



# Different thermal regimes and susceptibility to herbivory do not constrain seagrass seedling restoration

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## ABSTRACT

Recovering seagrass ecosystems through restoration has become impellent to re-establish their functionality and services. Although the use of seedlings may represent an appropriate solution, little information is provided on the seedling-based restoration effectiveness with influence of biotic and abiotic interactions.

Survival, morphological development and leaf total phenol content of transplanted *Posidonia oceanica* seedlings were evaluated under different origin, thermal regimes and herbivore pressure through a five-months field experiment in two MPAs, located on the west (cold) and east (warm) Sardinia coast to explore the effectiveness of seedling-based restoration.

Seedlings originated from the two coasts responded differently to thermal regime site and herbivory pressure, as the warm-adapted ones survived less but developed more (and *vice-versa*) and resisted to the herbivory pressure increasing their phenol content, thus showing compensating responses.

This study provided information on the *P. oceanica* seedling-based restoration by investigating abiotic and biotic interactions with the transplanted plants. It promotes the collection of beach-cast fruits from different coasts and their transplantation, regardless their origin, with no need of protecting seedlings from predators.

## 1. Introduction

Seagrass meadows are the most important coastal ecosystems that provide fundamental goods and services such as carbon sequestration, nutrient dynamics, sediment stabilization, providing habitats and nursery grounds, and preventing coastal erosion (Fourqurean et al., 2012; Hemminga and Duarte, 2000; Larkum et al., 2006; Macreadie et al., 2014). However, seagrasses are suffering a global decline due to direct and indirect anthropogenic threats, combined with the synergic effects of climate change (Bockelmann et al., 2012; Duarte et al., 2018; Giakoumi et al., 2015; Unsworth et al., 2015). Consequently, protection measures alone are not sufficient to guarantee the maintenance of seagrass beds (Griffiths et al., 2020) and relying on active conservation actions, such as restoration, has become impellent (Bastyan and Cambridge, 2008; Campbell, 2002; Kennish, 2022; Matheson et al., 2017; Orth and McGlathery, 2012; Paulo et al., 2019). In recent decades many seagrass restorations have been performed worldwide with a large

variety of techniques (Fraschetti et al., 2021; Tan et al., 2020; van Katwijk et al., 2016), so that the outcome of seagrass transplants remains quite controversial. Furthermore, several seagrass restorations are developed in Marine Protected Areas (MPAs) where the mitigation of the sources of human disturbance can be achieved, but the possible effect of the re-established species interactions on transplants success is largely ignored (Hopf et al., 2022).

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica* (L.) Delile, 1813 has been probably the main target species of restoration programs likely due to the ecosystem services it provides (Pergent-Martini et al., 1994; Rigo et al., 2021; Scanu et al., 2022; Vassallo et al., 2013), to the rapid decline it is facing (Marbà and Duarte, 2010; Telesca et al., 2015), and to the very slow growth rate that makes the rehabilitation extremely challenging (de los Santos et al., 2019; Greening et al., 2011; Hall et al., 2021; Vaudrey et al., 2010). In the last 40 years, some evidence has been gained about the methodologies (i.e., using different plant portions) and techniques (such as staples, grids,

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organic carpets) under different environmental conditions (transplanting depth and substrate, Boudouresque et al., 2021; Pansini et al., 2022). Overall, seeds and seedlings from beach-cast fruits may represent an appropriate solution to restoration (Alagna et al., 2020; Escandell-Westcott et al., 2023; Mancini et al., 2024; Terrados et al., 2013) because they allow increasing the genetic variability of meadows (Kendrick et al., 2017; Reynolds et al., 2012), contributing to seagrass productivity and recovery potential (Hughes and Stachowicz, 2004; Procaccini and Piazzini, 2001; Reusch et al., 2005; Reusch and Hughes, 2006). At the same time, a seedling-based restoration may minimize the impact of collecting cuttings from donor meadows (Mancini et al., 2021). The collection of seedlings could also be supported by an intensified sexual reproduction in *P. oceanica* with climate change (Stipcich et al., 2024a, 2024b; Tomas et al., 2024). However, seagrass recruitment is unobvious, since fruit production is highly variable (Stipcich et al., 2024a) and the rate of seedling establishment is very low (Pereda-Briones et al., 2020), since its success is limited to safe microsites, as on hard substrates in limited hydrodynamic forces (Alagna et al., 2020; Pereda-Briones et al., 2020). Therefore, understanding the mechanisms that lead to a favourable seed and seedling development has become of primary importance (Guerrero-Meseguer et al., 2018; Marín-Guirao et al., 2019). Although evidence on seedlings response to different types of substrata, transplanting depth, and anchoring techniques has been provided (Alagna et al., 2020; Domínguez et al., 2012; Escandell-Westcott et al., 2023; Mancini et al., 2024), only a few studies evaluated through field experiments their response to future conditions, such as increased temperature, acidification conditions (Pansini et al., 2023; Stipcich et al., 2022, 2024c), and interactions with other species (Alagna et al., 2013; Balestri et al., 2021). Mesocosm observations indicated that warming might hamper seedling survival, morphological and biochemical traits, according to temperature thresholds and age (Guerrero-Meseguer et al., 2018; Hernán et al., 2017), although other evidence showed a high resilience to acute climate change impacts, as high temperature and salinity conditions (Provera et al., 2024).

