



Poor success of seagrass *Posidonia oceanica* transplanting in a meadow disturbed by power line burial

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ARTICLE INFO

Keywords:

Rhizome fragments
Seedlings
Transplant
Ecological restoration
Mechanical impact
Environmental impact
Ecosystem change
Marine ecology
Coastal structure

ABSTRACT

Local disturbances drive the decrease of the area covered by *Posidonia oceanica* in the Mediterranean. Mechanical impacts during the development of coastal infrastructures alter sea floor and the recolonization of benthic community will depend on the recovery of pre-disturbance environmental conditions and on the intrinsic characteristics of the local community that was disturbed. We transplanted 468 rhizome fragments and 450 seedlings of *P. oceanica* in a meadow disturbed by the trenching and deployment of a power line to evaluate the suitability of the disturbed sea floor for rehabilitating *P. oceanica* meadows. We quantify and compare the survivorship and vegetative development of the transplanted/planted (i.e. fragments/seedlings) material in the two types of the unconsolidated substrata left after infrastructure deployment works finished: sand and burlap bags filled with coarse gravel. The latter was used as a corrective measure for topographic restoration. Three experimental plots with sixteen transplanted fragments or twenty-five seedlings were placed at each substratum type at three different depths (i.e. 15, 20 and 25 m). Our results show that the transplanting of *P. oceanica* rhizome fragments in the disturbed substrata had low survival rates (0–31%) after 40–48 months. The survivorship of seedlings was lower than that of fragments. Our results highlight the importance of substratum for *P. oceanica* recovery after mechanical impact; disturbed, non-consolidated substrata will preclude *P. oceanica* rehabilitation through planting. Preservation of meadow substratum (i.e. dead *matte*) is a critical element that coastal infrastructure projects should consider to enable future recovery of *P. oceanica* meadows.

1. Introduction

Seagrasses form valuable but threatened ecosystems in coastal waters worldwide (Orth et al., 2006; Waycott et al., 2009). Disturbance, whether natural or anthropogenic, is an intrinsic element of the dynamics of seagrass ecosystems (Clarke and Kirkman, 1989; Duarte et al., 2006). Seagrasses inhabit shallow sea floors, mostly unconsolidated, which are prone to disturbance by water dynamics (Preen et al., 1995; Fonseca and Bell, 1998). Trawl-fishing (González-Correa et al., 2005), hull grounding (Olesen et al., 2004), propeller scarring (Hammerstrom et al., 2007), anchor mooring (Walker et al., 1989; Milazzo et al., 2004) or dredging (Badalamenti et al., 2011) are examples of anthropogenic activities that disturb sea floor mechanically and drive seagrass loss.

Coastal infrastructures are altering coastal ecosystems, and seagrass meadows, throughout the world (Bugnot et al., 2020). Along Mediterranean coasts, human population increased by 46% between 1980 (84.5 million) and 2000 (123.7 million), and it is projected to nearly double between 2000 and 2025 (Airoldi and Beck, 2007). The predicted

increase of coastal populations for the next decades implies the proliferation of coastal infrastructures needed to sustain residential, commercial and touristic activities (Bulleri and Chapman, 2010). Such infrastructures may occupy the coast permanently and create new environments and substrata (e.g. docks, harbours or armouring structures) that often facilitate the establishment of non-indigenous species in the area (Connell, 2000; Bacchiocchi and Airoldi, 2003; Airoldi and Bulleri, 2011). They may also be buried infrastructures, such as some power or gas lines, which modify the sea floor during the construction and deployment but, afterwards, no artificial structure is located above sea bottom. Sea floor relief after impact can be naturally recovered or require topographic restoration measures to re-establish bottom profile and facilitate seagrass recovery (Hammerstrom et al., 2007). The altered sea floor after mechanical disturbances would undergo a recolonization process, which has been described for a few species only. Sediment filling combined with nutrient addition facilitated the recolonization of propeller scars in multispecific *Thalassia testudinum* *Halodule wrightii* and *Syringodium isoetifolium* meadows (Hammerstrom et al., 2007;

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<https://doi.org/10.1016/j.marenvres.2021.105406>

Received 29 January 2021; Received in revised form 29 June 2021; Accepted 1 July 2021

Available online 4 July 2021

0141-1136/© 2021 The Authors.

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Kenworthy et al., 2018; Furman et al., 2019). The addition of calcareous rubble for topographic restoration led to an improvement of natural recolonization in a dredged *Posidonia oceanica* meadow (Di Carlo, 2009; Badalamenti et al., 2011). With no further intervention (i.e., planting of the species lost), seagrass recolonization of mechanically disturbed meadows would likely depend on the recovery of pre-disturbance environmental conditions, especially those of the substratum, and on the intrinsic characteristics of the local community of seagrass species that was disturbed.

There are 24 Mediterranean marine ecosystems included in the Red List categories (plus 23 more with data deficient for assessment), only 8% of them are considered of least concern (Gubbay et al., 2016). One of the vulnerable habitats are *Posidonia oceanica* meadows, that provide important services, acting as carbon sinks (Duarte et al., 2005), coastal protectors (Infantes et al., 2012) and supporters of biodiversity (Duarte, 2000). The area covered by *P. oceanica* is decreasing throughout the Mediterranean, mostly after widespread local disturbances, although global impacts also threats *P. oceanica* persistence (Marbà et al., 2014). If no corrective action is taken, the forecast of increasing coastal infrastructures will aggravate this scenario.

Posidonia oceanica is a slow-growing clonal species (1–6 cm of rhizome year⁻¹) (Marbà and Duarte, 1998) which forms extensive meadows, characterized by a highly variable sexual reproduction, both in space and time (Balestri, 2004; Diaz-Almela et al., 2009). The natural recolonization of *P. oceanica* after a local impact (e.g. sea floor disturbance associated with buried coastal infrastructure) would be very slow either by clonal growth or recruitment of drifting plant fragments or seedlings (Di Carlo et al., 2005; Almela et al., 2008; Alagna et al., 2013). The effective recovery of the area could then take decades (Kendrick et al., 1995; Di Carlo, 2009). The few previous studies that evaluated the natural recolonization by vegetative fragments of *P. oceanica* after a mechanical impact, show the crucial importance of the presence of consolidated substratum (i.e. of low resuspendability by hydrodynamics) for propagule establishment (Di Carlo et al., 2005). However, *matte* is frequently disturbed and unavailable after coastal infrastructure works (i.e. after trenching for power line deployment). Similar to meadows, dead *matte* substratum may be affected by the sediment dynamic changes caused by coastal interventions, such as dredging or harbour construction (Ruiz and Romero, 2003; Erfteimeijer and Robin Lewis, 2006). Resuspended sediments by the building of coastal infrastructures may sediment on *matte* and change the upper layer of the substratum from consolidated into unconsolidated. The *matte* maximizes survival and improves growth of *Posidonia* propagules (Balestri et al., 1998; Piazzini et al., 1998). Regarding seedlings, the published evidence shows also the importance of consolidated substratum to maximize recruitment (Alagna et al., 2013; Pereda-Briones et al., 2020), survivorship and vegetative development (Meinesz et al., 1993; Piazzini et al., 1999; Terrados et al., 2013).

