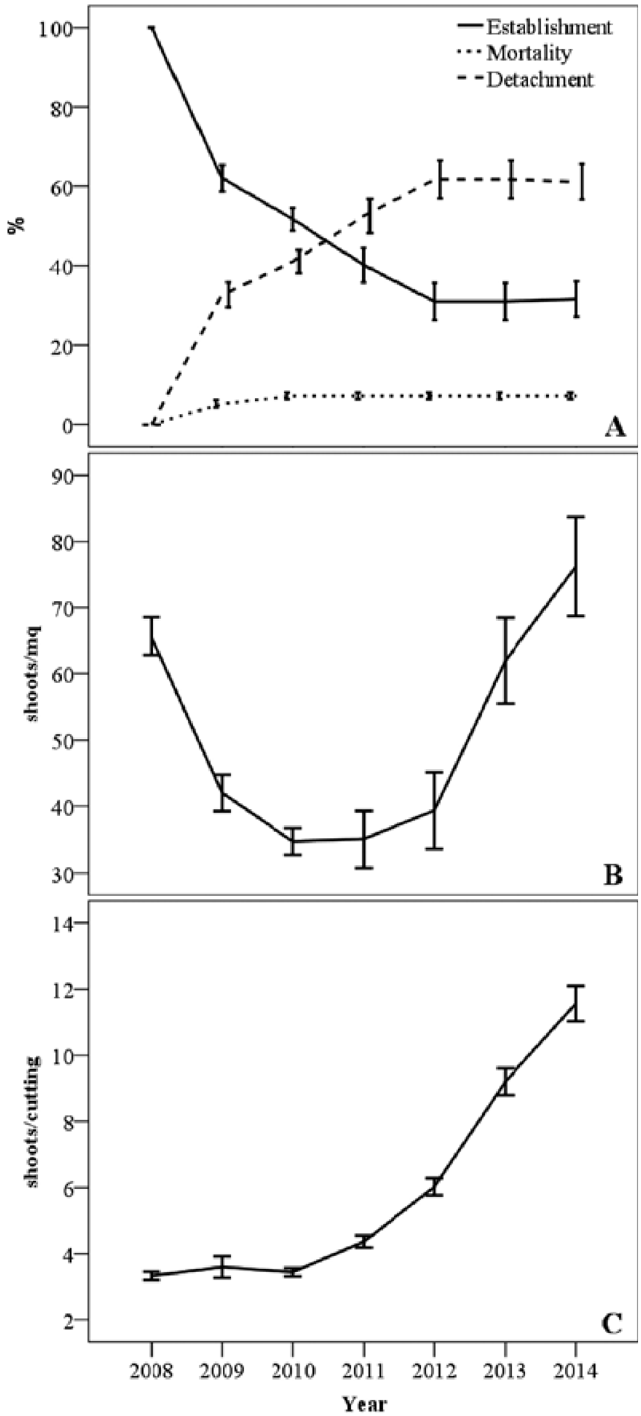

Fig. 7: Mean (± SE) percentages of establishment, detachment and mortality of cuttings (A), shoot density (B) and number of shoots per cutting (C) of the transplant (N=20).

frequented by boaters (marine protected and coastal urbanized areas), suggesting that there may be direct, adverse effects on meadow cover and shoot density (Milazzo *et al.*, 2004 and reference therein). For this reason, areas affected by these activities (e.g. harbours) were excluded *a priori* from our model. The impact of small-scale artisan fishing has not been documented extensively, perhaps because this activity is difficult to monitor, especially in unprotected areas. Therefore, transplant unit loss has been used as a proxy for anchorage and craft fishing pressure and it has been explicitly incorporated as a new parameter heavily weighted in our model, since the loss of one transplant unit makes a site unsuitable for restoration.

Posidonia oceanica transplantation

In this study, further focal results were obtained by extending the area and lengthening the monitoring period of *P. oceanica* transplantation.

Transplanting on the site chosen by the TSI model for *P. oceanica* showed, for the same temporal interval, an even higher establishment percentage (65.0±4.1%) than that measured for the corresponding test transplant of the model (57.2±2.3%). The small difference is possibly due to the fact that transplantation was carried out on dead *matte*, confirming this substratum as the most suitable for *P. oceanica* planting (Di Maida *et al.*, 2013; Terrados *et al.*, 2013). Such outcomes also increase the effectiveness of these kinds of models for *P. oceanica*. Our results clearly demonstrated that only one year of monitoring is insufficient to predict transplant performance, since a non-linear trend was detected, with percentage of cutting establishment decreasing below 50%, followed by an increase. Early transplant monitoring carried out for other seagrasses also showed low growth and structural seagrass performance in the first year after planting, because of planting stress (Bastyan & Cambridge, 2007; Bell *et al.*, 2008). Again, some loss of cuttings observed during the first months after transplantation was, in our opinion, likely due to intense fishing in the area, although previous test transplanting did not exhibit any loss. A larger sized transplant unit is necessary in order to obtain better information about the intensity of fishing activity on the seabed.