Herbivory, one of the main drivers of shoot condition and seagrass communities (Christianen et al., 2012; Planes et al., 2011; Unsworth et al., 2007; White et al., 2011), can also cause seedling mortality during the early development (Barton and Hanley, 2013; Moles and Westoby, 2004). This process could be exacerbated in MPAs, where fish herbivore assemblages are more structured (Raventos et al., 2009). At the same time, MPAs are likely to become participative in restoration considering this action as a management measure to seagrass conservation and climate change mitigation (Hu et al., 2021). Moreover, warmer conditions could increase seedling exposure to herbivores, likely due to the higher palatability, for the lower leaf thickness, fiber, and phenolic content (Hernán et al., 2017; Rotini et al., 2018; Stipcich et al., 2023; Zvereva and Kozlov, 2006), and to the increased grazer metabolism and thus higher predation rate (Carr et al., 2018; Svensson et al., 2017).

This study aimed at evaluating how the origin, temperature, and herbivory pressure affect the development of *P. oceanica* seedlings, hypothesising that differences could be attributed to acclimation vs. adaptative morphological and biochemical responses by using seedlings of different origin (Mediterranean biogeographic sectors). Therefore, changes in seedling survival, morphometry and total phenol content were evaluated in two MPAs of Sardinia (Italy) through a five-months field exclusion experiment where plants were cross-transplanted. The fish *Sarpa salpa*, the most common *P. oceanica* herbivore, is present at both MPAs (Guidetti et al., 2014), but differences in thermal regimes between the two sites are considerable. Overall, the results identify proper planting features promoting facilitative mechanisms in seedling-based *P. oceanica* restoration.

## 2. Materials and methods

### 2.1. Fruit collection and seed germination

During April and May 2023 *P. oceanica* beach-cast fruits (with open pericarps) were collected in several areas from the west and east coast of Sardinia, Italy (Fig. S1a). On each collection time, seeds extracted from fruits were immediately inserted in two tagged nets (Fig. S1b): one was transported to Capo Caccia – Isola Piana MPA (west coast) and the other to Tavolara Punta Coda Cavallo MPA (east coast), hereafter CC and TA sites, respectively. In both sites, the nets were fixed to the substrate (matte, a dead *P. oceanica* meadow patch) at 3 m of depth where the seeds were let to germinate for one month. Total seed germination percentage was overall 55.41%.

### 2.2. Experimental design and set-up

The effects of origin, development site, and herbivory pressure were evaluated in the same germination sites (Fig. 1). Despite the proximity and similar abiotic features, these sites are in two different Mediterranean biogeographic sectors: the Sardinian Sea and the Tyrrhenian Sea for the west and east coast, respectively (Bianchi et al., 2012). Temperature regimes are very different (Pansini et al., 2021), mainly due to currents and receiving winds, characterising the west coast with a colder and more variable regime, compared to the warmer and constant temperature of the eastern one (Olita et al., 2013; Pinardi et al., 2015).

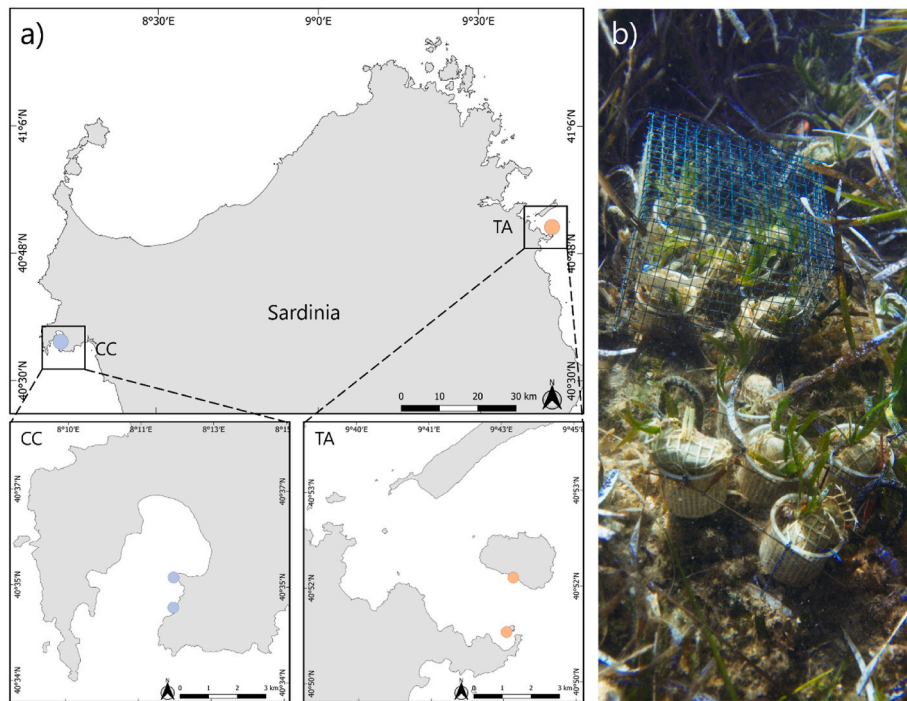
The cross-transplantation experiment was done on 10th and June 11, 2023 (hereafter T<sub>0</sub>, Fig. 2) for TA and CC sites, respectively. At each site 60 seedlings were selected from each germination nets, measured (see section 2.3) and placed in the field.