Once trenching and power line deployment are finished and the local environmental conditions (e.g. sedimentation and turbidity) recovered (Erfteimeijer and Robin Lewis, 2006), the disturbed sea floor is open to natural recolonization which will strongly depend on the surrounding species growth rates, propagule production and recruitment. Furthermore, the disturbed area can be also appropriate for initiating restoration initiatives to facilitate the recovery of the original benthic community (Clewett et al., 2004; McDonald et al., 2016). This is critical in case of slow-growing, poor sexual reproducers such as *Posidonia oceanica*. The introduction of corrective measures on the altered sea floor after infrastructure deployment may be essential to minimize impacts (Li et al., 2005; Naylor et al., 2011). After trenching on healthy *P. oceanica* meadows, there is a threat of collapse of the *matte* in trench margins, especially at those sections where *matte* thickness and slope are high. This substratum instability may lead to further sediment erosion and increase of the disturbed area (Boudouresque et al., 2006), prevent natural recolonization or reduce the success of *P. oceanica* planting. Sediment filling and substratum regrading have been shown to facilitate

seagrass, *Thalassia testudinum*, recolonization of mechanical disturbances that resulted in sediment excavation (Hammerstrom et al., 2007; Kenworthy et al., 2018; Furman et al., 2019). Except for the case a dredged *P. oceanica* meadow in SW Sicily (Di Carlo, 2009; Badalamenti et al., 2011) the suitability of the substrata introduced by topographic restoration measures for natural recolonization or transplanting of this species is unknown.

Different techniques and decisive design aspects need to be considered in *P. oceanica* rehabilitation projects. The transplantation techniques focus on rhizome fragments of adult plants (Piazzini et al., 1998) while planting techniques involve the use of seedlings obtained from shoreline drift fruits (Balestri et al., 1998; Domínguez et al., 2012). The production of seeds in *P. oceanica* is low and highly variable and questions the use of seedlings for large-scale rehabilitation projects. Regarding the transplantation of vegetative fragments, published evidence shows higher survival rates after transplantation of fragments with one plagiotropic (i.e. horizontal growth habit) and at least two orthotropic (i.e. vertical growth habit) shoots (Molenaar et al., 1993; Piazzini et al., 1998). Fall is apparently the best season for maximizing the survival rate of rhizome transplants (Meinesz et al., 1992). Rhizome fragments need an anchoring system to prevent being dragged before natural rooting occurs. In turn, artificial anchoring does not improve survival rate of seedlings planted in *matte* (Terrados et al., 2013). The anchoring systems may be individual for each fragment (e.g. Molenaar and Meinesz, 1995) or several fragments in one single anchoring structure (e.g. Augier et al., 1996; Piazzini et al., 1998). Traditionally, transplanting of *P. oceanica* adult plants involves their collection (destructive) from healthy beds but the future of *P. oceanica* transplanting should use drift material (Balestri et al., 2011) thus avoiding damage or degradation of the donor beds, a highly recommended guideline (Boudouresque et al., 2006).

In addition to substratum, transplanting technique and design, plant survival after transplanting can be affected by other factors such as sediment organic matter content (Cancemi et al., 2003), the surrounding algal community (Pereda-Briones et al., 2018, 2020) and carbohydrate or nutrient reserves of the transplants (plant fragments or seedlings) (Genot et al., 1994; Balestri et al., 2009). Transplanted *P. oceanica* shoots are considered unable to meet their nutrient requirements for growth in the short-term (Lepoint et al., 2004; Vangeluwe et al., 2004). Internal carbohydrate and nutrient content, particularly in transplant reserve structures (rhizomes), may affect transplant biomass production (Alcoverro et al., 1995), which is not physiologically integrated in the larger rhizome net of the natural meadow and cannot translocate resources (Alcoverro et al., 2000; Marbà et al., 2002). The comparison between the nutrient content of the fragments from the transplantation and from the surrounding natural meadow could indicate if nutrient up-take in transplants is fulfilling nutrient requirements.

Previous evidences show that the survivorship rate of *P. oceanica* transplants is quite variable. The translocation of meadow blocks (1 m² * 40 cm thickness of *matte*), before destruction by coastal works, in sandy meadow gaps resulted in very high mortality rates (85%) (Sánchez-Lizaso et al., 2009). The transplant of fragments on dead *matte* shows, in general, higher survivorship rates. The mean survivorship rate after three years for plagiotropic rhizomes transplanted on *matte* was of 76.4% (±5.7) in Piazzini et al. (1998) and between 69 and 85% in Molenaar and Meinesz (1995). The average survivorship was of the 67.2% (after two years of transplantation) and 77.6% (after 9 months of transplantation) in Meinesz et al. (1993, 1992), respectively. Regarding other substrata, Balestri et al. (2011) maintained shoreline drift fragments in tanks and then transplanted them on mounds of calcareous rubbles, obtaining a survivorship rate of 50% one year after transplanting. Alagna et al. (2019) reported fragment survivorship between 50% and 88% on artificial rocky substrata after 30 months. The reliability of the estimation of survivorship after restoration increases with the time of monitoring. Two years of monitoring after transplanting includes two adverse (winter) seasons and may be representative for

Table 1

Granulometry, organic matter content (mean \pm sd, N = 4) of sediment and shoot density (mean \pm sd, N = 10) in the surrounding non-impacted meadow at the three depths of transplanting.

Depth	% Clay (<0.063 mm)	% Sand (0.063 – 2 mm)	% Gravel (>2 mm)	% Organic matter	Shoot density. (shoots m^{-2})
15 m	0.4 ± 0.294	52.0 ± 13.356	43.8 ± 12.560	0.82 ± 0.294	320 ± 71.5
20 m	4.1 ± 1.727	92.7 ± 2.138	3.2 ± 3.799	0.35 ± 0.071	277 ± 41.6
25 m	3.4 ± 2.158	88.9 ± 7.036	7.3 ± 5.373	0.38 ± 0.134	357 ± 69.8

some seagrass species (van Katwijk et al., 2016), although extended monitoring is recommended for slow-growth seagrass species (Furman et al., 2019).