In the last two years (2012-2014), transplant stabilization was observed, which was likely due to an increase in the root structures, constituting direct evidence of real rooting that was already underway in the first year after implantation (Meinesz *et al.*, 1992).

However, the low establishment of cuttings detected does not necessarily entail lower efficacy in the entire transplant, since mortality or detachment may be offset in time by elongation and branching of the remaining cuttings (Calumpong & Fonseca, 2001). Seagrass clonal growth continuously speeds up space occupation; in fact, for several seagrass species, the space occupied by a seagrass clone increases exponentially in time, to the third power (Marbà & Duarte, 1998; Borum *et al.*, 2004; Sintes *et al.*, 2005). Much attention should be drawn to the final three years of *P. oceanica* transplantation monitoring, when an increase in shoot number per cutting was observed, which more than doubled, insomuch as it became difficult, *in situ*, to distinguish one cutting from the other. Ramification mean rate of cuttings was 36% with a maximum value of about 50% in 2013, comparable with those recorded for plagiotropic cuttings by Molenaar *et al.* (1993). This caused an increment of about 16% in

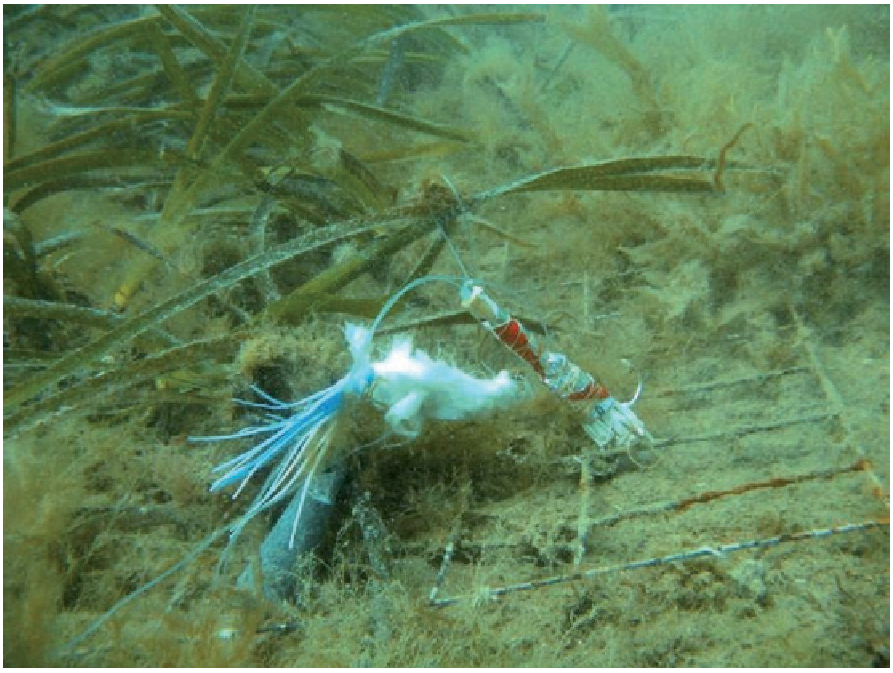

Fig. 8: Gear for octopus fishing trapped in the mesh of an implant grid.

transplant shoot density, which compensated the initial loss of cuttings.

Approximately two years after planting, stabilization of planted cuttings and an increase in the number of shoots was observed. As *P. oceanica* is a long-living species, its natural recovery is very slow (Duarte *et al.*, 2006; Pergent *et al.*, 2012) and the timeframe for understanding its resilience is often much longer than the conventional one to three years of most ecological studies (Hughes *et al.*, 2005; Cunha *et al.*, 2012).

Conclusion

The application of a site selection model, including historic and literature-based information, reference data and field measurements, is helpful in choosing suitable coastal areas for restoration with *P. oceanica*. Moreover, this model also helps us to control fishing and anchoring activities in the selected areas in order to reduce the risk of transplant failure. Limiting anchorage and craft fishing to protect transplants, at least until their stabilization, combined with more effective systems for fixing cuttings could considerably reduce the number of detached cuttings and increase transplantation success. Besides, to the best of our knowledge, our work is the first that provides quantitative data on the effects of tearing of cuttings itself and we believe that this will contribute to minimizing evaluation errors during transplantation monitoring. Finally, based on our findings, we advise that a monitoring period should last at least 3 years for the selection model outcome and at least 6 years for evaluating the effectiveness of restoration projects. Further lengthening of post-implementation monitoring would allow estimation of the actual recovery of the transplanted system. Documented seagrass restoration could provide strong evidence of the improvement in water quality in a degraded coastal system.

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