Seedlings were adhered to a pebble with an elastic net used to stimulate root adhesion (Alagna et al., 2015), then was attached to a 6 cm plastic pot which was fixed in 20 × 20 cm metal grids. In each grid, 15 randomly tagged seedlings (three seedlings/pot) were placed. Grids were anchored to the matte at a depth of 3 m using pegs, for a total of 16 grids used. To examine the effect of herbivory pressure on seedling development, half of the grids were covered with a predator exclusion cage (1 cm in mesh size, Fig. 1b). At each site, two areas distancing 1 km were selected, and four grids were placed in each area. Each combination of treatments, development site (CC and TA), origin (west and east), and herbivory (yes and no) included n = 15 seedlings, for a total of N = 240 (Fig. S2).

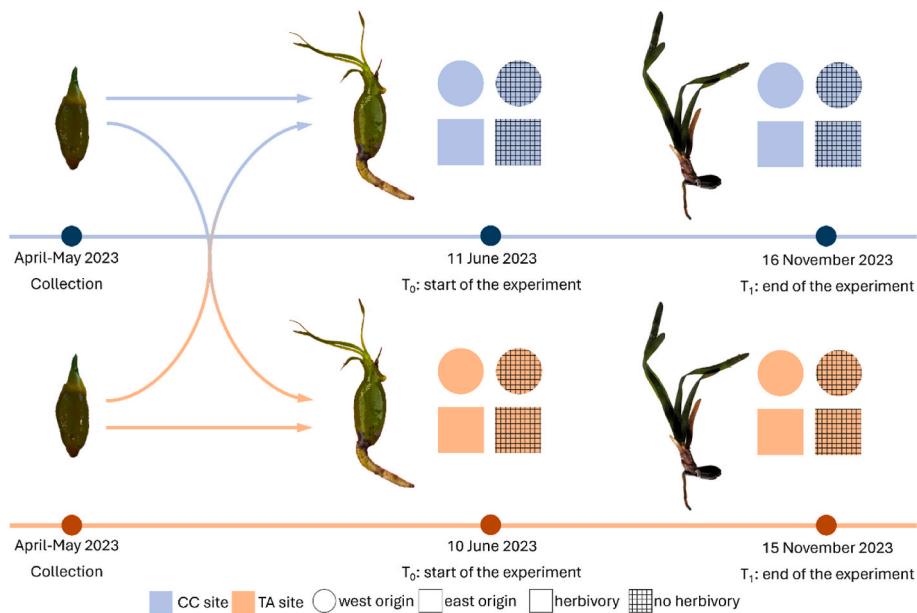
All grids were visited and cleaned from epiphytes every three weeks. Unfortunately, in November one grid of the CC site holding the seedlings from the east coast and not exposed to herbivory was lost. Temperature and light intensity were measured every hour with a logger (HOBO Pendant® Temp/Light UA-002-64) for the whole period (Fig. S3). In each area the herbivory presence was estimated at 3 m of depth on three times (second week of July, last week of August, and first week of October for each site) with the underwater visual census (UVC) along three linear transects of 25 × 5 m, where *S. salpa* individuals per size class (number of individuals/125 m<sup>2</sup> of <10 cm = small, 10–20 cm = medium, and >20 cm = large) were counted. No sea urchin *Paracentrotus lividus*, a further herbivore of *P. oceanica*, was found at the areas. At the end of the experiment (15th and 16th November for TA and CC sites, respectively, hereafter T<sub>1</sub>) all the material was removed and analysed.

### 2.3. Data collection

The number of leaves, the length of the three longest leaves (considered as the sum), the length of the necrotic tissue of the three longest leaves (ratio on the total leaf length), number of roots and length of the longest root were measured at each experimental time (Fig. S4), while survival rate was calculated at T<sub>1</sub>. Morphological development and survival rate were expressed as the ratio T<sub>1</sub>/T<sub>0</sub>. At T<sub>1</sub> the number of



**Fig. 1.** Study area. a) MPA sites located in the west (cold) and east (warm) coast of northern Sardinia (CC and TA, respectively) with two areas for each site. b) experimental setting: grids containing seedlings fixed to the dead matte with (upper) and without (lower) exclusion cage.