Our study assesses the suitability of extant substrata after power line burial (i.e. sand, gravel bags) in a *P. oceanica* meadow to perform actions for the rehabilitation of *P. oceanica*. To that end, we transplanted rhizome fragments and seedlings of *P. oceanica* in a meadow disturbed by the trenching and deployment of a power line. The assessed substrata were sand, the natural but disturbed substratum present after deployment works finished, and burlap bags filled with coarse gravel (average grain size 1–1.5 cm) used as a corrective measure to reduce the slope of trench margins (i.e. topographic restoration). The transplanting technique involved the use of seedlings (Terrados et al., 2013) and plagiotropic rhizome fragments with an individual anchoring system (Molenaar et al., 1993; Molenaar and Meinesz, 1995; Piazzzi et al., 1998), based on results of previous studies and the feasibility of implementing it in our study site. Our objective was to quantify and compare the mid-term (i.e. 4 years) survivorship and vegetative development of the transplanted/planted material in the two types of extant substrata in the disturbed sea floor.

2. Material and methods

2.1. The disturbance: the interconnection of the power line networks of Majorca and Ibiza, W Med

The 126 km long electrical connection between Majorca and Ibiza islands (Western Mediterranean) was deployed in 2014 with the aim of assuring the electrical power supply in Ibiza in a forecasted scenario of growing power demand. It is the longest underwater power line (alternating current) in the world with 118 km of line affecting marine habitats between 20 and 800 m depth. The trenching and deployment of the line affected 519 m^2 of *Posidonia oceanica* meadows in Santa Ponça Bay (Majorca, $39^\circ 30.771'N$, $2^\circ 27.470'E$). The width of the trench varied from 0.4 m to 2 m depending of the underlying substratum (wider in sand than in *matte*) (Suppl. material). The resulting disturbed sea floor consisted of a mixture of silt and clay, sand and gravel. Granulometry and organic matter content (percentage of sediment dry weight) data at each depth are presented in Table 1.

2.2. Collection and mesocosm period of seedlings and rhizome fragments

Posidonia oceanica adrift fragments were hand-collected by scuba divers during the winters of 2015 (287 fragments collected) and 2016 (355 fragments collected) in Majorca. Only fragments with one apical group (i.e. horizontal growth habit or plagiotropic) and two vertical (orthotropic) shoots were used in the study to maximize survivorship (Molenaar et al., 1993). The length of the rhizome of the fragments varied between 3 cm and 34 cm; 84% of the fragments had a rhizome length between 10 and 25 cm. Before planting, the fragments were kept in mesocosms under controlled conditions between 3 and 5 months at an average density of 350 shoots per m^2 in a gravel substratum (2–5 mm).

The mesocosm period allowed the selection of the fragments in good condition for the transplanting. Each selected-for-transplanting fragment was labelled and the number and type (i.e. orthotropic or plagiotropic) of shoots per fragment was counted. Approximately 1500 *P. oceanica* seeds were collected in Majorca beaches during the summer of 2015 and were kept in mesocosms under controlled conditions in gravel substratum (2–5 mm) during two months for germination and seedling development. There was not enough number of seeds collected for planting in 2016. Weekly, seedlings and rhizome fragments were cleaned from epiphytes, and degrading or dead items were removed from mesocosms.

The keeping period of rhizome fragments and seedlings was done in two indoor 5000 l mesocosms under similar to natural temperature and salinity conditions using a seawater flow-through system (i.e. water renewal rate 1.74 l/min). A number of white fluorescents provided illumination (minimum irradiance of 50 $\mu E\ m^{-2}\ s^{-1}$), with a light/dark cycle matching the seasonality of natural sunlight day/night photoperiod. The temperature of the water tanks was between 20.9 and 24 $^\circ C$. The nitrate concentration of seawater in the tanks was between $0.66 \pm 0.25\ \mu M$ and $4.20 \pm 0.38\ \mu M$ (mean \pm SD) in winter and summer, respectively. Phosphate concentration in seawater was between $0.13 \pm 0.11\ \mu M$ and $0.51 \pm 0.06\ \mu M$ (mean \pm SD) in winter and summer, respectively. Water nutrients were analysed in a continuous flow auto-analyzer (trAAcs-800, Bran + Luebbe, Inc., IL USA) as described by Arjonilla et al. (1991). Mesocosms functioning was controlled weekly.

2.3. Study site and experimental design

The plantation tests were performed during the spring of 2015 and 2016. 234 fragments of *P. oceanica* were transplanted at 15, 20 and 25 m of depth each year, in 2015 and 2016. The fragments were distributed in eighteen plots (i.e. 6 plots at each depth and 13 fragments per plot). The distance between fragments in a plot was 20 cm and they were planted in line along the trench track. Nine of these plots (i.e. 3 at each depth) were placed in the extant sandy seafloor (see disturbance description). The other nine plots (3 at each depth) were placed in trench sectors where burlap bags filled with gravel were placed to reduce the slope of trench margins. The minimal trench width in the area where the experimental plots were placed was 1 m. In addition, in 2015, 450 seedlings were planted at the same depths and substrata. At each depth, three plots of 25 seedlings were placed in sand and three plots were placed in burlap bags. The dimension of the seedling plots were 50*50 cm and the distance among seedlings in each plot was 10 cm.

Before transplanting, each fragment was knotted to a U-shaped iron piece (staple) to anchor the fragments in the substratum. The central part of the staple was covered with bee wax to avoid the potential damage of metal rust on the rhizome/root microenvironment and the fragment was tied with hemp rope of 4 mm section. The use of biodegradable materials was promoted throughout the study. Following the results of Terrados et al. (2013) where anchoring did not improve seedling survival rate in *matte*, the seedlings were planted by manually introducing seedling roots into the substratum, either sand or burlap bags filled with gravel, and leaving the seedling at substratum surface level. The roots of the seedlings to be planted in the gravel bags were introduced through small cuts done in the burlap.