**Fig. 2.** Experimental timeline and set-up. For each MPA, seedlings collected from both coasts were left to germinate, half of the seedling were cross-transplanted, and the other served as control. At  $T_0$ , half of the material were covered with an exclusion cage. For each experimental time, morphometry measures were collected, while at  $T_1$  all the seedlings were recollected for analyses.

*S. salpa* bites per seedling was also measured. In laboratory leaves were cleaned from epiphytes and total phenols content was extracted and quantified (Migliore et al., 2007): 100 mg of fresh weight of green leaf tissue were collected from the second to the sixth leaf of each seedling and grounded in liquid  $N_2$  with mortar and pestle. The powder was extracted twice in 4 mL of 0.1 N HCl, with the first extraction kept overnight at 4 °C under dark. After centrifugation (20 min; 4000 g), the 8 mL supernatants were pooled and 50  $\mu$ L of them were mixed with 475  $\mu$ L of 0.25 N Folin-Ciocalteu reagent and, after 3-min incubation, with

475  $\mu$ L of  $Na_2CO_3$  (7.5%), according to Booker and Miller (1998). After 60 min the absorbance was measured at 724 nm using a spectrophotometer ONDA© UV-30 SCAN. Total phenol content was quantified by the external standard plot method: a calibration curve was built by using five different concentrations (0, 25, 50, 100 and 200  $\mu$ g/mL) of chlorogenic acid ( $r^2 = 0.99$ ) and the phenol concentration was quantified as chlorogenic acid equivalents (mg/g) of leaf fresh weight.

## 2.4. Statistical analysis

Univariate statistics was used to assess the differences in seedling survival, morphological development (expressed as  $T_1/T_0$ ) and phenol content depending on the origin, development site and herbivory. Since data were not balanced (due to the grid loss and seedling mortality), data gained by the two areas were pooled and PERMANOVAs (Anderson, 2001) were run testing the effects of three fixed factors: development site (CC vs. TA), origin coast (west vs. east), and herbivory (yes vs. no). Differences on the number of bites per seedling due to the development site and origin was conducted by a two-way PERMANOVA. Before running the models, a resemblance matrix based on Euclidean distance was calculated and the factors were tested on each response variable using a permutation of residuals under a reduced model (9999 permutations) and a partial sum of squares (Type III). Pair-wise tests identified alternative hypotheses on significant interactions. Differences in temperature (between sites) and *S. salpa* abundance (between sites and fish size classes) were evaluated with PERMANOVAs, since data did not meet parametric assumptions. All analyses were performed using the Primer 6 v.6.1.13 & PERMANOVA + software (Clarke and Gorley, 2006).

## 3. Results

### 3.1. Environmental features

Temperature in CC site was lower than in TA site (Fig. 3a, Table S1) with a mean of the daily difference of 1.35 °C (1.95 °C for the summer period). The sites were also different in temperature range, since in TA and CC the difference between the maxima and minima were 8.2 °C and 9.0 °C, respectively. *S. salpa* abundance did not differ between the sites, but in both sites small sized individuals were more abundant than medium and large adults (Fig. 3b–Table S1), likely for the seasonal variability of the species (Buñuel et al., 2020).

### 3.2. Seedling survival and traits

*Posidonia oceanica* seedling survival was negatively influenced by herbivory ( $0.81 \pm 0.09$  and  $0.58 \pm 0.08$  mean  $\pm$  SE for no and yes herbivory, respectively). The survival depended also on the interaction between site and origin: in TA site, seedlings originating from the east coast survived almost half than the west seedlings, and on the contrary they survived more in CC than in TA site (Fig. 4; Tables 1 and 2).

Seedling morphology was consistently dependent on the origin, which strongly affected all the investigated traits (Table 1): seedlings originated from the east coast exhibited a higher morphological development, in terms of higher number of leaves and roots, leaf and root length, number of roots and lower leaf brown tissue. In addition, TA site positively affected the development of leaf and root length (Fig. 4;

Tables 1 and 2). No significant differences due to herbivory were found.

Seedling origin also affected herbivory pressure but differently depending on the site: in fact, the number of bites per seedling was more than threefold higher in plants originating from the east coast, but only in TA site, since in CC site the herbivory the seedlings regardless their origin (Fig. 5a; Tables 1 and 2).

Total phenol content was affected by the interaction between site, origin, and herbivory (Fig. 5b–Tables 1 and 2). Seedlings from the west coast exhibited higher phenol content, only in CC site. Conversely, seedlings originated from the east coast showed differences in herbivory pressure: when exposed to herbivory, they showed a higher phenol content, but only in TA site. Seedlings that were not cross-transplanted (i. e., west origin in CC and east origin in TA) showed higher phenol content.