2.4. Fragments and seedlings survival monitoring

The transplanted fragments in 2015 were monitored five times: after one, two, twelve, fifteen and forty-eight months after planting. Fragments transplanted in 2016 were monitored after one, eight and forty months after planting. The first survey (one month after planting) aimed to check the effectiveness of plantation work. The monitoring registered the presence/absence of fragments/seedlings in the plots and the number of shoots per fragment or the number of leaves per seedling. Fragments were labelled, therefore identifiable, and the comparison of the

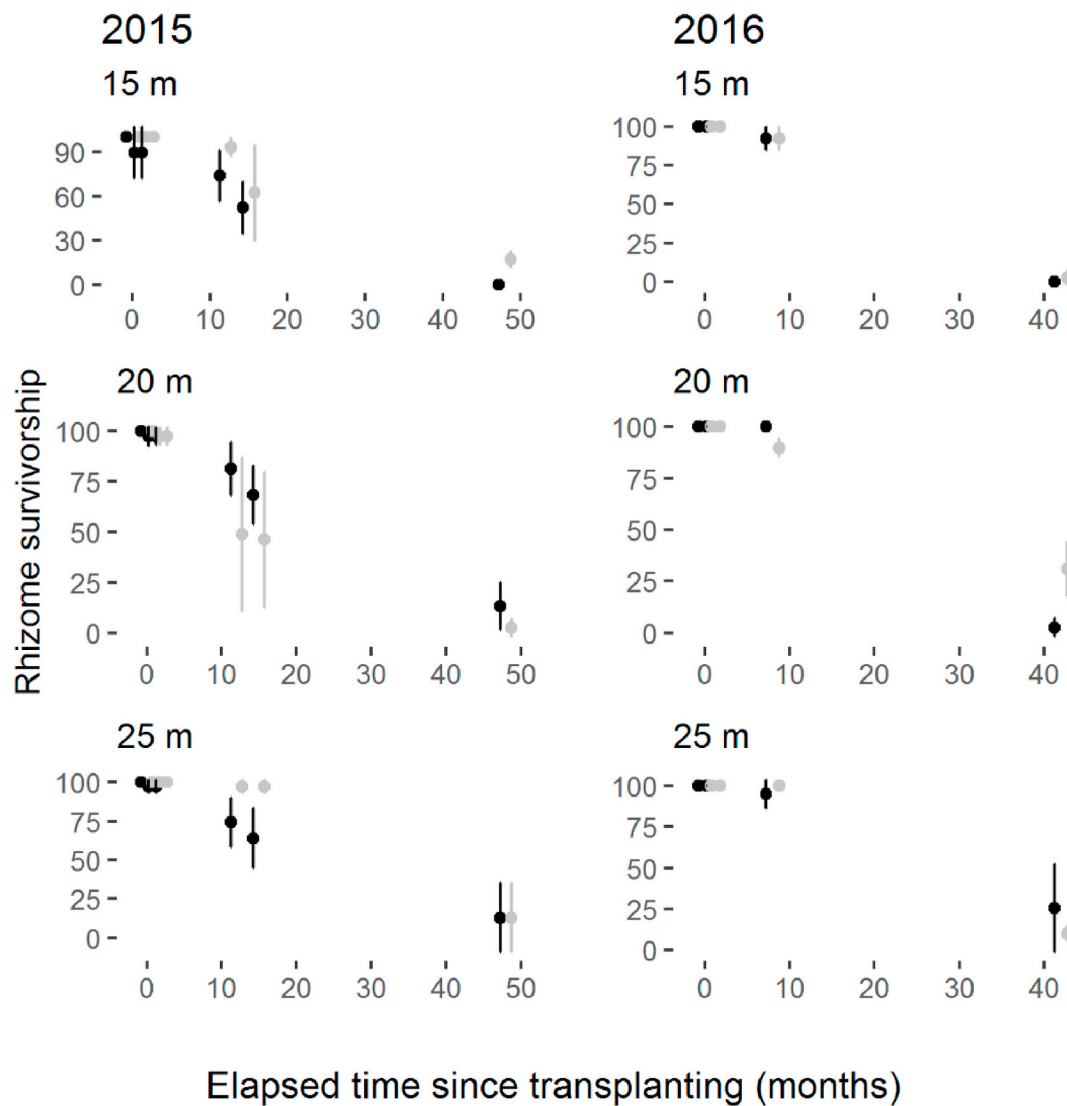


Fig. 1. Mean \pm SD of survivorship rate of fragments after plantation in 2015 and 2016 at different depths and substrata in Santa Ponça, Majorca. Circles show survivorship in plots established on sand (black) and on burlap bags filled with gravel (grey).

initial number of shoots per fragment with the number of shoots per fragment during monitoring was used to assess net production or loss of shoots by fragments.

2.5. Fragment nutrient condition, and features of plantation sites

Two sets of samples of the rhizome fragments was extracted, one in 2015 ($n = 5$) and one in 2016 ($n = 9$), to describe nutrient content of the plants just before transplanting in each field campaign. Carbon, nitrogen and phosphorus content were determined in the leaves and the rhizome of the apical group of shoots. The effect of the stay in the mesocosms on the nutrient content of the fragments was evaluated in 2016 only by analysing nutrient concentration in the apical group of shoots from two subsamples of fragments frozen just after collection ($n = 5$) and just before plantation ($n = 9$). Carbon and nitrogen content were analysed using a Carlo-Erba CNH elemental analyser. Phosphorous content was analysed as in Fourqurean et al. (1992). The nutrient content of vertical shoots in the natural meadow next to the transplantation sites was also determined for comparison with nutrient content in transplanted fragments ($n = 30$). At each depth, 4 sediment samples (i.e. corers) were collected to describe the unconsolidated substratum at the first 10 cm layer from surface (i.e. granulometry and organic matter content). Shoot

density in the surrounding meadow was estimated in summer 2016 by counting all seagrass shoots present inside ten quadrants of 20×20 cm placed randomly at each depth.