## 4. Discussion

The morphological development and leaf phenol content of transplanted *P. oceanica* seedlings were investigated under different thermal regimes and herbivory pressure through a cross-transplantation experiment, taking advantage of two MPA sites located in the west and east coast of Sardinia, colder and warmer, respectively. Overall, evidence here collected indicates that seedling performance is the result of both adaptive and acclimation features, assisting effective seagrass restoration. Seedlings originated from different coasts performed differently to thermal regime and herbivory pressure, as the warm adapted ones survived less but developed more (in both above and below ground structures) and resisted to the herbivory pressure increasing their phenol content. On the contrary, the cold-adapted plants survived more and developed less, showing compensating responses.

Results supported the importance of the seedling origin on their performance, as it affected all investigated variables. In both sites the fruits were collected in several surveys and beaches, granting a variety within each origin, so that a procedural bias could be excluded. Specifically, the west origin seedlings showed higher acclimation, as they survived at the development sites similarly regardless the different conditions, while at TA site the east origin seedlings had a lower survival than those coming from the west. A higher mortality for seedlings generated and developed in similar environmental features seemed to depend on genetic and intrinsic factors (Balestri and Lardicci, 2008; Micheli et al., 2010; Piazzini et al., 1999), and dispersal strategies (Howe, 1989). Seagrasses adopt the clonal growth as the main mechanism for meadow expansion (Migliaccio et al., 2005; Tomlinson, 1974) and likely to increase the resilience and adaptation to future environmental changes (Ehlers et al., 2008; Reusch et al., 2005). The mortality of east origin seedlings in TA site may be driven by the negative effects of within-population proximity, supporting the hypothesis formulated for terrestrial plants for which seedlings germinated far from parents are more likely to avoid natural stressors, as pathogens or herbivories

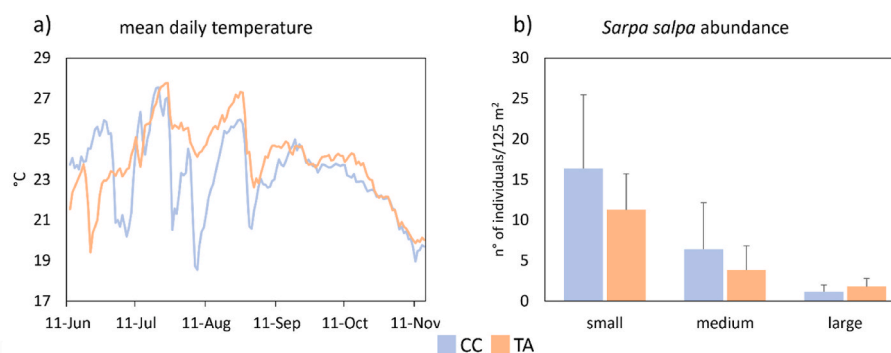


Fig. 3. a) Mean daily temperature at each site during the experiment (June 11, 2023–November 16, 2023). b) Mean (+SE) *S. salpa* abundance per size class (UVC, three surveys; small <10 cm; medium = 10–20 cm; large >20 cm).