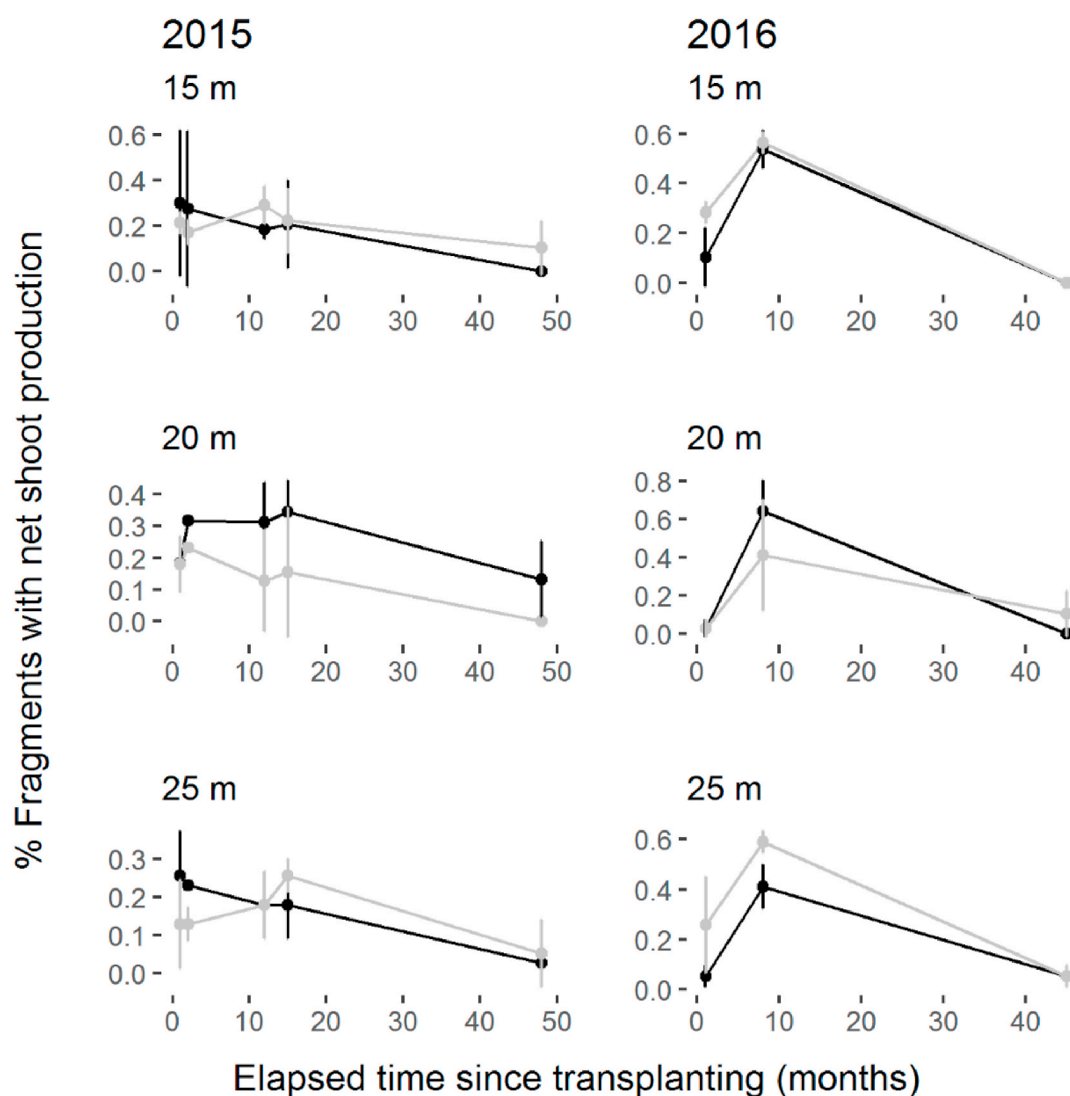
2.6. Statistical analysis

The differences between the initial size of the rhizome fragments transplanted in 2015 and 2016 and the differences in the size of the fragments assigned to each of the experimental plots prior to planting were tested using a linear model. The dependent variable used to quantify fragment size was the number of shoots per fragment. Depth, type of substratum and plot were used as factors to exclude a potential *a priori* effect of fragment size in the future response of transplanted fragments.

Data on final survivorship rate were considered binomial (i.e. dead = 0, alive = 1) and analysed by logistic regression (Zuur et al., 2009). The percentage of fragments showing net production of shoots was treated in the same way. Substrata and depth were additive factors for final survivorship analysis, and substrata, depth and time (i.e. months from transplantation) were the factors used for the assessment of differences of the percentage of fragments showing net production of shoots. The factors were treated as additive in the models because the

Table 2Logistic regression of the effect of substratum and depth on final survivorship in 2015 and 2016 plantations. Statistical significance ($p < 0.05$) are marked in bold.

	Plantation 2015				Plantation 2016			
	Estim.	SE	z-val.	P	Estim.	SE	z-val.	P
Intercept	−2.3630	0.4498	−5.254	<0.001	−4.6375	1.0409	−4.455	<0.001
Depth 20m	−0.2932	0.5667	−0.517	0.605	2.7445	1.0523	2.608	<0.01
Depth 25m	0.2530	0.5048	0.501	0.616	2.8350	1.0498	2.701	<0.01
Substratum	0.3619	0.4377	0.827	0.408	0.5217	0.4226	1.234	0.2170

**Fig. 2.** Mean \pm SD percentage of fragments with a net increase of shoot numbers, in sand (black) and in burlap bags filled with gravel (grey).

interactions between them were irrelevant. After testing the non-significant differences in the response variables (i.e. survivorship at the final time and fragments showing net production of shoots) between years, the analysis of 2015 and 2016 data were done independently.

Differences in element ratios and nitrogen and phosphorus content in leaves and rhizomes were analysed using three separate T-tests comparing: apical group from fragments collected vs transplanted (i.e. before/after mesocosm period) in 2016, apical group transplants from 2015 vs 2016, and transplanted vs natural vertical shoots in 2016. All the analysis and graphs were performed using R Core Team (2017).

3. Results

The initial size of the fragments of rhizome transplanted at different depths ($F_{2/430}=5.837$, $p > 0.05$), substrata ($F_{1/430}=0.0008$, $p > 0.05$) and plots ($F_{32/430}=4.551$, $p > 0.05$) in 2015 and 2016 was similar.

The survivorship of rhizome fragments was $>75\%$ during the first year after transplanting regardless the year of the test (Fig. 1). The survivorship after 15 months of plantation suffered a strong decrease, and after the 40th month the survivorship was between 0 and 25% in all plots. The gravel bags did not improve the final survivorship in 2015 or 2016, but higher depths registered slightly better survivorships in 2016 (Table 2, Fig. 2). During the first year of plantation, between 20% and 60% of the transplanted fragments showed a net increase of shoot

Table 3

Logistic regression of the effect of substratum, depth and time (i.e. months after transplantation) on the percentage of fragments with net production of shoots in 2015 and 2016 plantations. Statistical significance ($p < 0.05$) are marked in bold.