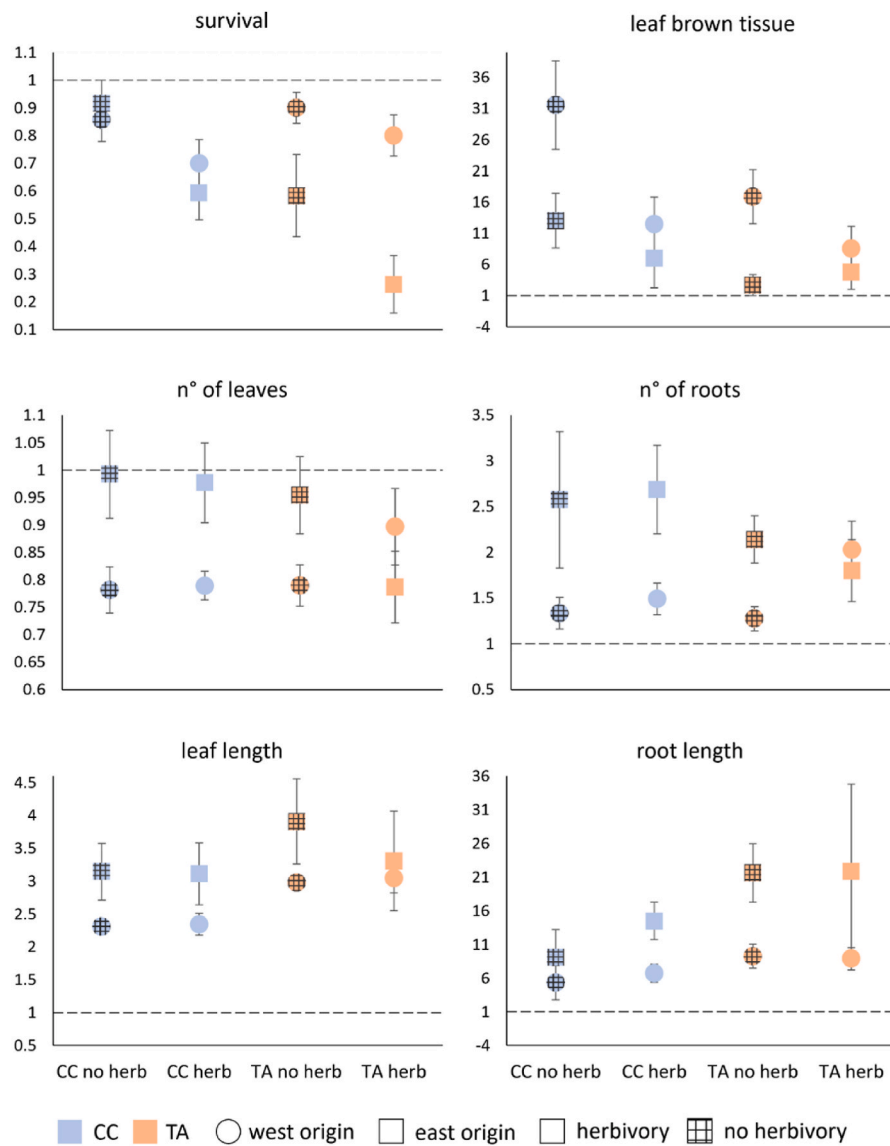


Fig. 4. Survival and morphological development ( $T_1/T_0$ , mean  $\pm$  SE) of *P. oceanica* seedlings for each site, origin, and herbivory. Value = 1 indicates no change; values > 1 indicate an increase; values < 1 indicate a decrease.

Table 1

PERMANOVA results on *P. oceanica* seedling survival, morphological development, number of bites and leaf total phenol content due to site, origin, and herbivory. Significant values ( $P_{(perm)} < 0.05$ ) are highlighted in bold.

PERMANOVA	Site	Origin	Herbivory	Site $\times$ Orig	Site $\times$ Herb	Orig $\times$ Herb	Site $\times$ Orig $\times$ Herb
<b>Survival</b>							
survival	<i>Pseudo-F</i> <sub>1,173</sub> 3.81	<i>Pseudo-F</i> <sub>1,173</sub> <b>11.44</b>	<i>Pseudo-F</i> <sub>1,173</sub> <b>11.44</b>	<i>Pseudo-F</i> <sub>1,173</sub> 9.14	<i>Pseudo-F</i> <sub>1,173</sub> 0.05	<i>Pseudo-F</i> <sub>1,173</sub> 2.11	<i>Pseudo-F</i> <sub>1,173</sub> 0.04
<b>Morphological variables</b>							
n° of leaves	<i>Pseudo-F</i> <sub>1,121</sub> 0.34	<i>Pseudo-F</i> <sub>1,121</sub> <b>5.39</b>	<i>Pseudo-F</i> <sub>1,121</sub> 0.12	<i>Pseudo-F</i> <sub>1,121</sub> 3.09	<i>Pseudo-F</i> <sub>1,121</sub> 0.07	<i>Pseudo-F</i> <sub>1,121</sub> 2.33	<i>Pseudo-F</i> <sub>1,121</sub> 1.67
leaf length	<i>Pseudo-F</i> <sub>1,121</sub> <b>6.23</b>	<i>Pseudo-F</i> <sub>1,121</sub> <b>8.93</b>	<i>Pseudo-F</i> <sub>1,121</sub> 0.32	<i>Pseudo-F</i> <sub>1,121</sub> 0.20	<i>Pseudo-F</i> <sub>1,121</sub> 0.33	<i>Pseudo-F</i> <sub>1,121</sub> 0.62	<i>Pseudo-F</i> <sub>1,121</sub> 0.40
brown tissue	<i>Pseudo-F</i> <sub>1,121</sub> 3.40	<i>Pseudo-F</i> <sub>1,121</sub> <b>6.13</b>	<i>Pseudo-F</i> <sub>1,121</sub> 3.40	<i>Pseudo-F</i> <sub>1,121</sub> 0.13	<i>Pseudo-F</i> <sub>1,121</sub> 1.23	<i>Pseudo-F</i> <sub>1,121</sub> 1.90	<i>Pseudo-F</i> <sub>1,121</sub> 0.02
n° of roots	<i>Pseudo-F</i> <sub>1,121</sub> 0.60	<i>Pseudo-F</i> <sub>1,121</sub> <b>8.01</b>	<i>Pseudo-F</i> <sub>1,121</sub> 0.39	<i>Pseudo-F</i> <sub>1,121</sub> 2.73	<i>Pseudo-F</i> <sub>1,121</sub> 0.02	<i>Pseudo-F</i> <sub>1,121</sub> 1.10	<i>Pseudo-F</i> <sub>1,121</sub> 0.93
root length	<i>Pseudo-F</i> <sub>1,121</sub> <b>8.68</b>	<i>Pseudo-F</i> <sub>1,121</sub> <b>17.28</b>	<i>Pseudo-F</i> <sub>1,121</sub> 0.61	<i>Pseudo-F</i> <sub>1,121</sub> 2.50	<i>Pseudo-F</i> <sub>1,121</sub> 0.54	<i>Pseudo-F</i> <sub>1,121</sub> 0.31	<i>Pseudo-F</i> <sub>1,121</sub> 0.11
<b>Biochemical variables</b>							
phenol content	<i>Pseudo-F</i> <sub>1,115</sub> 2.24	<i>Pseudo-F</i> <sub>1,115</sub> <b>7.75</b>	<i>Pseudo-F</i> <sub>1,115</sub> <b>17.63</b>	<i>Pseudo-F</i> <sub>1,115</sub> <b>24.82</b>	<i>Pseudo-F</i> <sub>1,115</sub> <b>8.50</b>	<i>Pseudo-F</i> <sub>1,115</sub> <b>4.90</b>	<i>Pseudo-F</i> <sub>1,115</sub> <b>11.10</b>
n° of bites/seedling	<i>Pseudo-F</i> <sub>1,62</sub> 0.53	<i>Pseudo-F</i> <sub>1,62</sub> 3.68	–	<i>Pseudo-F</i> <sub>1,62</sub> <b>6.18</b>	–	–	–

**Table 2**  
Comparison of means of *P. oceanica* variables for the significant interactions of site, origin, and herbivory.

PAIRWISE TEST	Site comparisons		Origin comparisons		Herbivory comparisons	
survival	WEST	CC =	TA	W >		
		TA		E		
	EAST	CC >	CC	W =		
		TA		E		
n° of bites/ seedling	WEST	CC =	WEST	W <		
		TA		E		
	EAST	CC =	EAST	W =		
		TA		E		
phenol content	WEST	CC >	CC NO	W >	CC	NO =
	NO	TA		E	WEST	YES
	WEST	CC >	CC	W >	CC	NO =
	YES	TA	YES	E	EAST	YES
	EAST	CC =	TA NO	W >	TA	NO =
	NO	TA		E	WEST	YES
	EAST	CC <	TA	W <	TA	NO <
	YES	TA	YES	E	EAST	YES

(Augspurger, 1984; Howe, 1989; Sugiyama et al., 2018). Indeed, less surprising is the effect of herbivory on seedling survival since the negative impact of grazing in seagrass beds (Larkum et al., 2006; Moles and Westoby, 2004) and transplanted plants (Charbonnel et al., 1995; Mancini et al., 2022) has been described. Although this finding is in contrast with patterns found in three-years old transplanted *P. oceanica* seedlings (Balestri et al., 1998), the use of very young (one-month old) germinated seedlings in this study could have increased susceptibility to grazing with respect to the older ones. In fact, their leaf phenol content is lower if compared to adult leaves (Rotini et al., 2023) and seagrass palatability is increased by a low phenol content (Rotini et al., 2018).

Furthermore, the influence of genetic and intrinsic factors on plant early-life stage is corroborated by seedling morphological development, as the east origin ones had a higher performance in number of leaves and roots, longest leaf, and root length, independently of the development site, suggesting an adaptive advantage of higher plant productivity when parent meadows are located in a warmer environment (Bennett et al., 2022). Such results are also corroborated by the higher leaf brown tissue (a sign of stress; Minina et al., 2013) in west origin seedlings at both sites and the lower phenol content in cross-transplanted seedlings, as if they have invested their energy on their establishment rather than in defence mechanisms (phenols). Interestingly, the seeds released by warm-adapted meadows are likely constrained on their survival at first, but then the germinated ones could foster the development of an appropriate leaf system to become independent from the seed reserves (Hernán et al., 2017; Stipcich et al., 2022). Moreover, warm-origin seedlings could be adapted to have a higher metabolic activity,

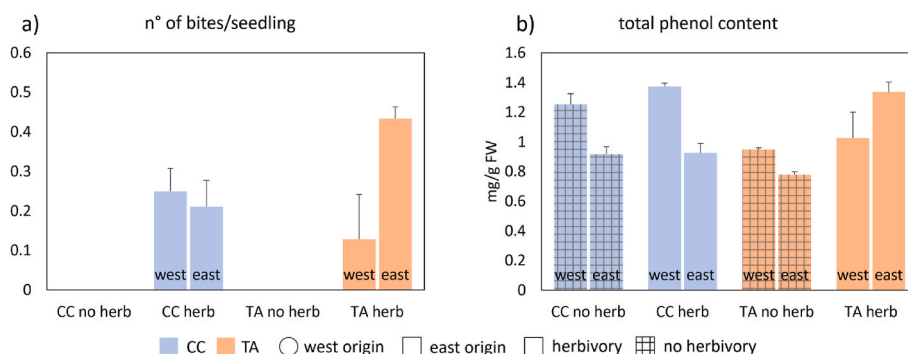
leading to produce larger root system, ensuring their anchoring capacity and nutrient acquisition during restoration actions. On the other hand, higher seedling performance could consequently increase palatability and vulnerability to herbivory.