	Plantation 2015				Plantation 2016			
	Estim.	SE	z-val.	P	Estim.	SE	z-val.	P
Intercept	-1.4914	0.4666	-3.196	<0.01	0.0111	0.3272	0.034	0.9730
Depth	0.0065	0.0208	0.312	0.748	-0.0031	0.0145	-0.218	0.8275
Substratum	-0.3417	.1719	-1.987	<0.05	0.1055	0.1186	0.889	0.3738
Time	-0.1059	0.0606	-1.746	0.080	-0.1018	0.0420	-2.423	<0.05

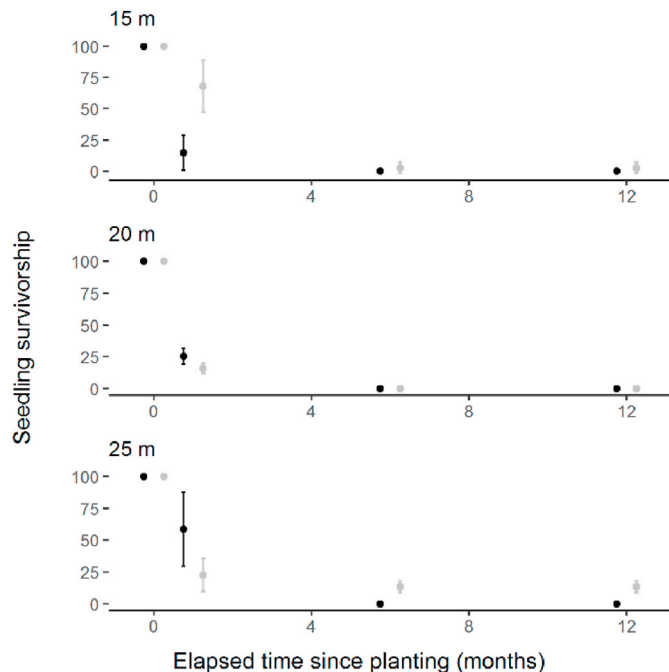


Fig. 3. Mean \pm SD of survivorship rate of seedlings after plantation in 2015 at different depth and substrata in Santa Ponça, Majorca: Sand (black circles), burlap bags filled with gravel (grey).

numbers (Fig. 2) suggesting good internal condition of the plants. In 2015 plantation, the number of fragments with net production of shoots were reduced in transplants on bags filled with gravel (Table 3). On the contrary, the overall effect of substratum was negligible in 2016 plantation, regardless the higher number of fragments with a net increase of shoot number in gravel bags at 25m after eight months of transplanting (Table 2, Fig. 2). Time had a negative effect on the number of fragments producing new shoots in both plantations (Table 3). The burlap bags started to degrade after one year from deployment and the gravel inside boiled over the surrounding sand and partly sunk in it.

Seedling survivorship drastically fell after 1 month from plantation regardless the substrata. Six months later, survivorship was null in all sand plots and lower than 15% in the plots on gravel bags (Fig. 3). Bioturbation and hydrodynamics caused the fast loss of seedlings.

Nutrient concentration in rhizomes and leaves did not change from collection to transplanting; except for the nitrogen concentration in the leaves, which was higher in the fragments after the period in the mesocosm than right after collection (Fig. 4A, Table 4). Carbon/nitrogen ratio in leaves and rhizome was slightly higher in the fragments transplanted in 2016 than in those transplanted in 2015 (Fig. 4B, Table 4). The vertical shoots of the transplanted fragments had higher concentration of nitrogen in leaves and rhizomes, and higher concentration of phosphorus in leaves but not in the rhizomes than vertical shoots in the adjacent meadow (Fig. 4C, Table 4).

4. Discussion

The disturbed substratum available after power line deployment and burial resulted inadequate for survival of transplants of *P. oceanica* (plagiotropic rhizome fragments) because it was low 40–48 months after transplanting. Topographic restoration measures to re-establish sea relief (i.e. burlap bags filled with gravel) did not result in better transplant survival rate. In the 2015 planting at 15 m depth and in the 2016 planting at 20 m depth final survivorship (i.e., 48 and 40 months after transplanting, respectively) in gravel bags was higher than the near-zero survivorship of fragments transplanted in sand. However, survivorship in the two substrata was not coherent during the study or among depths, probably because burlap bags filled with gravel changed with time: most of bags degraded after one year and the gravel and the surrounding sand became partially mixed. Previous published evidence showed successful natural recolonization of *P. oceanica* fragments on calcareous rubble mounds of particle size (average rubble measure: length 19.2 ± 9.5 SD cm, width 15.9 ± 2.3 SD cm, height 10.6 ± 1.9 SD cm) (Di Carlo et al., 2005; Di Carlo, 2009) bigger than the gravel used to fill the burlap bags. The transplanting of orthotropic fragments of rhizome on natural dead *matte* had survivorship rates between 25 and 73% after 36 months (Meinesz et al., 1993) and this rates rose to 76% (Piazzi et al., 1998) or 85% (Molenaar and Meinesz 1995) for plagiotropic fragments. The transplanting of fragments of *P. oceanica* rhizome in artificial mat substrata deployed in natural dead *matte*, show survival rates of 50% after three years of monitoring (Piazzi et al., 2021). This technique might improve substratum conditions before transplanting in areas where natural *matte* is lost or degraded. Non-consolidated sediments such as gravel and sand seem to detract natural recolonization of *P. oceanica* fragments (Badalamenti et al., 2011). However, Augier et al. (1996) show that orthotropic fragments anchored in groups by 50×50 cm cement plus metallic grid frames in sandy substratum were able to survive and increase in size 10 years after transplanting. Our results highlight the difficulty of recovering *P. oceanica* after mechanical impacts that eliminate the *matte* leaving a sandy substratum. The impact of coastal works on *P. oceanica* meadows is well documented (e.g. Ruiz and Romero, 2003; Badalamenti et al., 2006) and the need of minimizing coastal interventions to protect this endangered ecosystem is recognised by scientific community and managers. Our work highlights the need of minimizing interventions that affect not only the living meadows but also the remaining dead *matte* areas after an impact. Protecting *matte* seems a cost effective measure to facilitate future recovery of degraded or lost *P. oceanica* meadow. In the case of unavoidable interventions that will affect *matte* areas, it would be necessary to avoid transition to a non-consolidated substratum. In this sense, the addition of near to consolidated substrates (e.g. calcareous rubbles, stones of sizes that make their mobility by waves difficult) can facilitate natural recruitment (Di Carlo et al., 2005; Badalamenti et al., 2011) or future transplanting of fragments of rhizome (Alagna et al., 2019). However, Calvo et al. (2020) mentioned the economic cost and the introduction of an important quantity of exogenous material (i.e. rubbles, stones) in the environment as inconveniences of this measure. Intervention in current heavily degraded environments, former meadows, where only un-consolidated substratum is available might be feasible only if the remaining conditions (e.g. light, sedimentation rates) in the area are