In fact, although *S. salpa* abundance was similar at the sites, herbivory patterns were influenced by both the seedling origin and the development site, indicating that at TA site the east origin seedlings, corresponding to the more developed ones, were more grazed than the west origin ones. On land, studies have shown higher seed and seedling predation near parent plants (Harms et al., 2000; Janzen, 1970). Higher palatability under warmer conditions would likely be combined with the increasing feeding rates of herbivores due to increased metabolic requirements at higher temperatures (Burnell et al., 2013; Lemoine et al., 2014) and the absence of structural defences, therefore making plants further susceptible to grazing. This could also be corroborated by the higher impact of *S. salpa* on seagrasses inside MPAs: denser populations of grazers may consume more forage, first reducing the epiphyte biomass, which might induce higher grazing on seagrass leaves (Jiang et al., 2023; Raventos et al., 2009). Moreover, warming favours the decrease of biochemical components invested as defence to herbivores in marine vegetation (Hernán et al., 2017; Mannino et al., 2016). Indeed, here in seedlings inaccessible to herbivores the leaf phenol content changed depending on the origin, being lower in the east origin than in the west and regardless the development site. Nevertheless, grazed seedlings respond to grazing by increasing their total phenol content (i.e., the west origin seedlings in CC site and the east origin ones in TA site), although values remain low. Seagrasses show a variety of responses to herbivore damage, involving both resistance responses by developing traits to reduce leaf palatability and compensation-tolerance responses (Strauss and Zangerl, 2002). Particularly, increasing phenolic compounds in leaf tissue can act as a resistance-based response, lowering plant palatability and decreasing herbivory preference or performance (Kumar et al., 2020; Martínez-Crego et al., 2015), consistently to other seagrasses (Jiménez-Ramos et al., 2024). On the other hand, seagrasses can tolerate herbivory pressure by increasing morphometrical traits as a compensation response, as grazing can stimulate the production of new photosynthetic tissue (Sanmartí et al., 2014; Valentine et al., 1997; Vergés et al., 2008). Interestingly, here the seedlings more grazed had a higher leaf and root length development, also suggesting the acclimation response to compensate the loss of material (Jiménez-Ramos et al., 2024; Vergés et al., 2008).

## 5. Conclusion

### 5.1. Restoration implications

Considering that seagrass restoration does not deal only with plant success *per se*, but also with the interactions with the habitat-forming species (Balestri et al., 2021), results here gained indicated that the herbivory in the early-life stages of *P. oceanica* seedlings may not



**Fig. 5.** a) number of bites/seedling (mean ± SE) and b) total phenol content of *P. oceanica* seedlings (mean ± SE) for each site, origin, and herbivory.

necessarily influence their development because of the heterogeneous grazing which probably depends on plant palatability due to the seed origin. Evidence here gained supports the importance of the plant early-life stage structure remodelling as acclimation response to different environments and potential stressors (Hernán et al., 2017; Pansini et al., 2023; Stipcich et al., 2022, 2024c). Seedling-based restorations can take advantage of germinating the seeds directly in the field, without the use of aquaria. This emerging low-cost technique could become popular among stakeholders, considering that although the seeds were collected by beach-cast material, seed germination percentage was high (Mancini et al., 2024). Moreover, it could imitate natural ecological processes and guaranteeing genetic variability necessary for the species persistence and adaption to environmental changes (Kendrick et al., 2017; Reynolds et al., 2012). Here, both cold- and warm-adapted seedlings responded differently to thermal regime and herbivory pressure, but showing compensating responses, as the warm-adapted ones survived less but developed more, and *vice-versa*. Although defining the success of *P. oceanica* restoration requires several years of evaluation after transplanting (Boudouresque et al., 2021; Pansini et al., 2024) and further investigations on seedling performance under other ecological conditions are needed, the evidence here reported has provided indications about some restoration practical features.

In a context of changing Mediterranean Sea where *P. oceanica* sexual reproduction is intensified by climate change and seeds availability is increasing, this study promotes the collection of *P. oceanica* beach-cast fruits from different coasts, their germination in the field and their transplantation regardless their origin. Considering also that restoration would be integrated more frequently as a conservation management measure inside MPAs, where grazers are more abundant, this study also highlights the no need of protecting seedlings from predators with anti-herbivory cages, at least in the first stages of development.

#### CRedit authorship contribution statement

**Arianna Pansini:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Patrizia Stipcich:** Writing – review & editing, Methodology, Investigation. **Sara Frasca:** Writing – review & editing, Writing – original draft, Investigation. **Luciana Migliore:** Writing – review & editing, Resources. **Giulia Ceccherelli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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#### 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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106918>.

#### Data availability

Data will be made available on request.

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