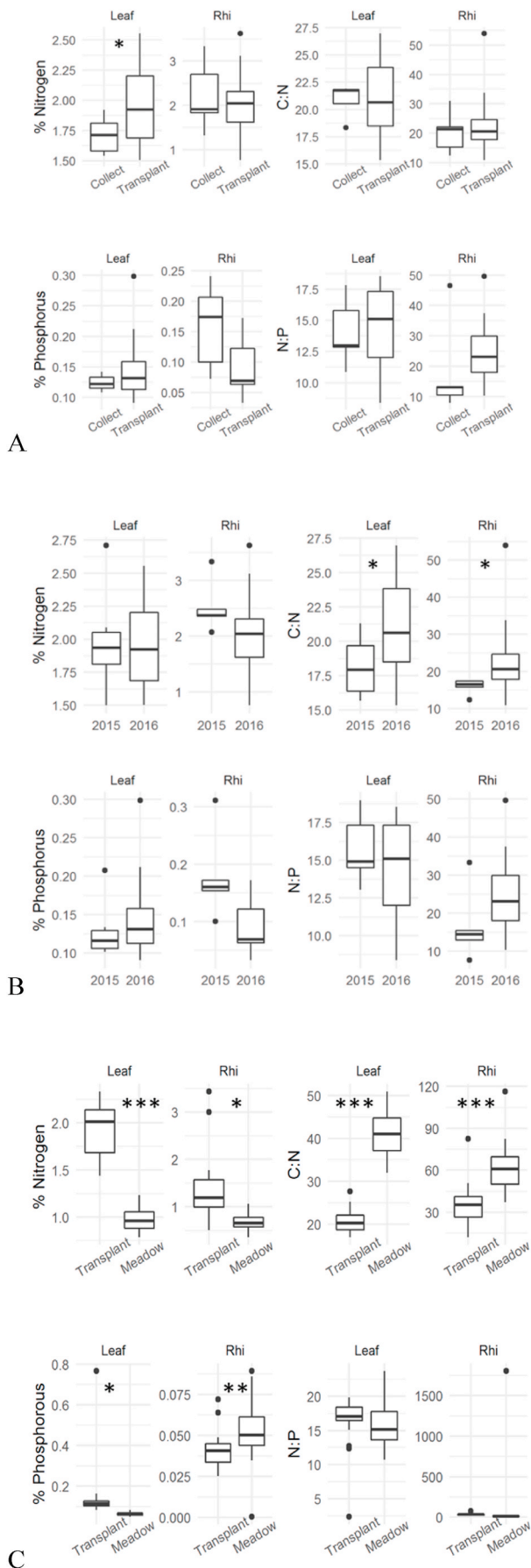


Fig. 4. Nitrogen and phosphorus content and CN and NP ratios in leaves and rhizome of A) the apical group from a subset of samples of fragments from Mallorca before ("Collect") and after ("Transplant") mesocosm period in 2016, B) of the apical group from a subset of samples of fragments from Mallorca just before planting in 2015 and 2016, C) of vertical shoots in a subset of samples of fragments from Mallorca just before planting ("Transplant") in 2016 and in the surrounding natural meadow. Significant differences are marked in each plot: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

appropriate for *P. oceanica* re-establishment and development (Clewett et al., 2004; van Katwijk et al., 2016). In these cases, testing the performance of artificial mat (Piazzi et al., 2021) can be of interest in sheltered environments once data about degradability of the geomat at sea are available. The addition of calcareous stones (Alagna et al., 2019) could be an alternative to facilitate natural recruitment and transplant in exposed areas or in those areas where sediment regrading is necessary to avoid further erosion (i.e. trench).

In addition, the good status of the fragments during the first year after transplanting, with high proportion of them with net increase of shoot number, did not mirrored the survival rate after 40–48 months since transplanting, which felt drastically between the 15th and the 40th month after transplant. Thus, a minimum 4-year monitoring of fragment survival and vegetative development after active transplanting is crucial to evaluate truthfully the transplantation success especially in an extremely slow growth species such as *Posidonia oceanica*. Fragment size and shoot density in transplanted *P. oceanica* patches surpasses values at transplanting after 6 years (Pirrotta et al., 2015).

The loss of seedlings planted in both substrata was almost total in just 10 months. Vertical growth of *P. oceanica* roots seems to be favoured in sandy sediment (Balestri et al., 2015), that was interpreted as a plant response to live in mobile sediment and get an effective anchoring. However, our results corroborate previous published evidence: seedlings do not tolerate sediment motion (Balestri et al., 1998; Piazzi et al., 1999; Infantes et al., 2011; Pereda-Briones et al., 2020) and therefore seedling planting in non-consolidated substratum should be discouraged. Total loss of planted seedlings suggests that the development of the adhesive root hairs that facilitate seedling anchoring in rocky substrata (Badalamenti et al., 2015) was not enough to provide an effective anchoring in the two unconsolidated substrata tested.

The percentage of organic matter in the first 10 cm of sediment was below 1.5% at all the stations, which suggests suitable sediment features for the development of *P. oceanica* (De Falco et al., 2000; Cancemi et al., 2003). Regarding grain size, *P. oceanica* distributes preferably on sandy bottoms (De Falco et al., 2000) and sand dominated the sediments in all the plantation sites. During the second year of monitoring, the hemp rope of the fragment anchoring system started to show signs of degradation, at the fourth year of monitoring the rope had disappeared. At this time, the few fragments alive were rooted naturally close to the staple that once served as the anchoring system. Most of the staples had no dead rhizome fragments or pieces of them attached and there were no dead rhizomes next to them. This points out to the degradation of hemp rope before the development of an effective adventitious root system by the rhizome fragments as a possible cause of the fragment loss in the last two years of the study. Root development is essential for the transplants to cope with hydrodynamics (Infantes et al., 2011) and to provide nutrient supply for the plant requirements and assure fragment long term survival (Lepoint et al., 2004; Vangeluwe et al., 2004; Balestri and Lardicci, 2006). The development of roots in fragments of rhizome requires at least 3–12 months and occurs in summer/spring (Meinesz et al., 1992; Balestri et al., 2011). Our results point out that the development of an effective root system in transplanted fragments may be slower than previous published evidence. Limited availability of reserves in the transplanted fragments of rhizome could have slowed root formation (Lepoint et al., 2004; Vangeluwe et al., 2004). The nitrogen content (%DW) in leaves in the transplanted fragments (mean \pm sd; 2015, 1.99% \pm 0.40; 2016, 1.96% \pm 0.32) was above the previous

(caption on next column)

Table 4

T-test results. Nitrogen and phosphorus content and CN and NP ratios in leaves and rhizome of the apical group from a subset of samples of fragments from Mallorca before ("Collect") and after ("Transplant") mesocosm period in 2016, of the apical group from a subset of samples of fragments from Mallorca just before planting in 2015 and 2016 and of vertical shoots in a subset of samples of fragments from Mallorca just before planting ("Transplant") in 2016 and in the surrounding natural meadow.

Comparison	Nutrient	t-value	df	P
Fragments before and after mesocosm period. 2016 plantation	N Leaf	2.5031	13.64	<0.05
	P Leaf	1.5839	22.52	Ns
	C:N Leaf	0.16785	15.52	Ns
	N:P Leaf	0.3378	7.36	Ns
	N Rhi	-0.4348	7.02	Ns
	P Rhi	-1.9776	5.33	Ns
	C:N Rhi	0.68145	11.47	Ns
	N:P Rhi	0.9354	5.48	Ns
	N Leaf	0.137	7.03	Ns
	P Leaf	-0.731	10.32	Ns
Fragments just before transplant: 2015 plantation vs 2016 plantation	C:N Leaf	-2.396	13.03	<0.05
	N:P Leaf	0.9881	12.30	Ns
	N Rhi	1.606	11.91	Ns
	P Rhi	2.372	5.07	Ns
	C:N Rhi	-2.357	13.96	<0.05
	N:P Rhi	-1.6644	8.08	Ns
	N Leaf	14.536	22.72	<0.001
	P Leaf	2.426	18.07	<0.05
	C:N Leaf	-18.412	48.13	<0.001
	N:P Leaf	0.7593	27.80	Ns
Natural meadow vs. Transplant fragment. 2016 plantation	N Rhi	3.730	15.73	<0.01
	P Rhi	-2.646	40.76	<0.05
	C:N Rhi	-5.063	30.41	<0.001
	N:P Rhi	0.6350	30.22	Ns

values reported in Balearic Islands natural meadows (e.g. Fourqurean et al., 2007, $1.63\% \pm 0.39$; Terrados et al., 2008, $0.07\text{--}1.3\% \pm 0.03$; Castejón-Silvo et al., 2012, $1.50\% \pm 0.18$). Leaf phosphorous content in transplanted fragments (2015, $0.13\% \pm 0.04$; 2016, $0.14\% \pm 0.05$) was also above previously reported content in Balearic Islands meadows (Fourqurean et al., 2007, $0.12\% \pm 0.04$; Terrados and Medina-Pons, 2011, $0.05\text{--}0.08\% \pm 0.003$; Castejón-Silvo et al., 2012, $0.09\% \pm 0.017$). Thus, leaf nutrient content does not suggest nutrient limitation of the fragments at the time of transplanting, although most of bibliography refers nutrient content of orthotropic shoots and our results correspond to the nutrient content of the apical group of shoots, that is, a plagiotropic shoot. The comparison of orthotropic shoots from transplanted fragments and from natural meadow revealed higher nitrogen and phosphorous %DW in leaves and rhizomes in the transplanted fragments. The N:P atomic ratio of the transplanted fragments (2015, 34.82; 2016, 32.21) indicates that nitrogen is at higher availability than phosphorus (N:P ratio of nutrient-added plants = 31.5: Alcoverro and Romero, 1997). The drastic fall of survival after the first year of transplantation could be explained by a slow development of the adventitious roots after transplantation and the obsolescence of the artificial anchoring system. In any case, our results show that artificial anchoring systems should provide efficient anchoring for between 1.5 and 4 years in underwater conditions for achieving success. Our results suggest that sandy and gravel sediments disturbed after underwater works do not favour the re-establishment of *P. oceanica*. This conclusion is consistent with the natural recolonization pattern found by Badalamenti et al. (2011): calcareous rubbles were more favourable than boulders, sand or even dead *P. oceanica* matte for the establishment of adrift rhizome fragments.

Published evidence demonstrates that *P. oceanica* vegetative fragments can stay in mesocosm conditions for at least two years with acceptable mortality rates (<40%) (Meinesz et al., 1991; Balestri et al., 2011). We found no evident changes in the nutrient content (leaves or rhizome) of the fragments during the three month stay in mesocosms. The nutrient concentration in the mesocosm sea water was

representative of the oligotrophic Balearic waters (Puigserver et al., 2010, nitrate: 0.04–5.53 μM , phosphate: <0.02 μM). This is the first assessment of the effects of a maintenance period in mesocosms on the nutrient content of *P. oceanica*, and it did not significantly change the nutrient content of the fragments. Maintenance of fragments in mesocosm before transplanting allows discarding the *P. oceanica* fragments in bad condition without compromising the condition of the rest of fragments. Strikingly, the nitrogen and phosphorus content in the leaves and nitrogen content in rhizome from the fragments cut from the natural meadow were lower than those measured in the adrift fragments maintained and transplanted. Thus, low nutrient availability for transplanted fragments is not likely to explain fragment loss.

5. Conclusions

In summary, this is the first experience of active replanting of *P. oceanica* vegetative fragments on a substratum disturbed after power line deployment and sea floor trenching to bury a power line and the success was low. *P. oceanica* is a slow-growing species and, without active initiatives to facilitate recovery, the natural recolonization would last for decades. This study confirms the crucial role of the extant substratum after underwater works for *P. oceanica* recolonization. Our results highlight the need for implementation of mitigation measures that provide substratum suitable for recolonization after dredging activities or any other mechanical impact that changes substratum features in a *P. oceanica* meadow. The findings described here allow concluding that a sandy substratum or burlap bags filled with gravel are not appropriate for *P. oceanica* establishment and development. Preservation of meadow substratum (i.e. dead matte) is a critical element that coastal infrastructure projects should consider to enable future recovery of *P. oceanica* meadows. Finally, the future transplantation of fragments of rhizome of *P. oceanica* needs to consider the obsolescence of anchoring system to allow the development of an effective root system before it degrades. In the case of *P. oceanica* the anchoring system of rhizome fragments should be effective for at least four years. We consider the effect of applying chemicals to stimulate root formation (Balestri and Lardicci, 2006) a pertinent line of research to improve *P. oceanica* transplanting techniques.

Declaration of competing interest

The authors 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

The work was funded by the Red Eléctrica de España through the contract "Use of seeds and fragments of *Posidonia oceanica* for the recovery of areas affected by Red Eléctrica de España's activity" awarded to CSIC. We thank the Interpretation Center of Cabrera National Park for the use of their mesocosm facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2021.105406>.

CRedit author statement

Inés Castejón-Silvo: Methodology, Formal analysis, Investigation, Writing – original draft, Project administration, Visualization, Jorge Terrados: Conceptualization, Methodology, Investigation, Writing – review & editing, Funding acquisition, Supervision.

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